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R. Z. Sayyed *Editor*

Plant Growth Promoting Rhizobacteria for Sustainable Stress Management

Volume 2: Rhizobacteria in Biotic Stress Management

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Volume 13

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Plant Growth Promoting Rhizobacteria for Sustainable Stress Management

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Foreword

त्रिलोचन महापात्र, पीएच.डी.
एफ एन ए, एफ एन ए एस सी, एफ एन ए ए एस
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Crop plants are subject to various types of biotic stresses right from the stage of seed germination till the harvesting stage. Attacks by a wide variety of already known and newly emerging pests, nematodes, and microorganisms are some of the major threats to the crop plants and therefore to the agriculture productivity. Plant diseases caused by different pathogens are known to cause loss of more than 30% crop yield, resulting in decreased agriculture produce of the country thus increasing the economic hardships of the farmers. Traditionally these plant diseases have been managed so far using various agrochemicals. However, the liberal, untargeted, and nonspecific use of these agrochemicals increases the cultivation cost of crops, besides posing threat to the health of human beings, soil, useful soil microflora, and environment. With increasing awareness of demerits of agrochemicals and benefits of organic agriculture and food safety, the use of plant bioinoculants that serves as biocontrol agents (against a wide variety of phytopathogens) besides plant growth promotion activity is now gaining significance as the best and eco-friendly alternative to the hazardous agrochemicals. Chemical-free management of pests and diseases, agro-ecosystem well-being, and health issues in humans and animals have become the need of the hour. The use of plant growth promoting rhizobacteria (PGPR) as biotic stress managers offers good management of plant diseases (biotic stress). They also provide induced systemic resistance (ISR) and systemic acquired resistance (SAR). Application of PGPR as bioinoculants can help in reducing the loss of crop yield due to the attack by various phytopathogens, and hence PGPR has gained considerable attention among researchers, agriculturists, farmers, and policymakers and consumers.

The book entitled ***Rhizobacteria in Biotic Stress Management*** contains 16 book chapters contributed by eminent researchers, scholars, and academicians from around the globe. It deals with the various mechanisms and strategies adopted by PGPR in managing the biotic stress, i.e., plant disease. Various mechanisms adopted by PGPR for the lysis of phytopathogens have been discussed in this book. The principal mechanisms, namely production of antibiotics, production of antifungal metabolites, induction resistance, seed biopriming, and plant small RNAs, have been encompassed in this book. This book highlights salient features on the application of PGPRs as effective managers of biotic stress (plant diseases) in agricultural crop plants to lend a hand to scientists working in this field. ***Rhizobacteria in Biotic Stress Management*** is a timely effort for sustainable agriculture. I compliment the authors and hope that the teachers and researchers working in this area would make use of this publication.

Dated the 19th February, 2019 **(T. Mohapatra)**
New Delhi

Preface

Achieving sustainable agricultural production while keeping the environmental quality and agro-ecosystem function and biodiversity is a real challenge in current agricultural practices. Crop plants are subject to a wide range of biotic stresses, and plant pathogens are the major biotic threats to the agriculture crops affecting quality and yield of crops. It is estimated that about 30% of crops are lost due to phytopathogen infestations. Phytopathogens also cause deficiency of variety of micronutrients in crops, and consumption of such staple crops has been one of the principal causes of micronutrient deficiency diseases. Traditional use of chemical inputs (fertilizers, pesticides, nutrients, etc.) poses serious threats to crop productivity, soil fertility, and the nutritional value of farm produce. Global concern over the demerits of chemicals in agriculture has diverted the attention of researchers towards sustainable agriculture by utilizing the potential of plant growth promoting rhizobacteria (PGPR). Therefore, management of pests and diseases, agro-ecosystem well-being, and health issues for humans and animals has become the need of the hour. The use of PGPR as biofertilizers, plant growth promoters, biopesticides, and soil and plant health managers has gained considerable attention among researchers, agriculturists, farmers, and policymakers and consumers.

Application of PGPR as a bioinoculant mitigating the biotic stresses can help in plant growth promotion and disease control thus leading to more crop yield and can help in meeting the expected demand for global agricultural productivity to feed the world's booming population, which is predicted to reach around 9 billion by 2050. However, to be a useful and effective bioinoculant, PGPR strain should possess high rhizosphere competence, safety to the environment, plant growth promotion and biocontrol potential, compatibility with useful soil rhizobacteria, and broad-spectrum activity and be tolerant to various biotic and abiotic stresses. In the light of the above properties, the need for a better PGPR to complement increasing agro-productivity as one of the crucial drivers of the economy has been highlighted.

PGPR-mediated biotic stress management is now gaining worldwide importance and acceptance as eco-friendly and effective bioinoculants for sustainable agriculture. However, the performance of PGRR is subject to various abiotic factors such as salinity, temperature (high/low), drought, metal ions, and presence of various toxic compounds. Only those PPGR that establish themselves and can manage such abiotic stress can perform better as plant growth-promoting and biocontrol agents.

The prime aim and objective of this book is to highlight salient features on the application of PGPRs as biotic stress managers of agricultural crop plants to lend a hand to scientists throughout the world working in this field. PGPR in biotic stress management is a timely effort for sustainable agriculture. PGPR also provide excellent tools for understanding the stress tolerance, adaptation, and response mechanisms that can be subsequently engineered into crop plants to cope with climate change-induced stresses.

This book is composed of 19 chapters encompassing the influence of various abiotic factors on the performance of PGPR to comprehend the information that has been generated on the abiotic stress alleviating mechanisms of PGPR and their abiotic stress alleviation potential. Agricultural crops grown on saline soils suffer on an account of high osmotic stress, nutritional disorders and toxicities, poor soil physical conditions, and reduced crop productivity. The various chapters in this book focus on the enhancement of productivity under stressed conditions and increased resistance of plants against salinity stress by application of PGPR.

It has been an immense pleasure to edit this book, with continued cooperation of the authors. We wish to thank Dr. Mamta Kapila, Ms. Raman Shukla, and Mr. Sivachanrda Ravanan at Springer, India, for their generous cooperation in the completion of this book.

Shahada, Nandurbar, Maharashtra, India R. Z. Sayyed

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Naveen Kumar Arora, PhD in Microbiology, Professor and Head of the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh, India, is a renowned researcher in the field of environmental microbiology and biotechnology. His specific area of research is rhizosphere biology and plant growth-promoting rhizobacteria (PGPR). He has more than 60 research papers published in premium international journals and several articles published in magazines and dailies. He is editor of 15 books, published by Springer, and a member of several national and international societies, member of the editorial board of four journals, and reviewer of several international journals. He is also the Editor in Chief of the journal *Environmental Sustainability* published by Springer Nature. He has delivered lectures in conferences and seminars around the globe. He has been advisor to 118 postgraduate and 9 doctoral students. He has also received awards for excellence in research by the Honorable Governor of Uttar Pradesh, Asian PGPR Society, and Samagra Vikas Welfare Society. Although an academician and researcher by profession, he has a huge obsession for the wildlife and its conservation and has authored a book, *Splendid Wilds*. He is President of the Society for Conservation of Wildlife and is also Secretary of the Society for Environmental Sustainability (website: www.ses-india.org).

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Biosynthesis of Antibiotics by PGPR and Their Roles in Biocontrol of Plant Diseases

1

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Abstract

Plant growth-promoting rhizobacteria (PGPR) plays an essential role when it comes to protection of crop, promoting growth, and improvement on soil health status. There are some prevalent PGPR strains such as *Pseudomonas*, *Bacillus*, *Azospirillum*, *Rhizobium*, and *Serratia* species. The key mechanism of biocontrol by PGPR is the involvement of antibiotics production such as phenazine-1-carboxylic acid, 2,4-diacetyl phloroglucinol, oomycin, pyoluteorin, pyrrolnitrin,

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kanosamine, zwittermicin-A, and pantocin. The cascade of endogenous signals such as sensor kinases, N-acyl homoserine lactones, and sigma factors regulates the synthesis of antibiotics. The genes which are responsible for the synthesis of antibiotics are greatly conserved. The antibiotics of this PGPR belong to polyketides, heterocyclic nitrogenous compounds, and lipopeptides which have broad-spectrum action against several plant pathogens, affecting crop plants. Though antibiotics play a vibrant role in disease management, their role in biocontrol is questioned due to limitations of antibiotic production under natural environmental conditions. In addition to direct antipathogenic action, they also serve as determinants in prompting induced systemic resistance in the plant system.

Keywords
PGPR · Antibiotics · Secondary metabolites · Biocontrol · Plant disease

1.1 Introduction

Biological control is the utilization of variously beneficial microorganisms that are biological enemies, neutral or antagonistic of a pest or pathogen, to suppress or kill its harmless results on plants or products. Nowadays, the agricultural industry faces challenges, for example, reduction of soil fertility, climate change, and increased pathogen and pest attacks (Gopalakrishnan et al. 2015). In this manner, environmentally sound crop protection techniques are our future core interest. Expanding worries over the utilization of chemical and synthetic fertilizers and pesticides. Demand for ecologically stable and sustainable approaches for crop production. Sustainability and environmental safety of horticulture business depend on eco-accommodating methodologies like biofertilizers, biopesticides, and crop residue return. Plant growth-promoting rhizobacteria (PGPR) assume an essential part in crop protection, in growth promotion, and in the change of soil well-being (Liu et al. 2017; Beneduzi et al. 2012). Some outstanding PGPR strains are *Pseudomonas*, *Bacillus*, *Azospirillum*, *Rhizobium*, and *Serratia* species which show a major role to inhibit or kill pathogenic microorganism by producing specific or mixtures of antibiotics. Usage of microbial antagonist has been proposed as another way to combat against plant pathogens in agriculture crops aside from chemical pesticides. PGPR is known to control an extensive variety of plant pathogens like bacteria, fungi, viruses, bug irritations, and nematodes. PGPR is a standout among the best and environmental friendly for the plant disease management (Coy 2017; Liu et al. 2017).

PGPR as biocontrol specialists were preferred over conventional chemical control strategy, on the grounds that PGPR are nontoxic naturally occurring microorganisms, their application is feasible, and they can stimulate plant development and soil health, but they are also involved in abiotic and biotic stress tolerance. Another favorable position of PGPR is that they have different scopes of methods of activity,

namely, they are involved in antibiosis; act as cell divider debasing compounds, biosurfactants, and volatiles; and furthermore prompt fundamental obstruction in plants. The utilization of PGPR inoculants as biofertilizers is because of the creation of some plant development advancing substances, production of compounds, and generation of some antifungal and antibacterial secondary metabolites and as antagonists of phytopathogens is because of discharge of antibiotics which gives a promising method to chemical fertilizers and pesticides. Antibiotic is described as a heterogeneous grouping of low-molecular-weight organic complex that is harmful to the development or metabolic exercises of different microorganisms (Kumar et al. 2015). The antibiotics were more effective in smothering the development of target pathogen in vitro and in situ. The creation of at least one antibiotic production is the most imperative component of plant development advancing rhizobacteria which encourage the opposing against numerous phytopathogens (Glick, et al. 2007). The antibiotics are categorized into volatile and nonvolatile complexes. The volatile antibiotics include alcohols, aldehydes, ketones, sulfides, and hydrogen cyanide, and the nonvolatile antibiotics are polyketides, cyclic lipopeptide amino polyols, phenylpyrrole, and heterocyclic nitrogenous compound (Gouda et al. 2017; Fernando et al. 2018). This antibiotic production has antiviral, antimicrobial, insecticidal, antihelminthic, phytotoxic, antioxidant, and cytotoxic effect and promotes plant growth (Ulloa-Ogaz et al. 2015; Fernando et al. 2018).

1.2 Intrinsic Antibiotic Resistance

The soil is an oligotrophic environment, which is an excellent habitat for the growth of microorganisms and maintaining their biodiversity. As the microbial load gets bigger, microbes usually compete for nutrients and strive trying to colonize their habitat (ecosphere) (Song et al. 2005; Demanèche et al. 2008; Allen et al. 2009; Philippot et al. 2010; Arora et al. 2013a). Therefore, different species have developed varied strategies to secure their needs and ensure their survival. The production of antibiotics, which are heterogeneous, low-molecular-weight, and toxic organic compounds that affect the activities of other microorganisms, is one important strategy and an important means of competition among different microbial strains (Duffy 2003). These metabolites have shown diverse properties such as antimicrobial, antihelminthic, phytotoxic, antiviral, antioxidant, cytotoxic, antitumor, and plant growth-promoting compounds (Kim 2012). Furthermore, the development of intrinsic antibiotic resistance (IAR) was a crucial mechanism to encounter the effect of another aggressive microorganism. Both strategies determine the fitness of a strain in a population and secure its survival (Nesme and Simonet 2015). The production of one or more antibiotic is usually detrimental for the competition between microorganisms in any ecosphere including plant growth-promoting rhizobacteria (PGPR) in their rhizosphere, allowing for better colonization and enhancing microbial efficiency (Sharma et al. 2017). In addition, PGPR antibiotics are produced as important antagonistic agents against phytopathogens (Glick et al. 2007; van Loon 2007; Sharma et al. 2017).

As the IAR pattern of a bacterial strain, generated by testing it against low concentrations of antibiotics, was found to be stable property, many researchers have used IAR as a classification method in order to differentiate between closely related isolates. The strain-specific IAR profile was widely accepted to group the closely related bacterial isolates that belong to the same serological group of the same species as IAR profile was found to be strain specific rather than a species-specific feature (Amarger et al. 1997). For example, different populations of PGPR rhizobial isolates were studied using numerical taxonomy, and the isolates were grouped using IAR profile (Atta et al. 2004; Atta 2005; Degefu et al. 2018). In addition, IAR profiling technique was also used to characterize rhizobial strains that nodulate *Trifolium alexandrinum* and *Phaseolus vulgaris* according to their resistance to different antibiotics (Nassef 1995). The diversity of rhizobia associated with *Amorpha fruticosa* isolated from Chinese soils was investigated using different phenotypic and genotypic techniques using the IAR patterns analysis. As a result, *Mesorhizobium amorphae* was described as a new species (Wang et al. 1999).

Several classes of antibiotics were found to be produced in the soil by PGPRs such as phenazines, phloroglucinol, pyoluteorin, pyrrolinitrin, cyclic lipopeptides, and volatile HCN (Hass and Defago 2005). In addition, the biosurfactants of *Pseudomonas* and *Bacillus* species were used as biocontrol agents against plant diseases (Raaijmakers et al. 2010). The mechanisms by which these antibiotics are working are partly understood; the main effects of antibiotics include inhibition of cell wall synthesis, the arrest of ribosomal RNA formation, deformation of cellular membranes, and inhibition of protein biosynthesis (Maksimov et al. 2011).

1.3 Major Antibiotics of PGPR

Antibiotics production (antibiosis) by PGPR plays an important role in the management of plant diseases. The process has been defined as the inhibition or suppression of pathogenic microorganisms via the production of low-molecular-weight compounds (antibiotic) by other microorganisms.

Bacillus species and fluorescent pseudomonas are playing active roles in the suppression of pathogenic microorganisms by producing extracellular metabolites that have inhibitory and antagonistic effects against their competitors. Additionally, to the direct antagonistic action, antibiotics have a vital role in induced systemic resistance (ISR) mechanism in plants.

Different microorganisms have the ability to produce different antibiotics, for example, PGPR (*Bacillus* species) produces several antibiotics that comprise iturins, mycosubtilin, bacillomycin D surfactin, fengycin, and zwittermicin A, whereas antibiotics produced by fluorescent pseudomonads include 2,4-diacetyl phloroglucinol (DAPG), pyoluteorin, phenazines, pyrrolnitrin, oomycin A, viscosin, and massetolide A.

1.3.1 Polyketides

1.3.1.1 2,4-Diacetyl Phloroglucinol (DAPG or Phl)

DAPG or Phl is a phenolic polyketide compound that is produced by many fluorescent pseudomonads and has antifungal, antibacterial, antihelminthic, and phytotoxic activities (Harrison et al. 1993; Gaur 2002).

Phl is a major determinant in the biocontrol activity of plant growth-promoting rhizobacteria. Take-all diseases of wheat caused by *Gaeumannomyces graminis* var. *tritici* can be naturally suppressed by take-all decline (TAD) caused by strains of *P. fluorescens* that produce the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG) (Raaijmakers and Weller 1998; Weller et al. 2002; Weller et al. 2007). Some strains of *P. fluorescens* inhibit several soil-borne pathogens that cause diseases such as damping off, root rot, take-all, and other wilting diseases (McSpadden Gardener 2007). 2,4-Diacetylphloroglucinol (DAPG) produced from some strains of *P. fluorescens* had a nematicidal effect (Meyer et al. 2009; Siddiqui and Shaukat 2003). Production of DAPG by *Pseudomonas* sp. LBUM300 plays a vital role in the biocontrol of bacterial canker of tomato caused by *Clavibacter michiganensis* subsp. *michiganensis* (Lanteigne et al. 2012).

The mode of action of Phl is still unclear, although it is known that the interaction between Phl-producing root-associated microorganisms and the pathogens is a major reason for disease suppression. Phl also elicits ISR in plants. Thus, Phl-producing microorganisms can act as specific elicitors for the production of phytoalexins and other similar molecules in plant-disease biocontrol (Dwivedi and Johri 2003).

The molecular basis for the production of Phl has been studied, and five complete open reading frames (ORFs) and one partial ORF with a molecular size of 6.8 kb were found responsible for the biosynthesis of Phl (Bangera and Thomashaw 1996). The genes phlA, phlC, phlB, and phlD are located within a large transcriptional unit transcribed in the same direction. phlD is the polyketide synthase gene that is necessary for the synthesis of the DAPG precursor monoacetylphloroglucinol (Bangera and Thomashaw 1996). phlE gene secretes a red pigment, which is responsible for transportation of Phl out of the cell and is placed downstream of phlD (Delany et al. 2000). Another divergently transcribed gene, phlF, is located 421 bp upstream of biosynthetic genes and consists of an ORF of 627 bp with a corresponding protein of 209 amino acids, with the expected molecular mass of 23,570 Da. The Phl operon is regulated by a repressor molecule of PhlF that exhibits a helix–turn–helix DNA binding motif. phlO is a specific sequence of 30 bp that exists downstream of phlA. The interaction between PhlF repressor protein and this sequence results in repression of Phl operon (Cook et al. 1995; Bangera and Thomashaw 1996; Delany et al. 2000).

Biosynthesis of a polyketide Phl occurs by condensation of three molecules of acetyl CoA with one molecule of malonyl CoA to produce the precursor monoacetylphloroglucinol (MAPG), which is subsequently transacetylated to generate Phl (Dwivedi and Johri 2003).

1.3.1.2 Pyoluteorin (Plt)

Pyoluteorin (Plt) is a phenolic polyketide with a resorcinol ring. The ring is coupled to a bichlorinated pyrrole moiety (Fernando et al. 2005). Several strains of *Pseudomonas* sp. that produce Plt suppressed plant diseases caused by phytopathogenic fungi (Maurhofer et al. 1994; Kraus and Loper 1995). Most of oomycete pathogens such as *Pythium ultimum* were inhibited by Plt. Nowak-Thompsan et al. (1999) reported that the severity of *Pythium* damping-off decreased when Plt-producing pseudomonads were applied to seeds. Pyoluteorin produced by *P. putida* was more effective in reducing symptoms of red root rot disease caused by *Glomerella tucumanensis* in sugar cane (Hassan et al. 2011).

Ten open reading frames, pltLABCDEFGMR, are involved in the biosynthesis of Plt with a molecular size of 24 kb in *P. fluorescens* Pf-5. Among these ten genes, pltB and putC are responsible for the synthesis of type 1 polyketide synthase, pltG synthesizes thioesterase, and pltA, pltD, and pltM are involved in the biosynthesis of three halogenases (Dwivedi and Johri 2003).

Plt biosynthesis starts from proline, which acts as a precursor for dichloropyrrole moiety of Plt. Proline condenses with three acetate equivalents linked to chlorination and oxidation. The action of a single multienzyme complex is responsible for the formation and cyclization of the C-skeleton (Cuppels et al. 1986; Nowak-Thompsan et al. 1999).

1.3.2 Heterocyclic Nitrogenous Compounds

Heterocyclic pigments containing nitrogen known as phenazines, which are low-molecular-weight metabolites, are produced by a restricted number of bacterial genera including *Pseudomonas*, *Burkholderia*, *Brevibacterium*, and *Streptomyces* (Leisinger and Margraff 1979; Turner and Messenger 1986; Budzikiewicz 1993; Huang et al. 2011; Chen et al. 2014; Dasgupta et al. 2015). Greater than 50 naturally occurring phenazine compounds have been studied. Some bacterial strains are capable of producing mixtures of different phenazine derivatives at one time (Turner and Messenger 1986; Smirnov and Kiprianova 1990; Guttenberger et al. 2017). For instance, *P. fluorescens* 2–79 produces essentially PCA (phenazine-1-carboxylic acid), whereas *P. aureofaciens* 30–84 not only produces PCA but also minor amounts of 2-hydrozyphenazine. Pyocyanin (1- hydroxy-5-methyl phenazine) is a major phenazine biosynthesized by *P. aeruginosa* (Wienberg 1970); also *P. aeruginosa* has the ability to biosynthesize other phenazine compounds, including phenazine-1-carboxylic acid (PCA), 1-hydroxyphenazine (1-OH-PHZ), and phenazine-1-carboxamide (PCN).

Phenazines produced by several strains of PGPR pseudomonads have antibiotic and antitumor properties; they are involved with their capability to control plant pathogenic fungi and nematodes (Chin-A-Woeng et al. 2000; Mavrodi et al. 2001, 2006; Pierson and Pierson 2010; Cezairliyan et al. 2013; Zhou et al. 2016). Phenazine-1-carboxylic acid (PCA) produced by *P. fluorescens* 2–79 and *P. aureofaciens* 30–84 plays a vital role in biocontrol of take-all disease of wheat caused by *G. graminis* var. *tritici* (Thomashow and Weller 1988; Ju et al. 2018). Tomato foot and root rot are caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* and rice pathogens, *Rhizoctonia solani Kühn* and *Xanthomonas*

oryzae pv. *oryzae*, suppressed by phenazine-1-carboxamide (PCN) produced from *P. chlororaphis* PCL1391 and *P. aeruginosa* MML2212 (Chin-A-Woeng et al. 2000; Shanmugaiah et al. 2010). Phenazine-1-carboxylic acid and phenazine-1-carboxamide produced by *P. aeruginosa* PNA1 (wild type) are essential compounds in the control of root rot of cocoyam caused by *P. myriotylum* (Tambong and Hofte 2001). Phenazine-1-carboxylic acid and pyocyanin produced by *P. aeruginosa* revealed antagonistic activity against *Aspergillus niger* NCIM 1025, *F. oxysporum* NCIM 1008, *Sclerotium rolfsii* NCIM 1084, *R. solani*, and several other phytopathogens (Rane et al. 2007; Abo-Zaid 2014). Yu et al. (2018) reported that phenazine derivatives produced by *P. chlororaphis* 30–84 are necessary for their ability to inhibit plant pathogenic fungi.

1.3.3 Mode of Action of Phenazine

The wide-range activity demonstrated by phenazine pigments against fungi and other bacteria is not clear. However, it is assumed that pyocyanin can accept electrons that produce a relatively stable anion radical, which readily enters the redox cycle. Mn-containing superoxide dismutase (MnSOD) is a major enzyme that causes an increase in the production of O•2⁻ (superoxide radical), as illustrated in Fig. 1.1. There is a distinct possibility that the antibiotic action of pyocyanin is actually a result of toxicity of O•2⁻ and H2O2 produced in increased amounts in its presence (Mavrodi et al. 2001, 2006).

1.3.4 Phenazines Biosynthesis

Seven genes phzABCDEFG are involved in the synthesis of PCA that represents a 6.8 kb fragment in *P. fluorescens* 2–79 (Mavrodi et al. 1998). The precursor for phenazine biosynthesis is shikimic acid (Jin et al. 2016; Guo et al. 2017). The

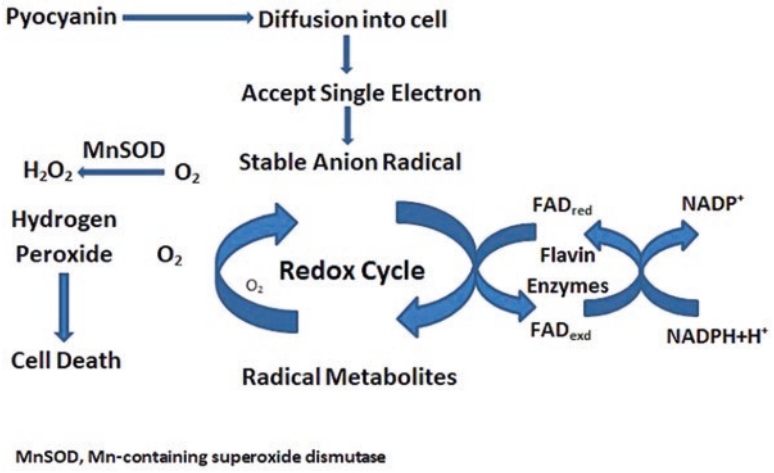


Fig. 1.1 Mode of action of pyocyanin (Abo-Zaid 2014)

symmetrical condensation of two molecules of chorismic acid forms phenazine nucleus (Chang and Blackwood 1969; Herbert et al. 1976), in which the amide nitrogen of glutamine serves as the immediate source of N in the heterocyclic nucleus. The first step is amination of chorismic acid to aminodeoxyisochorismate (ADIC) which is catalyzed by aminodeoxyisochorismate (ADIC) synthase (Fig. 1.2). The second step is the elimination of pyruvate and aromatization to form 3-hydroxyanthranilic acid, which is catalyzed by ADIC lyase (Morollo and Bauerle 1993). The products of phzF and phzG are involved in the condensation of two molecules of 3-hydroxyanthranilate to generate the phenazine nucleus. Spontaneous non-enzymatic decarboxylation is responsible for the conversion of phenazine-1,6-dicarboxylic acid to PCA probably by Mavrodi et al. (1998). Minor amounts of 2-hydroxyphenazine-1-carboxylic acid (2-OH-PCA) and small quantities of

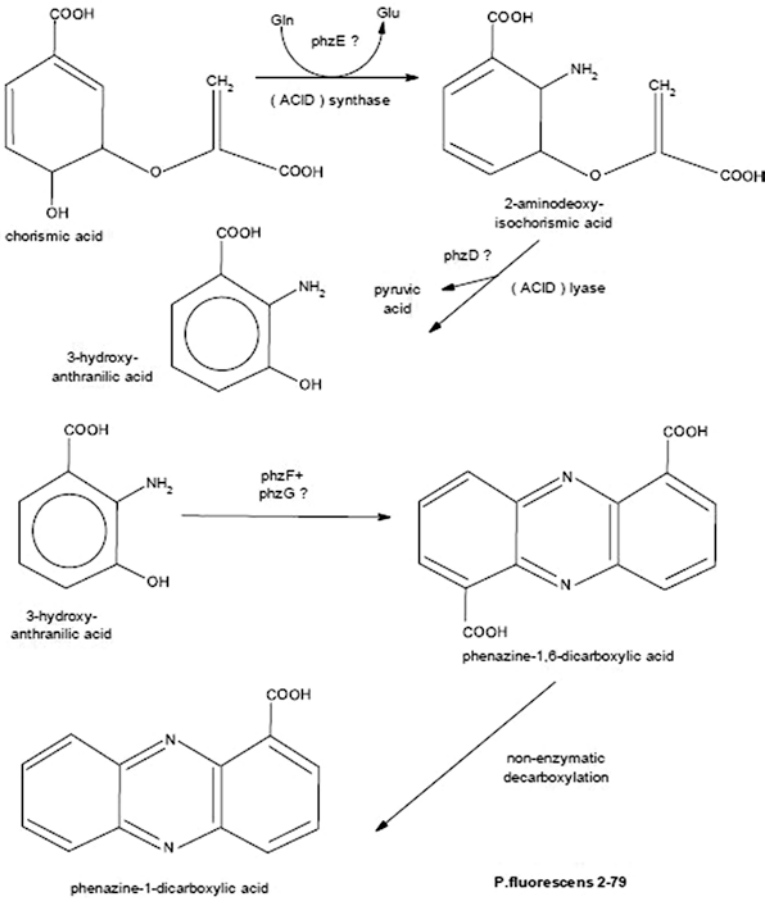


Fig. 1.2 The proposed biosynthetic pathway for the synthesis of phenazine-1-carboxylic acid in *Pseudomonas fluorescens* 2–79 (Abo-Zaid 2014)

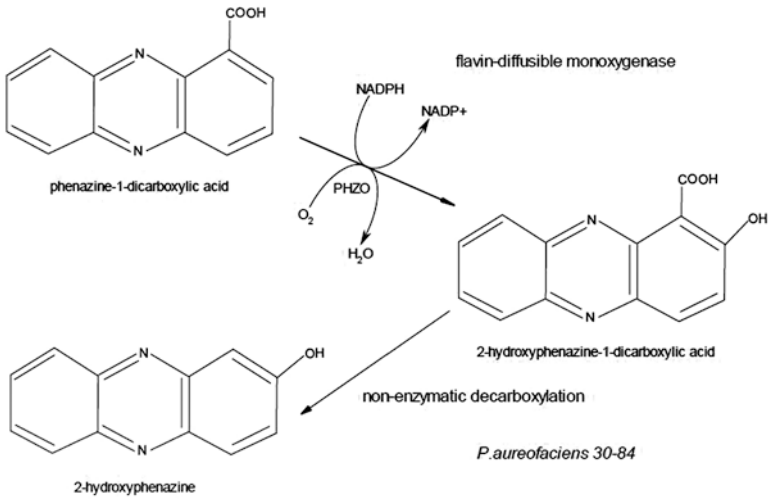


Fig. 1.3 The proposed biosynthetic pathway for the synthesis of 2-hydroxyphenazine in *Pseudomonas aureofaciens* 30–84 (Abo-Zaid 2014)

2-hydroxyphenazine are produced by *P. aureofaciens* 30–84 and *P. chlororaphis* GP72 in addition to PCA. phzO gene that codes flavin-diffusible monooxygenase is responsible for conversion of PCA to 2-OH-PCA in strain 30–84 which adds a hydroxyl group to PCA at ortho-position relative to carboxyl group (Fig. 1.3) (Delaney et al. 2001; Pierson and Pierson 2010; Huang et al. 2011; Chen et al. 2014). *P. aeruginosa* contains two operons (phzA1B1C1D1E1F1G1 and phzA2B2C2D2E2F2G2), which are responsible for the biosynthesis of PCA and three genes (phzM, phzS, and phzH) coding a set of enzymes that converts PCA to 5-methyl-phenazine-1-carboxylic acid (5MPCA), 1-hydroxy-phenazine (1OHPZ), PCN, and pyocyanin (Fig. 1.4) (Mavrodi et al. 2001, 2006; Greenhagen et al. 2008; Abo-Zaid 2014; Jin et al. 2016).

1.3.5 Phenylpyrroles

Many fluorescent and non-fluorescent strains of the genus *Pseudomonas* can produce pyrrolnitrin [3-chloro-4-(2′-nitro-3′-chloro-phenyl) pyrrole] that is a broad-spectrum antifungal metabolite. Prn was first studied and utilized as a clinical antifungal agent against dermatophyte fungus *Trichophyton* skin mycoses. Consequently, Prn was expanded as an agricultural fungicide (Elander et al. 1968). Its antifungal activity against *R. solani* and *F. graminearum* was reported (El-Banna and Winkelmann 1988; Huang 2017). Post-harvest diseases of apple and pear caused by *Botrytis cinerea* are suppressed by Prn (Janisiewicz and Roitman 1988; Evensen and Hammer 1993). In addition, Prn produced by *P. fluorescens* strains was sufficient in the reduction of the take-all decline of wheat (Tazawa et al. 2000).

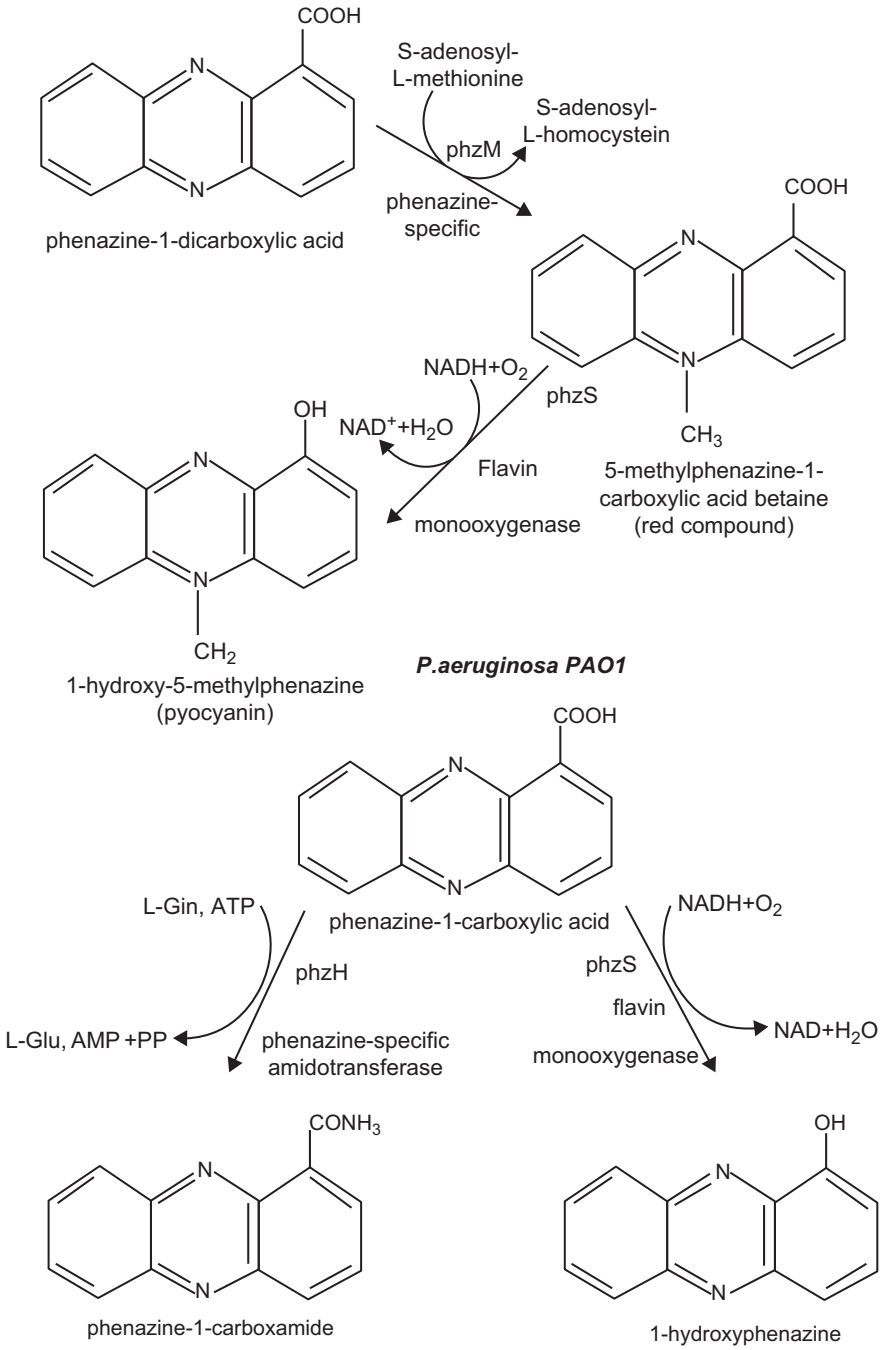


Fig. 1.4 The proposed biosynthetic pathway for the synthesis of pyocyanin, 1-hydroxyphenazine, and phenazine-1-carboxamide in *Pseudomonas aeruginosa* PAO1 (Abo-Zaid 2014)

Qing-Xia et al. (2016) illustrated that Prn produced by *P. fluorescens* FD6 isolated from the canola rhizosphere was able to inhibit *Monilinia fructicola*, the causal agent of peach brown rot. Prn of *P. chlororaphis* PA23 used as a biocontrol agent against the model nematode, *Caenorhabditis elegans* (Nandi et al. 2015).

Pyrrolnitrin inhibited the growth of *Saccharomyces cerevisiae*, *Penicillium atrovenetwn*, and *P. oxalicwn*. The primary site of action of Prn on *S. cerevisiae* was the terminal electron transport system between succinate or reduced nicotinamide adenine dinucleotide (NADH) and coenzyme-Q. At growth inhibitory concentrations and after its addition to the system, Prn inhibited endogenous and exogenous respiration immediately. In mitochondrial preparations, the antibiotic inhibited succinate oxidase, NADH oxidase, succinate-cytochrome C reductase, NADH-cytochrome C reductase, and succinate-coenzyme-Q6 reductase (Tripathi and Gottliep 1969).

The biocontrol agent, *P. fluorescens* BL915, containing one operon consists of four genes that are implicated in the biosynthesis of Prn from the precursor tryptophan (Hamill et al. 1970; Chang 1981; Xiaoguang et al. 2018). The prn operon with 5.8 kb (prnABCD) has been fully sequenced. It includes four ORFs, prnA, prnB, prnC, and prnD, which are localized on a single transcriptional unit (Qing-xia et al. 2016).

The first step in the biosynthesis of Prn is chlorination of tryptophan to result in 7-chlorotryptophan (7-CT). This step is catalyzed by a tryptophan halogenase enzyme that is synthesized by prnA gene. 7-CT is a catalyzed by-product of prnB to phenylpyrrole and decarboxylate to monodechloroamino pyrrolnitrin (MDA). The third step includes second chlorination in the three positions of pyrrole ring to form amino-pyrrolnitrin that is catalyzed by MDA halogenase synthesized by the prnC gene. The fourth step comprises of oxidation of amino group to a nitro group to form pyrrolnitrin that is catalyzed by enzyme coded by prnD (Fig. 1.5) (Van Pee et al. 1980).

1.3.6 Cyclic Lipopeptides of Pseudomonas sp.

Cyclic lipopeptides are adaptable metabolites produced by different genera of bacteria such as *Pseudomonas* and *Bacillus* (Nybroe and Sorensen 2004; Ongena and Jacques 2008; Raaijmakers et al. 2006). Fluorescent pseudomonades produce different kinds of CLPs (Nielsen et al. 2002). CLPs play an important role in seeds and roots colonization (Nielsen et al. 2005; Tran et al. 2007), in protection from competing microorganisms and predatory protozoa (Mazzola et al. 2009), and in swarming motility and biofilm creation (Raaijmakers et al. 2010). CLP biosynthesis is managed by large multi-modular non-ribosomal peptide synthetases (NRPS) through a thiotemplate process (Finking and Marahiel 2004; Raaijmakers et al. 2006; Zhao et al. 2018a, b). The composition of CLPs produced by *Pseudomonas* spp. including a fatty acid tail is linked to a short oligopeptide, which is formed in a lactone ring between two amino acids in the peptide chain (Raaijmakers et al. 2006; Zhao et al. 2018a, b). CLPs of *Pseudomonas* spp. were classified into four major groups (viscosin, amphisin, tolaasin,

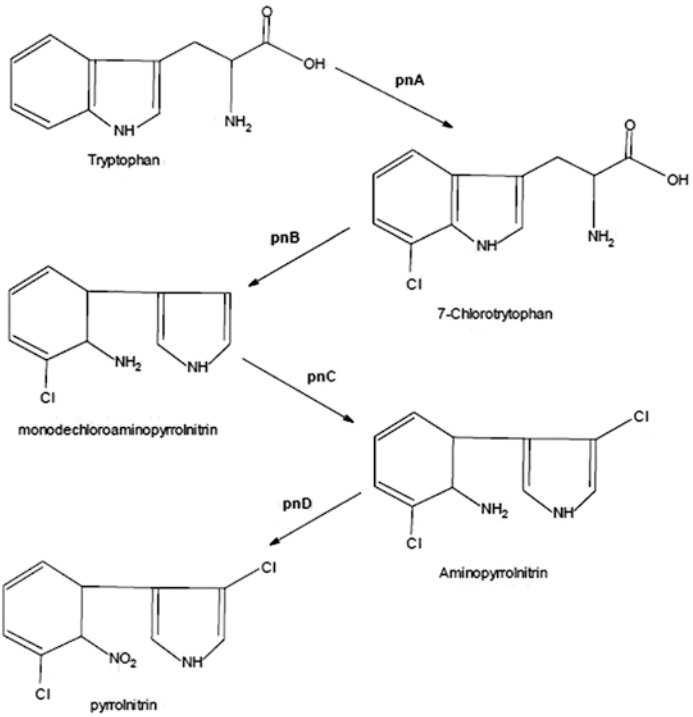


Fig. 1.5 The proposed biosynthetic pathway for the synthesis of pyrrolnitrin

syringomycin) according to the length and composition of the fatty acid tail as well as the number, type, and configuration of the amino acids in the peptide moiety.

1.3.7 Viscosin Group

Viscosin group contains CLPs with nine amino acids linked at the N-terminus, in most cases, to the 3-hydroxy decanoic acid (3-HDA) (De Bruijn et al. 2008). For example, viscosin has been described and identified for pectolytic strains of *P. fluorescens* causing head rot of broccoli (Hildebrand et al. 1998). In addition, massetolide A was first identified in a marine *Pseudomonas* species isolated from Masset Inlet, BC, Canada (Gerard et al. 1997). Zoospores of multiple oomycete plant pathogens are destructive when treated by massetolide A produced from PGPR *P. fluorescens* SS101 (De Bruijn et al. 2007; De Souza et al. 2003). Furthermore, massetolide A plays a vital role in the induction of systemic resistance response in tomato plants and root colonization by strain SS101 (Tran et al. 2007). Massetolide A is produced in the early exponential growth phase and is essential for swarming motility and biofilm formation of strain SS101 (De Bruijn et al. 2008). Three nonribosomal peptide synthetases, designated MassA, MassB, and MassC, is responsible for biosynthesis of massetolide A in strain SS101 (De Bruijn et al. 2008).

1.3.8 Amphisin Group

Amphisin group consists of 11 amino acids in the peptide part attached to 3-HDA. This group includes amphisin and tensin (Henriksen et al. 2000; Sorensen et al. 2001; Raaijmakers et al. 2006), which had antagonistic effects against *P. ultimum* (Thrane et al. 2000) and *R. solani* (Nielsen et al. 2002).

1.3.9 Tolaasin Group

There are multiple variations in the composition and length of the peptide chain (19 to 25 amino acids) and the lipid tail (3-HDA or 3-hydroxyoctanoic acid [3-HOA]) in the tolaasin group, which are different from viscosin and amphisin groups. The peptide part of the CLPs in this group includes several unusual amino acids, such as 2,3-dihydro-2-aminobutyric acid (Dhb) and homoserine (Hse). Five to eight amino acids are involved in the composition of the cyclic part of the peptide moiety, and the lactone ring is formed between the C-terminal amino acid and the all-Thr residue (Raaijmakers et al. 2006). Few tolaasin-like CLPs produced by plant-pathogenic strains of *Pseudomonas* are working as virulence factors.

1.3.10 Syringomycin Group

CLPs in the syringomycin group have similar structure to the CLPs in the viscosin group. On the other hand, syringomycin contains unusual amino acids, including Dhb, 2,4-diamino butyric acid (Dab), and C-terminal 4-chlorothreonine (Thr [4-Cl]), the latter being effective for the antifungal activity of syringomycin (Grgurina et al. 1994). Furthermore, the lactone ring is formed between the N-terminal Ser and the C-terminal Thr(4-Cl); being different from members of the viscosin group, the ring usually is formed between the C-terminal amino acid and the D-allo-Thr at the third amino acid position in the peptide chain. 3-Hydroxy or 3,4-dihydroxy fatty acid composed of 10–14 carbon atoms represents the fatty acid tail of CLPs in the syringomycin group (Bender et al. 1999; Bender and Scholz-Schroeder 2004; Raaijmakers et al. 2006).

1.3.11 Cyclic Lipopeptides of Bacillus sp.

Bacillus sp. produce small peptides with a long fatty moiety, the so-called cyclic lipopeptide antibiotics. Based on the structural relationship, the lipopeptides that have been identified in *Bacillus* spp. are generally classified into three groups: iturin group, surfactin group, and plipastatin-fengycin group (Zhao et al. 2014).

1.3.12 Iturin Group

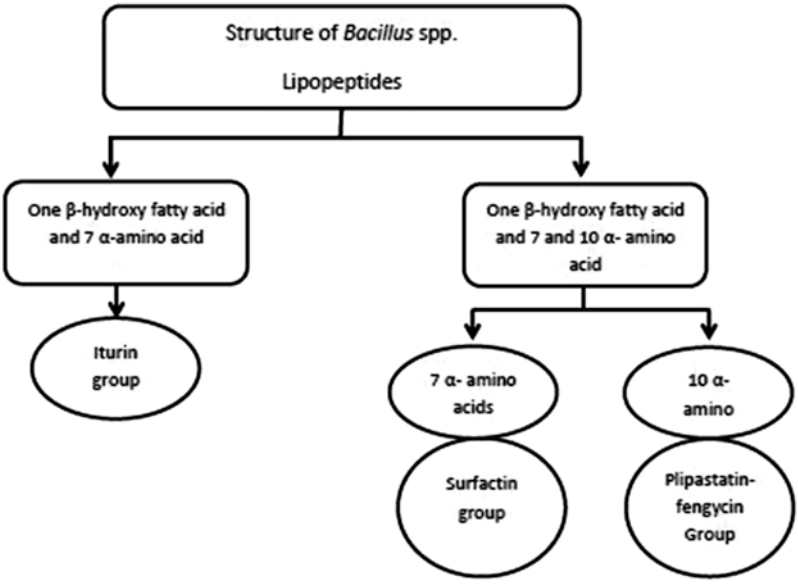
This group includes iturin A, bacillomycin L, bacillomycin D, bacillomycin F, and mycosubtilins. Iturin A as a molecule has a low molecular weight of ~ 1.1 kDa. Iturin A consists of a peptide chain composed of 7 amino acid residues linked to the hydrophobic tail of *β*-amino fatty acid chain that can vary from C-14 to C-17 carbon molecules (Fig. 1.6) (Meena and Kanwar 2015). Members of this group are produced from all strains of *Bacillus subtilis.* Four open reading frames, namely, ItuA, ItuB, ItuC, and ItuD, are responsible for the synthesis of iturin A that are located in one operon with a molecular size of 38–40 kb (Tsuge et al. 2001). Iturin A produced by *B. subtilis* RB14 was effective in reduction of damping-off of tomato caused by *R. solani*. Also, iturin A showed suppressing effect against *P. ultimum, F. oxysporum, S. sclerotiorum, M. phaseolina*, and *Podosphaera fusca* (Asaka and Shoda 1996; Constantinescu 2001; Romero et al. 2007). Overexpression of mycosubtilin in *B. subtilis* ATCC 6633 is involved in the reduction of seedling infection by *P. aphanidermatum* (Leclère et al. 2005).

1.3.13 Surfactin Group

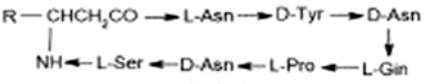
This group includes surfactin, esperin, lichenysin, and pumilacidin. Surfactin is a biosurfactant molecule with a molecular mass of 1.36 ~ kDa that is produced by several strains of *B. subtilis*. Surfactin consists of a peptide chain of 7 amino acids (Glu-Leu-Leu-Val-Asp-Leu-Leu) linked to *β*-hydroxy fatty acid of the chain length of 12 to 16 carbon atoms to form a cyclic lactone ring structure (Fig. 1.6) (Seydlova et al. 2011; Meena and Kanwar 2015). The type of surfactin might also vary based on amino acids and the size of lipid portion (Korenblum et al. 2012). Three large open reading frames (ORFs), namely, srfA-A, srfA-B, and srfA-C, encoding surfactin synthetases are responsible for biosynthesis of surfactin (Peypoux et al. 1999). Additionally, a fourth gene called srfA-D stimulates the initiation of the biosynthesis (Steller et al. 2004). Surfactin was able to reduce infection of *Arabidopsis* with *P. syringae* (Bais et al. 2004).

1.3.14 Fengycin Group

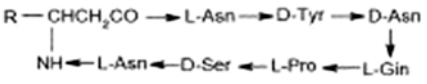
This group includes fengycin A, fengycin B, plipastatin A, and plipastatin B. Fengycin is a bioactive molecule that contains a peptide chain of 10 amino acids linked to *β*-hydroxy fatty acid chain that can vary from C-14 to C-17 carbon atoms with lactone ring (Fig. 1.6) (Akpa et al. 2001; Meena and Kanwar 2015). Five open reading frames, namely, fenC, fenD, fenE, fenA, and fenB, are responsible for the synthesis of fengycin that are located in one operon with a molecular size of 37 kb (Lin et al. 1999). Both iturins and fengycins showed an antagonistic effect against *P. fusca* infecting melon leaves (Romero et al. 2007).



- **Iturin group**

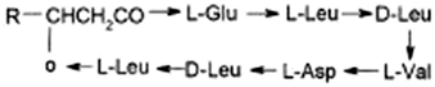


Iturin A



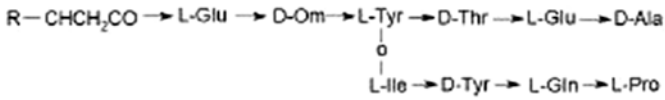
Mycosubtilin

- **Surfactin group**



Surfactin

- **Plipastatin-fengycin group**



Fengycin

R indicates an alkyl moiety (generally C_{14} to C_{17})

Fig. 1.6 Different types of Bacillus spp. lipopeptides of biological control activities

1.3.15 Aminopolyols (Zwittermicin A)

Zwittermicin A is known as an aminopolyol antibiotic produced by *B. cereus* group and has structural similarities to polyketide antibiotics with a wide range of actions against various microorganisms (Silo-Suh et al. 1998; Elizabeth et al. 1999; Sansinenea and Ortiz 2012). Zwittermicin A is used as antifungal against oomycete plant pathogens. Also, zwittermicin A produced by *B. thuringiensis* had insecticidal activity (Emmert et al. 2004). Kevany et al. (2009) reported 22 open reading frames (ORFs) with a molecular size of 62.5 kb related to ZmA biosynthesis by gene mapping the zma16Bc cluster from *B. cereus* UW85.

1.3.16 Volatile Antibiotics

There are several volatiles antibiotics such as hydrogen cyanide (HCN), aldehydes, alcohols, ketones, and sulfides, but HCN is the most important metabolite among them.

1.3.16.1 Hydrogen Cyanide (HCN)

Cyanide is a secondary metabolite produced by gram-negative *P. fluorescens, P. aeruginosa*, and *Chromobacterium violaceum* (Askeland and Morrison 1983). Hydrogen cyanide secreted by *P. chlororaphis* O6 demonstrates nematicidal activity against *Meloidogyne hapla* (Lee et al. 2011; Anderson and Kim 2018; Kang et al. 2018). In alfalfa, *P. putida* produced HCN to stop the infection by *F. solani* (Sarhan and Shehata 2014). Production of hydrogen cyanide (HCN) is an important biocontrol determinant (Haas and Defago 2005). The characterized set of genes hcnABC were found to be responsible for the biosynthesis of HCN in *P. fluorescens* strains Q2–87 and CHA0 (Haas and Defago 2005).

1.4 Regulation of Antibiotic Biosynthesis

1.4.1 GacS/GacA System

GacS/GacA double constituent signal transduction system manages essential pathogenicity and virulence mechanisms in numerous gram-negative bacteria (Zhang et al. 2018). Research of Gac-defective mutants has shown that several traits are controlled by these two constituent systems which comprise of motility, siderophores, pigment production, and lesion formation (Cha et al. 2012). De la Torre-Zavala et al. (2011) reported that phaseolotoxin biosynthesis includes elements within and outside the Pht cluster and that the GacS/GacA system regulates them. In that case, tox-phenotype gacA- and gacS- mutants were found and gacA- transcriptome analysis showed that this response activator regulates expression of genes within the Pht cluster and other gene placed in a different area in the bacterial chromosome and it has shown to be directly involved in the biosynthesis of phaseolotoxin.

1.4.2 Quorum Sensing

Quorum sensing (QS) is a molecular mechanism whereby bacteria is adapting their self according to cell density and neighboring atmosphere (Rémy et al. 2018). Normally, bacteria constantly produce signal beginning at a low concentration in a fresh culture and the signal gathers in the initial location as the population concentration upsurges (Abisado et al. 2018). The signal interrelates with a receptor protein triggering a synchronized alteration in gene expression in the population when a threshold concentration is reached. This will allow bacteria to perform processes that are expensive and non-effective at small cell concentration but that turn into valuable for the entire community at high cell density such as biofilm formation, virulence factor synthesis, protease, and production of siderophore (Heilmann et al. 2015). Gram-positive bacteria possess peptide-based QS systems agr system where *Staphylococcus aureus* is the most studied species. Effector purposes of agr are mainly regulated by RNAIII in which a regulatory RNA encoded by this operon and the phenotype and expression significantly affect the chronicity of an infection (Singh and Ray 2014).

1.4.3 Type VI Secretion System (T6SS)

Bacterial cells are able to interact with their surrounding atmosphere through secretion systems. Type VI secretion system (T6SS) is one of the most lately learned secretion systems, which is dispersed widely in gram-negative bacteria such as *Pseudomonas aeruginosa* (Chen et al. 2015). It was reported that the gene expression of H2-T6SS *P. aeruginosa* PAO1 WT strain is upregulated by the Las and Rhl QS systems (Sana et al. 2013). They concluded that T6SS is important for the survival of *P. aeruginosa* by bringing toxins to its surrounding pathogens, translocating protein effectors into the host cells, acting as a virulence factor, and taking part in biofilm formation. In general, T6SS is regulated at transcriptional, posttranscriptional, and posttranslational levels by diverse mechanisms in *P. aeruginosa* (Sana et al. 2013).

1.4.4 Virulence Factor Regulator

Virulence factor regulator (Vfr) is an associate of the cyclic 3′,5′-adenosine monophosphate (cAMP) receptor proteins that manage the expression of many vital virulence genes (Taguchi and Ichinose 2013). Regulation by Vfr permits the organized production of related virulence functions, such as type IV pili and type III secretion that are necessary for adherence to and intoxication of host cells, respectively (Marsden et al. 2016). Biochemical studies showed that antibiotics production of 2,4-diacetylphloroglucinol, pyrrolnitrin, and pyoluteorin was distinctly improved in the vfr mutant *P. fluorescens* FD6 (Zhang et al. 2016). These outcomes show that Vfr regulates the expression of several important traits and production of essential

antibiotics involved in the biocontrol potential of *P. fluorescens* FD6. It was also reported that vfr mutation improved swimming motility and biofilm production and exopolysaccharide-associated gene (pelA, pslA, and pull) transcripts expression (Taguchi and Ichinose 2013; Ventre et al. 2006).

1.5 Outer Membrane Protein Gene oprF

Soil-borne pathogen antagonization depends mainly on the production of secondary metabolites, such as pyoluteorin, siderophores, 2,4-diacetylphloroglucinol (2,4-DAPG), pyrrolnitrin, hydrogen cyanide, phenazines, and several lipopeptide compounds (Raaijmakers et al. 2010). Survival in harsh environments obliges bacteria to use their outer membrane to sense and response quickly to the extracellular environments. OprF of *Pseudomonas* spp. is the major OM protein involved in forming nonspecific channels for passive diffusion of ions, small polar nutrients, and even antibiotics (Nestorovich et al. 2006). Expression of the 2,4-DAPG biosynthesis enzymes, which are encoded by the phlACBD locus, is under the control of a delicate regulatory network. The previous study by Li et al. (2018) shows a novel role for the outer membrane protein gene oprF, in negatively regulating the 2,4-DAPG production by using random mini-Tn5 mutagenesis. SigX, a sigma factor gene, was located on the upstream of oprF gene revealed to be a positive regulator for oprF transcription and 2,4-DAPG production.

1.6 Control of Soil-Borne Disease Using PGPR Antibiotics

Administration of soil-borne disease relies upon an exhaustive learning of the pathogen, the host plant, and the natural conditions that support the infection. The diseases that are initiated by pathogens which stay in the soil and in residues on the soil surface are defined as soil-borne diseases (Veena et al. 2014; Landa et al. 2013). Soil-borne diseases are hard to manage since they are caused by a pathogen which can live for long times in the absence of the normal crop host. In this way, these diseases may not be seen until over-the-ground (foliar) parts of the plant are influenced by extremely indicating side effects, for example, hindering, shrinking, chlorosis, and demise. There are a few different sorts of disease caused by soil-borne plant pathogens, for instance, root rots; *Rhizoctonia* root rot disease; stem, collar, and head rots; wilts; shrinks; seedling blights; and damping-off diseases, pythium damping-off disease, and *Phytophthora* damping-off (Veena et al. 2014).

Crop losses due to plant diseases represent the main risk to food security worldwide. The outcome of losses ranges from a modest reduction of plant development measurements to more significant damage leading to plant death and decreased yield (Savary et al. 2012). To avoid or control such pathogenic microorganism and their pervasions, numerous methodologies have been attempted, including the improvement of resistant varieties through plant breeding, the invention of genetically modified resistant plants, and the practice of chemical enrolments such as

fungicides. However, all have restrictions. In addition, the existence of pesticide and fungicide leftovers on food may affect human well-being, which has also elevated significant concerns. The importance of antibiotic in biocontrol and in microbial antagonism has been addressed due to the imperatives to antibiotic production in regular habitat. Every one of these antibiotics has a different method of activity, some assault the cell layers, and others affect the ribosome or other cell constituents.

Antibiotic production by rhizobacteria species is one of the real components hypothesized for antifungal and plant development advancement. These antibiotics have appeared to assume a part in disease concealment in numerous biocontrol frameworks by mutant investigations and biochemical examinations utilizing purified antibiotics. These antimicrobial mixes may follow up on plant pathogenic microbes or growths by inducing fungistasis, inhibition of spore germination, and lysis of fungal mycelia (Adhya et al. 2018; Ulloa-Ogaz et al. 2015). Usage of microbial antagonists has been proposed as another way to combat against plant pathogens in horticulture crops aside from chemical pesticides. The importance of antibiotics application in biocontrol and in microbial antagonism has been addressed as a result of the requirements of antibiotics production in natural environments. Recuperation and discovery might be hampered by biotic and abiotic intricacy, substance precariousness of the compound, irreversible authoritative to soil colloids or natural issue, or microbial decay. The primary line of proof of the expansive range of activity of antibiotic agents by PGPR was gotten from purified antibiotics (Fernando et al. 2005). In numerous biocontrol frameworks, at least one antibiotic has appeared to assume a part in disease control or suppression. Molecular tool or genetic engineering has been successful here, because mutants faulty in antibiotics creation are effortlessly acquired, and in vitro examinations are helpful tests. The most broadly examined gathering of rhizospheric microbes as for the generation of antibiotics is that of the fluorescent pseudomonads (Fernando et al. 2005).

Antibiotics produced by PGPR include phenazine, 2,4-diacetylphloroglucinol (DAPG), surfactin, iturin, fengycin, bacilysin, pyrrolnitrin, pyoluteorin, hydrogen cyanide, iturin A, iturin D, bacillomycin D, fengycin A, pyrrolnitrin (3-chloro-4-[2′-nitro-3′ chlorophenyl]-pyrrole) pyrrolnitrin, viscosinamide, tensin, amphisin, triterpenoid soyasapogenol, bacillomycin, subtilin, and subtilosin (Table 1.1). The important antibiotic DAPG produced by *Pseudomonas fluorescens* has efficiently affected membrane destruction to *Meloidogyne incognita* and *Fusarium oxysporum f.* sp. *niveum. Pseudomonas* is biocontrol bacteria that presented antagonistic action including fungi, bacteria, protozoa, and nematodes by producing lipopeptide biosurfactant (Zihalirwa Kulimushi et al. 2017; Nielsin et al. 2003). *Pseudomonas* has the capacity to produce phenazine, 2,4-diacetylphloroglucinol (DAPG), and antibiotic, showing antagonistic activity against plant pathogen in watermelon, which are *Meloidogyne incognita* and *Fusarium oxysporum f.* sp. *niveum (*Meyer et al. 2016). A study done by Caulier et al. (2018) showed that antagonistic mixed soil bacteria can substitute the indiscriminate use of pesticide in potato farming. For example, the discovery of genes involved in bacilysin biosynthesis was associated with the strong antagonism of *Bacillus pumilus* strains toward *P. infestans*. The production of cyclic

Table 1.1 Antibiotics production by PGPR microorganism for management of soil-borne diseases

Antibiotics/functions	PGPR	Pathogen/disease	Crop/function	References
Phenazine, 2,4-diacetylphloroglucinol (DAPG)	*Pseudomonas fluorescens*	*Meloidogyne incognita* *Fusarium oxysporum f.* sp. *niveum*	Watermelon	Meyer et al. (2016)
Surfactin Iturin Fengycin	*-Bacillus velezensis*	*Ralstonia solanacearum* *Fusarium oxysporum*	Banana	Cao et al. (2018)
Volatile antibiotics	*Bacillus amyloliquefaciens subsp. plantarum XH-9*	*Fusarium oxysporum*	Wheat	Wang et al. (2018)
Bacilysin	*B. subtilis*	*Phytophthora infestans*	Potato	Caulier et al. (2017)
Phenazine, 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin, pyoluteorin, and hydrogen cyanide	*Fluorescent pseudomonads*	*Pythium aphanidermatum*	Turmeric	Prabhukarthikeyan and Raguchander (2016)
Pyrrolnitrin	*Pseudomonas aeruginosa*	*Rhizopus microsporus, Fusarium oxysporum, Alternaria alternata, Penicillium digitatum*	Plant growth promotion	Uzair et al. (2018)
Bacillus Peptide Antibiotics	*Bacillus*	*Fusarium graminearum*	Cereal crops	Khan et al. (2017)
Surfactin Iturin A Iturin D Fengycin Bacillomycin D.	*Bacillus subtilis*	*Wilt and root rot*	Chickpea	Smitha et al. (2017)
Bacillomycin D Fengycin A	*B. subtilis*	*Sclerotinia sclerotiorum*	Tomato	Abdeljalil et al. (2016)
Pyrrolnitrin (3-chloro-4-[2′-nitro-3′ chlorophenyl]-pyrrole)	*Pseudomonas fluorescens*	*Rhizoctonia solani*	Cotton seedlings	Howell and Stipanovic (1979).

Antibiotics/functions	PGPR	Pathogen/disease	Crop/function	References
Pyrrolnitrin Hydrogen cyanide	*Pseudomonas chlororaphis*	*Sclerotinia sclerotiorum.*	*Caenorhabditis elegans*	Nandi et al. (2015)
Viscosinamide Tensin Amphisin)	*Pseudomonas fluorescens*		Sugar beet	Nielsen and Sørensen (2003)
Triterpenoid soyasapogenol	*Rhizobium leguminosarum* bv. *viciae*	*Didymella pinodes*	Pea (*Pisum sativum*)	Ranjbar Sistani et al. (2017)
Rhizobium japonicum filtrate	*Rhizobium japonicum*	*Fusarium solani* *Macrophomina phaseolina*	Soybean	Al-Ani et al. (2012)
Surfactin Fengycin Iturin A Bacillomycin	*Bacillus subtilis*	*Podosphaera fusca*	Cucurbits	Romero et al. (2007)
Iturin Bacilysin Bacillomycin Surfactin Subtilin Subtilosin	*B. amyloliquefaciens*	*Sclerotinia sclerotiorum*	Carnation	Vinodkumar et al. (2017)
Fengycin	*Bacillus amyloliquefaciens* subsp. *plantarum*	*Rhizomucor variabilis*	Maize cobs	Zihalirwa Kulimushi et al. (2017)

lipopeptides (CLPs) with antibiotic and biosurfactant properties has been found in various microorganisms isolated from different habitat. Lipopeptides, such as viscosinamide, tensin, amphisin (Nielsen and Sørensen 2003), as well as fengycin, were isolated from *Pseudomonas fluorescens* and *Bacillus amyloliquefaciens* subsp. *plantarum*, respectively. Furthermore, Zihalirwa Kulimushi et al. (2017) found that a strain belonging to *Bacillus amyloliquefaciens* subsp. *plantarum* clade has the ability to generate varied antimicrobial compounds that participate in their effectiveness as biocontrol agents against plant fungal pathogens. In that context, the function of cyclic lipopeptides (CLPs) has been reported, but still little is known about the impact of interactions with other soil-inhabiting microbes on the expression of these molecules. In this work, the antagonistic activity is created by this bacterium against *Rhizomucor variabilis*, a pathogen isolated from diseased maize cobs.

Cao et al. (2018) isolated *Bacillus velezensis* from banana, which was found to suppress *Ralstonia solanacearum* and *Fusarium oxysporum*. The antibiotic compound was identified as surfactin, iturin, and fengycin. *Fluorescent pseudomonad* was isolated from turmeric; in soil naturally it can inhibit *Pythium aphanidermatum*. It was found to produce a variety of secondary metabolites, for example, phenazine, 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin, pyoluteorin, and hydrogen cyanide, which protect from diseases. This exploration expects to assess the execution of *fluorescent pseudomonads* against rhizome rot disease in turmeric plants. *Fluorescent pseudomonads* were screened against *Pythium aphanidermatum* utilizing double culture. Chosen strains were assessed for the execution of development advancing properties and the nearness of antimicrobial qualities through PCR examination (Prabhukarthikeyan and Raguchander (2016).

Pseudomonas chlororaphis strain PA23 is a biocontrol agent talented to inhibit the growth of the fungal pathogen *Sclerotinia sclerotiorom*. This bacterium generates several antibiotics including pyrrolnitrin, phenazine, hydrogen cyanide, and enzymes. Production of these mixtures of exometabolites is regulated at both the transcriptional and posttranscriptional levels by the Gac-Rsm system, RpoS, PsrA, and the Phz quorum-sensing system. Commonly, these outcomes demonstrated that PA23 is capable to recognize the presence of *C. elegans* and it can kill the nematodes, which ought to encourage natural ingenuity and eventually biocontrol (Nandi et al. 2015). Table 1.1 gives a few examples of antibiotics production by PGPR microorganism for management of soil-borne diseases.

1.7 Involvements of PGPR Antibiotics in Induced Systemic Resistance (ISR)

The rhizosphere, ecosphere of plant roots, is a complicated ecosystem, which is colonized by diverse groups of organisms including arthropod, fungi, bacteria, and nematodes (Venturi and Keel 2016). All of these organisms are interconnected through a coherent network of biochemical signals that link them to each other as well as to plants growing in the same rhizosphere (Mhlongo et al. 2018). PGPRs are essential for plant growth in either direct or indirect mechanisms. Several

publications have shed the light on these mechanisms, and it has been proven that the direct mechanisms including nitrogen fixation, nutrient acquisition, iron chelation, phytohormone production, and phosphate solubilization could indirectly help the plant. Similarly, the indirect mechanisms such as antibiotics for biocontrol and induced systemic resistance (ISR) execute other direct functions in favor of plant thriving. In other words, the two types of mechanisms function simultaneously reducing the boundaries between them (Zahir et al. 2004; van Loon 2007; Arora et al. 2013a). Recently, commercial microbial inocula (either single or a consortium) have been developed based on the advancement in plant-microbe interactions to enhance plant growth and development (Mhlongo et al. 2018). The common PGPR includes *Acinetobacter, Arthrobacter, Burkholderia, Bacillus, Enterobacter, Paenibacillus,* and *Pseudomonas* (Finkel et al. 2017; Sasse et al. 2017; Zhang et al. 2017; Mhlongo et al. 2018).

Induced resistance is a state of enhanced defensive capability that develops by a plant upon stimulation via biotic or abiotic cues (van Loon et al. 1998). The pathogen-related systemic acquired resistance (SAR) and rhizobacteria-mediated induced systemic resistance (ISR) are the two major components of plant-induced resistance (Pieters and Van Loon 1999; Bakker et al. 2003; Bakker et al. 2013). The two mechanisms have been integrated into the biological control process of plant diseases. The two mechanisms are mediated through Jasmonic acid, ethylene, and salicylic acid biosynthesis pathways (Dempsey and Klessig 2012; Denance et al. 2013). These hormones interact either antagonistically or synergistically to adjust the defense system (Koornneef and Pieterse 2008; Verhage et al. 2010; Nassem and Dandekar 2012). The production of a plethora of secondary metabolites that possess antibiotic activities (small phenolic molecules, flavonoids, alkaloids, cyaniding glycosides, etc.) in non-infected plants after receiving chemical signals from infected plants was documented as an ISR mechanism and the signal was the volatile methyl salicylic acid (Dempsey et al. 2011; Dempsey and Klessig 2012). Phenolic compounds have antimicrobial activity and can suppress microbial growth, and different phenolic metabolites are accumulated in the plant cells as phenolic glycosides such as salicylic acid glycosides and flavonoid glycosides, which are less toxic to plant cells than the aglycone. Upon infection, hydrolysis occurs releasing the aglycone, which is toxic for both plant cells and microbes (Kenawy 2016). In plant system, the defense responses may initiate cell wall thickening and lignification, deposition of callose, accumulation of antimicrobial low-molecular-weight substances (e.g., phytoalexins), and synthesis of various enzymes (chitinases, glucanases, peroxidases, and other stress-related proteins) that help plants to resist the pathogen (Hammerschmidt and Kuc 1982; Hammerschmidt et al. 1984; Kessmann et al. 1994; Sticher et al. 1997).

Several examples in the literature illustrate the role of PGPRs in ISR initiation in plants. Ongena et al. (1999) found that the induced resistance elicited by fluorescent pseudomonads could protect cucumber against pythium root rot, and two of the tested strains were found to increase cucumber growth. Peer et al. (1991) also found increased amounts of phytoalexins in *P. fluorescens* strain WCS417r inoculated plants when compared to the control plants. Leeman et al. (1996) have also found

that the lipopolysaccharide with the O-antigenic side chain produced by *P. fluorescens* strain WCS374 is involved in the induction of systemic resistance in radish against *Fusarium* wilt. In addition, the antibiotic pyocyanin induced ISR in radish against *Fusarium* wilt of tomato (Leeman et al. 1995; Audenaert et al. 2001, 2002). However, salicylic acid or pyocyanin mutant of wild-type *P. aeruginosa* 7NSK2 was defective in inducing plant resistance against *B. cinerea* (Audenaert et al. 2001). Similarly, bacteria in the genera *Streptomyces, Pseudomonas, Burkholderia*, and *Agrobacterium* suppress plant disease through production of antibiotics and induction of systemic resistance (Tenuta and Beauchamp 2003). Both pyoluteorin and DAPG negatively affected the growth of sweet corn, cress, and cucumber, and the stress response triggered by these antibiotics might cause plant resistance (Maurhofer et al. 1992). *P. fluorescens* protected tomato from wilt disease by accumulating the pool of DAPG in tomato root rhizosphere and might act as a signal to trigger ISR (Aino et al. 1997; Haas and Keel 2003).

N-Acyl homoserine lactones (AHLs) are signaling molecules that were reported to affect plant physiology and initiate plant defense via the accumulation of plant secondary metabolites. For example, in barley endophytic *Acidovorax radicis* N35 rhizobacteria producing 3-hydroxydecanoyl-homoserine lactone induced defense responses and caused accumulation of flavonoids such as saponarin and lutonarin (Pierson et al. 1998; Han et al. 2016). The growing understanding of the signaling role of AHL in the production of antimicrobial metabolites through quorum sensing and the identification of promoters that can be induced in the rhizosphere can open new areas for the development of novel biocontrol agents. The development of a formulation of PGPR consortia possess compatible signaling mechanism between the bacterial strains and sensitive receptors in the plant rhizosphere, which can perceive the signals and will elicit resistance in the plant against pathogens (Pierson et al. 1998; Arora et al. 2013b).

Several in vitro studies showed that antibiotic-producing PGPRs are efficient in suppressing plant pathogens. However, the antibiotics are produced in very low concentrations in the rhizosphere and below the minimal inhibitory concentration. Nevertheless, the antibiotic producers are still able to control plant diseases, and this might be via the involvement of very low concentration antibiotic-mediated systemic resistance or due to the interaction of antibiotics with other extracellular metabolites that may trigger ISR (Fernando et al. 2005). More studies are needed to explore the interaction between antibiotics and other components of ISR.

1.8 Conclusions

Nature is the most precious gift because it is rich in different kinds of PGPR. Some of the well-known microfloras that are present in the PGPR community are *Pseudomonas* sp. and *Bacillus* spp. Many research on this PGPR over the decades resulted in the introduction of many well-characterized *Pseudomonas* spp. This ironically helps to have a deep understanding of the regulation and organization of the biosynthetic gene clusters that involved predominantly in antibiotics production.

Broad knowledge on the regulation of antibiotics can help in the development of PGPR with improved efficiency and reliability. On top of that, the molecular communication between the different species of PGPR helps much when it comes to the selection of the compatible strains that can be released under some field conditions.

Research about the communication between different types of antibiotic and its interaction with the abiotic environment, plant pathogens, and the plant is still at its beginning stage. But the intensification of the research in the field can help in the better understanding level about the interaction of PGPR, pathogen, plant, and the abiotic environment around the rhizosphere. This will be very helpful for the fellow researchers to make a good conclusion on figuring out the best biocontrol agents which overcome the negative crosstalk in the environment around the rhizosphere. Moreover, the knowledge on the antibiotic genes and the ecology of these organisms in its natural environment can help to introduce the non-indigenous strains, and in addition to that, it also helps to select the biocontrol strains which can be suitable for different ecological conditions and for different species of the crop.

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2 Effect of Substrates on *Azotobacter chroococcum*-Enriched Vermicompost for Growth of *Phaseolus*

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Abstract

Inoculation of *Azotobacter chroococcum* in preparing organic compost by vermicomposting using *Eisenia fetida* (common names: red worm, brandling worm, panfish worm, trout worm, tiger worm, red wiggler worm, red Californian earthworm) can promote the growth of the *Phaseolus* bean. Various abiotic stresses, such as drought and salinity, are among the major environmental constraints that limit growth, productivity, and quality of plants. The growth promotion of *Phaseolus* bean with inoculation of *A. chroococcum* in the presence of vermicompost using different substrate combinations was assessed by a number of nodules, shoot length, root length, dry shoot weight, dry root weight, and nitrogen, phosphorus, and potassium (NPK) content of the plant. Among different substrates used, cow dung only and cow dung plus straw could be enriched with *A. chroococcum* with survival period up to 5 weeks. However, cow dung plus chopped grass and cow dung plus ground grass couldn't be enriched with *A. chroococcum*. A significant positive response was noted in all growth parameters when the plant was inoculated with *A. chroococcum* in the presence of vermicompost compared to the untreated control plants. Cow dung plus straw had been used as a substrate for the enrichment of vermicomposting with *A. chroococcum*.

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Keywords
Vermicomposting · *Azotobacter* · Abiotic stress · Nodules · Untreated · Enrichment

2.1 Introduction

Optimal growth of plants requires nutrients in sufficient and balanced quantities in available form (Atlas and Bartha 2000). Soil contains a natural reservoir of essential nutrients for plants, but these nutrients are not directly available to the plants (Khan et al. 2009). As a result, primary nutrients nitrogen (N), phosphorus (P), and potassium (K) are utilized by crops in large amounts and are commonly made available in the form of biofertilizers nowadays. Application of biofertilizers for crop production is environmentally friendly and sustainable for the ecological system. Several types of biofertilizers have been developed from bacteria and used in the growth of various plants (Prasad et al. 2015). *Azotobacter* is one of the commonly used biofertilizers that has the ability to fix nitrogen providing beneficial effects on plants and increase soil fertility. The application of *Azotobacter chroococcum* as microbial inoculant has shown a positive effect on the plant with marked enhanced crop production (Manandhar et al. 2017). Despite the availability of beneficial biofertilizer, the adverse effects associated with various abiotic stresses, such as drought and salinity, are among the major environmental constraints that limit growth, productivity, and health of plants.

Vermicomposting can be a cost-effective process for the enhanced growth and yield of various plants (Acharya 1997; Saha et al. 2018). In vermicomposting, microbes are responsible for the biochemical degradation of organic matter where the earthworms drive the process, conditioning the substrate and altering its biochemical activity (Edwards and Burrows 1988; Sharma and Garg 2017). The nature of the substrate used for vermicomposting greatly determines the quality of vermicompost (Borges et al. 2017). The application of vermicompost using various substrate combinations for the cultivation of the bean plant inoculated with *A. chroococcum* was aimed to enhance the growth, productivity, and nutritional content of the plant.

2.2 *Azotobacter chroococcum* as a Biofertilizer

Azotobacter is a free-living obligatory aerobic heterotrophic Gram-negative bacterium that belongs to the family *Azotobacteriaceae*. The first described species of the genus was *A. chroococcum* (Beijerinck 1901). Cells appear as blunt rods to ellipsoid forms, 1.6–2.5 μm in diameter and 3–5 μm in length. Occasionally, cells in some strains appear in chains. They are motile with peritrichous flagella having a wavelength of 2.0–2.9 μm and an amplitude of 0.40–0.59 μm (Kennedy et al. 2015). *Azotobacter* has the ability to fix nitrogen non-symbiotically with at least 10 μg of nitrogen fixed per gram of glucose consumed. The pH range for growth in the

presence of combined nitrogen is 4.8–8.5 with optimum pH for growth and nitrogen fixation to be 7.0–7.5 (Garrity et al. 2005).

The use of *Azotobacter* as a biofertilizer was first described by Gerlach and Voel in 1902 for their ability to fix atmospheric nitrogen (Gerlach and Voel 1902). *Azotobacter* has also been reported to play a role in promoting the growth of plants by synthesizing biologically active substances such as vitamins, amino acids, auxins, gibberellins, etc. (Barea and Brown 1974). Furthermore, the fungistatic compounds have been reported to be synthesized by this organism which inhibits the growth of fungi like *Alternaria* (Bhattarai 2001). These attributes of *Azotobacter* explain the observed beneficial effects of the bacteria in improving seed germination and plant growth.

2.3 Environmental Stresses Affecting Nitrogen Fixation in *Phaseolus* Bean

Beans (*Phaseolus vulgaris*) are the members of the *Leguminosae*, family *Phaseoleae*, subfamily *Papilionoideae* (Bressani 1993). Common bean is a nutritionally and economically important food crop. *Phaseolus* associates with rhizospheric and other microorganisms and fixes atmospheric nitrogen in the soil thereby benefiting the crop (Kay 1979). However, several environmental conditions limit the growth and activity of these plants. Environmental stresses faced by the common bean and their symbiotic partners typically include photosynthate deprivation, water stress, salinity, soil nitrate, temperature, heavy metals, and biocides (Walsh 1995). One stress may also have multiple effects; for example, salinity may also act as water stress, which in turn affects the rate of photosynthesis or metabolism. The initial interaction between the common bean and *Azotobacter* and subsequent sustainability of this association is greatly influenced by the environmental factors.

2.4 Effect of Substrates Used for Vermicomposting on the Survival of *Azotobacter chroococcum*

Vermicomposting is the process of biooxidation and stabilization of organic matter under aerobic and mesophilic conditions through the combined activity of earthworm (*Eisenia foetida)* and microorganisms (Hait and Tare 2011). Vermicompost is rich in nitrogen, phosphorus, and potassium (NPK) and important plant growth hormones and thus enhance the biomass production of plants (Tuladhar et al. 2013).

Microorganisms play a key role in the biodegradation of organic matter and the transformation of nutrients during vermicomposting (Prajapati et al. 2010). Inoculation of suitable microorganisms could accelerate the vermicomposting process and improve compost quality. Microbial inoculants, also known as biofertilizers, are the carrier-based preparations containing beneficial microorganisms in a viable state intended for seed or soil application and designed to improve soil fertility and help plant growth by increasing the number and biological activity of desired

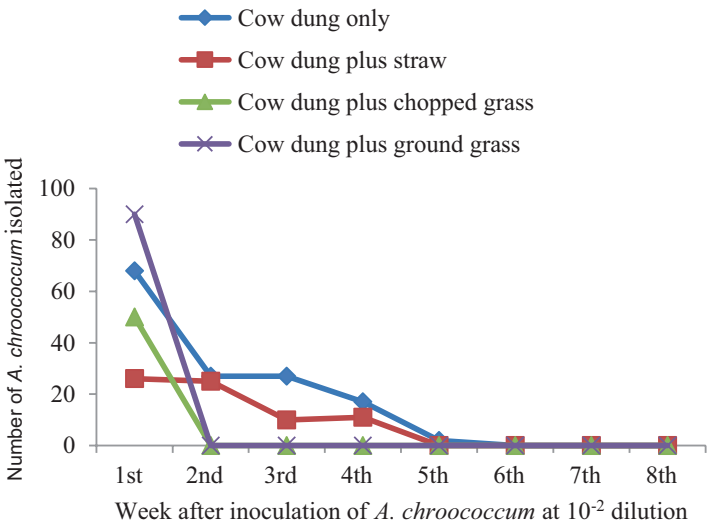

Fig. 2.1 Number of *A. chroococcum* isolated from 1st to 8th week from vermicompost using different substrates

microorganisms in the root environment (Bhandari and Somani 1990). Earthworms have been found to proliferate a variety of microorganisms such as actinomycetes, *Azotobacter, Rhizobium, Nitrobacter*, and phosphate-solubilizing bacteria significantly (Singh and Sharma 2002). However, the survival of these microbes depends on various environmental factors such as pH, temperature, availability of nutrients, oxygen concentration, etc.

An experiment was carried out by enriching *A. chroococcum* at 10^{-2}, 10^{-4}, and 10^{-6} dilutions into four different types of substrates for vermicomposting, i.e., cow dung only, cow dung plus straw, cow dung plus chopped grass, and cow dung plus ground grass. Enumeration of *A. chroococcum* was done at every week interval for up to 8 weeks. *A. chroococcum* was recovered from vermicompost using cow dung only and cow dung plus straw up to 5 weeks. The bacteria couldn't survive in vermicompost using cow dung plus chopped grass and cow dung plus ground grass. The counts at 10^{-2} dilution were the most representative ones for the evaluation of the viability of *A. chroococcum* (Fig. 2.1).

2.5 Inoculation of *Azotobacter* into Vermicompost Enhances the Growth of *Phaseolus* Bean

Improvement in crop production due to *Azotobacter* inoculation has been reported in a number of crops including bean, corn, potato, wheat, clove, oat, etc. A significant positive response in plant growth with inoculation of *A. chroococcum* is

attributed to their ability of nitrogen fixation (Manandhar et al. 2017). Nitrogen is one of the important nutrients required for production of crops (Deacon 2006).

Phaseolus vulgaris inoculated with *A. chroococcum* and grown in soil supplemented with vermicompost increased the length and dry weight of shoot and root compared to the plant treated with vermicompost alone (Fig. 2.2). The experiment was carried out in an earthen pot filled with soil supplemented with vermicompost using different substrates. The plants were harvested at the flowering stage, i.e., 42nd day after sowing of the seeds (Fig. 2.2).

The number of nodules per plant was highest in the plant grown in *Azotobacter*-enriched vermicompost prepared with cow dung only in comparison to vermicompost prepared with other substrate combinations (Fig. 2.3). The least number of nodules per plant was observed when chemical fertilizers were used for the experiment.

The symbiotic relationship between *Piriformospora indica* and *Rhizobium leguminosarum* in the presence of vermicompost further enhances the productivity of *Phaseolus vulgaris* (Singh 2004; Tuladhar et al. 2013; Varma et al. 1999). The symbiosis between *P. indica* and *A. chroococcum* has also been reported to improve the growth and development of *Oryza sativa* in the presence of vermicompost (Das et al. 2014; Prajapati et al. 2010).

2.6 Nitrogen, Phosphorus, and Potassium (NPK) Content in the Plant

NPK is most essential for plant's growth. Nitrogen is the key building block of the protein and present in the nucleic acid. Phosphorous is present in biomolecules like nucleic acid, phospholipids, and ATP. Potassium promotes the growth of root in plants and enhances the absorption of minerals. The efficient uptake of these essential nutrients plays a crucial role in the growth of plants. The increase in uptake of NPK by plants has been reported in the presence of *Azotobacter* (Biswas et al. 2000). This experiment was conducted to determine the percentage of NPK content in *Phaseolus* grown on vermicompost using various substrates and enriched with *A. chroococcum.* The highest percentage of NPK was observed in vermicompost of cow dung enriched with *A. chroococcum* as compared to vermicompost using other substrates (Fig. 2.4).

2.7 Conclusion

Inoculation of *Phaseolus* bean with *A. chroococcum* enhances the overall growth performances resulting in an increase of NPK content. Vermicompost using cow dung serves as the superior substrate for the viability of *A. chroococcum*. This synergistic interaction can be applied in the field to promote sustainable agriculture.

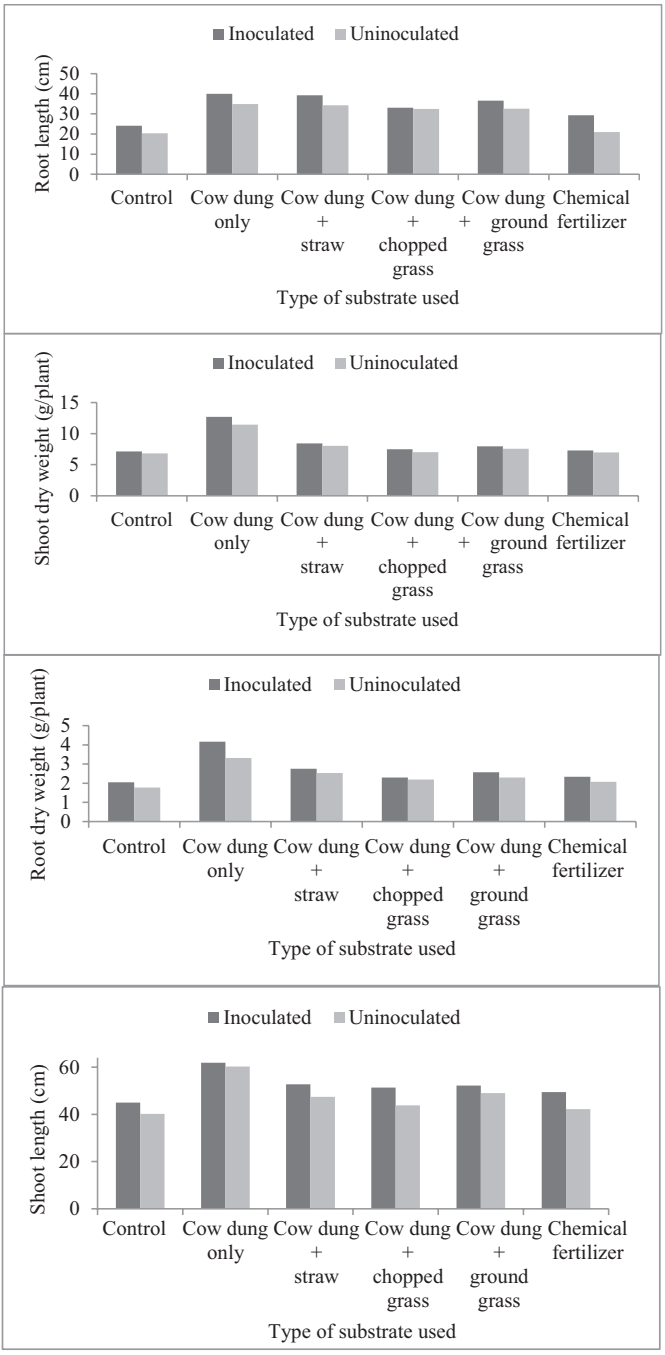

Fig. 2.2 Impact of vermicompost using different substrates on vegetative growth of *Phaseolus* with and without inoculation of *A. chroococcum*

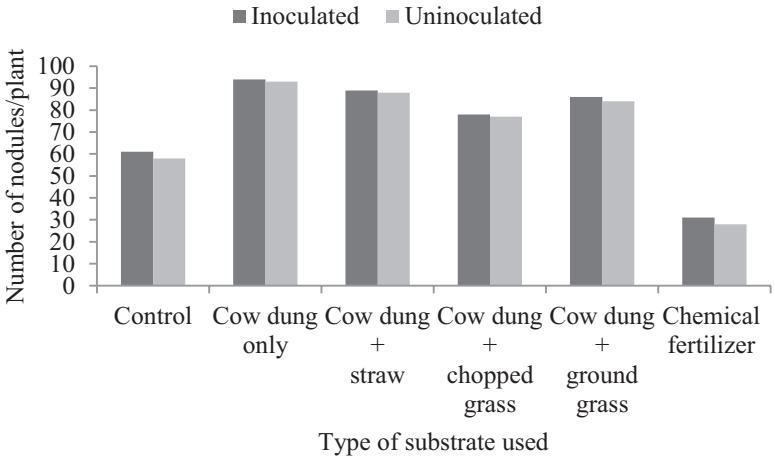

Fig. 2.3 Impact of vermicompost using different substrates with and without inoculation of *A. chroococcum* on the number of nodules on the 42nd day after sowing of seed, i.e., flowering stage

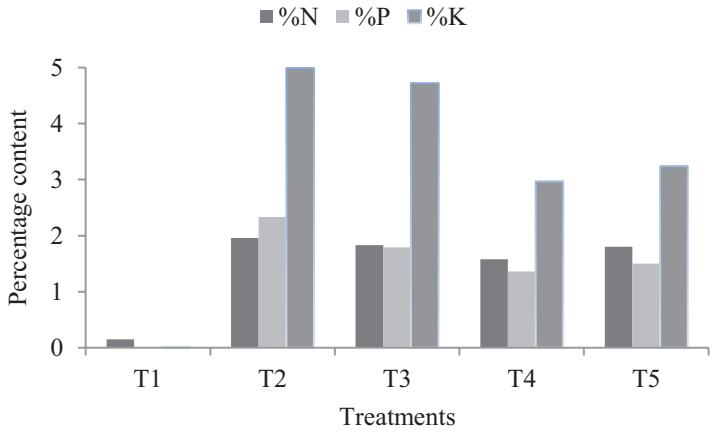

Fig. 2.4 Percentage of NPK content in a plant in different treatments. T1 vermicompost only; T2 *A. chroococcum*-enriched vermicompost of cow dung; T3 *A. chroococcum*-enriched vermicompost of cow dung plus straw; T4 *A. chroococcum*-enriched vermicompost of cow dung plus chopped grass; T3 *A. chroococcum*-enriched vermicompost of cow dung plus ground grass

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3 Biological Control of Some Plant Diseases Using Different Antagonists Including Fungi and Rhizobacteria

Samah Abd El-Kader El-Debaiky

Abstract

Among the different causes of plant diseases, microbes are considered the most important and serious. From which, the fungal pathogens occupy the first place in distribution between numerous plant hosts, including economically important plants. There are a huge number of fungal genera affecting the foliar of the plants including leaves, stems, branches, and flowers while others attacking only roots. Also, wood-decaying fungi are another group affecting trunks of different trees. Many fungal pathogens are opportunistic, where they are invading their hosts through pruning wounds and newly cut surfaces. Beside all the previous fungal pathogens, an important group of fungi responsible for decaying fruits and vegetables after harvest and at storage are recognized.

Fungal pathogens are highly distributed and very specific in their infection process where there are fungal genera able to invade many host plants while other genera are specific only for one host. Throughout history, trials for controlling these aggressive pathogens were increased including several ways such as cultural, physical, chemical, and biological methods. In this chapter, some fungal diseases of various host plants will be introduced with special demonstrations of the biological control of them using several antagonistic microorganisms.

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Keywords
Fungal diseases · Biological control · Foliar diseases · Pruning wounds · Post-harvest diseases

3.1 Introduction

The plant disease is usually defined as any disruption of the normal status of the plant that modifies its vital functions. There are several causal agents, either biotic or abiotic, which result in abnormal physiological activities that interrupt the plant's normal structure, growth, function, and other activities that appear in characteristic pathological conditions or symptoms (Horst 1950; Agrios 2005; Leonberger et al. 2016). The biotic agents responsible for infectious plant diseases include nematodes, fungi, bacteria, mycoplasmas, viruses, viroids, and parasitic higher plants. These diseases can be spread between diseased and healthy members of the host plant (Lucas et al. 1992; Sinclair and Lyon 2005). Detailed explanation is given in this chapter dealing with plant diseases caused by fungal pathogens. Fungi are the most abundant and distributed pathogens causing plant diseases where there are thousands of them capable of causing various plant diseases. This wide spread of fungi may be illustrated due to formation of numerous reproductive structures such as spores, sclerotia, and rhizomorphs. From these structures, spores are found everywhere, in soil, air, water, plant debris, etc. that facilitate its transformation among host plants and between diseased and healthy members (Leonberger et al. 2016). In detail, when fungal spore contacts a plant surface at favorable environmental conditions, it germinates, forming hyphae which are capable of infecting plants via stomata, through wounds, or by direct penetration of the plant epidermis. After infection has happened, the fungal hyphae began to utilize nutrients from their hosts, consequently leading to host weakness and appearance of disease symptoms (Fry 2012; Leonberger et al. 2016).

The control and management of plant diseases aim to keep disease intensity below an economic injury threshold (Zadoks 1985; Nutter Jr et al. 1991) and thereby prevent losses in yield and quality of the crop (Nutter and Guan 2001; Nutter 2007). The disease control relies on five fundamental principles: exclusion, eradication, protection, resistance, and therapy (Horst 1950; Leonberger et al. 2016). For long time, chemical fungicides have been used for control of fungal plant diseases. But, harmful effects on the environment, especially in long-term usage of fungicides, had appeared because they cause pollution and leave harmful residues and resistant strains of the pathogen may be developed with repeated use (Belete et al. 2015). As a result, searching for ecofriendly alternatives for plant disease management became a serious issue. Accordingly, using of biological control agents is considered as potential alternative method to control fungal diseases where it is safe for environment and organisms (Tewari and Bhanu 2003; Barakat and Al-Masri 2005).

The present chapter will introduce several plant diseases caused by fungi. For instance, different types of foliar diseases affect shoot system including leaves, stems, branches, and flowers. Also, wound diseases and post-harvest diseases will be explained here. A special spotlight will be focused on controlling of these

diseases by numerous antagonists such as fungi, bacteria, mycophagous insects, and commercial products prepared using these antagonists.

3.2 Biological Control of Some Foliar Diseases

3.2.1 Leaf Spots

This type of fungal diseases affects leaves of several plants, is distributed worldwide and is caused by different fungal pathogens: *Septoria* (Hansen 2009), *Cercospora* (Shane and Teng 1992), *Curvularia* (Ou 1985), etc. One of the most common diseases of this group is *Septoria* leaf spot, also called *Septoria* blight. It infects several hosts especially plants of family Solanaceae: tomatoes, potatoes, and eggplant by the fungus *Septoria lycopersici.* The fungus, *Septoria* spreads rapidly and can quickly defoliate and weaken the plants, let them unable to bear fruits to maturity. Disease symptoms usually occur in the older, lower leaves and also appear on the stems as well as the flowers of the host plant but rarely affect the fruits (Blum 2000). Figure 3.1 illustrates the symptoms which appeared as brown spots that develop

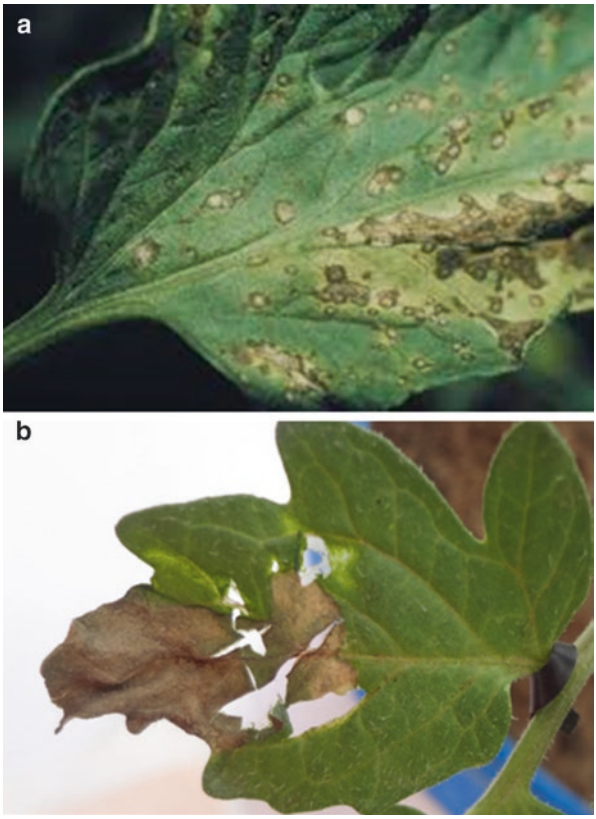

Fig. 3.1 Differences in symptoms between (**a**) leaf spot disease caused by *Septoria lycopersici* and (**b**) early blight disease caused by *Alternaria solani* (Citation: Photo (**a**) from (Gleason 1995) and photo (**b**) from (El-Debaiky 2018)

light tan to white in the center as they age and then the leaves turn yellow and brown and finally die (Gleason 1995). Biological control of different *Septoria* species has been investigated. *S. lycopersici* was effectively controlled by some isolates of *Trichoderma harzianum* (Sain and Pandey 2016). The pathogen *S. musiva*, the causal agent of leaf spot of poplar, was inhibited by spore suspension and culture filtrate of *Phaeotheca dimorphospora* (Yang et al. 1994) and the gram-positive bacteria *Streptomyces* spp. under laboratory and greenhouse conditions (Shimizu 1994). Also, the blotch disease of wheat which is caused by *S. tritici* is diagnosed by necrotic lesions on leaves and stems and is considered the most damaging disease of wheat (Ponomarenko et al. 2011). *The bacterium Bacillus megaterium* was tested as a biocontrol agent of this disease and found to consistently retard the disease development on the adult wheat plants up to 80% (Kildea et al. 2008). Moreover, fungal antagonists have been recorded with promising results in reducing the disease where *T. harzianum* and *T. koningii* reduced the incidence and severity of the leaf blotching of wheat using two techniques: spore suspension and the coated seed. But, these antagonists were effective only at early stages of the disease (Perelló et al. 2009).

Another fungus causing leaf spot disease is *Cercospora* (Saccardo 1876) such as *Cercospora beticola* which is considered the most destructive of foliage of sugar beet worldwide (Weiland and Koch 2004). This pathogen was successfully controlled by the antagonistic bacterium *Bacillus subtilis* (Collins and Jacobsen 2003) and the fungus *Penicillium frequentans* which has been recorded to inhibit the pathogen in vitro via secretion of pectinase and cellulase enzymes. It also, viewed a marked reduction in the disease incidence in field experiment (El-Fawy et al. 2018). Moreover, *Curvularia lunata* was found to cause brown leaf spot of rice plant. Spores production of this pathogen was markedly inhibited by *Chaetomium cupreum* in the dual culture. Also, in the pot experiment, *C. cupreum* significantly reduced the incidence of brown leaf spot, while in a field trial, the chemical fungicide recorded the best results in all plant parameters (Tann and Soytong 2016). In addition, leaf spot of yam caused by *C. eragrostidis* was successfully reduced by the antagonist *Trichoderma* (Michereff et al. 1995).

3.2.2 Blights

Blights are considered another type of disease rather than leaf spots invading the plant leaf blade. The difference between a leaf spot and a leaf blight refers to the degree of damage happening to the leaf blade, viz., if the spots are clearly separated from each other by green tissues, the disease is considered a leaf spot. But, when these spots occur suddenly and fuse together to form a large area of diseased tissue, the disease is referred to as a blight (Fig. 3.1) (Elliott 2005). Early and late blight are widely distributed serious diseases of some vegetable plants such as potato and tomato. The terms “early” and “late” refer to the relative time of their appearance in the field, although both diseases can occur at the same time. Early blight is caused by the fungus, *Alternaria solani*, and potentially distributed by high humidity and

warm weather, firstly, on older leaves. While late blight is caused by *Phytophthora infestans* (Mercure 1998). Along time, several researchers and studies were concerned by controlling both diseases even by chemical fungicides or biologically. Both early and late blight diseases were successfully suppressed by some isolates of the bacterium *B. subtilis* and the antagonistic fungus *T. harzianum* (Chowdappa et al. 2013). On the other hand, the late blight infection of potatoes was inhibited by *Chaetomium globosum* (Shanthiyaa et al. 2013). Recent study indicated that both antagonistic fungi *Aspergillus piperis* and *T. harzianum* attacked the hyphae of *A. solani* by different mechanisms illustrated in Fig. 3.2, for example, mycoparasitism and antibiosis (El-Debaiky 2017). Also, the antagonistic fungus *A. piperis* exhibited

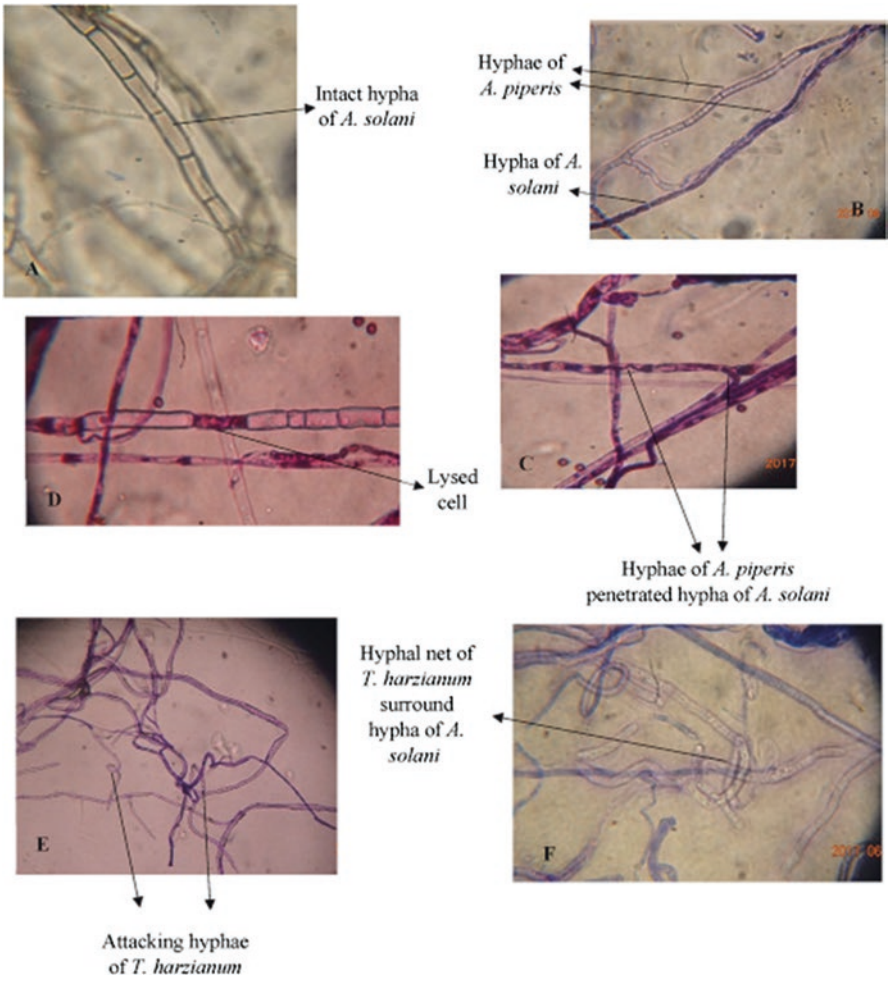

Fig. 3.2 Antagonism and hyphal interactions between *A. piperis* and *T. harzianum* against *A. solani*. Control (**a**), with *A. piperis* (**b**–**d**) and with *T. harzianum* (**e** and **f**). (Photos by Samah El-Debaiky (El-Debaiky 2017)

a slight reduction in early blight incidence of tomato leaflets, caused by *A. solani*, in vivo, related to the control treatment (El-Debaiky 2018).

The chestnut blight is a fungal disease caused by *Cryphonectria parasitica* affecting the chestnut tree causing economically losses in the trees where in the first half of the twentieth century it destroyed about 4 billion trees. The biological control of this fungus is depending on a phenomenon called hypovirulence where there is a viral pathogen that acts as a hyperparasite of the fungal pathogen that weakens the fungus and helps the tree survive by inducing its own resistance (Anagnostakis 1982; Milgroom and Cortesi 2004). Also, the biological control of chestnut blight was obtained with different degrees by some antagonists such as hypovirulent isolates of *C. parasitica*, *Trichoderma* sp., *Penicillium* sp., and *Bacillus* sp. Where *Trichoderma* sp. was the best antagonist followed by the hypovirulent isolates (Akilli et al. 2011).

3.2.3 Rust Diseases

Rusts are group of plant diseases caused by obligate parasitic fungal species belonging to order *Pucciniales* (formerly: *Uredinales*) where more than half of which of genus *Puccinia*. Several host plants from ferns to advanced monocots and dicots are affected by rust fungi with high specificity where each species has a very narrow range of hosts and cannot be transmitted to non-host plants. Moreover, rust fungi affected economically important plants such as cereals, legumes, composites, and many trees. Some species of rust fungi were able to complete their life cycle on two different host plants and produce different types of spores, viz., spermatogonia, aecia, uredinia, telia, and basidiospores. So, the rust fungi derive their name from the rust color of urediniospores (Kolmer et al. 2009; Mohanan 2010).

The biological controlling microorganisms showed effective results against the rust fungi. Some bacterial strains of *Pantoea agglomerans* and *Stenotrophomonas maltophilia* were effective as antagonists in many experiments in reducing bean rust disease caused by *Uromyces appendiculatus* (Yuen et al. 2001). Another example of bacterial antagonism of rust fungi was adopted by *B. subtilis*. The spore germination of *Puccinia pelargonii-zonalis*, the causal agent of geranium rust, was inhibited by some strains of the bacterium *B. subtilis*; consequently, the incidence of rust pustules on the host leaves was reduced. The culture filtrate of this bacterium contains some inhibitory agents to the pathogen so it is most effective than treatment by the washed bacterial cells (Rytter et al. 1989). Moreover, the rust fungus of wheat, *Puccinia recondite* f. sp. *Tritici*, was suppressed by the *Pseudomonas putida* strain BK8661, which produces siderophores, antibiotics, and low levels of hydrogen cyanide (Flaishman et al. 1996).

In the past, the entomopathogenic fungus *Verticillium lecanii* (recently *Lecanicillium*) showed a double potentiality in protection of chrysanthemum plant from both insect pests and white rust disease caused by *Puccinia horiana* (Whipps 1993). Moreover, the hyperparasitic activity against *Puccinia coronata* on oat seedlings was tested using some fungal species. Under the experimental conditions,

Acremonium implicatum and *Verticillium* spp. colonized the uredialsori of *P. coronata*. The hyperparasitic activity of these fungi was investigated microscopically where their mycelia penetrated the uredospores, often forming appressoria- like structures, and then the spore walls and internal contents were degraded due to chitinolytic activity (Leinhos and Buchenauer 1992).

The geneticists also have a very important role in the field of biological control of plant diseases by induction of the self-resistance of the host plants against the pathogens or by breeding of new generations unsusceptible to the disease occurrence. For instance, two new genotypes of pearl millet (*Pennisetum glaucum*) plant, which is resistant to rust disease caused by *Puccinia substriata*, were produced by cDNA encoding the antifungal protein AFP from the mold *Aspergillus giganteus* (Girgi et al. 2006).

3.2.4 Powdery Mildews

Powdery mildews are from the most common, widespread, and destructive groups of plant pathogens worldwide (Braun et al. 2002). They are easily recognizable foliar diseases caused by some obligate parasitic fungi belonging to *Ascomycota*. The infection and the fungal growth are favored by high humidity. Each fungal species of powdery mildew has a specific host where it tends to grow superficially or epiphytically on the plant surfaces except some endophytic genera which spread internally among the host tissues. There are many fungal genera responsible for causing powdery mildews of several plant hosts, such as *Erysiphe*, *Leveillula*, *Phyllactinia*, *Podosphaera* (Heffer et al. 2006). Many trials for biological control of powdery mildews were conducted using the mycoparasitic fungi such as *Ampelomyces quisqualis*, *Meirageula konigii* (Kiss 2003; Szentiványi and Kiss 2003; Kiss et al. 2004; Sztejnberg et al. 2004), or *Lecanicillium lecanii* (Dik et al. 1998; Verhaar et al. 1999). These mycoparasites invade and degrade structures of fungal pathogen, providing adequate control of the disease mainly under greenhouse conditions and moderate pathogen density (Paulitz and Bélanger 2001). For example, the powdery mildew of grape caused by *Uncinula necator* was reduced by the antagonist *A. quisqualis* which parasitizes the cleistothecia of the pathogen (Falk et al. 1995). The mycoparasite *Ampelomyces* sp. also was observed to parasitize and destroy the rubber powdery mildew (Liyanage et al. 2018). The details of this mycoparasitism were illustrated in Fig. 3.3.

The efficacy of the biological control agents *A. quisqualis*, *L. lecanii*, and *Sporothrix flocculosa* was investigated against cucumber powdery mildew caused by *Sphaerotheca fuliginea*. This experiment indicated that *S. flocculosa* recorded the best result in controlling the disease (Dik et al. 1998). In addition, the commercial product of *Lecanicillium longisporum*, Vertalec®, has a potential dual role as a microbial control agent of both aphids and powdery mildew in cucumber caused by *S. fuliginea* (Kim et al. 2008). Also, other reports have demonstrated the ability of the commercial products of the mycoparasitic fungi, *A. quisqualis* (AQ10®) and *L. lecanii* (Mycotal®), as well as three *B. subtilis* strains, UMAF6614, UMAF6639,

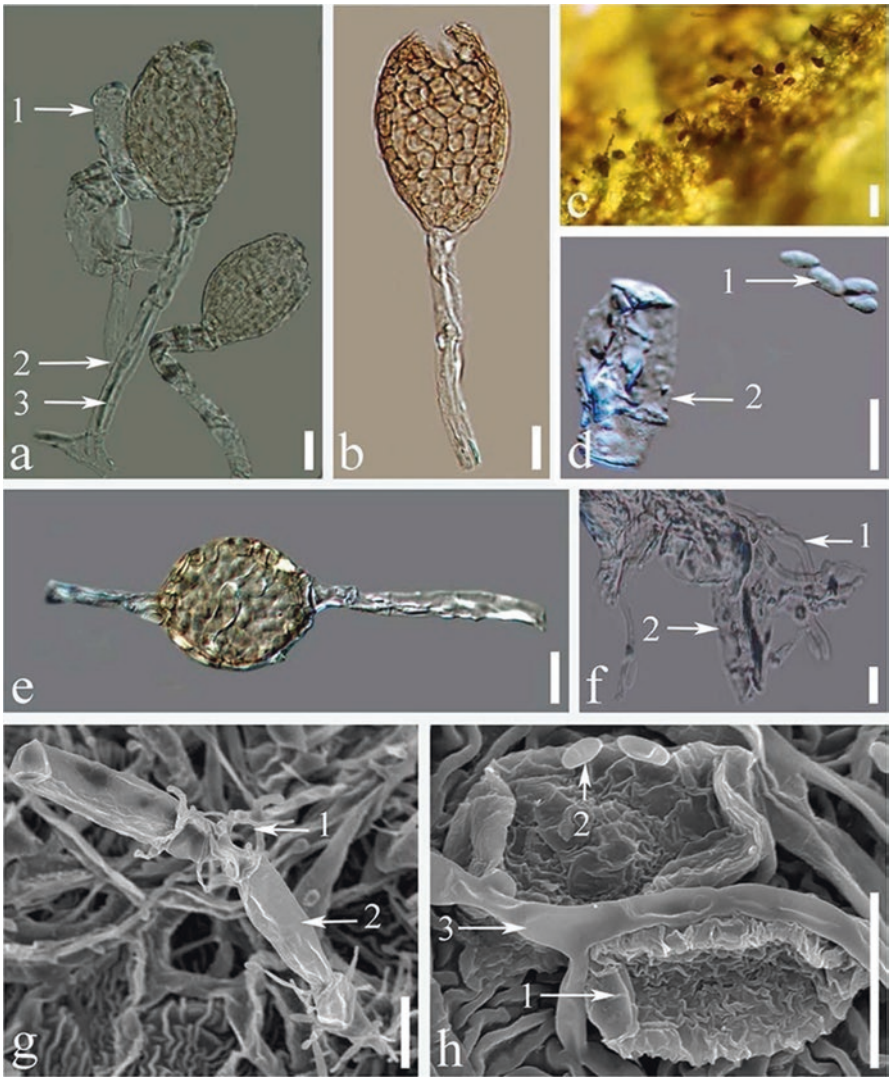

Fig. 3.3 Mycoparasitism of *Ampelomyces* sp. on rubber powdery mildew: (**a**) pycnidia of *Ampelomyces* produced on the conidiophores of rubber powdery mildews (*1*. conidia of rubber powdery mildew; *2*. conidiophores of rubber powdery mildew; *3*. intracellular hyphae of *Ampelomyces*); (**b**) broken pycnidium by apical rupture; (**c**) pycnidia on the surface of a rubber leaf; (**d**) conidia of (*1*) *Ampelomyces* and (*2*) rubber powdery mildew; (**e**) pycnidia produced inside the hyphae of rubber powdery mildews; (**f**) superficial mycelia of (*1*) *Ampelomyces* and (*2*) rubber powdery mildew; (**g**) hyphae of *Ampelomyces* coiled around the catenate-type conidia of rubber powdery mildew (*1*. hyphae of *Ampelomyces*; *2*. catenated conidia of rubber powdery mildew); (**h**) mycelium and conidia of *Ampelomyces* (*1*. Non-catenate conidia (*Erysiphe quercicola*), *2*. conidia of *Ampelomyces*, *3*. mycelium of *Ampelomyces*) (scale bars: figures **a**, **b**, **d**–**h** = 10 μm, figure **c** = 20 μm). (Cited from Liyanage et al. 2018)

and UMAF8561, in controlling the powdery mildew disease caused by *Podosphaera fusca* on melon seedlings (*Cucumis melo*) (Romero et al. 2007).

Alternatively, yeast-like fungi belonging to the genera *Pseudozyma* (Gafni et al. 2015) and *Tilletiopsis* (Urquhart et al. 1994) and bacteria from the genus *Bacillus* (Romero et al. 2004) have been described as biocontrol agents of cucurbit powdery mildew by production and release of antifungal compounds that affect the viability of powdery mildew conidia and hyphae. Although, the success of many fungal and bacterial species as biocontrol agents, the process of biological control is not restricted on them, where there are some mycophagous insects that can also help in the biological control of some plant fungal diseases. For example, the mycophagous mites, *Orthotydeus lambi*, can suppress the development of powdery mildew of grape by feeding the fungal mycelia (English-Loeb et al. 1999).

3.2.5 Downy Mildews

Although there is similarity in names, confusion between downy and powdery mildews must be avoided. Fungi causing powdery mildews are belonging to *Ascomycota*; on the other hand, downy mildews are from Oomycota. The disease symptoms which are characteristic of this group of diseases appear to the naked eye as grayish, fuzzy-looking carpet or "down" of mycelia, conidiophores, and spores on the leaves of the host plant (Beckerman 2009; Slusarenko and Schlaich 2003).

Few reviews have illustrated the biological control of downy mildew diseases. Unexpected suppression of downy and powdery mildew diseases was developed after spraying by *T. harzianum* (strain T39) (Elad 2000). Also, this strain induces the plant-mediated resistance as well as reduces the severity of downy mildew caused by *Plasmopara viticola* in susceptible grapevines (Palmieri et al. 2012). In addition, sporulation of *P. viticola* was completely inhibited by the endophytic fungus *Alternaria alternate* (Musetti et al. 2006). The ultrastructural analyses and cytological observations of cellular interactions between *P. viticola* and *A. alternate* showed a toxic effect of *P. viticola* cells. This toxicity appeared in the form of severe ultrastructural alterations, such as the presence of enlarged vacuoles or vacuoles containing electron-dense precipitates. Also, necrotic and irregularly shaped haustoria appeared. Therefore, a toxic action of *A. alternata* against *P. viticola* was discovered to be due to three diketopiperazines: cyclo(L-phenylalanine-trans-4-hydroxy-L-proline), cyclo(L-leucine-trans-4-hydroxy-L-proline), and cyclo(L-alanine-trans-4-hydroxy-L-proline). On the other hand, the mycoparasitic action of some strains of *Fusarium proliferatum* against *P. viticola* was also investigated, where the hyphae of this antagonist coiled and penetrated the hyphae of the pathogenic fungus (Bakshi et al. 2001).

The efficacy of various environmentally friendly products was tested for controlling some diseases in grapes over several years. The tested products were JMS Stylet Oil (paraffinic oil), Serenade (*B. subtilis*), Croplife (citrus and coconut extract) + Plant food (foliar fertilizer), Armicarb (potassium bicarbonate), Elexa (chitosan), Milsana (giant knotweed extract), and AQ10 (*A. quisqualis*). The results indicated that each of JMS Stylet Oil, Armicarb, Serenade, AQ10, Elexa, and Milsana provided moderate control of downy and powdery mildews (Schilder et al. 2002).

3.3 Biological Control of Pruning Wounds and Wood-Decay Diseases

Trunk wounds developed from broken bark, which is considered the first line of defense of the tree against wood-decaying microorganisms, so the underlying tissues are exposed to the pathogens (e.g., fungi and bacteria). There are several causes leading to wounds like mechanical factors, human activities, insect pests, or animals (Gauthier et al. 2015). In addition, pruning wounds and newly cut surfaces of tree trunks and vines are leading to entrance of different plant pathogens, consequently leading to death of limbs or the entire host plant (Stirling and Stirling 1997). But, it is not necessary that all trunk wounds lead to wood decay or destruction of the trees. Frequently, trees are able to compartmentalize the wounded tissues by formation of internal barriers and wound wood/callus which can prevent spreading of the pathogens. Mainly, this self-defense depends on the plant and microbe species, vigor and age of the tree, and season (Gauthier et al. 2015). *Armillaria*, *Fomes*, *Ganoderma*, *Polyporus*, *Trametes*, and *Xylaria* represented some examples of wood-decay fungi (Gauthier et al. 2015).

Also, there are various examples of wound diseases which in some cases are destructive and causing a lot of economical loses. Some important examples of pruning wounds are represented in trunk diseases of grapevine including dieback, black dead arm, esca, Petri disease, and dead arm (Munkvold et al. 1994; Gubler et al. 2005). Numerous fungal pathogens are able to invade these pruning wounds of grapevine such as *Eutypa lata*, *Phaeomoniella chlamydospora*, and *Botryosphaeria*, *Phomopsis*, and *Phaeoacremonium* (Kotze et al. 2011).

Wound prevention or protection of wounds from fungal pathogens is critical, where, once the infection has begun by any of these fungi, there are no controls or cures (Gauthier et al. 2015). Thus, protection of wounds have been performed using various fungicides and/or biological control agents (Halleen et al. 2010). Many antagonists such as *Trichoderma atroviride* and *B. subtilis* exhibited successful protection of pruning wounds of grapevine (Kotze et al. 2011). Moreover, the fungal pathogen, *Eutypa armeniacae*, which causes gummosis or dieback of apricot trees and dead arm of grapevine, was prevented biologically by *Fusarium lateritium*. This antagonist colonized the newly cut surfaces and produces a nonvolatile, water-soluble antibiotic which inhibits spore germination and growth of *E. armeniacae* (Stirling and Stirling 1997).

The basidiomycete *Heterobasidion annosum* (formerly *Polyporus annosum*) is considered the most economically important forest pathogen. It causes the annosus root rot disease of conifers where the infection occurs through wounds such as freshly cut stumps. The fungus is transferred among diseased and healthy trees via root grafts (Asiegbu et al. 2005). This pathogen is excluded by *Phlebiopsis gigantea*, which competes for nutrients and space, and also, it attacks the hyphae of the pathogen and suppresses it by production of antibiotics (Stirling and Stirling 1997). This antagonist shows a fully protective effect of the stumps of *Pinus pinea* against spore infection by *H. annosum* (Annesi et al. 2005).

Neonectria ditissima (syn. *Neonectria galligena*) is another fungal plant pathogen that causes cankers of apple and beech trees where it can kill branches of the trees by choking them off (Castlebury et al. 2006). Research has indicated that some fungal and bacterial antagonists (e.g., *Alternaria* sp., *T. viride*, and *B. subtilis*) were used for biological control of *N. ditissima* where they colonize the leaf scar throughout winter and early spring, inhibit the entry of the pathogen, and consequently reduce the number of shoots that are susceptible to infection (Aldwinckle and Jones 1990).

Armillaria root disease is distributed worldwide in tropical warm regions. It is one of the most destructive diseases of many species of trees and shrubs in natural forests, plantations, orchards, and gardens throughout the world. The fungus *Armillaria mellea* causes mortality, wood decay, and growth reduction of the host trees. They infect and kill either weak or healthy trees. The pathogen either kills the host directly or predisposes it to secondary attacks by other fungi or insects. The disease transfers from tree to tree through rhizomorphs which grow from infected roots through the soil to the adjacent healthy roots or by direct root contact (Fig. 3.4). In addition, the fungus can be spread by basidiospores in which they first colonize dead stumps or woody material and then the rhizomorphs radiate from these, to living roots directly or through wounds (Morrison 1981). Several studies indicated that antagonistic fungi especially *T. harzianum* (Wargo and Shaw III 1985) and *Chaetomium olivaceum* (Raziq and Fox 2005) were effective in attacking and

Fig. 3.4 Honey fungus or boot-lace fungus, *Armillaria mellea*. (**a**) Fruit bodies and rhizomorphs (photo by David Moore). (**b**) Emerged rhizomorphs from beneath the bark of a felled log. (**c**) Enlarged rhizomorphs shown in **b** (photos **b** and **c** by Elizabeth Moore) (Moore et al. 2011)

killing the hyphae of *A. mellea*-infected different hosts with root rot such as strawberry (Wargo and Shaw III 1985), cherry, and almond trees (Asef et al. 2008). In these studies, the antagonists exhibited several mechanisms in attacking the pathogen such as hyperparasitism and antibiosis via volatile metabolites.

3.4 Biological Control of Post-Harvest Diseases

Post-harvest diseases are referred to spoilage of fruits and vegetables after harvest which affect the crop and cause losses as great as 25–50% (Wilson et al. 1991). Synthetic fungicides (El Ghaouth et al. 2004; Korsten 2006; Singh and Sharma 2018) and ultraviolet radiation (Stevens et al. 1997) are primarily used to control post-harvest diseases of fruits and vegetables. But, there is a strong public and scientific desire to search about safer and ecofriendly alternatives for reducing these diseases (Mari et al. 2007). Consequently, usage of the microbial antagonists like yeasts, fungi, and bacteria is quite a successful solution for post-harvest diseases (Eckert and Ogawa 1988; Droby et al. 1991; Wisniewski and Wilson 1992; Droby 2005; Korsten 2006). The biological control of post-harvest diseases depends on either using of normal microflora which occur naturally on the fruit surface or those which can be introduced to it artificially (Sharma et al. 2009). The major mechanism by which antagonists suppress the pathogens, causing fruit and vegetable decay, is competition for nutrition and space (Droby et al. 1989; Wilson and Wisniewski 1989).

The infection of the fruits and vegetables may occur at pre-harvest stage and continue after post-harvest during transportation or storage of fruits and vegetables. Therefore, pre-harvest application of microbial antagonists to fruits and vegetables is recommended to protect the wounds inflicted during harvesting from the entrance and colonization of the pathogens (Ippolito and Nigro 2000; Janisiewicz and Korsten 2002; Ippolito et al. 2004; Irtwange 2006). However, the application of microbial antagonists in the post-harvest stage is better, practical, effective, and useful than pre-harvest application (Barkai-Golan 2001; Irtwange 2006). Also, the formulation process of the biocontrol agent is very important in the protection of the fruits and vegetables. For example, lyophilized cells of *Erwinia amylovora* were more effective in colonizing pear flowers than bacterial cells harvested from fresh cultures (Stockwell et al. 1998). Moreover, protection of variety of fresh fruits from post-harvest diseases caused by *Rhizopus stolonifer*, *Botrytis cinerea*, and *Penicillium expansum* was evaluated with an invert emulsion formulation of *T. harzianum*. The conidia of *T. harzianum* in an invert emulsion reduced the occurrence of *R. stolonifer* on apple, pear, peach, and strawberry; *B. cinerea* on grape, pear, strawberry, and kiwifruit; and *P. expansum* on grape, pear, and kiwifruit (Batta 2007). In the meantime, combination between the antagonists and other treatments such as essential oils improve the suppression of post-harvest pathogens. Combination between the antagonistic bacterium *Bacillus amyloliquefaciens* PPCB004 and thyme and lemongrass essential oils has potentially controlled the post-harvest spoilage of peach fruits caused by *B. cinerea*, *P. expansum*, and *R. stolonifer* (Arrebola et al. 2010).

In the last decades, many studies are interested in production of antimicrobial films which are used for packaging of food products and saving them from microbial spoilage (Cha and Chinnan 2004). Therefore, in a recent study, antimicrobial cloth films were prepared by immobilization of the degrading enzymes of *T. harzianum*: chitinase, cellulase, and glucose oxidase on polyester cloth films separately. Then, these antimicrobial films were used as coverage of tomato fruits to protect them from black mold disease caused by *A. alternata* (El-Badry and El-Debaiky 2018). The best protection of tomatoes in this study was obtained using polyester cloth films immobilized by cellulase enzyme (Fig. 3.5) after 4 and 7 days. Another trial to protect the fruits using the antimicrobial films was adopted when the strawberries were covered by biofilm containing *Cryptococcus laurentii* in combination with alginate, glycerol, palmitic acid, glycerol monostearate, and β-cyclodextrin. This biofilm containing *C. laurentii* as antagonist aided inhibition of mold growth, protected the strawberries intact throughout storage, and improved the fruit quality (Fan et al. 2009).

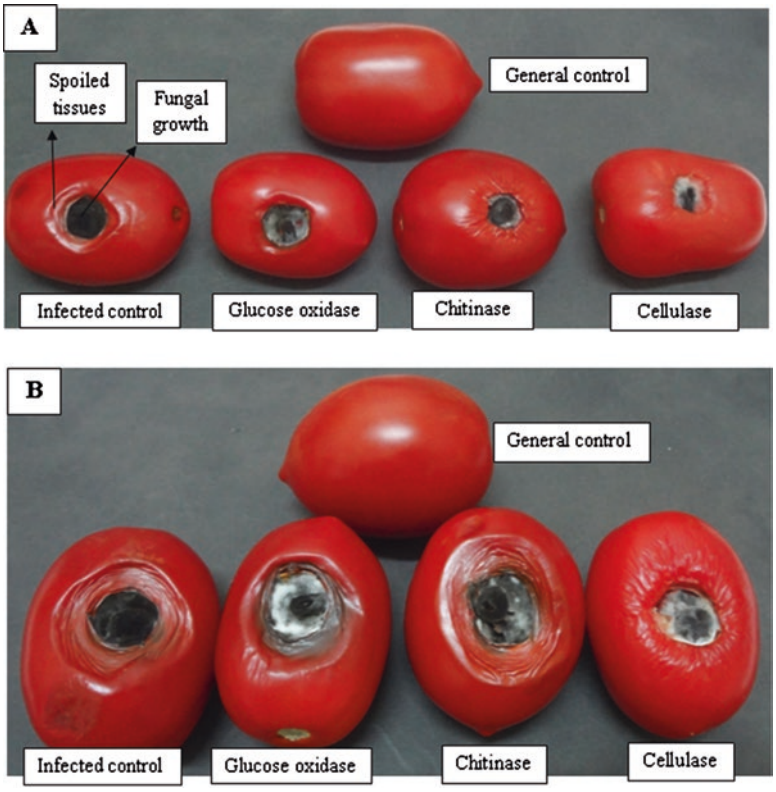

Fig. 3.5 Effect of polyester films of enzymes of *T. harzianum* on the growth of *A. alternata* and black rot incidence on tomato fruits after 4 days (**a**) and 7 days (**b**). Photos by Samah El-Debaiky (El-Badry and El-Debaiky 2018)

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4 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.

Keywords
PGPR · Biotic stress management · Biocontrol · ISR · Blister blight of tea

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 orbiculare* (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 antibiotic-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. solanacearum* (Aliye et al. 2008); and *P. fluorescens* strain FP7 against mango anthracnose 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 extracellular 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 systemic resistance (ISR). The siderophore-producing *B. subtilis* strain CAS 15 competed 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 exhibited 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 conditions, 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 provide 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 prescreened 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,

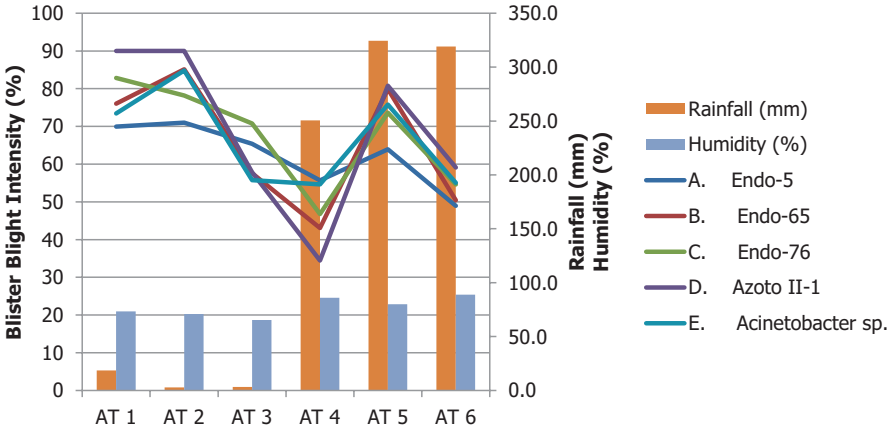

Fig. 4.1 Rainfall, humidity, and intensity of blister blight during the experiment

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%

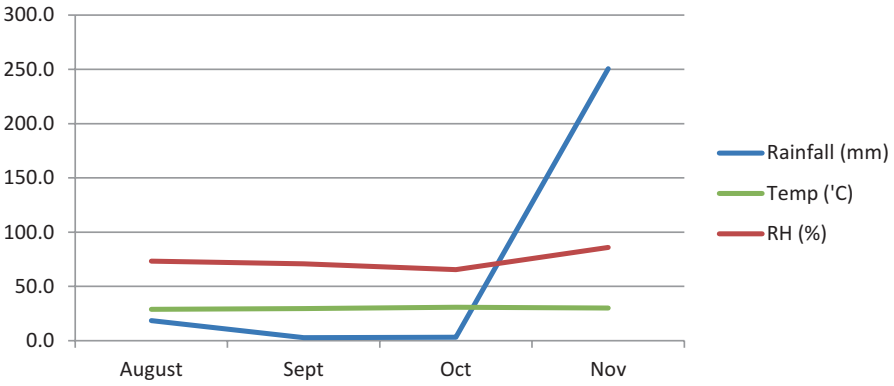

Fig. 4.2 Climate condition during experiment

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 distict 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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5 Amelioration of Biotic Stress by Application of Rhizobacteria for Agriculture Sustainability

Satyavir S. Sindhu and Ruchi Sharma

Abstract

Increase in agriculture crop yields is needed to feed the ever-growing human population. But, the biotic and abiotic stresses are major constraints for plant growth, crop yield, food quality, and global food security. Different pathogens, weeds, and insects collectively contribute to biotic stress. Biotic stress causes adverse impacts on plants, including hormonal and nutritional imbalance, physiological disorders, susceptibility to diseases, etc., and results in reduced economic yield. The application of plant growth-promoting rhizobacteria (PGPR) offers a cost-effective and eco-friendly mechanism for protecting plants against the stress conditions. These microbial populations in the rhizosphere may benefit the plant by increased recycling, solubilization, and uptake of mineral nutrients; by synthesis of vitamins, amino acids, auxins, and gibberellins; and by antagonism with potential plant pathogens. Certain PGPR strains also protect the plants against pathogens through a mechanism associated with induced systemic resistance (ISR) or systemic acquired resistance (SAR). Recent progress in our understanding on the diversity of rhizobacteria in the rhizosphere, their colonization ability, and their mechanism of action in amelioration of biotic stress will facilitate their application as a reliable component in the management of a sustainable agricultural system. In this chapter, the effects of rhizobacteria on plant susceptibility/resistance to potential deleterious organisms, including root and shoot pathogens, pathogens, weeds, and phytophagous insects, will be discussed. The application of these rhizobacteria as biofertilizers and biopesticides may become a feasible and potential technology in the future to feed the global population with reduced impact on environmental quality.

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Abiotic and biotic stresses · Pathogen · Insect · Weed · Amelioration of biotic stress · Microbiome engineering

5.1 Introduction

Soil and plant roots are the habitat for colonization of a variety of soil-borne pathogens and beneficial microorganisms. The interactions of microbes with plants in natural habitats are crucial for proper growth and development of plants. Plant root exudates and other chemicals released by plants attract the microbial population. Abiotic and biotic stresses are the major challenges to the crop yield and cause vast economic loss (Ramegowda and Senthil-Kumar 2015). Biotic stress is caused by different pathogens, such as bacteria, viruses, fungi, nematodes, protists, and insects. The common impacts of these biotic factors include imbalanced hormonal regulation, nutrient imbalance, and physiological disorder resulting in a significant reduction in agricultural yield (Haggag et al. 2015). Biotic stress also has adverse impacts on plant co-evolution, population dynamics, ecosystem nutrient cycling, natural habitat ecology, and horticultural plant health (Gusain et al. 2015). Global crop yields are reduced by 20 to 40% annually due to pests and diseases (Strange and Scott 2005; FAO 2012).

For the control of phytopathogens and insect pests in agriculture, farmers have mostly relied on the application of synthetic pesticides, and the global pesticide market is presently growing at a rate of 3.6% per year (Lehr 2010). However, indiscriminate use of chemical pesticides to control the pathogens/insects has generated several problems including resistance to insecticides/fungicides, an outbreak of secondary pests, as well as safety risks for humans and domestic animals. Moreover, the long persistence of applied pesticides in soil leads to contamination of groundwater and soil, and the residual toxic chemicals enter into the food chain. Excessive pesticide application also decreases the biodiversity due to the destruction of nontarget entomofauna. Sustainable agriculture in the twenty-first century will rely increasingly on alternative interventions for pest management that are environment-friendly and will reduce human contact with chemical pesticides. Therefore, microorganisms are currently being explored for their possible use as biocontrol agents in the integrated pest management programs.

Over the past few decades, attempts have been made to understand the molecular mechanisms implicated in abiotic and biotic stress tolerance (Tripathi et al. 2015, 2017; Pontigo et al. 2017; Singh et al. 2017). Several microorganisms including bacteria, actinomycetes, fungi, viruses, protozoa, and nematodes obtained from the rhizosphere of crop plants have been found to control various root, foliage, and postharvest diseases of agricultural crops (Glick and Bashan 1997; Sindhu et al. 2016). Many microorganisms have been found to act as potential entomopathogens (Vega and Kaya 2012; Mascarin and Jaronski 2016; Sindhu et al. 2017). Among the various bacterial control agents (BCAs), *Bacillus thuringiensis* (*Bt*), *Pseudomonas*

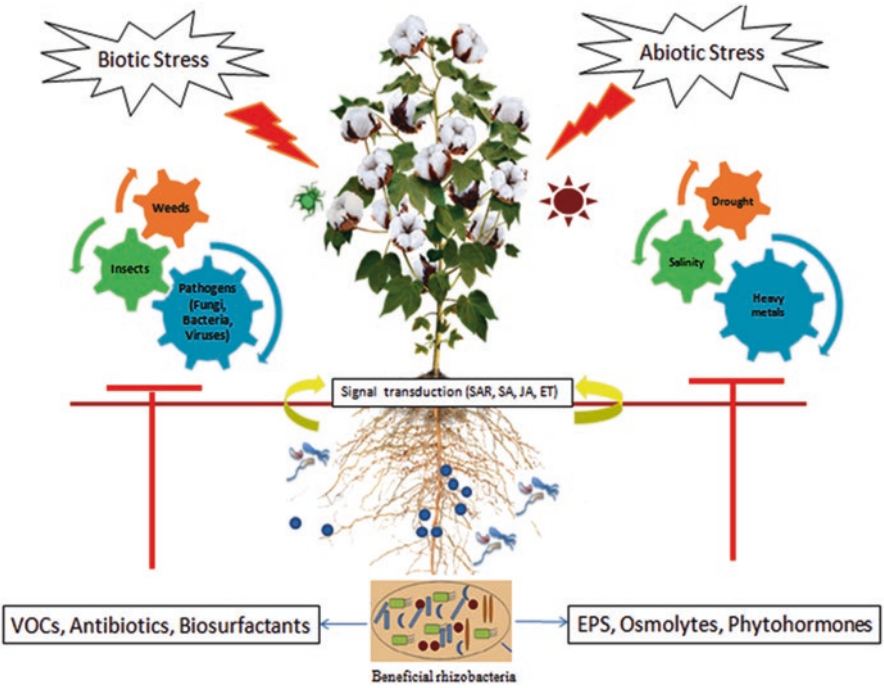

Fig. 5.1 Beneficial rhizobacteria in the rhizosphere of plants contribute toward amelioration of plant stress through various mechanisms

fluorescens, *Serratia marcescens*, and *Streptomyces* sp. are predominantly used in plant protection (Mascarin and Jaronski 2016; Sindhu et al. 2016).

Interaction of microbes with plants causes the release of different elicitors and triggers physiological and biochemical changes in plants. Plants inoculated by soaking their roots or seeds overnight in cultures of PGPR exhibited enormous resistance to different forms of biotic stress (Ngumbi and Kloepper 2016). Some of the non-pathogenic microbes have shown the ability to suppress the diseases caused by these pathogens. Therefore, the use of beneficial microbes as biological control agent has been viewed as an alternative and sustainable approach to replace chemical pesticides (Fig. 5.1). Plant growth-promoting microorganisms (PGPM) have been considered as an eco-friendly and cost-effective means for control of diseases. The defense-related hormones, i.e., jasmonic acid (JA), ethylene, and salicylic acid (SA), have been found to play a primary role in signal transduction and defense mechanism (Bari and Jones 2009; Verhage et al. 2010). Co-inoculation of PGPR with mycorrhizae also ameliorates the harmful impact of biotic stress and protects plants from pathogens by enhancing growth attributes and reducing the susceptibility for disease (Dohroo and Sharma 2012). Biopesticides are nowadays extensively applied in controlled and predictable environmental conditions such as greenhouse crops to produce tomatoes, cucumbers, and sweet peppers (Chandler et al. 2011; Xu et al. 2011) and postharvest control of fruits, vegetables, and grains (Liu et al. 2013), whereas their use in open fields is still limited.

5.2 Rhizosphere Biology

The plant-soil interface, termed as rhizosphere, around living roots is a narrow zone of soil that is overwhelmingly influenced by root activities and provides a niche to various microorganisms including fungi, bacteria, actinomycetes, algae, and nematodes. Microbial cell count up to 10^{11} per gram of soil in the rhizosphere has been reported, and the microbial population contains about 30,000 prokaryotic species (Egamberdieva et al. 2008; Badri and Vivanco 2009). These microbial populations of rhizosphere markedly affect interactions between plants and the soil environment (Mendes et al. 2013). Thus, the root system in plants is populated by a complex microbial community termed as the root microbiome (Hacquard et al. 2015). Some plants shape their rhizosphere microbiome with the recruitment of beneficial bacteria or fungi (Berendsen et al. 2012), and host genotype has also been found to influence the overall composition of these microbial communities (Badri et al. 2013; Bulgarelli et al. 2015). Moreover, edaphic and environmental factors also affect the composition of microbiome (Chaparro et al. 2012; Hacquard et al. 2015). Nearly 5 to 21% of all photosynthetically fixed carbon is being transferred to the rhizosphere through root exudates (Marschner 1995; Flores et al. 1999). The population and functional dynamics of soil microorganisms differ from rhizospheric to non-rhizospheric zone due to the release of a multitude of organic compounds (e.g., exudates and mucilage) derived from photosynthesis and other plant processes (Khalid et al. 2004; Lee et al. 2016; Kumar et al. 2017). The particular types of root exudates released by different plant species either attract or repel specific microbes (Grayston et al. 1998; Bertin et al. 2003; Marschner et al. 2011). For example, some plants use root exudates to attract symbiotic microbes, which can improve their nutrient supply (Parniske 2008; Marschner et al. 2011; Oldroyd 2013). Some microbes in the rhizosphere produce siderophores to increase the amount of soluble iron for uptake. Plants profit from this increased Fe^{II} availability and therefore select for these beneficial microbes through their root exudates in order to improve the availability of iron (Hartmann et al. 2009; Carvalhais et al. 2013). Some plant roots release strigolactones to attract mycorrhizae for improving phosphate and nitrogen supply (Akiyama et al. 2005). Legumes secrete specific kind of flavonoids to establish symbiosis with nitrogen-fixing rhizobia, respectively (Bertin et al. 2003; Hassan and Mathesius 2011). Recently, the changing climatic conditions were found to alter the rhizosphere biology by modifying root exudation rate, resource availability, and biogeochemical cycling (Liu et al. 2017). In the era of sustainable crop production, the plant-microbe interactions in the rhizosphere play a pivotal role in transformation, mobilization, and solubilization of nutrients from a limited nutrient pool and subsequent uptake of essential nutrients by plants. These rhizosphere bacteria (i) supply nutrients to crops; (ii) stimulate plant growth, e.g., through the production of plant hormones; (iii) inhibit the activity of plant pathogens; (iv) improve soil structure; and (v) exhibit bioaccumulation or microbial leaching of inorganics (Ehrlich 1996). More recently, bacteria have also been used in the soil for the mineralization of organic pollutants, i.e., bioremediation of polluted soils (Zhuang et al. 2007; Zaidi et al. 2012).

Recently, the use of biological approaches is becoming more popular as an additive to chemical fertilizers for improving crop yield in an integrated plant nutrient management system. In this regard, the use of PGPR has found a potential role in developing sustainable systems in crop production (Sturz et al. 2000; Shoebitz et al. 2009). A variety of symbiotic (*Rhizobium* sp.) and non-symbiotic bacteria (*Azotobacter*, *Azospirillum*, *Bacillus*, *Klebsiella* sp., etc.) are now being used worldwide with the aim of enhancing plant productivity (Cocking 2003; Sindhu et al. 2016). Interactions of plant roots with beneficial rhizosphere microorganisms influence plant growth and development (Berendsen et al. 2012; Panke-Buisse et al. 2015), because microbes play an important role in nutrient cycling and aid in the acquisition of mineralized nutrients (Mishra et al. 2012; Bulgarelli et al. 2013; Sindhu et al. 2016). Endophytic bacteria belonging to *Klebsiella*, *Enterobacter*, *Bradyrhizobium*, *Alcaligenes*, *Azospirillum*, *Herbaspirillum*, *Ideonella*, *Acetobacter*, and *Acinetobacter* have been isolated from wild rice (*Oryza alta*) plants, which supply nitrogen to their host plants (Baldani et al. 2000; Chaudhary et al. 2012). The composition of microbial communities around the roots also has significant impacts on plant growth through stress tolerance under field conditions (Yang et al. 2008; Mendes et al. 2011; Panke-Buisse et al. 2015). In natural ecosystems, equilibrium develops between utilization of metabolites in root exudates by microorganisms and uptake of mineralized nutrients by the roots of the plant and microorganisms that is affected further by seasonal changes in the environment (Whipps and Lynch 1986). Therefore, understanding of the interactions of plants with microbial communities is increasingly relevant in the context of increased demand for food by an expanding human population, coupled with reductions in cultivable land and recent effects of climate change on agricultural productivity (Alexandratos and Bruinsma 2012). Therefore, research efforts are required in understanding the rhizosphere biology under changing climatic conditions for harnessing beneficial interactions as low-input biotechnology for sustainable agriculture (Dubey et al. 2016).

Plant species usually recruit their own microbiome from the soil, which influences plant competitiveness, health, and productivity (Berg et al. 2014; Hardoim et al. 2015; Agler et al. 2016). Species of *Pseudomonas*, *Streptomyces*, and *Bacillus* spp. have been found to inhibit the proliferation of pathogens (Bhattacharyya and Jha 2012; Sindhu et al. 2016). Challenging of plants with a pathogen has been found to alter the composition of soil microbiome via shifts in root exudation profile (Chaparro et al. 2013). For example, the presence of the pathogenic fungus *Fusarium graminearum* in the rhizosphere of barley triggers the exudation of many phenolic compounds that prevented spore germination (Lanoue et al. 2009). Similarly, alterations of phenolic compound exudation in barley infected with the oomycete *Pythium ultimum* induced expression of antibiotic-related genes in *Pseudomonas protegens* (Jousset et al. 2011). Two *Arabidopsis* mutants which were disrupted in different branches of the jasmonate pathway, namely, myc2 and med25, showed distinct exudation patterns and increased the abundance of *Streptomyces*, *Bacillus*, and *Lysinibacillus* taxa in the med25 rhizosphere and *Enterobacteriaceae* population in the myc2 rhizosphere (Carvalhais et al. 2015). Thus, many commonalities, as well as differences, exist in defense strategies employed by roots and foliar tissues

during pathogen attack (De Coninck et al. 2015). Infection with foliar pathogen *Pseudomonas syringae* pv. *tomato* (Pst DC3000) caused selective recruitment of the beneficial rhizobacterium *Bacillus subtilis* FB17 by *Arabidopsis thaliana* (Rudrappa et al. 2008). The secretion of L-malic acid by roots was shown to recruit the rhizobacterium in response to infection of foliage with Pst DC3000. Transcriptome analyses revealed that the interaction with *B. subtilis* FB17 caused an alteration in the expression of *Arabidopsis* genes involved in regulation of auxin production, metabolism, defense, and stress responses and also caused modifications in the cell wall (Lakshmanan et al. 2012). The populations of beneficial *B. subtilis* were also increased in response to aphid attack of foliage in *Capsicum annuum*, and it was correlated with reduced populations of the pathogen *Ralstonia solanacearum* (Lee et al. 2012). The significance of the hormones involved in plant immunity has also been highlighted in shaping the root microbiome (Lebeis et al. 2015). These compounds enhance the availability of chemical compounds in the soil, which provide nutrient sources for microbes in the rhizosphere (Bever et al. 2012; Miransari 2013).

5.3 Abiotic and Biotic Stresses

Agriculture is considered to be the most vulnerable sector that is often exposed to the plethora of climate change. The abrupt change in climatic conditions increases the incidence of abiotic and biotic stresses that become a major cause for the stagnation of productivity in agriculture and horticulture crops (Grover et al. 2011).

5.3.1 Abiotic Stresses

Among abiotic factors, inter-seasonal climatic variability is a concern, which is usually reflected in year-to-year fluctuations in crop yields. Global warming and changes in precipitation patterns lead to several abiotic stresses such as extreme temperatures, drought, flooding, salinity, metal stress, and nutrient stress that cause adverse effects on food production (Pandey et al. 2007; Barrios et al. 2008; Selvakumar et al. 2012). The probability of occurrence of extreme climatic events has increased in the last couple of decades, and farmers lack the management options to sustain the agricultural productivity (Kalra et al. 2013). Climate change models have predicted that warmer temperatures and increase in the frequency and duration of drought during the twenty-first century will have net negative effects on agricultural productivity (Clair and Lynch 2010). In the developing countries, it has been estimated that, on an average, nearly two-thirds of the soils are prone to climatic constraints that significantly reduce crop yields (Lal 2001). Abiotic stress hampers growth and production of the crop, causing land degradation by making soil nutrient deficient and more stress-prone. In one way or another, abiotic stresses are intermingled and correlated with one another. For example, climatic variability such as increase or decrease in rainfall and rise or fall in temperature brings drought stress. Drought stress ultimately gives rise to salinity stress (Munns 2002). Salinity

stress causes alkalinization of soil. In alkaline saline soil, the nutrients remain unavailable to the plant and it leads to the nutrient-deprived situation or nutrient stress (Maheshwari et al. 2012). Humidity is another climatic variability. In humid areas, the rate of precipitation is high, and soil leaching decreases soil pH due to the reduction of basic cations. The adverse effects of soil pH result in acidification stress and the acidification stress makes nutrient unavailable to plants and further leads to nutrient stress platform. The abiotic stresses thus are interconnected with one another and function as a chain due to climatic variations (Grover et al. 2011).

Inoculation with beneficial rhizosphere bacteria has recently been found to alleviate the abiotic stress. Some bacterial species such as *Paenibacillus polymyxa*, *Achromobacter piechaudii*, and *Rhizobium tropici* confer tolerance to drought stress in *Arabidopsis*, tomato (*Solanum lycopersicum*), and common bean (*Phaseolus vulgaris*), respectively, by accumulation of abscisic acid and due to degradation of reactive oxygen species and ACC (1-aminocyclopropane-1-carboxylate) (Timmusk and Wagner 1999; Mayak et al. 2004b; Figueiredo et al. 2008; Yang et al. 2008). Salinity tolerance in plants is conferred by inoculation of *Achromobacter piechaudii* and *B. subtilis* (Mayak et al. 2004a; Zhang et al. 2008; Choudhary and Sindhu 2016).

Maxton et al. (2018) studied *Burkholderia cepacia* and *Citrobacter freundii* possessing the maximal and the least plant growth-promoting efficacy under salt and drought stress. ACC deaminase activity of purified *B. cepacia*, *C. freundii*, and *Serratia marcescens* was 12.8 ± 0.44, 12.3 ± 0.56, and 11.7 ± 0.53 mM αKB (ketobutyrate) mg^{-1} min^{-1}, respectively. Under drought stress, *B. cepacia* showed maximum tolerance as it produced 4.893 ± 0.06 mg/mg protein of exopolysaccharide, followed by *C. freundii* and *S. marcescens* that produced 4.23 ± 0.03 and 3.46 ± 0.05 mg/mg protein, respectively. Thus, bacterial inoculation mitigated the effects of salinity by the proliferation of root system and increasing plant biomass, thus proving to be potential bioinoculum for alleviating abiotic stress.

Treatment of pea plants with *Pseudomonas* sp. containing ACC deaminase partially eliminated the effects of drought stress (Arshad et al. 2008). Similarly, treatment of tomato (*Solanum lycopersicum* L.) and pepper (*Capsicum annuum* L.) seedlings with *Achromobacter piechaudii* ARV8 reduced the production of ethylene (ET), which may have contributed to the observed drought tolerance (Mayak et al. 2004b). Lim and Kim (2013) showed that pepper plants treated with PGPR *Bacillus licheniformis* K11 tolerated drought stress and had better survival compared to non-treated plants. The observed drought tolerance was attributed to ACC deaminase production by PGPR that reduced ET concentrations by cleaving ACC.

5.3.2 Biotic Stresses

Plants being sessile, their growth and yield are strongly influenced by biotic stress caused by an infestation of insect, pathogenic fungi, weeds, etc. Microbial diseases cause a malfunction in plants which results in the reduced capability of the plant to survive and maintain its ecological niche. Plant diseases either result in death or

may greatly impair the growth and yield of the plant. Pathogenic microorganisms usually weaken or destroy plant tissues and reduce crop yields varying from 25% to 100% (Choudhary and Sindhu 2015). Among the different kinds of diseases, root diseases are estimated to cause 10–15% yield losses annually in the world.

5.3.3 Disease Development on Plants

Plant diseases are caused by pathogenic bacteria, fungi, protozoa, and viruses, and these diseases cause an estimated US$40 billion of losses annually worldwide (Roberts et al. 1994). At least 20–40% of losses in crop yield are caused by pathogenic infections (Savary et al. 2012). Some plant diseases can be highly destructive and catastrophic on a large scale. In the 1840s, the potato late blight pathogen *Phytophthora infestans* caused a major destructive disease that caused food shortages resulting in a million deaths and migration of 1.5 million people from Ireland (Donnelly 2002). The annual losses of potato crops due to late blight are conservatively estimated at the US$ 6.7 billion per year (Evers et al. 2007; Pimentel 2011). Another historic example, the brown leaf spot of rice caused by *Helminthosporium oryzae*, resulted in severe devastation by reducing rice yields which caused the death of two million people in Bengal in the 1940s as the direct result of calamitous famine (Tatum 1971; Ulstrup and Figenschou 1972). *Helminthosporium maydis* was the causal agent of a severe epidemic of southern corn leaf blight in 1970 in the USA that destroyed 15% of the US corn crop with losses estimated at US$1 billion (Tatum 1971; Ulstrup and Figenschou 1972). There are many more historical examples of the fungal, oomycete, bacterial, and viral plant pathogens and plant-parasitic nematodes, respectively, that are considered most significant for molecular plant pathology (Dean et al. 2012).

5.3.4 Effect of Pathogens on Plant Protein Contents, Photosynthesis, and Cell Structure

After entry into plant tissue, microbial pathogens disrupt normal plant function by producing toxins, degradative enzymes, and growth regulators. Plant pathogens produce pectinases, cellulases, and hemicellulases that result in degeneration of the plant structure, producing soft rots and other lesions. Destruction of plant growth regulators by plant pathogens results in dwarfism, whereas microbial production of IAA, gibberellins, and cytokinins by some plant pathogens results in gall formation and excessive elongation of plant stems. Toxins produced or induced by pathogenic organisms in plants interfere with normal metabolic activities of the plant. The toxin produced by *Pseudomonas syringae* pv. *tabaci*, which causes tobacco wildfire disease, has been characterized as β-hydroxy-diaminopimelic acid, and it interferes with the metabolism of methionine. Plants develop a variety of morphological or metabolic abnormalities as a result of microbial infections and develop various kinds of diseases such as necrosis (rots), wilt, chlorosis, hypoplasia, hyperplasia, gall, scab, canker, and blight.

Stress caused after infection of pathogen results in altered gene expression, leading to qualitative and quantitative changes in protein content (Corthals et al. 2000; Langham and Glover 2005). The increase in protein concentration could be due to activation of some genes which confer resistance under stress conditions. PR proteins are a category of plant proteins which are produced in plants in the event of a pathogen attack. Seventeen families of PR proteins have been discovered and classified as PR-1 to PR-17 (Swarupa et al. 2014). Pathogen recognition receptors (PRRs) are the most deliberated recognition proteins. These are cell exterior receptors and resistance genes (R-genes). Some of these proteins are cell surface receptors, but many of them are cytoplasmic proteins of the nucleotide-binding leucine-rich repeat (Swarupa et al. 2014). Due to stress, biomolecules undergo conformational changes, oxidation, and rupture of covalent bonds and formation of free radicals such as the hydroxyl and superoxide anion (Variyar et al. 2004). Molecular properties of proteins are modified by free radicals resulting in oxidative modifications of the proteins (Wilkinson and Gould 1996). Stress causes RNA synthesis failure and subsequent protein synthesis collapses (Bajaj 1970). The covalent bonds of polypeptide chains are broken due to stress, and this brings irreversible changes in conformation of protein at the molecular levels (Kume and Matsuda 1995).

Moreover, plants have evolved a cellular strategy that involves the activation of various enzymatic antioxidants to combat against pathogen toxicity (Krishna et al. 2013). Many plants are known to produce small molecular antioxidants, for example, phenolic compounds, ascorbate, glutathione, and tocopherols, for cellular protection (Shohael et al. 2006; Margesin et al. 2007). Under normal conditions, there is regulation of the scavenging process and the production of both enzymes and antioxidants (Yordanova et al. 2004). Antioxidant system modulation could reflect a defense response to the cellular damage provoked by pathogen toxins (Singh and Upadhyay 2014). Plant-pathogen interactions are affected by peroxidase and it interferes with the growth of plant cells (Passardi et al. 2004). Peroxidase in the plants is affected by special in vitro conditions including limited space, metabolic waste products, limited exchange of gases, and medium nutritive substance content (Svábová et al. 2011).

Infection by species of *Fusarium* adversely affects light as well as dark reaction of photosynthesis (Ayres et al. 1996; Pshibytko et al. 2006). Necrosis and leaf wilting were observed due to the reduction in the chlorophyll content. The concentration of chlorophyll a was higher than chlorophyll b in untreated plants. However, fungal-attacked plants showed higher concentrations of chlorophyll b compared to chlorophyll a (Dehgahi et al. 2015a, b). The infectious agent also consumes fixed carbon which could have been used for plant growth (Ayres et al. 1996). A drop in the uptake of minerals (e.g., magnesium) required for chlorophyll synthesis will indirectly reduce chlorophyll content in pathogen-infected plants and interfere with the photosynthesis reaction (Murkute et al. 2006; Sheng et al. 2008). The activity of enzymes involved in carbon assimilation including ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Ruiz-Lozano et al. 2012) may also be damaged by pathogen infection (Dehgahi et al. 2015a, b).

Inoculation with fungal toxin or culture filtrate causes plant cells to appear abnormal, shrunk, and irregular with broken cell walls in comparison to untreated plant cells (Dehgahi et al. 2014). Fungal-attacked cells showed symptoms of plasmolysis, denser cytoplasm density, shrinkage, and cell wall rupture. Plant cells attacked by fungi show the presence of storage materials which may contain protein and starch reserves around the nucleus (Das et al. 2008). The plant cells are ruptured and there is spillage of cytoplasmic components into the intercellular space (Dehgahi et al. 2015a, b). In fungal toxin-treated plant cells, the chloroplasts, mitochondria, vacuoles, cell walls, and plasma membrane structure appear damaged in comparison to untreated control plant cells (Wang et al. 2014; Dehgahi et al. 2015a, b). Fungal-infected cells showed damaged plasma membrane and distorted chloroplasts. The swelling of the chloroplast outer membrane leads to finally rupture after the fungal attack (Dehgahi et al. 2014). Larger plastoglobuli were found in the stromal regions of swollen chloroplasts. There is a separation of plasma membrane from the cell wall, and numerous small vacuoles are formed in the cytoplasm of the fungal-attacked cells. Cell death is caused due to an increase of vacuole number and later clearance of cytoplasm (Jiao et al. 2013).

5.3.4.1 Insect Infestation and Biotic Stress

Insects are among the most diverse living organisms as compared to other animals on Earth, comprising of more than a million identified species, and these represent more than half of all recognized living organisms (Chapman et al. 2012). Insects have certainly adapted to live in all the terrestrial situations, and they are present in numbers greater than other living animals. Less than 0.5% among total insect species are considered as pests, and only some of these can become a direct threat to humans and crops (Salam 2008). Insects destroy almost one-fifth of the total world's agricultural food productivity (Salam 2008) by chewing leaves; absorbing plant juice; boring within the roots, fruits, stems, and leaves; and spreading various plant diseases (Aetiba and Osekre 2015). Certainly, insecticides have improved the quality and yield of crops; however, their extensive and continuous applications are responsible for the rapid development of resistance in many insects.

5.3.5 Weed Occurrence and Biotic Stress

Weeds are unwanted useless plants that compete with crop plants for space, nutrients, water, sunlight, and other elements (Ferreira and Reinhardt 2016). Weeds usually cause in average ~20–37% losses of the world's agricultural output, and therefore, weed control is indispensable in every crop production system. About 1800 weeds species have been reported to cause serious economic losses in crop yield, and about 300 species are found in cultivated crops throughout the world (Ware and Whitacre 2004). Weeds are the silent robbers of plant nutrients, soil moisture, and solar energy and also occupy the space which would otherwise be available to the main crop. Weeds harbor insect-pests and disease-causing organisms leading to a reduction in the quality of farm produce and increased cost of production.

5.4 Characterization of Rhizobacteria Involved in Amelioration of Biotic Stress

Among the alternatives, biological control of plant pests and pathogens appears to be the best option for the development of low-cost, eco-friendly, and sustainable management approaches for protecting plants and crops. Biologicals, including biocontrol microbes, are now accepted as significant tools for the control of plant diseases in sustainable agriculture (Azcón-Aguilar and Barea 1997). However, a better understanding of the complex interaction between plants, environment, and pathogens is necessary for further exploration of rhizobacteria.

There are several methods for controlling weeds (Sindhu et al. 2018). Weed management forces the use of large amounts of human labor and technology to prevent crop losses (Fickett et al. 2013). Recently, labor has become unavailable and costly due to intensification and diversification of agriculture and urbanization. Therefore, chemical herbicides are applied under field conditions for successful weed management. However, more health and environmental hazards have been created in nature with the application of chemical herbicides (Soares and Porto 2009). The application of chemical herbicides to control weed population causes spray drift hazards and adversely affects the environment. Residual toxicity of these xenobiotics has resulted in high incidences of cancer, hormonal and immunological disorders, and allergies apart from the effects on reproductive ability. Moreover, continuous herbicide use may lead to a shift in weed flora and the evolution of resistant weed biotypes (Singh 2007a, b), threatening the efficacy of weed management in agriculture. These problems necessitated the search for an alternate eco-friendly and cost-effective method of weed management through the biological approach in which microorganisms or their products could be used to suppress the growth or population of the weed species (Templeton 1988; Kremer and Kennedy 1996; Gnanavel 2015).

5.4.1 Suppression of Pathogens

In agricultural soils, populations of beneficial microbes must be selectively recruited and maintained in the rhizosphere to suppress the growth of pathogens (Doornbos and van Loon 2012). Disease suppression by biocontrol agents occurs due to interactions among the biocontrol agents with pathogenic members of the rhizosphere or phyllosphere community. Several rhizosphere bacteria including *Pseudomonas* and *Bacillus* species possess many traits that make them well suited as biocontrol agents. Satisfactory biocontrol was achieved with *Pseudomonas* antagonists in sugar beet (Georgakopoulos et al. 2002). Better disease biocontrol in cucumber was achieved when bacterial antagonists were applied by drenching or by coating seed with bacteria in a peat carrier. *Pseudomonas* antagonists were found superior to *Bacillus* antagonists in controlling damping-off disease in cucumber and sugar beet. Ramette et al. (2006) found that *Pseudomonas* populations growing in the rhizosphere soil of tobacco produced biocontrol compounds, viz., 2,4-diacetylphloroglucinol (DAPG)

and hydrogen cyanide, which were suppressive to root-rot disease. *P. fluorescens* strain CHA0 was found to produce several secondary metabolites, notably HCN, 2,4-diacetylphloroglucinol, pyoluteorin, and indole acetic acid (Keel et al. 1992). The combined application of *P. fluorescens* and *B. subtilis* exhibited the highest reduction of tomato wilt disease and increased the dry weight of tomato plants up to 27% in comparison to the non-bacterized control (Sundaramoorthy and Balabaskar 2013).

Bacillus subtilis strain E1r-j isolated from wheat roots inhibited mycelium growth in vitro of numerous plant pathogenic fungi, especially of *Gaeumannomyces graminis* var. *tritici* (*Ggt*), *Coniothyrium diplodiella*, *Phomopsis* sp., and *Sclerotinia sclerotiorum* (Liu et al. 2009). Jiang et al. (2015) reported that *Brevibacillus laterosporus* strain JX-5 isolated from the poplar rhizosphere demonstrated significant growth inhibition of several pathogenic fungi in vitro. The fermentation broth of *B. laterosporus* JX-5 and its main antifungal component, designated as component B, reduced *B. dothidea*-associated canker of the excised poplar branch by 70 and 90%, respectively. Bioactive metabolic product inhibited *Botryosphaeria dothidea* by permeating the fungal membrane, fracturing the nuclei, damaging the cell wall, and eventually killing the pathogenic fungus.

Strains of *Bacillus* have been found to produce several antifungal compounds with significant inhibitory activity against *Ceratocystis ulmi* (Shigo et al. 1986), *Colletotrichum musae* (Mahadtanapuk et al. 2007; Alvindia and Natsuaki 2009), *Colletotrichum gloeosporioides* (Demoz and Korsten 2006), and *Fusarium moniliforme* (Agarry et al. 2005). Moreover, application of biomix of PGPR strains consisting of *Bacillus pumilus*, *B. subtilis*, and *Curtobacterium flaccumfaciens* to cucumber seeds enhanced the biological control of several cucumber pathogens and also increased the plant growth (Raupach and Kloepper 1998). The presence of AMF has also been associated with reductions in bacterial foliar pathogens (Parniske 2008). The inoculation of the prairie legume *Amorpha canescens* with AMF and rhizobial bacteria produced greater increases in plant biomass than inoculation with AMF or rhizobia alone (Larimer et al. 2014), suggesting synergistic effects of rhizobia and AMF on the growth of *A. canescens*. Usually, a combination of PGPR strains has been found more effective than single treatment in either suppressing disease or improving the plant growth (Pérez-Piqueres et al. 2006; Ahemad and Khan 2011; Yang et al. 2011). For example, the co-inoculation of *Cicer arietinum* (chickpea) with *P. indica* and *P. striata* showed that the presence of *P. indica* resulted in short-term increases of *P. striata* in the rhizosphere (Meena et al. 2010). The inoculation of *C. arietinum* with the *Glomus intraradices*, *Pseudomonas alcaligenes*, and *Bacillus pumilus* reduced the combined impact of *M. phaseolina* (root-rot fungus) and *M. incognita* (root-knot nematode), when compared to single-strain inoculants, dual-strain inoculants, and controls, indicating synergism between AMF and bacterial strains for control of *Macrophomina phaseolina* and *Meloidogyne incognita* in *C. arietinum* (Akhtar and Siddiqui 2008). Treatments with PGPR, mycorrhizal fungi, and 50% fertilizer exhibited a greater yield in the field than the control (100% fertilizer) (Hernández and Chailloux 2004), and this combination of beneficial microbes also showed the additive effect in stimulation of plant N and P adsorption (Table 5.1).

Table 5.1 List of rhizobacteria involved in the control of plant pathogens

Plants	Diseases/ pathogens	Biological control microbes	Mechanism/effect	References
Green gram (*Vigna radiata* L.)	Fungicide-induced phytotoxicity	*Pseudomonas aeruginosa*	Solubilized phosphate and produced IAA siderophores, exopolysaccharides, HCN, and ammonia	Ahmad et al. (2011)
Cabbage (*Brassica oleracea*)	Black rot (*Xanthomonas campestris*)	*Paenibacillus* sp.	Induced systemic resistance	Ghazalibigla et al. (2016)
Cucumber	Cucumber mosaic cucumovirus (CMV)	*Bacillus subtilis*, *P. fluorescens*, *A. chroococcum*	Higher peroxidase and β-1,3-glucanase enzyme activities	El-Borollosy and Oraby (2012)
			Production of pathogen-related (PR) protein	
Panax ginseng	Root diseases (*Phytophthora cactorum*)	*Bacillus amyloliquefaciens* HK34	Induced systemic resistance	Lee et al. (2015)
Rice	Bacterial leaf blight (*Xanthomonas oryzae*)	*Bacillus* sp.	Increased accumulation of phenylalanine ammonia lyase, peroxidase, and polyphenol oxidase	Udayashankar et al. (2011)
Pepper	Gray leaf spot disease (*Stemphylium lycopersici*)	*Brevibacterium iodinum* KUDC1716	Enhanced expression of pathogenesis-related (PR) protein genes	Son et al. (2014)
Arabidopsis thaliana	*Pseudomonas syringae* pv. tomato DC3000	*Bacillus cereus* AR156	ISR, systemic acquired resistance (SAR)	Niu et al. (2011, 2016)
Cucumis sativus	*Sphaerotheca fuliginea*	*Bacillus amyloliquefaciens* LJ02	SAR	Li et al. (2015)
Potato	Potato bacterial wilt (*Ralstonia solanacearum*)	*Bacillus amyloliquefaciens* and *B. subtilis*		Ding et al. (2013)
	Scab (*Streptomyces* spp.)	*B. amyloliquefaciens*		Meng et al. (2013)

(continued)

Table 5.1 (continued)

Plants	Diseases/ pathogens	Biological control microbes	Mechanism/effect	References
Cotton	*Verticillium* wilt	*Paenibacillus xylanilyticus* YUPP-1, *Paenibacillus polymyxa* YUPP-8, and *Bacillus subtilis* YUPP-2		Yang et al. (2013)
	Cotton leaf curl virus disease	*P. aeruginosa*, *Burkholderia* sp., and *Bacillus* spp.		Ramzan et al. (2016)
Pearl millet (*Pennisetum glaucum*)	*Sclerospora graminicola* (downy mildew)	*Bacillus pumilus* strains T4, SE34, INR7, *B. amyloliquefaciens* strains IN937a, *Bacillus subtilis* strains IN937b, GB03 *Brevibacillus brevis* strain IPC11		Raj et al. (2003)
Several crops		*Paenibacillus elgii*, *P. lentimorbus*, *P. polymyxa* strain E681, JSa-9, OSY-DF, and SQR-21	Production of antimicrobial compounds, enzymes, and polysaccharides	Choi et al. (2008), He et al. (2007), Canova et al. (2010) and Deng et al. (2011)

5.4.2 Biological Control of Insect Pests

Several microorganisms inhabiting the soil or plant rhizosphere have been identified to act as entomopathogens (Borneman and Becker 2007; Lacey et al. 2015). *Bacillus thuringiensis* (*Bt*) is the most studied entomopathogenic species for biological control of insect pests, and some of the toxin-producing strains have shown high mortality against specific insects compared to conventional insecticides used in the microbial pest management (Vega and Kaya 2012). The insecticidal proteins produced by *B. thuringiensis* are highly specific insect gut toxins and do not affect the non-target organisms (Lacey and Goettel 1995). *B. thuringiensis* subsp. *kurstaki* strain HD-1 (De Barjac and Lemille 1970) is most widely used for the management of lepidopteran pests in agriculture and forestry. Strains of *B. thuringiensis* subsp. *aizawai* (*Bta*) (i.e., ABTS-1857) are used against armyworms and diamondback moth larvae. Similarly, *Bacillus* strains belonging to the subsp. *israelensis* (*Bti*) and *tenebrionis* (*Btt*) have been employed for the management of mosquitoes and simulids and against coleopterans, respectively (Glare and O'Callaghan 2000). Other entomopathogenic bacteria that possess potential against diverse insect pests include

B. popilliae with *B. lentimorbus*, the causal agents of milky disease in phytophagous scarab larvae (Zhang et al. 1997). *Serratia entomophila* contains a specific plasmid (pADAP) encoding genes implicated in pathogenicity against the grass grub, *Costelytra zealandica* (White) (Jackson et al. 1992).

Another group of entomopathogenic bacteria includes the endosymbionts of insecticidal nematodes, especially the members of the genera *Xenorhabdus* and *Photorhabdus* (Burnell and Stock 2000). These entomopathogenic bacteria and the nematodes produce a variety of metabolites that enable them to colonize and reproduce in the insect host. The metabolites include enzymes such as proteases, lipases, and phospholipases to maintain a food supply during reproduction (Bowen et al. 2000). Bowen (1995) reported that a soluble protein fraction purified from *P. luminescens* culture medium possessed sufficient insecticidal activity to kill *Manduca sexta* upon injection. The novel protein toxin secreted by bacterium *Xenorhabdus nematophila* was found effective against *Galleria mellonella* and *H. armigera*, cabbage white caterpillar *Pieris brassicae*, mosquito larva *Aedes aegypti*, and mustard beetle *Phaedon cochleariae* (Sergeant et al. 2006). These bacteria were found effective on most of the economically important lepidopteran, dipteran, and coleopteran insect orders, suggesting the wide scope of these organisms for application in insect pest management.

Bacillus cereus has also been found pathogenic to insects and has been isolated from several insect species (Kuzina et al. 2001; Sezen et al. 2005). Various bacterial isolates, i.e., *B. cereus* (Ags1), *Bacillus* sp. (Ags2), *B. megaterium* (Ags3), *Enterobacter aerogenes* (Ags4), *Acinetobacter calcoaceticus* (Ags5), *Enterobacter* sp. (Ags6), *Pseudomonas putida* (Ags7), *Enterococcus gallinarum* (Ags8), and *Stenotrophomonas maltophilia* (Ags9), were identified from the flora of *Agrotis segetum* (Sevim et al. 2010), and these isolates caused 60% insect mortality after eight days of application. *B. cereus*, *B. sphaericus*, *Morganella morganii*, *Serratia marcescens*, and *Klebsiella* species isolated from the predatory larvae of the antlion species *Myrmeleon bore* (Neuroptera: Myrmeleontidae) were found to kill 80% or more cutworms *S. litura* (Nishiwaki et al. 2007). The bacterial flora *Leclercia adecarboxylata* of Colorado potato beetle showed highest insecticidal effect (100% mortality) within five days (Muratoglu et al. 2009) and thus showed potential for the control of several coleopteran pests. *Pseudomonas entomophila* showed insecticidal properties against insects in different orders and triggered a systemic immune response in *Drosophila melanogaster* Meigen after ingestion (Vodovar et al. 2006). Similarly, biopesticidal potential of *Brevibacillus laterosporus* Laubach has been reported against insects, such as those belonging in the orders Coleoptera (Boets et al. 2004) and Lepidoptera (Oliveira et al. 2004), mosquitoes and black flies (Rivers et al. 1991), and house flies (Ruiu et al. 2006), and against nematodes (Singer 1996). *Chromobacterium subtsugae* showed its insecticidal potential after ingestion against diverse insect species in different orders (i.e., Coleoptera, Lepidoptera, Hemiptera) (Martin et al. 2007; Hoshino 2011).

Khan et al. (1985) reported that a commercial preparation of *B. thuringiensis* (Thuricide-HP concentrate) exhibited 100% mortality within 6 days of exposure against subterranean termites, i.e., *H. indicola*, *M. championi*, and *Bifiditermes beesoni* (Gardner) (Kalotermitidae). Similarly, the colonies of *M. championi*, *H. indicola*, and *B. beesoni* exposed to suspensions of the spore-forming bacterium *Serratia marcescens* Bizio succumbed completely 7–13 days following infection (Khan et al. 1977). Khan et al. (1992) showed that mortality of *M. championi*, *H. indicola*, and *Coptotermes heimi* (Wasmann) (Rhinotermitidae) termites ranged from 25–52% after 7 days post-inoculation to 84–100% 25 days post-inoculation due to the pathogenicity of *P. aeruginosa* (Schroeter). Osbrink et al. (2001) isolated biological control agents from dead termites and revealed the presence of 15 bacteria and 1 fungus in dead termites. Bacteria isolated from termite substrata included *Corynebacterium urealyticum* Pitcher, *Acinetobacter calcoaceticus/baumannii/Gen2* (Beijerinck), *S. marcescens*, and *Enterobacter gergoviae* Brenner. Devi et al. (2007) observed killing of *Odontotermes obesus* subterranean termites under in vitro conditions by three HCN-producing rhizobacterial species, i.e., *Rhizobium radiobacter*, *Alcaligenes latus*, and *Aeromonas caviae*. Rakshiya et al. (2016) reported that 63 bacterial isolates obtained from termite mound soils killed the termites under Petri plate conditions at 2 days of observation. Killing frequency of different bacterial isolates was found to vary from% 40 to 90%.

5.4.3 Microorganisms Having Bioherbicidal Properties

Biological control of weeds represents an effective and innovative means to manage troublesome weeds (Harding and Raizada 2015). It utilizes the naturally occurring rhizosphere microorganisms with deleterious/phytotoxic activity toward the seedling growth of weed due to the production of secondary metabolites (Khattak et al. 2014; Sayed et al. 2014; Boyette and Hoagland 2015; Lakshmi et al. 2015). These compounds either kill or retard the growth of weeds so that beneficial plant species can gain a competitive advantage (Olesen et al. 2004). Biological control of weeds has several advantages including higher selectivity, the capacity to inhibit plant growth, the diminished potential for resistance, lower production costs, and the introduction of environment-friendly practices (Boyetchko and Rosskopf 2006; Sforza and Jones 2007; Sindhu and Sehrawat 2017). Rhizobacteria have been demonstrated as a novel, nonchemical approach for suppressing the weed growth (Kennedy et al. 1991, Kremer and Kennedy 1996; Kremer 2006). Several deleterious rhizosphere bacteria (DRB) such as *Enterobacter*, *Klebsiella*, *Grimontella*, *Novosphingobium*, *Microbacterium*, *Acinetobacter*, *Pantoea*, *Variovorax*, *Asticcacaulis*, *Chryseobacterium*, *Herbaspirillum*, *Mitsuaria*, *Moraxella*, *Serratia*, *Shinella*, *Sphingobium*, *Xanthomonas*, *Alcaligenes*, *Pseudomonas*, etc. have been found to inhibit weed germination and growth of seedlings (Imaizumi et al. 1997; Mejri et al. 2013). These rhizosphere microorganisms have been found to suppress the growth of weeds by reducing weed density, biomass, and seed production. Many of the microorganisms have been released as commercial bioherbicides for different

crops, and there are immense possibilities for characterizing and developing novel microbial bioherbicides that could reduce the application of chemical herbicides for weed control in sustainable agriculture.

Kennedy et al. (1991) screened 1000 isolates of pseudomonads for differential inhibition of downy brome (*Bromus tectorum*) and winter wheat. The filtrate obtained from eight percent of the isolates inhibited root growth of downy brome on agar but did not affect root growth of winter wheat. When applied to soil (10^8 CFU mL^{-1}) in the field, two isolates (0.2%) suppressed downy brome by ~31 to 53%, and this treatment increased winter wheat yield by ~18 to 35%. *P. fluorescens* strain D7 was found to selectively inhibit growth and germination of a number of grassy weeds (Kennedy et al. 1991, 2001; Gealy et al. 1996). *Pseudomonas fluorescens* and *P. syringae* pv. *tabaci* and *tagetis* have also been reported to be potential biological agents for weeds (Daigle et al. 2002; Zidack and Quimby 2002; Zdor et al. 2005). The strain *X. campestris* pv. *poae* (JT-P482) was registered in Japan in 1997 for control of annual blue grass (*Poa annua*) under the product name Camperico (Imaizumi et al. 1997; Tateno 2000).

Rhizosphere microorganisms and their metabolites have been evaluated as weed control agents in different crop systems (Norman et al. 1994; Mazzola et al. 1995; Gealy et al. 1996). For example, live cultures of *Pseudomonas syringae* strain 3366 were found to reduce weed root growth in a controlled environment (Johnson and Booth 1983) and in field studies (Kennedy et al. 1991). Inoculation with *Bacillus* strain was found to suppress the growth of *Phalaris minor* weed species more effectively (Phour 2012), and inoculation of bacterial isolate WHA87 caused 21–81% decrease in root dry weight and 33–43% decrease in shoot dry weight of *Chenopodium album* at different stages of plant growth under pot house conditions (Khandelwal 2016). Inoculation of the *Pseudomonas trivialis* strain X33d caused the growth suppression of great brome weed and promoted the growth of durum wheat (Mejri et al. 2010).

Serratia plymuthica strain A153 showed strong growth-suppressing activities against a range of broad-leaved weeds after foliar spraying (Weissmann et al. 2003). In field tests of *S. plymuthica* strain in spring wheat, spring barley, and potatoes, variable effects were achieved on a range of weeds including *Chenopodium album*, *Stellaria media*, *Polygonum convolvulus*, and *Galeopsis speciosa*. At one site, good suppression of *C. album* was observed when the strain was applied in a tank mix with another bacterial isolate or with reduced doses of herbicide. Li and Kremer (2006) demonstrated that *P. fluorescens* strain G2-11 inoculated to wheat and soybean crops suppressed the growth of *Ipomea* sp. and *Convolvulus arvensis* weeds, while it promoted the growth of agricultural crops. Zermane et al. (2007) reported that *P. fluorescens* has the potential for controlling *Orobanche crenata* and *O. foetida* (broomrape) in Northern Tunisia. Fifteen potential deleterious rhizospheric bacteria were characterized from the rhizosphere of *Sida acuta* (Patil 2014). Five of these bacterial isolates significantly reduced the root and shoot lengths of weed seedlings compared to the crop plants on agar plate bioassay. *Xanthomonas* sp. was found to inhibit root and shoot length of crop plants in a range of ~25–36% and 8–34%, respectively. Sayed et al. (2014) isolated actinobacterium *Streptomyces*

levis strain LX-65 from cultivated soil, and it was found to produce an extracellular metabolite that exhibited effective antibacterial, antifungal, and herbicidal activity against some weeds associated with winter wheat (*Triticum aestivum* L.) and maize (*Zea mays*). The virulence and host range of a bacterial pathogen *Xanthomonas campestris* isolate LVA987 were evaluated as a bioherbicide against *Xanthium strumarium* L. (common cocklebur) under greenhouse conditions (Boyette and Hoagland 2013a, b, 2015). Rosette leaf stage plants were found more susceptible than older plants, and increasing inoculum from 10^5 to 10^9 cells mL^{-1} caused significantly greater plant mortality and biomass reduction of plants in both the rosette and bolting growth stages. Strain Ha1 isolated from brine in Bohai, China, showed the highest herbicidal activity, and it was identified as *Serratia marcescens* based on 16S rDNA sequencing (Juan et al. 2015).

5.5 Mechanisms Involved in Amelioration of Biotic Stress

The contributions of PGPR include the production of hydrolytic enzymes (chitinases, cellulases, proteases, etc.) and various antibiotics in response to plant pathogen or disease resistance, induction of systematic resistance against various pathogen and pests, production of siderophores and VOCs, etc. (Gupta et al. 2014).

5.5.1 Antibiotic Production

Some rhizosphere bacteria such as *P. fluorescens* and *Bacillus amyloliquefaciens* contain large gene clusters, which are involved in detoxification and production/release of antibiotics and siderophores (Paulsen et al. 2005; Chen et al. 2007). These antibiotics inhibit the growth of the pathogens and cause suppression of pathogens in soils (Raaijmakers and Mazzola 2012). The production of antibiotics by PGPR against several plant pathogens has become one of the most effective and most studied biocontrol mechanisms over the past two decades (Ulloa-Ogaz et al. 2015). Most *Pseudomonas* species produce a wide variety of antifungal antibiotics, i.e., phenazines, phenazine-1-carboxylic acid, phenazine-1-carboxamide, pyrrolnitrin, pyoluteorin, 2,4-diacetylphloroglucinol (2,4-DAPG), rhamnolipids, oomycin A, cepaciamide A, ecomycins, viscosinamide, butyrolactones, *N*-butylbenzene sulfonamide, and pyocyanin (Ramadan et al. 2016). *Bacillus* sp. also produces a wide variety of antifungal and antibacterial antibiotics. The ribosomal originating antibiotics include subtilosin A, subtilintas A, and sublancin, and those of the non-ribosomal origin include chlorotetain bacilysin, mycobacillin, rhizocticins, difficidin, bacillaene, etc. *Bacillus* sp. also produced a wide variety of lipopeptide antibiotics, such as surfactin, iturins, bacillomycin, etc. (Wang et al. 2015).

These antimicrobial compounds and secondary metabolites play an important role in establishing microbial communities in the rhizosphere, which may help in competitive niche exclusion (Bulgarelli et al. 2013). For example, secretion of antibiotic phenazine-1-carboxylic acid and 2,4-DAPG by the *Pseudomonas* spp. caused the

suppression of the soil-borne pathogen *Rhizoctonia solani* (Raaijmakers et al. 1997; Mendes et al. 2011). Similarly, the production of lipoproteins by *Pseudomonas* and *Bacillus* spp. inhibited the growth of a wide range of pathogens (Raaijmakers et al. 2010; Watrous et al. 2012; Zachow et al. 2015). Inoculation with *Pseudomonas* spp. that synthesized 2,4-DAPG inhibited the growth of *Gaeumannomyces graminis* var. *tritici* and resulted in control of take-all disease (TAD) in wheat (Weller et al. 2002). Thus, microbes that produce secondary metabolites, i.e., antibiotics and toxins, may outcompete pathogens to occupy similar niches and may establish in the rhizosphere or inside roots (Thomashow and Weller 1988; van Loon and Bakker 2006; Kim et al. 2011). Kataryan and Torgashova (1976) reported that antibiotic 2,4-DAPG showed phytotoxic activity resembling that of 2,4-dichlorophenoxyacetate (2,4-D) herbicide. Phytotoxic metabolites geldanamycin and nigericin were obtained from a strain of *Streptomyces hygroscopicus*, and geldanamycin showed significant pre-emergence activity on proso millet, barnyard grass, garden cress, and giant foxtail.

5.5.2 Toxin and Phytotoxin Production

Bacillus thuringiensis has been found to produce various virulence factors including insecticidal parasporal crystal (Cry) toxins, δ-endotoxin, vegetative insecticidal proteins, phospholipases, immune inhibitors, and antibiotics (de Maagd et al. 2003). Most of the insecticidal activity of *B. thuringiensis* is associated with the proteinaceous toxins located in the parasporal crystals which are collectively referred to as δ-endotoxins. The activated Cry I protein after ingestion in insect gut causes pore formation, membrane transport disruption, and cell lysis leading to the death of the insect (Bravo et al. 2007). Vegetative insecticidal proteins (Vips) produced by *B. cereus* and *B. thuringiensis* also showed similar activity to endotoxins. Vip1 and Vip2 are toxic to coleopteran insects and Vip3 is toxic to lepidopteran insects (Zhu et al. 2006). Vips have excellent activity against black cutworms and armyworms (Yu et al. 1997), *S. frugiperda* (Barreto et al. 2005), *S. litura* and *Plutella xylostella* (Bhalla et al. 2005), *Heliothis zea*, *Trichoplusia* sp., and *Ostrinia nubilalis* (Fang et al. 2007; Sellami et al. 2011). *Lysinibacillus sphaericus* produced insecticidal toxins during the vegetative phase of growth, and mosquitoes have been found to be the major targets of the bacterium. Sphaericolysin, a toxin from the *L. sphaericus*, was also found lethal to the common cutworm *S. litura* (Nishiwaki et al. 2007).

Yersinia entomophaga isolated from diseased larvae of the New Zealand grass grub, *Costelytra zealandica* White (Coleoptera: Scarabaeidae), secreted a multisubunit toxin complex (Yen-Tc) (Hurst et al. 2011). It showed homology with toxin complexes produced by *Photorhabdus* sp. Tc-like proteins also identified in other entomopathogenic bacteria such as *Serratia entomophila* and the nematode symbiont *Xenorhabdus nematophila* (Morgan et al. 2001). Recently, the insecticidal activity of formulations containing *Y. entomophaga* against the pasture pest porina (*Wiseana* sp. larvae) has been reported under the field conditions (Ferguson et al. 2012). Khan et al. (1985) employed a commercial preparation of Bt (Thuricide-HP concentrate) that exhibited 100% mortality of *H. indicola*, *M. championi*, and

Bifiditermes beesoni (Gardner) (Kalotermitidae) within 6 days of exposure. Grace and Ewart (1996) constructed recombinant cells of the bacterium *P. fluorescens* that expressed the δ-endotoxin genes of *B. thuringiensis* (Bt). Two commercial agricultural formulations prepared by the CellCap process were evaluated for palatability to the *C. formosanus* termite. The MVP formulation, active against Lepidoptera, contained the *P. fluorescens* encapsulated δ-endotoxin of Bt var. *kurstaki*. Similarly, the M-Trak™ formulation, active against Coleoptera, contained the δ-endotoxin of Bt var. *san diego*.

Similarly, bacteria and fungi produced various phytotoxins with the potential to be used as herbicides (Duke et al. 1991). Rhizobitoxine produced by some *Bradyrhizobium* strains (Duke et al. 2011) is phytotoxic enough to act as a commercial herbicide (Giovanelli et al. 1973). Since the synthesis of the essential plant hormone ethylene is dependent on methionine, therefore, it is expected that ethylene synthesis would be greatly inhibited in plants treated with rhizobitoxine. *P. syringae* pv. *tagetis* (Pst) produced the phytotoxin tagetitoxin, which caused the symptom of apical chlorosis in infected plants. *P. syringae* strain CT99 isolated from *Cirsium arvense* (Canada thistle) was found to act as a biological control agent for this invasive weed and other weeds in the family *Asteraceae*. Alternatively, tagetitoxin may be of value as a natural herbicide because of its impact on chloroplasts (Lydon and Patterson 2001).

5.5.3 Production of Extracellular Enzymes

Rhizosphere bacteria produce enzymes such as β-1,3-glucanase and chitinase, which are generally involved in lysing cell walls and killing pathogens (Goswami et al. 2016). Most of the fungal cell wall components are comprised of β-1,4-*N*-acetyl-glucosamine and chitin; hence, β-1,3-glucanase- and chitinase-producing bacteria inhibit their growth. *Pseudomonas fluorescens* LPK2 and *Sinorhizobium fredii* KCC5 produced β-glucanases and chitinase and suppressed *Fusarium* wilt caused by *Fusarium oxysporum* and *Fusarium udum* (Ramadan et al. 2016). *Phytophthora capsici* and *Rhizoctonia solani*, regarded as the most catastrophic crop pathogens in the world, are also inhibited by PGPR (Islam et al. 2016).

Lysenko and Kucera (1971) showed that *Serratia marcescens* produced extracellular proteases that could be a mode of pathogenicity of these bacteria in termites. Osbrink et al. (2001) examined 15 bacteria and 1 fungus associated with dead termites as possible biological control agents against Formosan subterranean termites, *Coptotermes formosanus* Shiraki. Bacterial isolates obtained from dead termites were primarily *Serratia marcescens* Bizio that caused septicemia in *C. formosanus* and found to contain proteolytic enzymes. Singh (2007a, b) reported chitinolytic activity in some of the bacterial isolates that killed the termites. Bahar et al. (2011) identified chitinase-producing *Serratia marcescens* which was found effective in killing the coleopteran insects with more chitin in their exoskeleton. Jafri et al. (1976) observed microsporidians in the body cavity and proventriculus of *Microcerotermes championi* collected from the roots of *Saccharum munja*. These

organisms passed into the midgut after ingestion with the food attacked fat body tissues and caused the death of termites, indicating the role of lipolytic enzymes in termite killing. Rakshiya et al. (2016) reported that some of the bacterial isolates found effective in termite killing possessed all the three enzyme activities, i.e., lipase, protease, and chitinolytic activity.

1-Aminocyclopropane-1-carboxylate (ACC) deaminase-containing plant growth-promoting rhizobacteria catalyze the conversion of ACC, the immediate precursor of ethylene in higher plants, into ammonia and α-ketobutyrate. ACC deaminase has been widely reported in numerous microbial species of Gram-negative bacteria, Gram-positive bacteria (Ghosh et al. 2003), rhizobia (Uchiumi et al. 2004), endophytes (Pandey et al. 2005; Sessitsch et al. 2005), fungi (Jia et al. 1999; Minami et al. 1998), *Burkholderia* (Blaha et al. 2006), *Pseudomonas* (Belimov et al. 2001; Blaha et al. 2006), *Ralstonia solanacearum* (Blaha et al. 2006), *Sinorhizobium meliloti* (Belimov et al. 2005), and *Variovorax paradoxus* (Belimov et al. 2001). The plants treated with bacteria containing ACC deaminase showed relatively extensive root growth due to less ethylene (Burd et al. 2000; Shaharoona et al. 2006) and can better resist various stresses (Burd et al. 2000; Safronova et al. 2006). PGPR containing ACC deaminase activity have been found to promote plant growth both under stress and normal conditions, and genetic manipulation of cultivars with genes expressing this enzyme has attracted much attention among the scientists (Safronova et al. 2006; Sergeeva et al. 2006). The ACC deaminase-containing bacteria *Pseudomonas putida* (WPTe) reduced the downy mildew disease severity and significantly improved the growth and yield of *P. somniferum* plants (Barnawal et al. 2017). Reduced synthesis of ethylene precursor (ACC) and abscisic acid (ABA) and enhanced production of indole acetic acid (IAA) in *P. somniferum* plants were observed upon WPTe treatments. Moreover, WPTe treatment reduced proline and lipid peroxidation in plant leaves. These results highlighted that the ACC deaminase-containing plant growth-promoting rhizobacteria (PGPR) enhance the tolerance of *P. somniferum* plant against downy mildew.

5.5.4 Production of Secondary Metabolites and Volatile Organic Compounds

Hydrogen cyanide (HCN) is known to be produced by many rhizosphere bacteria and has been demonstrated to play a role in the biological control of pathogens and pests. HCN-producing *P. aeruginosa* was found to have lethal effects on nematodes (Darby et al. 1999; Gallagher and Manoil 2001). Devi et al. (2007) tested three different species of HCN-producing rhizobacteria for their potential to kill subterranean termite *O. obesus*. *Rhizobium radiobacter* and *Alcaligenes latus* caused 100% mortality of the termites following one-hour incubation. *Aeromonas caviae*, which produced significantly lower amounts of HCN, caused only 70% mortality. Termites exposed to exogenous HCN showed 80% mortality at cyanide concentrations of up to 2 μg ml^{-1}. The observed HCN toxicity in termites could be correlated with the inhibition of the respiratory enzymes.

Pseudomonas aeruginosa (HM195190) strain KC1 was isolated from the rhizosphere of castor plants (*Ricinus communis*) indigenous to agricultural fields of Bihar (Lakshmi et al. 2015). Strain KC1 was found to produce cyanide (4.78 nmol L^{-1}) over a period of 36 h. Seed bacterization with strain KC1 exhibited a reduction in root and shoot length of *Amaranthus spinosus* and *Portulaca oleracea* weed seedlings in both laboratory and glasshouse experiments. Biomass was also significantly reduced for the weed seedlings in glasshouse experiments. However, KC1-inoculated crop seedlings (*Triticum aestivum*) were found to be less inhibitory as compared to weed seedlings. *P. fluorescens* strain BRG100 produced pseudophomin A and B, which are cyclic lipodepsipeptides that showed suppressive activity on the grassy weed green foxtail (*Setaria viridis*) (Quail et al. 2002; Caldwell et al. 2011). This strain can reduce the root growth in green foxtail by 73 to 79% and is able to colonize root hairs and the root of green foxtail (Caldwell et al. 2011). Gostatin, a product of *Streptomyces sumanensis* (Amagasa et al. 1994), is a potent aminotransferase inhibitor that is phytotoxic (Nishino et al. 1984). The germination-inhibiting activity of *P. fluorescens* strain WH6 has been attributed to the production of a compound originally referred to as germination-arrest factor (GAF) (Banowetz et al. 2008). The active component of GAF was identified as 4-formyl aminooxy-L-vinylglycine (McPhail et al. 2010). The effects of cell-free supernatants (S) and anionic fractions (Q) obtained from three different strains of *Bacillus subtilis*, i.e., DN, Car13, and a non-promoting strain PY79, were evaluated on seed germination on pigweed (*Amaranthus hybridus* L.) and Johnson grass (*Sorghum halepense* L. Pers) (Mendoza et al. 2012). The application of anionic fractions QCar13, QDN, and QPY caused a drastic decrease in the germination rates of both pigweed and Johnson grass seeds in comparison to controls. *P. fluorescens* strain D7, which was isolated from roots of winter wheat, showed a reduction of downy brome (*Bromus tectorum* L.) biomass production of 18–54% in the field when the strain was applied to the soil (Ibekwe et al. 2010). This strain produced a complex of chromopeptides, peptides, fatty acids, and a lipopolysaccharide matrix.

Volatile organic compounds (VOCs) that are produced by biocontrol strains were found to promote plant growth, inhibit bacterial and fungal pathogens and nematodes, and elicit induced systemic resistance in plants against phytopathogens (Raza et al. 2016a, b). VOC emissions are a common characteristic of a wide variety of soil microorganisms and include cyclohexane, 2-(benzyloxy)-1-ethanamine, benzene, methyl, decane, 1-(*N*-phenyl carbamyl)-2-morpholinocyclohexene, dodecane, benzene (1-methylnonadecyl), 1-chlorooctadecane, tetradecane, 2,6,10-trimethyl, dotriacontane, and 11-decyldocosane, although the quantity and identity of the VOCs emitted vary among species (Kanchiswamy et al. 2015). Particular bacterial species from diverse genera, including *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Stenotrophomonas*, and *Serratia*, produced VOCs that affected plant growth. 2,3-Butanediol and acetoin produced by *Bacillus* spp. are the most effective VOCs for inhibiting fungal growth and improving plant growth (Santoro et al. 2016). It has been reported that bacterial VOCs are determinants for eliciting plant ISR (Sharifi and Ryu 2016). The VOCs from PGPR strains directly or indirectly mediate increased disease resistance, abiotic stress tolerance, and plant biomass.

5.5.5 Production of Hormones

Auxins and cytokinins have been demonstrated to act in defense responses either depending on other defense-related hormones such as salicylic acid and jasmonic acid or independently (Naseem and Dandekar 2012). The synthesis of auxin- and cytokinin-like molecules by some root pathogens (Estruch et al. 1991; Argueso et al. 2009) indicated that the production of these two hormones is not restricted to either beneficial (symbiotic) or detrimental (pathogenic) microorganisms (Chen et al. 2014).

5.5.5.1 Indole Acetic Acid Production

Indole acetic acid (IAA) production stimulates plant growth in lower concentrations, and in contrast, if the concentration becomes higher, the elongation of root and shoot is inhibited (Grossmann 2010). In addition, application of auxin promotes the susceptibility of the plant to bacterial pathogens and increases disease symptoms (Spaepen and Vanderleyden 2011). Natural auxins have modes of action similar to many herbicides that interfere with plant growth such as 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid (Patten and Glick 1996). Sarwar and Kremer (1995) reported that auxins are produced in high concentrations in the rhizosphere by deleterious rhizobacteria (DRB) that may contribute to reduced root growth of weeds. An *Enterobacter taylorae* isolate with high auxin-producing potential (72 mg L^{-1} IAA-equivalents) was found to inhibit root growth of field bindweed (*Convolvulus arvensis* L.) by ~91% when combined with 10^{-5} M L-tryptophan compared with non-treated control. IAA production in *Bacillus japonicum* isolates GD3 resulted in suppression of morning glory growth (Kim and Kremer 2005). The specificity assay showed the suppressive activity of *P. trivialis* X33d against great brome (*Bromus diandrus* Roth), and it caused growth-promoting effect on most of the considered crops, especially durum wheat (*Triticum durum* Desf.) (Mejri et al. 2010). The production of indole acetic acid by *P. trivialis* X33d was suggested to cause growth suppression of great brome and growth promotion of durum wheat.

Park et al. (2015) reported that two bacterial strains, I-4-5 and I-3, significantly reduced the seedling growth of radish in comparison to their controls. The highest rate of seedling growth inhibition was observed in bacterial isolate I-3 treatment in lettuce and radish. In vitro study revealed that culture filtrate obtained from I-3 bacterial isolate and combined with tryptophan significantly decreased leaf length, leaf width, and root length and increased the number of lateral roots of lettuce. Similarly, ten rhizobacterial isolates, obtained from wheat rhizosphere soil, showed maximum retardation on 5th and 10th day of seed germination of *Phalaris minor* on 0.8% water agar plates (Phour 2012). At 10th day of seed germination, ~15% of bacterial isolates showed retardation of shoot growth and ~19% of bacterial isolates retardation of root growth. Screening of these rhizobacterial isolates for production of indole acetic acid showed that two isolates HWM49 and HWM35 produced 11.10 and 14.07 μg mL^{-1} IAA, respectively, and significant production of IAA (>than 25 μg mL^{-1}) was observed in isolates CPS67, CP43, and HWM13.

5.5.5.2 Aminolevulinic Acid Production

δ-Aminolevulinic acid (ALA) has recently been used as a favorable biodegradable herbicide and insecticide, and it is harmless to crops, humans, and animals (Sasikala et al. 1994; Bhowmick and Girotti 2010; Kang et al. 2012). Zhang et al. (2006) reported that ALA at low concentrations of 0.3–3 mg L^{-1} promoted development and growth of potato microtubers in vitro and enhanced protective functions against oxidative stresses, but ALA at 30 mg L^{-1} and higher concentrations may induce oxidative damage. Khandelwal (2016) showed that 96 rhizobacterial isolates obtained from the rhizosphere of wheat and mustard showed significant retardation effect on seed germination of weed *Chenopodium album* and *Asphodelus tenuifolius* on 0.8% water agar plates. Rhizobacterial isolates WSA38, MSA57, WSA68, WSA56, MSA42, MSA39, WHA98, and MSA11 showed >11.0 μg ml^{-1}δ-aminolevulinic acid production, which contributed to growth retardation of *C. album* and *A. tenuifolius*. Forty-five isolates showed root growth inhibition on the 5th day of seed germination in *C. album*. Nine rhizobacterial isolates caused shoot growth inhibition on the 5th day, and seven bacterial isolates caused shoot growth inhibition at 10th day of *C. album*. In *Asphodelus tenuifolius*, 34 isolates showed root growth inhibition on the 5th day, and 27 rhizobacterial isolates showed root growth inhibition at 10th day of seed germination.

5.5.6 Plant Defense Mechanism

Innate immunity in plants is of two types, namely, effector-triggered immunity (ETI) and microbial-associated molecular pattern-triggered immunity (MTI; also called PTI). Callose deposition, reactive oxygen species production, salicylic acid (SA) accumulation, and expression of pathogenesis-related (PR) genes take place in PAMP-triggered immunity (PTI) (Yang and Huang 2014). However, successful pathogens produce protein effectors to suppress PTI, leading to effector-triggered susceptibility (ETS) (Feng and Zhou 2012). To counter the pathogen, plants have evolved a secondary immune response, called as effector-triggered immunity. Resistance (R) proteins trigger ETI, and these proteins can recognize specific pathogen effectors and suppress them. R proteins trigger hypersensitive response (HR) and death of cells at the infection site to limit pathogen growth is mediated by HR (Huang et al. 2016). Microbe-pathogen-associated molecular patterns (MAMPs/PAMPs) are molecular signatures typical of whole classes of microbes. The recognition of these signatures plays a key role in innate immunity. Fungal chitin, xylanase or bacterial flagellin, lipopolysaccharides, and peptidoglycans are examples of PAMP. Damage-associated molecular patterns (DAMPs) respond to a compromised "self" and are recognized as endogenous elicitors (Boller and Felix 2009), and the other that responds to a compromised "self" (Malinovsky et al. 2014) is recognized by plants (Zvereva and Pooggin 2012). Transmembrane pattern recognition receptors (PRRs) are involved in PAMP and DAMP recognition (Onaga and Wydra 2016).

Interkingdom biochemical signaling between microorganisms (prokaryotes) plays a significant role in pathogen-host specificity, host defense response induction, and antagonism between pathogens and biocontrol microorganisms (Venturi and Fuqua 2013; Clinton and Rumbaugh 2016; Kan et al. 2017). Plants trigger the mitogen-activated protein kinase (MAPK) cascades on the perception of the pathogens, or their associated signals by specific plant receptors and hormone (jasmonates and ethylene)-dependent and hormone-independent signaling are activated, which results in mounting of a defense response against the invading necrotrophs. This response involves the activation of specific transcription factors that result in the production of antifungal proteins (plant defensins) or the accumulation of defensive secondary metabolites (phytoalexins). The perception and communication mechanisms triggered by pathogen-associated molecular patterns and the hormones are coordinated by the MAPK signaling cascades which integrate various aspects of the multi-layered plant defense response (Pandey et al. 2016).

Defense responses are more evident in the plant's production of pathogen-related (PR) proteins that are induced in pathological or related situations (Antoniw et al. 1980). The major families of PR proteins have been organized into 11 different classes, primarily on the basis of their amino acid sequence identity (van Loon et al. 1994). Many PR proteins have been shown to possess antimicrobial activity. In vitro studies of chitinases (PR-3 class) and β-1,3-glucanases (PR-2 class) showed that these proteins can inhibit fungal growth (Mauch et al. 1988; Sela-Buurlage et al. 1993), presumably by hydrolytic degradation of fungal cell walls. In addition, transgenic studies with constitutively upregulated expression of various PR proteins such as chitinases, β-1,3-glucanases, tobacco PR-1, and type I barley ribosome-inactivating protein (Alexander et al. 1993; Jach et al. 1995) resulted in plants having decreased disease severity after infection by fungal pathogens. These results demonstrated that PR proteins are important for active defense against disease. Following pathogen attack, PR-10 proteins are also induced in a wide variety of plant species including pea (Barral and Clark 1991), potato (Matton and Brisson 1989), soybean (Crowell et al. 1992), and sorghum (Lo et al. 1999). These PR-10 proteins share homology to a ribonuclease (RNase) isolated from phosphate-starved ginseng cells (Moiseyev et al. 1994), suggesting that PR-10 proteins may possess such activity.

5.5.6.1 Pathogen- or Microbe-Associated Molecular Pattern-Triggered Immunity

Communication between plants and microbes takes place by using different signaling molecules during their interaction (Kan et al. 2017). Plants recognize certain compounds released by microbes and mount first line of active plant inducible defense PTI (Schwessinger and Zipfel 2008). In PTI, conserved microbial elicitors known as PAMPs are recognized by membrane-bound extracellular receptors PRRs consisting of either the receptor-like proteins (RLPs) or receptor-like kinase (RLK) families (Nurnberger and Kemmerling 2009). In direct recognition of pathogens, plants can detect extracellular molecules referred to as PAMPs/MAMPs, e.g., bacterial flagellin, EF-Tu proteins, lipopolysaccharides, and peptidoglycans (Boller and

Felix 2009; Freeman and Beattie 2008), and/or intracellular effector proteins, e.g., Avr3a, Avrk, and Avra10 proteins, or tissue damage using pattern recognition receptor (PRR) proteins located on the cell surface or intracellularly (Rivas and Thomas 2005; Boller and Felix 2009).

The bacterial flagellum is composed of flagellin which is so far the best characterized PAMP in plants. The N-terminal part of the flagellin of *Pseudomonas syringae* has 22-amino-acid (flg22) peptide-spanning regions in the N-terminal part. This region elicits a typical immune response in a broad variety of plants (Felix et al. 1999). Flagellin perception in the model plant *Arabidopsis thaliana* is due to the leucine-rich repeat receptor-like kinase (LRR-RLK) FLAGELLIN-SENSING 2 (FLS2) PRR. In some species of plants, flagellin appears to be recognized by other means. In rice, the PRR activation is not allowed by flg22 epitope, but flagellin causes cell death (Takai et al. 2008). Another flagellin, flgII-28, has been identified in Solanaceae (Cai et al. 2011), though the corresponding PRR is yet to be identified. A stretch of 33-amino-acid residues physically links both flg22 and flgII-28, indicating that detection of both molecules is brought about by the same receptor, FLS2 (Clarke et al. 2013).

Elongation factor Tu (EF-Tu) is the most prevalent bacterial protein. It was first isolated from *Escherichia coli*. It plays the role of PAMP in Brassicaceae family including *A. thaliana* (Kunze et al. 2004). Defense responses in plants are triggered by the conserved N-acetylated epitope elf18 (first 18 amino acids of the protein). As an elicitor, the shorter peptide, elf12 (first 12 N-terminal amino acids), comprising the acetyl group is inactive but acts as a specific antagonist for EF-Tu-related elicitors. EF-Tu is recognized by the LRR-RLK EF-Tu RECEPTOR (EFR) of the same subfamily (LRRXII) as FLS2 (Zipfel et al. 2006).

The major constituent of fungal cell walls is chitin which is a homopolymer of (1,4)-linked N-acetylglucosamine (GlcNAc) unit and is a classical PAMP (Dodds and Rathjen 2010). Breakdown of microbial chitin polymers by plant chitinases (hydrolytic enzymes) takes place when pathogen comes in contact with the host. Different plants employ mechanisms that have common factors to perceive chitin, and this could be the possible reason for the evolution of pathogen's countermeasures, e.g., in the fungal pathogen *Cladosporium fulvum* (Jashni et al. 2015). The lysine motif (LysM)-RLP was the first chitin-binding PRR that was identified in rice and named chitin elicitor-binding protein (CEBiP) (Shimizu et al. 2010). CEBiP is a glycoprotein that is localized in the plasma membrane. After binding with chitin, CEBiP homodimerizes and there is the formation of a hetero-oligomeric complex with the chitin elicitor receptor kinase 1 (OsCERK1), the rice ortholog of *Arabidopsis* AtCERK1. A sandwich-type receptor system for chitin is formed due to binding (Hayafune et al. 2014), and the mechanism of perception, however, varies between plant species.

Plants can sense DAMP molecules and they are available for recognition only after cell/tissue damage. DAMP perception in plants bears striking similarities to DAMP perception in animals (Lotze et al. 2007). Cell wall components derived from the enzymatic activity of highly specific microbial homogalacturonan (HGA) are a good example of DAMPs (Liu et al. 2014a, b). The enhanced production of

oligogalacturonic acid (OGA) fragments from plant cell walls potentially acts as DAMP, which is perceived by receptors such as RLK THESEUS1 (THE1), ER, and WAK1. Thus, a good approach to have a strategy to improve plant protection is to study the expression of endogenous molecules and microbial cell wall-degrading enzymes and their inhibitors, e.g., polygalacturonases (PGs) and polygalacturonase-inhibiting proteins (PGIPs) (Schacht et al. 2011).

The Ca^{2+} and mitogen-activated protein (MAP) kinase signaling cascades and transcriptome reprogramming are triggered by these PAMPs (Boller and Felix 2009), leading to defense responses such as oxidative burst, ethylene production, and plant cell wall modifications (Asai et al. 2002). As a countermeasure, plants have acquired additional receptors, known as resistance (R) proteins, which recognize pathogen effectors to induce a response called ETI, which ultimately triggers HR cell death in plants (Liu et al. 2007). In addition, the induction of defense signaling is mediated by plant hormones such as jasmonic acid, ethylene, or salicylic acid on perception of the pathogen or its associated pattern (Broekaert et al. 2006; Meng and Zhang 2013), and these plant hormones act as secondary messengers in signaling networks triggered during PTI and ETI in the plant cell (Jones and Dangl 2006; Meng and Zhang 2013). For example, host innate immunity to *Pythium* is conferred by the jasmonic acid (JA) and ethylene (ET) signal pathways in roots, and the triggers of these pathways include cell surface components of the pathogen, metabolites, and protein effectors (Okubara et al. 2016). Roots also can mount chemical (metabolite-based) defenses against specific *Pythium* spp., and reciprocally, *Pythium* can degrade defense metabolites. In contrast, *P. oligandrum* is a mycoparasite of other *Pythium* species and also sends signals that trigger defense responses in plants.

5.5.6.2 Effect of Hormones on Defense Signaling

Two mutually antagonistic hormones, salicylic acid (SA) and jasmonic acid (JA), control the defense responses in plants in response to infection by different types of pathogenic microbes, and they orchestrate a different and complex transcriptional reprogramming that eventually leads to plant resistance. The attack of insect herbivores on the plant roots and leaves imposes different selection pressures on plants, which in turn produces contrasting responses in terms of gene expression and production of secondary metabolites and wound hormones (Johnson et al. 2016). Different kinds of plant defenses are reported against root herbivores as compared with foliar herbivores (Johnson and Rasmann 2015). Following herbivore recognition, plants configure their metabolism through changes in the phytohormonal networks (Johnson et al. 2016). Jasmonates, which are widely viewed as the master regulators of plant responses to herbivores, are less inducible in the roots than the leaves (Erb et al. 2012; Lu et al. 2015). Salicylic acid signaling can buffer the jasmonic acid response aboveground (Gilardoni et al. 2011). Root herbivore attack induces a different signal signature compared with leaf attack. For instance, attacked rice roots do not increase the biosynthesis of abscisic acid and ethylene (Lu et al. 2015), two important synergistic signals in the wound response of leaves. The difference may be explained by the fact that both hormones strongly influence root growth and architecture. Plants may, therefore, be able to maintain root

development under herbivore attack by maintaining abscisic acid and ethylene homeostasis. Thus, it is apparent that roots respond to pathogen or insect attack differently than shoots and regulate the defenses through modulating their phytohormonal networks in a tissue-specific manner.

5.5.6.3 Salicylic Acid

Characterization of genes functioning in SA biosynthesis, conjugation, accumulation, signaling, and cross-talk with other hormones has justified its role in the finely tuned immune response network (An and Mou 2011). Salicylic acid has also been found important in providing a basal defense to *Solanum tuberosum* against *Phytophthora infestans* (Halim et al. 2007). Transduction of the SA signal leads to the activation of genes encoding pathogenesis-related (PR) proteins, some of which have antimicrobial activity (van Loon et al. 2006). The regulatory protein nonexpressor of PR genes 1 (NPR1) was required for transduction of the SA signal because mutations in the NPR1 gene rendered the plant largely unresponsive to pathogen-induced SA production (Dong 2004). SA-mediated suppression of JA-inducible gene expression was blocked in *npr*1 mutant plants, demonstrating a crucial role for NPR1 in the cross-talk between SA and JA signaling (Spoel et al. 2003, 2007). A similar function of NPR1 in the cross-talk was reported in rice (*Oryza sativa*) (Yuan et al. 2007). Overexpression of cytosolic OsNPR1 suppressed JA-responsive transcription and enhanced the level of susceptibility to insect herbivory. Interestingly, NPR1-silenced wild tobacco (*Nicotiana attenuata*) plants demonstrated that these transgenic plants accumulated increased levels of SA upon insect herbivory and were highly susceptible to herbivore attack (Rayapuram and Baldwin 2007). Therefore, it was proposed that in wild-type plants NPR1 is required to negatively regulate SA production during herbivore attack and thus it suppressed SA/JA cross-talk to allow induction of JA-mediated defenses against herbivores.

Many plant pathogens manipulate host auxin biosynthesis to interfere with the normal developmental process of the host (Chen et al. 2007), and conversely, plants have evolved mechanisms to repress auxin signaling during pathogenesis. SA application caused global repression of auxin-related genes, resulting in stabilization of the Aux/IAA repressor proteins and inhibition of auxin responses (Wang et al. 2007). Application of exogenous ABA prevented SA accumulation and suppressed resistance to *P. syringae* in *Arabidopsis* (Mohr and Cahill 2003). A loss-of-function mutation in the *Arabidopsis MPK*4 gene, which encodes a mitogen-activated kinase, was found to impair JA signaling and simultaneously conferred enhanced resistance against bacterial and oomycete pathogens due to constitutive activation of SA signaling (Petersen et al. 2000).

Most wilt-causing pathogen strains of the *R. solanacearum* species were found to degrade SA via gentisic acid to pyruvate and fumarate. *R. solanacearum* strain GMI1000 expressed this SA degradation pathway during tomato pathogenesis (Lowe-Power et al. 2016). Transcriptional analysis revealed that subinhibitory SA levels induced the expression of the SA degradation pathway, toxin efflux pumps, and some general stress responses. Interestingly, SA treatment repressed expression of virulence factors, including the type III secretion system, suggesting that this

pathogen may suppress virulence functions when stressed. These results suggested that *R. solanacearum* degrades plant SA to protect itself from inhibitory levels of this compound and also to enhance its virulence on plant hosts like tobacco that uses SA as a defense signal molecule (Lowe-Power et al. 2016).

5.5.6.4 Jasmonic Acid

Jasmonic acid plays a key role in modulating many physiological processes and is a key cellular signal involved in the activation of immune responses to most insect herbivores and necrotrophic microorganisms (Glazebrook 2005). Cyclic precursors of jasmonic acid, the cyclopentenones, have also been reported to function as potent signals of plant defense responses (Farmer and Ryan 1992). Similarly, volatile derivatives of JA, such as methyl jasmonate (meJA) and cis-jasmone, can act as airborne signals stimulating plant defenses and repelling insects (Birkett et al. 2000). Together, JA and ethylene are required for defense against necrotrophic pathogens (Thomma et al. 2001) and associated gene expression (Xu et al. 1994; Lorenzo et al. 2003). The transcription factor ethylene response factor1 (ERF1) has been proposed to act as a convergence point in synergistic signaling of JA/ethylene (Lorenzo et al. 2003). Therefore, a good understanding of the interaction of plant roots with the microorganisms in the rhizosphere would be important to engineering resistance against root pathogens without negatively altering root-beneficial microbe interactions. The understanding and exploitation of the signals between plant and microorganisms could become the basis for crop improvement and protection.

5.5.6.5 Inducible Defense

Plants have the ability to induce both local and systemic resistance to subsequent attack by the same or different pathogens (Walters et al. 2005). This induced resistance (IR) may control the pathogens or damaging factors, completely or partially (Kuc 1982; Chen et al. 2014). Genes expressed during IR responses produce proteins with chitinase, glucanase, and other enzymatic activities that are involved in defense reactions to a wide array of pathogens (van Loon et al. 2006). Production of reactive oxygen species (ROS) and oxidative burst is an important mechanism for biotic stress tolerance (Miller et al. 2010). There are two common ways to manage the activation of defense mechanism in the plant, which are called induced systemic resistance (ISR) and systemic acquired resistance (SAR) (Pieterse et al. 2012). Their differentiation is done on the basis of regulatory pathways involved and the nature of the elicitor as demonstrated in model plant system (Uknes et al. 1992; Pieterse et al. 1998; Knoester et al. 1999; Yan et al. 2002).

Induced systemic resistance triggered by *P. aeruginosa* 7NSK2 was found to be iron-regulated and involved three siderophores, i.e., pyoverdine, pyochelin, and salicylic acid. SA is also a precursor in the production of SA-containing siderophores such as pseudomonine in *P. fluorescens* WCS374 and pyochelin in *P. aeruginosa* 7NSK2 (Audenaert et al. 2002). A mutant of 7NSK2 that lacked SA and pyochelin production no longer induced resistance, and a mutant defective in pyocyanin biosynthesis could not trigger ISR in tomato against *B. cinerea*. On the other hand, treatment with the mixture of two mutants showed significant suppression of

B. cinerea (Audenaert et al. 2002). The production of the volatile 2,3-butanediol triggered *Bacillus*-mediated ISR in *Arabidopsis* (Kloepper et al. 2004). However, the signaling pathway activated in *Bacillus* was found dependent on ethylene, but it was found independent of salicylic acid and jasmonic acid signaling (Ryu et al. 2004). Induced ethylene biosynthesis and subsequent intracellular signaling were found to induce the expression of a cascade of transcription factors consisting of primary EIN3-like regulators and downstream ERF-like transcription factors (Broekaert et al. 2006).

The ISR may be strengthened by non-pathogenic root-associated plant growth-promoting microbes, while plant exposure to virulent, avirulent, and non-pathogenic microbes can trigger SAR. SAR involves a change in molecular gene expression and is associated with pathogenesis-related (PR) protein and SA accumulation, and the time required for this accumulation depends on the plant and elicitors. Induction and expression of the gene in both ISR and SAR are different which depend on elicited and regulatory pathway (Nawrocka and Małolepsza 2013). ISR relies on pathways regulated by jasmonate and ethylene under biotic stress (Bari and Jones 2009; Salas-Marina et al. 2011). Reactive oxygen species and nitrogen oxygen species (NOS) highly influence SA, JA, or ET production and form a complex network to modulate pathogens (Bari and Jones 2009; Choudhary and Johri 2009). The elicitors released by non-pathogenic microbes and interaction of these molecules determine the induction of resistance in plants.

Induced systemic resistance (ISR) by rhizobacteria is activated upon colonization of roots by selected strains of non-pathogenic rhizobacteria (van Loon et al. 1998), and wound-induced resistance is typically elicited upon tissue damage such as that caused by insect feeding (Kessler and Baldwin 2002; Howe 2004). Specific strains of *B. amyloliquefaciens*, *B. subtilis*, *B. pasteurii*, *B. cereus*, *B. pumilus*, *B. mycoides*, and *B. sphaericus* caused significant reductions in the incidence or severity of various diseases on a diversity of hosts under greenhouse or field conditions (Kloepper et al. 2004). These strains induced systemic resistance (ISR) in tomato, bell pepper, muskmelon, watermelon, sugar beet, tobacco, *Arabidopsis* sp., cucumber, loblolly pine, and two tropical crops (long cayenne pepper and green kuang futsoi). Moreover, ISR induced by *Bacillus* spp. protected the plants against leaf-spotting fungal and bacterial pathogens, systemic viruses, a crown-rotting fungal pathogen, root-knot nematodes, and a stem-blight fungal pathogen as well as damping-off, blue mold, and late blight diseases. ISR elicited by several strains of *Bacillus* spp. was found independent of salicylic acid, but it was dependent on jasmonic acid, ethylene, and the regulatory gene *NPR1*.

ISR is also induced by strains belonging to genus *Pseudomonas* that cause no apparent damage to the plant's root system (van Loon and Glick 2004). Unlike SAR, ISR does not involve the accumulation of salicylic acid or pathogenesis-related proteins but jasmonate and ethylene signaling molecules (Pieterse et al. 2002; Yan et al. 2002). Lee et al. (2015) reported that root-associated *B. amyloliquefaciens* strain HK34 effectively induced resistance against *P. cactorum*. In addition, *Pseudomonas* and *Bacillus* strains manage plant disease in many crops through induced systemic resistance. *Paenibacillus* P16 performed as an effective biological

control agent in cabbage for black rot (*Xanthomonas campestris*) disease and has the potential ability to confer induced systemic resistance (Ghazalibigla et al. 2016).

5.5.6.6 Engineering of Plants and Microbes

The rhizosphere is the zone of soil around roots that is influenced by root activity. The intimacy of this interface between plants and their environment is essential for the acquisition of water and nutrients and for beneficial interactions with soil-borne microorganisms. Yet, this same intimacy increases the vulnerability of plants to a range of biotic and abiotic stresses. Plants have evolved a variety of strategies to modify the rhizosphere to lessen the impact of these environmental stresses, and an understanding of the involved processes will suggest ways in which the rhizosphere can be manipulated to improve plant health and productivity. Rhizosphere engineering may ultimately reduce our reliance on agrochemicals by replacing their functions with beneficial microbes, biodegradable biostimulants, or transgenic plants. Rhizosphere can be engineered through appropriate selection of crop species and varieties, by the introduction of microorganisms or soil amendments, and by genetic modification of plant and microbial biological activities. Plants could be selected by breeders with favorable traits or microorganisms can be engineered that increase nutrient accessibility, minimize biotic and abiotic stresses, suppress pathogenic microbes, or encourage the persistence of beneficial microorganisms (Weller 2007; Dey et al. 2009; Sindhu et al. 2009a, b). The emergence of molecular techniques now allows the direct manipulation of genes that influence rhizosphere functions, and continuing advances in biotechnology ensure more progress for the future. Metagenomics approach will benefit from the remarkable development of mass sequencing procedures and will enable us to explore the microbial diversity of the rhizosphere more rapidly and in greater detail (Rup Lal 2011).

5.5.6.7 Transgenic Plants

Transgenic plants have been produced with genes involved in different pathways to enhance disease resistance against fungal pathogens. An approach would be the expression of pathogenesis-related genes and defensins for controlling diseases. Defensins are small cysteine-rich peptides which have antimicrobial activity. The transgenic expression of plant defensins protects vegetative tissues against pathogen attack (Sanghera et al. 2011). Enhanced resistance in tobacco plants against *Rhizoctonia solani* has been shown by the *chit*1 gene from the entomopathogenic fungus *Metarhizium anisopliae*, encoding the endochitinase Chit42 (Kern et al. 2010). Three genes, *ech*42, *nag*70, and *gluc*78, encoding hydrolytic enzymes from a biocontrol fungus *Trichoderma atroviride* were introduced in rice. Gluc78-overexpressing transgenic plants showed enhanced resistance to *Magnaporthe grisea* (Sanghera et al. 2011). Rizhsky and Mittler (2001) used the *Halobacterium halobium* bacterio-opsin (bO) gene under the control of the wound-inducible promoter Pin2 to develop transgenic tobacco plants resistant to *Pseudomonas syringae* pv. *tabaci* via *Agrobacterium*-mediated transformation. Bacterio-opsin activates the self-defense mechanisms in plants by enhancing proton pumping across the cell membrane (Mittler et al. 1995). Transgenic tobacco plants produced a

hypersensitive response (HR) due to the expression of the bO gene, and there was enhanced expression of different types of defense-related proteins such as chitinase, glucanase, and salicylic acid. The transgenic tobacco plants expressing the bO gene, when challenged with *P. syringae* pv. *tabaci*, slowed down the pathogen growth (Sanghera et al. 2011).

Agarwal and Agarwal (2016) highlighted the significance of a pathogenesis-related gene, JcPR-10a, from the biofuel crop *Jatropha curcas* L. toward stress/defense tolerance. The JcPR-10a recombinant protein exhibited RNase and DNase activity, and the protein also possessed antifungal activity against collar rot-causing fungus *Macrophomina phaseolina*. Furthermore, the overexpression of *JcPR-10a* gene resulted in improved shoot regeneration, salinity tolerance, and reduced fungal susceptibility in transgenic tobacco. The transgenics also showed enhanced endogenous cytokinin level as compared to wild-type plants, which further increased with salinity. Therefore, *JcPR-10a* gene can serve as an important candidate to engineer stress tolerance in *Jatropha* as well as other plants susceptible to collar rot by *Macrophomina.*

The release of organic anions such as citrate and malate has been reported to improve the availability of poorly soluble organic and inorganic phosphorus (Richardson et al. 2001; Ryan et al. 2001). Citrate as many other forms of dissolved carbon (e.g., glucose) is also an important source of energy for most microorganisms. Accordingly, when soluble carbon is available for microbial respiration and growth, P immobilization by microbes can directly affect P availability by removing PO^{4-} from the soil solution (Bünemann et al. 2004; Olander and Vitousek 2004). A bacterial citrate synthase gene was reported to increase exudation of organic acids and P availability to the plant when expressed in tobacco roots (Lopez-Bucio et al. 2000). Citrate-overproducing plants yielded more leaf and fruit biomass when grown under P-limiting conditions and required less P fertilizer to achieve optimal growth. This showed the putative role of organic acid synthesis genes in P uptake in plants.

5.5.7 Microbiome Engineering

Individual microbes or entire beneficial microbial consortia could be engineered to improve the growth of crop plants in different soil types. As a result, plant-/soil-optimized microbes can be used as inoculum for different crops in different soils. There is evidence that soil microbiomes adapt to their crops over time leading to improved plant-microbe interactions (Berendsen et al. 2012). Substantial evidence supports the major role of the naturally occurring plant microbiome in disease development and suppression in plants (Bulgarelli et al. 2013).

Among the nitrogen-fixing systems, the legume-*Rhizobium* symbiosis alone accounts for 70–80% of the total N fixed biologically on a global basis per annum and one-third of the total N input needed for world agriculture. The symbiotic rhizobia have been found to fix N ranging from 57 to 600 kg ha^{-1} annually (Elkan 1992). Annual inputs of fixed nitrogen are calculated to be 2.95 million tons (Tg) for

the pulses and 18.5 Tg for the oilseed legumes (Herridge et al. 2008). Due to host specificity characteristics of rhizobia, attempts have been made to broaden the host range of rhizobia by transfer of cloned nodulation genes, symbiotic plasmids, and mutational approaches as well as through protoplast fusion (Sindhu and Dadarwal 1985, 1993; Sindhu et al. 2003). Manipulations of common nodulation genes to improve the bacterial competition have usually resulted in either no nodulation, delayed nodulation, or inefficient nodulation (Devine and Kuykendall 1996). Mendoza et al. (1995) enhanced NH_4^+-assimilating enzymes in *R. etli* through genetic engineering, by adding an additional copy of glutamate dehydrogenase (GDH), which resulted in total inhibition of nodulation on bean plants. However, nodule inhibition effect was overcome when *gdh*A expression was controlled by NifA and thereby delaying the onset of GDH activity after nodule establishment (Mendoza et al. 1998).

Biotechnological approaches used to enhance N_2 fixation and crop productivity (Hardarson 1993; Sindhu et al. 2009a, b) under field conditions have been of limited use. Attempts to develop self-fertilizing crops for N have also been a failure, mainly because of the complexity of the nitrogenase enzyme complex to be expressed in absence of an oxygen protection system in eukaryotes (Dixon et al. 1997). Moreover, induction of nodule-like structures or pseudonodules using lytic enzymes or hormone treatment in wheat (*Triticum aestivum*) and rice (*Oryza sativa*) showed nitrogenase activity and $^{15}N_2$ incorporation, but the activity expressed was >1% of the value observed for legumes (Cocking et al. 1994). Fox et al. (2016) expanded the nitrogen-fixing ability to major cereal crops. The use of the efficient nitrogen-fixing rhizobacterium *Pseudomonas protegens* Pf-5 X940 was demonstrated as a chassis to engineer the transfer of nitrogen fixed by BNF to maize and wheat under non-gnotobiotic conditions. Inoculation of maize and wheat with Pf-5 X940 largely improved nitrogen content and biomass accumulation in both vegetative and reproductive tissues, and this beneficial effect was positively associated with high nitrogen fixation rates in roots. ^{15}N isotope dilution analysis showed that maize and wheat plants obtained substantial amounts of fixed nitrogen from the atmosphere. Pf-5 X940-GFP-tagged cells were always reisolated from the maize and wheat root surface but never from the inner root tissues. Confocal laser scanning microscopy confirmed root surface colonization of Pf-5 X940-GFP in wheat plants, and microcolonies were mostly visualized at the junctions between epidermal root cells. Genetic analysis using biofilm formation-related *Pseudomonas* mutants confirmed the relevance of bacterial root adhesion in the increase in nitrogen content, biomass accumulation, and nitrogen fixation rates in wheat roots.

Ortiz-Marquez et al. (2014) studied the biological nitrogen fixation carried out by some bacteria and archaea. The effect of controlling the maximum activation state of the *Azotobacter vinelandii* glutamine synthase by a point mutation at the active site (D49S mutation) was compared. Strains bearing the single D49S mutation were more efficient ammonium producers under carbon-/energy-limiting conditions and sustained microalgae growth at the expense of atmospheric N_2 in synthetic microalgae-bacteria consortia. However, citrate as many other forms of dissolved carbon (e.g., glucose) is also an important source of energy for most

microorganisms. Accordingly, when soluble carbon is available for microbial respiration and growth, P immobilization by microbes can directly affect P availability by removing PO^{4-} from the soil solution (Bünemann et al. 2004; Olander and Vitousek 2004).

Expression of the mineral phosphate-solubilizing (*mps*) genes in a different host could be influenced by the genetic background of the recipient strain, the copy number of the plasmids present, and metabolic interactions. Thus, genetic transfer of any isolated gene involved in MPS to induce or improve phosphate-dissolving capacity in PGPB strains is an interesting approach. An attempt to improve MPS in PGPR strains, using a PQQ synthase gene from *E. herbicola*, was carried out (Rodriguez et al. 2001). This gene was subcloned in a broad-host-range vector pKT230. The recombinant plasmid was expressed in *E. coli* and transferred to PGPR strains of *Burkholderia cepacia* and *Pseudomonas aeruginosa*. Several of the ex-conjugants that were recovered in the selection medium showed a larger clearing halo in medium with tricalcium phosphate as the sole P source. This indicates the heterologous expression of this gene in the recombinant strains and gave rise to improved MPS ability in these PGPR.

P. fluorescens strain BL915 synthesized the antifungal compound pyrrolnitrin. In one derivative, the regulatory gene *gac*A was constitutively expressed on a multicopy plasmid in BL915. This regulatory derivative produced about 2.5-fold more pyrrolnitrin than the parent strain (Ligon et al. 2000). A second derivative in which the entire four-gene *prn*ABCD operon was constitutively expressed from a plasmid produced fourfold more pyrrolnitrin. When both the plasmids were expressed in the same cells, antibiotic production was increased to tenfold level over those of the parental strain. In greenhouse trials, the derivative strains were protective of cucumber in soil infested with *R. solani* and *P. ultimum*, while on cotton protection was better than that provided by BL915 and not significantly different from chemically treated and healthy controls.

In another study aimed at improving strain efficacy, a cassette containing the PCA operon from *P. fluorescens* strain 2-79, expressed from a *tac* promoter, was transposed into random sites in the genome of *P. fluorescens* SBW25 (Timms-Wilson et al. 2000), which itself has no antibiotic activity. PCA-producing derivatives of SBW25 gave significantly better control of damping-off disease of pea caused by *Pythium ultimum* than did SBW25. Moreover, pre-treatment of the soil with the engineered strain effectively decontaminated it and reduced the disease incidence. In *P. fluorescens* Q8r1-96, a superior root colonizer that produces the unrelated antibiotic 2,4-diacetylphloroglucinol and controls take-all disease of wheat (Raaijmakers and Weller 2001), and recombinants expressing the PCA cassette produced more DAPG than did wild-type Q8r1-96 and more PCA than *P. fluorescens* 2-79. The recombinant strains suppressed not only take-all disease but also *Rhizoctonia* root rot and were effective at only 10^2 CFU per seed, an inoculum dose one to two orders of magnitude less than the dose of Q8r1-96 required for comparable control (Huang et al. 2004). In 3 years of field trials, wheat treated with the recombinant strains consistently had yields 8–20% greater than those from treatments with Q8r1-96.

P. fluorescens strain CHA0 transformed with ACC deaminase gene from *P. putida* UW4 (formerly classified as *Enterobacter cloacae*) provided improved protection of cucumber against *Pythium*, demonstrating the involvement of ethylene in this plant-pathogen interaction (Wang et al. 2012). Moreover, transformed *Pseudomonas* also increased root length of canola seedlings. Recombinant strains of *R. meliloti* have been constructed which carry genes to produce chitinase and express it during symbiosis in alfalfa roots (Sitrit et al. 1993). Downing et al. (2000) transformed cloned *chi*A genes of *Serratia marcescens* and *cry1*Ac7 genes of *Bacillus thuringiensis* in the sugarcane-associated endophytic bacterium, *Herbaspirillum seropedicae.* Expression of the genes resulted in biocontrol of sugarcane borer *Eldana saccharina*.

A study using appropriate mutant strains of *Bacillus amyloliquefaciens* FZB42 was performed recently, demonstrating that difficidin and bacilysin are efficient against two different *Xanthomonas oryzae* pathovars, causative agents of damaging rice diseases (bacterial blight and bacterial leaf streak). Agar diffusion tests performed with several FZB42 mutant strains revealed that the inhibitory effect of mutant CH8 *(Δdfn)* deficient in production of difficidin was clearly reduced compared to wild-type FZB42. The double mutant RS06 (*Δsfp Δbac*) was completely unable to suppress *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* suggesting that difficidin and bacilysin act as antagonists of the pathogenic *Xanthomonas* strains (Wu et al. 2015).

5.6 Conclusion

With the increase in the world's population, the demand for agriculture crop yield has increased tremendously. The use of fertilizers and pesticides in the agricultural fields has caused degradation of soil quality and fertility; thus the availability of agricultural land with fertile soil is limited. Reliable environment-friendly techniques are needed to sustainably meet growing global food demands. On the other hand, stressful environments deteriorate soil structure and also affect crop productivity. Increasing concerns for a safe environment and minimizing the use of agrochemicals in modern agriculture necessitate the search for the eco-friendly alternatives. Therefore, there is now a strong push to develop low-input and more sustainable agricultural practices that include alternatives to chemicals for providing nutrients and controlling pests and plant pathogens. Rhizobacteria have been found to enhance plant growth by a wide variety of mechanisms like biological nitrogen fixation, phosphate solubilization, siderophore production, production of ACC deaminase, phytohormone production, exhibition of antifungal activity, production of volatile organic compounds (VOCs), induction of systemic resistance, promotion of beneficial plant-microbe symbioses, and interference with pathogens by antibiotic or toxin production. Some plant-microbe interactions can alleviate stress, with the application of PGPR. New bacterial traits conferring strain survival in the rhizosphere have been found and opened a way to better understand specific signaling and the regulatory processes governing the plant-beneficial bacterial

association (Matilla et al. 2007). Use of molecular techniques in genetic modification of microbial and plant biological activities allows their better functioning in the rhizosphere (Ryan et al. 2009) leading to substantial improvement in the sustainability of agricultural systems.

The multipartite interactions in the rhizosphere involving microbes, crop plants, and weeds lead to assembly and maintenance of highly complex and specific root microbiome (Lareen et al. 2016; Rasmann and Turlings 2016). In addition to pathogens, plant roots interact with a plethora of non-pathogenic and symbiotic microorganisms. A good understanding of how plant roots interact with the microbiome would be particularly important to engineering resistance to root pathogens without negatively altering root-beneficial microbe interactions. Therefore, it is important to understand the role of these microbes in promoting growth (as biofertilizers) and controlling diseases (as biopesticides) under the field conditions, whose success in the field is still inconsistent. Farming methods that support the recruitment and maintenance of beneficial microbial communities in the rhizosphere could provide benefits to agriculture in the form of enhanced crop yields and suppression of diseases and growth of the weeds. Many more plant-microbe interactions remain to be uncovered, and a good understanding of the mechanisms and ecological implications could become the basis for exploitation and manipulation of these interactions for weed, pest, and disease control leading to improved crop productivity for sustainable agriculture. This review focuses on how biocontrol agents modulate plant defense mechanisms, deploy biocontrol actions in plants, and offer new strategies to control plant pathogens, weeds, and pests. In particular, new approaches of using "plant-optimized microbiomes" (microbiome engineering) and establishing the genetic basis of beneficial plant-microbe interactions will enable breeding of "microbe-optimized crops." The integration of microbial biofertilizers, biocontrol microbes, optimized microbiomes, soil amendments, and microbe-optimized crops for different soil types would be the ultimate goal to benefit most from positive plant-microbe interactions. This largely untapped area holds the promise to improve crop yields and address food security in an environment-friendly and sustainable manner.

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Role of *Serratia* sp. as Biocontrol Agent and Plant Growth Stimulator, with Prospects of Biotic Stress Management in Plant

6

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Abstract

Serratia species is a member of the *Enterobacteriaceae* family and found to be ubiquitous in the environment. There are several plants associated with *Serratia* sp. that are reported as endophytes or thriving in the rhizosphere of host plants. Many such isolates are known to have plant growth-promoting (PGP) abilities and/or biocontrol potential based on the antibiosis (production of prodigiosin and pyrrolnitrin) and production of lytic enzymes (chitinases and β-1,3-glucanases) against soilborne fungal pathogens that infect various crops. *Serratia* sp. colonized plant roots and within the plant tissues and induced plant growth. Among the mechanisms by which the genus *Serratia* exerts beneficial effects on plants are facilitating the uptake of nutrients such as phosphorus via phosphate solubilization and siderophore production (secretes catecholate siderophore enterobactin) and synthesizing stimulatory phytohormones like indole-3-acetic acid (IAA) (both auxin-dependent and auxin-independent signaling pathways) that are involved in plant growth promotion. *Serratia* sp. also elicits induced systemic resistance (ISR) where enhancement of the plant's defensive capacity against diverse plant pathogens and pests is acquired after appropriate stimulation. Bacteria of the genus *Serratia* have created tremendous interest in researchers as such strains showed high potential for biofertilization and plant growth promotion, contributing better yield of the diverse field and agricultural crops. Some of the species such as *S. plymuthica*, *S. liquefaciens*, *S. proteamaculans*, *S. grimesii*, *S. nematodiphila*, and *S. rubidaea* had acquired the attention of researchers due to their benefits to plants. Some other uncommon species of

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Serratia, like *S. ficaria*, *S. fonticola*, *S. odorifera*, *S. entomophila*, and *S. quinivorans*, have been recognized for their role in plant growth stimulation. With the continuation of interest and research on *Serratia* as PGPR and biocontrol agents, the formulations based on *Serratia* sp. will be instrumental for sustainable agriculture.

Keywords
Serratia · Biocontrol · Endophytes · PGPR · Indole-3-acetic · Siderophore

6.1 Introduction

The global necessity to enhance agricultural yields to meet the requirement of an incessantly increasing population has placed considerable strain on the fragile ecosystem. To enhance agricultural productivity, the utilization of biological inoculants has been increased to reduce chemical fertilizer inputs. Beneficial microorganisms are used with the aim of improving crop yields because these are believed to augment nutrient availability, enhance plant growth, and provide protection to plants from diseases and pests. The bacteria that colonize in the rhizosphere of plants and enhance growth and yield of crop plants are considered as plant growth-promoting rhizobacteria (PGPR). The large-scale application of PGPR to crops as inoculants is an attractive alternative as it would substantially reduce the use of chemical fertilizers and pesticides, which often pollute the environment. Kloepper and Schroth (1981) introduced the term "rhizobacteria" to the soil bacteria which flourish in the rhizosphere of plants that competitively colonized plant roots and stimulated growth and thereby reducing the incidence of plant diseases. They termed these beneficial rhizobacteria as plant growth-promoting rhizobacteria (PGPR). PGPR can be defined as the indispensable part of rhizobacteria biota that when grown in association with host plants can stimulate the growth of the host. Plant roots produce secondary metabolites, indicating the presence of the roots in the soil and activating the bacterial genes and the bacterial movement toward the roots (Lutenberg and Kamilova 2009).

Martinez-Viveros et al. (2010) went a step further and classified PGPR into extracellular plant growth-promoting rhizobacteria (ePGPR) and intracellular plant growth-promoting rhizobacteria (iPGPR). The ePGPRs may exist in the rhizosphere, on the rhizoplane, or in the spaces between the cells of the root cortex; on the other hand, iPGPRs are generally located inside the specialized nodular structures of root cells. The bacterial genera such as *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, and *Serratia* belong to ePGPR (Gray and Smith 2005). The iPGPR includes the endophytes and *Frankia* species, both of which can symbiotically fix atmospheric N_2 with the higher plants (Verma et al. 2010).

The genus *Serratia* comprises Gram-negative bacterium (family *Enterobacteriaceae*), which are ubiquitous and can be found in water, soil, plants, and animals (including humans). Some of the species are plant-associated that comprise both endophytes and free-living species in the rhizosphere (Hallmann et al. 1997). It has also been reported to promote plant growth by inducing resistance against plant pathogens (Kloepper et al. 1993), production of antagonistic substances (de Queiroz and de Melo 2006), and solubilization of phosphates (Tripura et al. 2007).

Several PGPR inoculants including *Serratia* sp. and PGPR-based biofertilizers have been commercialized and achieved consistent results in terms of crop productivity under field conditions and/or provide protection to the crop from pests and diseases. Several rhizobacterial inoculants are able to supply the important part of required nutrients like nitrogen, phosphorus, potassium, sulfur, iron, etc. to the plant, which is of environmental and economic significance. The PGPR strains belonging to the genera such as *Agrobacterium*, *Azospirillum*, *Burkholderia*, *Pseudomonas*, *Serratia*, and *Streptomyces* can affect plant health and are also used for the production of several commercial products, which are generally being applied against several target pathogens like *Botrytis cinerea*, *Penicillium* sp., *Mucorpyroformis*, *Geotrichum candidum*, *Erwinia amylovora*, russet-inducing bacteria, *Fusarium* sp., *Phytophthora* sp., and *P. tolassi* (Nakkeeran et al. 2005; Berg 2009). These organisms suppress plant disease by the production of antibiotics and siderophores or by induction of systemic resistance or any other mechanism (Tenuta 2003). Further, improved understanding on the way by which PGPRs promote plant growth can lead to expanding exploitation of these "biofertilizers" to reduce the potential negative environmental effects associated with food and fiber production (Denton 2007). According to Miransari and Mackenzie (2011a, b), the adverse effects of chemical fertilization on the environment (Miransari 2011a, b) can be alleviated using biological fertilization. Soil microbes are important to the health of the ecosystem and it is of particular significance to determine their efficiency on plant growth, especially when combined with inorganic products. Hence, the use of biological fertilization can be economically and environmentally sustainable.

6.2 Exploration of Microbial Diversity of *Serratia* Species for Their Role as PGPR

The rhizosphere of a plant is the introductory defense in contradiction to the outbreak by pathogenic fungi (Weller 1988). The rhizosphere is profoundly occupied by diverse microorganisms comprising both useful and harmful groups. Therefore, there is an outstanding prospect to study the potential biocontrol agents. Genus *Serratia* has been found to be often associated with rhizosphere. Some strains of *S. plymuthica, S. marcescens*, and *S. liquefaciens* have been documented to ease infection rigorousness to an anticipated extent with the help of specific application tactics. Antibiotics such as the red pigment prodigiosin and pyrrolnitrin can be harvested from few strains of *Serratia*; in addition, they also produce chitinases and

siderophores which help to limit fungal growth. *Serratia* sp. has been isolated from the rhizosphere of wheat, oat, cucumber, maize, strawberry, oilseed rape, and potato (Alstrom and Gerhardson 1988; Grimmont and Grimmont 1992; Kalbe et al. 1996). Certain bacterial strains have the ability to inhibit plant diseases in natural environments and can be used as a replacement to chemical control measures which is potentially hazardous in nature. In many fungi, insect exoskeletons, and crustacean shells, chitin assists as a major cell wall component. Chitinolytic bacteria as biocontrol agents have exhibited potential antagonistic activity against pathogenic fungi by degrading chitin of fungal cell walls (Someya et al. 2011a, b). *Serratia marcescens* has been recognized for their ability to producing multiple chitinases enzyme (ChiA, ChiB, and ChiC) which are capable of degrading the chitin in the cell walls of fungi and the exoskeletons of insects (Someya et al. 2011a, b). Strain B2 of *Serratia marcescens* has been testified as biocontrol representative of *Rhizoctonia solani, Fusarium oxysporum*, and *Botrytis cinerea* and rice sheath blight disease (Someya et al. 2000 and 2005).

Plant growth-promoting *Serratia plymuthica* strain HRO-C48 was found to control *Verticillium* wilt and *Phytophthora* root rot in strawberry (Kurze et al. 2001). In one more instance, Shen et al. (2002) observed 100% control of *Phytophthora* blight rate in pepper by *S. plymuthica* strain A21-4 in pot trials and substantial disease suppression in greenhouse studies. Kamensky et al. (2003) witnessed that *S. plymuthica* IC14 isolated from the rhizosphere of melon protected cucumber against *Botrytis cinerea* gray mold and *Sclerotinia sclerotiorum* white mold diseases of leaves upon foliar application under greenhouse conditions. *S. marcescens* strain N1–14 provided substantial conquest of *R. solani* and *P. ultimum* causing damping-off disease of cucumber (Roberts et al. 2005). The 3Re4–18 strain of *S. plymuthica* isolated from the rhizosphere of *Solanum tuberosum* found to be effective in controlling soilborne pathogens *Verticillium dahlia* and *Rhizoctonia solani* (Berg et al. 2005). For controlling root rot disease in citrus, De Queiroz and de Melo (2006) reportedly used *S. marcescens* strain R-35 isolated from washed root surface of healthy citrus plants. Jaiganesh et al. (2007) found that foliar spraying (2.5 kg/ha) of talc-based formulations of *S. marcescens* in the field showing extreme disease drop of rice blast caused by *Pyricularia oryzae*. Müller and Berg (2008) used *S. plymuthica* HRO-C48 against *V. dahlia* in oilseed rape which indicated statistically substantial biocontrol. One of the studies reported the suppression of rice sheath blight caused by the pathogen *R. solani* using *S. marcescens* B2. *Serratia grimessi* and *S. plymuthica* were studied for suppressing dry rot of potato caused by *Fusarium sambucinum* (Gould et al. 2008). Yazici et al. (2011) observed the biocontrol potential of *S. plymuthica* IK-139, *S. marcescens*, and some other bacteria in whole plant test for protecting tomato plants against early blight disease caused by *Alternaria solani*.

Bacterial strains with an advantageous effect on plant growth and expansion are referred to as plant growth-promoting bacteria (PGPB) (Andrews and Harris 2003). Bacteria-induced growth promotion is accomplished either by fixation of atmospheric nitrogen, solubilization of minerals, and production of siderophores and plant growth regulators (hormones) or by a combination of any of these mechanisms (Kloepper 1997). *Serratia marcescens* has been reported to stimulate the

development of a plant by induction of resistance against plant pathogens (Kloepper et al. 1993), production of antagonistic substances (de Queiroz and de Melo 2006), and solubilization of phosphates (Tripura et al. 2007). Plant growth is affected by a plethora of abiotic and biotic factors. Most plant growth-promoting rhizobacteria (PGPRs) increase plant growth indirectly either by suppression of well-established diseases caused by major pathogens or by reduction of the deleterious effects of minor pathogens. Alternatively, PGPRs may directly affect plant metabolism resulting in increased plant growth, seed emergence, or improved crop yield (Whipps 2001). Several strains of *S. plymuthica* have been demonstrated to exert plant. *S. grimesii* has been previously isolated from the rhizosphere of several plant species (Grimmont et al. 1981). It was commonly associated with maize roots, inducing strawberry, oilseed rape, and non-transgenic potato (Berg et al. 2002). Saïdi et al. (2013) confirmed that 55% of nodule isolates including *S. odorifera* were putative endophytes, and high frequency of endophytes in *V. faba* root nodules exerts their effect on plant growth. The *S. fonticola* was included in the composition of bacterial communities found in the end root and exoroot and associated exoroot (root zone soil) in potatoes (*Solanum tuberosum* L.) against *Phytophthora erythroseptica* Pethyb. (causal agent of pink rot of potatoes), *Streptomyces scabies* (Thaxt.) Waksm. and Henrici (causal agent of potato common scab), and *Fusarium oxysporum* Schlecht Emend. Snyder and Hansen (causal agent of *Fusarium* potato wilt) (Sturz et al. 2005). Neupane et al. (2013) studied the ability of *S. proteamaculans* isolated from the rhizosphere of wild *Equisetum* sp. for plant growth enhancement and for suppressing the growth of several soilborne fungal pathogens like *Verticillium dahliae* and *Rhizoctonia solani*. Shuhegger et al. (2006) first reported that AHL signal produced by *S. liquefaciens* MG1 from the rhizosphere of tomato increased its systemic resistance of tomato plant in the rhizosphere against the *Alternaria alternata* (fungal leaf pathogen).

The plant growth-promoting effects of *Serratia* spp. have been experimented in phytochamber, green house, and/or field conditions (Faltin et al. 2004; Kurze et al. 2001; Berg et al. 2001). The plant growth-stimulating ability of such strains has often been linked to their capacity to produce the auxin phytohormone indole-3-acetic acid (IAA) in vitro. IAA is the core auxin in plants, governing many essential physiological courses including cell enlargement and division, tissue differentiation, and responses to light and gravity (Teale et al. 2006; Woodward and Bartel 2005).

6.3 *Serratia* sp. as Biocontrol Agent of Plant Diseases

Interest in biological control of plant pathogens has increased in recent years fueled by trends in agriculture toward greater sustainability and public concern over the use of hazardous pesticides in the environment. There are several mechanisms by which PGPR brings about the control of plant diseases. *Serratia* sp. is one of the most efficient bacteria which is known for the production of metabolites and competition with the pathogens in the soil. The metabolites include antibiotics,

siderophores, HCN, cell wall-degrading enzymes, quorum-sensing molecules, and N-acyl homoserine lactones (AHLs) (Ebebak et al. 1998; Kloepper 1993; Someya et al. 2002, 2003, 2005; Liba et al. 2006; Tripura et al. 2007; Koo and Cho 2009; Chakraborty et al. 2010; Zahir et al. 2011; Ryu et al. 2013). Further, Kloepper et al. (1992) mentioned two types of resistance in plants. Induced systemic resistance (ISR), or systemic acquired resistance (SAR), is defined as the activation of chemical and physical defenses of plant host by an inducer which could be a chemical or a microorganism, leading to the control of several pathogens. A positive role for *Serratia* in growth promotion of several plant species, including oilseed rape, tobacco, lentils, wheat, tomato, sorghum, rice, soybean, and summer squash, has been documented (Kalbe et al. 1996; Gyaneshwar et al. 2001; Pan et al. 2002; Zhang et al. 2002; Selvakumar et al. 2008; Zahir et al. 2009; Zahir et al. 2011; Almaghrabi et al. 2013; Gujral et al. 2013). The interaction of *Serratia* spp. with the pathogens in the rhizosphere and the role of antagonistic metabolites are illustrated in Fig. 6.1.

According to Beattie (2006), bacteria that reduce the incidence or severity of plant diseases are often referred to as biocontrol agents, whereas those that exhibit antagonistic activity toward a pathogen are defined as antagonists. The following rhizospheric environment and bacterial antagonistic activities can be highlighted: (1) synthesis of hydrolytic enzymes, such as chitinases, glucanases, proteases, and lipases, which can lyse pathogenic fungal cell (Neeraja et al. 2010; Maksimov et al. 2011); (2) competition for nutrients and suitable colonization of niches at the root surface (Stephens et al. 1993; Kamilova et al. 2005); (3) regulation of plant ethylene

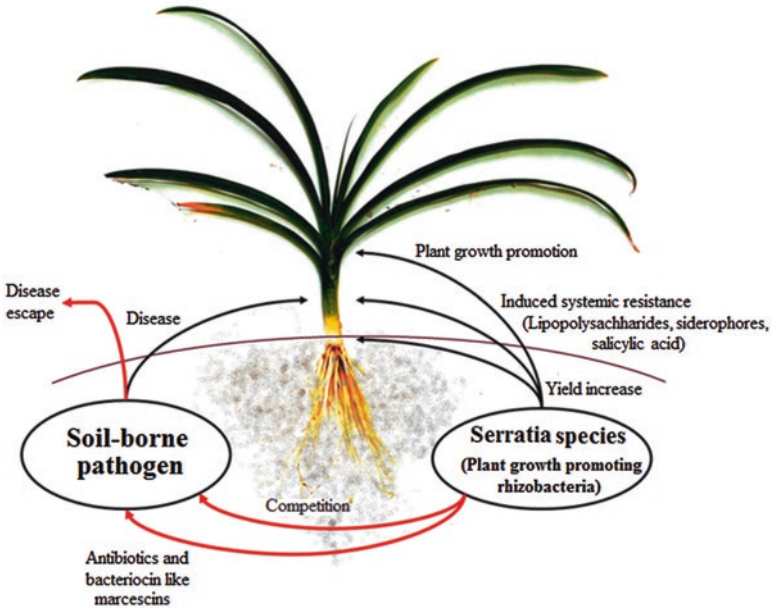

Fig. 6.1 Modes of action of antagonist *Serratia* species in the rhizosphere

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 and Bashan 1997; Van Loon 2007); and (4) production of siderophores and antibiotics. The *Serratia* sp. has been explored well for its potential as a promising antagonist and strikingly inhibited soilborne pathogens as well as foliar fungal diseases in a wide variety of crops including rice (Jaiganesh et al. 2007), potato (Berg et al. 2005; Gould et al. 2008), citrus (de Queiroz and de Melo 2006), cucumber (Kamensky et al. 2003; Roberts et al. 2005), tomato (Yazici et al. 2011), strawberry (Kurze et al. 2001), pepper (Shen et al. 2002, Kim et al. 2008), and oilseed rape (Müller and Berg 2008). Mostly *Serratia* is isolated from the rhizosphere where it helps to control plant diseases associated with the roots, but few species of *Serratia* can also be isolated as endophytes.

6.4 Different *Serratia* spp. as Biocontrol Agents

6.4.1 *Serratia marcescens*

Serratia marcescens is characterized by its ability to produce the red pigment prodigiosin (Khanafari et al. 2006) which is produced under both aerobic and anaerobic conditions (Wai and Chen 2005). Prodigiosin is known to exhibit antioxidant, antitumor, and antibiotic properties (Green et al. 1956; Gerber 1975; Cang et al. 2000). *S. marcescens* is very efficient in the degradation of chitin because of its ability to produce different chitinolytic enzymes (Brurberg et al. 1995). Chitin is a component of the fungal cell wall and an important factor among various attributes of antagonism present in *Serratia*. Chitinase production and its activity depend on a number of limiting factors, viz., culture state, temperature, pH of media, etc. The production of three types of chitinases (*ChiA*, *ChiB*, and *ChiC*) and a chitobiosidase by *S. marcescens* has been demonstrated, and these enzymes are able to degrade the chitin present in the cell walls of fungi and the exoskeletons of insects.

S. marcescens effectively inhibits the growth of several phytopathogenic fungi and suppresses some crop diseases (Okamoto et al. 1998; Someya et al. 2000). It also shows pesticidal effects against a number of plant pathogens including *Rhizoctonia solani* and *Fusarium oxysporium* causing wilt disease (Someya et al. 2000). Strains of *S. marcescens* have been reported to be recovered from other tephritids such as *Ceratitis capitata* Weidermann and *Dacus* (*Bactrocera*) *dorsalis* hendel flies (Grimont and Grimont 1978), and these bacteria may possess some utility as insect control agents. *S. marcescens* has been employed as a biological control agent of phytopathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Phytophthora parasitica*, and *Phytophthora capsicii* in temperate climates (Akutsu et al. 1993; Someya et al. 2000). Chakraborty et al. 2010 studied the in vivo biocontrol and plant growth-promoting prospective of *S. marcescens* (TRS-1) in tea plants as aqueous suspensions or as bioformulations in different carriers. The bacterium was reported to reduce brown root rot of tea caused by *Fomes lamaoensis*. Further, Cascales et al. (2007) reported the production of bacteriocin, namely, marcescins,

from *S. marcescens* is increasingly becoming more important due to its 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). *S. marcescens* R-35 isolated from the citrus rhizosphere have been reported as the biological control agent for pathogens such as *Phytophthora parasitica* that causes serious, widespread, and difficult to control root rots in warmer regions (de Queiroz and de Melo 2006). Kobayashi et al. (1995) investigated that chitinolytic bacteria *S. marcescens* 9 M5 isolated from soils known to harbor *Magnapothe poae*, the causal agent of summer patch on Kentucky bluegrass, have the ability as biocontrol agents to suppress summer patch symptom development in Kentucky bluegrass cv Baron by more than 50%. Plant growth-promoting rhizobacteria (PGPR) strain *S. marcescens* 90–166 demonstrated induced systemic resistance in cucumber against some fungal and bacterial diseases and proved their capacity to protect *Cucumis sativus* L. cv. Straight 8 from disease development of cucumber mosaic cucumovirus (CMV) (Raupach et al. 1996). The potential *S. marcescens* has been reported as indigenous strain isolated from the date palm compost as a biocontrol agent of *Rhizoctonia solani* that are associated with stem canker and black scurf diseases of potato, one of the destructive pathogens in Tunisia (Khaldi et al. 2015). *S. marcescens* SR1 strain was isolated as a source of chitinase from the local soil of a cultivated farm with the potential to be used as a biocontrol agent against *Fusarium oxysporum*, *Rhizoctonia solani*, *Helminthosporium*, and *Xanthomonas putida* (Parani et al. 2011). Patil et al. (2011) investigated the mosquito-larvicidal potential of microbial pigment prodigiosin produced by *S. marcescens* NMCC46 against *Aedes aegypti* and *Anopheles stephensi*. Further, the active compound prodigiosin produced by this species was more useful against vectors responsible for diseases of public health importance. The *S. marcescens* strains isolated from tropical regions in Mexico had the potential as a biocontrol agent for plant pathogens by inhibiting the growth of mycelial and conidial germination of *Colletotrichum gloeosporiodes*, the causal agent of fruit anthracnose (Guitiérrez-Román et al. 2012).

6.4.2 *Serratia plymuthica*

Serratia plymuthica is an ubiquitous bacterium that has been preferentially recovered from rhizospheres all over the world, both as a free-living and endophytic organism. Specific strains of *S. plymuthica* produce a broad palette of antimicrobial compounds and might hold great potential as broad-spectrum biocontrol agents. In the *Serratia* genus, *S. plymuthica* was introduced as a biocontrol agent because of its high chitinolytic activity (Frankowski et al. 2001). *S. plymuthica*, however, is most frequently associated with plants. The plant-associated *S. plymuthica* has been isolated from the rhizosphere (Grimmont and Grimmont 1992; Kalbe et al. 1996; Berg 2000) or as endophytes (Benhamou et al. 2000) of several crops. Some of these isolates have been shown to be able to suppress several fungal plant pathogens, including *Fusarium culmorum*, *Pythium ultimum*, *Rhizoctonia solani*,

S. sclerotiorum, and *Verticillium dahliae* (Kalbe et al. 1996; McCullagh et al. 1996; Liu and Morrell 1997; Frankowski et al. 1998; Benhamou et al. 2000; Thaning et al. 2001). This organism has been isolated from the rhizosphere of grass (Alstrom and Gerhardson 1987), wheat (Alstrom and Gerhardson 1988), maize (Lucon and Melo 2000), oilseed rape (Kalbe et al. 1996), grape (Chemin et al. 1995), melon (Kamensky et al. 2003), onion (Park and Shen 2002), *Brassica* sp. (Carlot et al. 2002), *Cichorium intybus* (Stock et al. 2003), sugar beet (Tenning et al. 1987), and tomato (Frommel et al. 1991) and as an endophyte from the endorhiza of potato (Berg et al. 2005). It has been found on the edible parts of green onion, carrot, and lettuce, on the phyllosphere of spring wheat (Legard et al. 1994), and on *Brassica* sp. (Leifert et al. 1993) and as a contaminant in a raw vegetable processing line (van Houdt et al. 2005). The mechanisms of fungi suppression may be based on antibiosis and production of lytic enzymes (chitinases and β-1,3-glucanases) and siderophores. In addition, *S. plymuthica* strains may secrete the plant growth hormone indole acetic acid (IAA), which can directly promote root growth (Kalbe et al. 1996). *S. plymuthica* AS12 and *S. plymuthica* AS13 isolated from the roots of rapeseed plants promote host plant growth (Neupane et al. 2012a, b). Similarly, *S. plymuthica* HRO-C48 has been used as a successful biocontrol agent against soilborne fungal diseases in strawberries and rapeseed (Müller and Berg 2008). Bacterium *S. plymuthica* isolated from the soil around melon roots shows that suppression of a wide range of phytopathogenic fungi such as *Botrytis cinerea* gray mold and *Sclerotinia sclerotiorum* white mold diseases of leaves under greenhouse condition by foliar application of strain protected the plant cucumber (Kamensky et al. 2003).

6.4.3 *Serratia liquefaciens*

S. liquefaciens is one of the potential biocontrol bacterial agents (possess antifungal properties) and a typical rhizobacteria which were mostly isolated from the rhizosphere of the plants. *S. liquefaciens* from carnation rhizosphere has been used to protect root cutting (Sneh et al. 1985). It has also been reported to have bacterially mediated plant tolerance to abiotic stress in the plant soybean (*Glycine max*) (Zhang et al. 1997). *S. liquefaciens* plays an important role in biofertilization that increases up to 14% maize yield (dry weight) in plant maize when they were inoculated with respect to controls (Lalande et al. 1989). Bai et al. (2002c) reported that the co-inoculation of *S. liquefaciens* with other rhizobacteria at their optimal dose increased nodule number, plant dry weight, and fixed nitrogen in the field and greenhouse condition. Co-inoculation studies with rhizobia and PGPR are becoming a frequent practice in the development of sustainable agriculture. Many experiments are focused on the improvement of soybean yield production by increasing the nitrogen fixed by rhizobia. PGPR tested as co-inoculants with rhizobia includes *Bacillus subtilis*, *B. thurigiensis*, *Azospirillum brasiliensis*, *S. proteomaculans*, *S. liquefaciens*, and *Pseudomonas aureofaciens*, and the commonly used rhizobia strain has been *Bradyrhizobium japonicum* (Pérez-Montano et al. 2014). Quorum sensing has been studied in depth in Gram-negative than in Gram-positive bacteria. Quorum

sensing through the production of AHLs (N-acyl homoserine lactones) is widely detected in *S. liquefaciens* than any other root-colonizing bacteria. Usually, AHLs are synthesized by a member of LuxI protein family and act as a signal molecule. AHL-mediated cell-to-cell communication is a widespread phenomenon among plant-associated bacteria. GFP-based AHL reporter system *S. liquefaciens* was observed to sense AHLs from each other in tomato rhizosphere (Steidle et al. 2001). Dashti et al. (1997) reported that *S. liquefaciens* has benefits as it is increased in the grain yield and grain protein yield in soybean. Shuhegger et al. (2006) first reported that AHL signal produced by *S. liquefaciens* MG1 in the rhizosphere increases systemic resistance of tomato plant against the fungal leaf pathogen *Alternaria alternata* and systemic induction of salicylic acid (SA)- and ethylene (ET)-dependent defense gene. Analysis of the reaction of tomato to the ISR-eliciting strain *S. liquefaciens* MG1, using a microarray containing DNA probes of 70 defense-related and signaling genes, revealed enhanced expression of 12 genes. Seven of those are coded for PR genes, whereas the others are involved in oxidative stress, ethylene signaling, or metabolism. Co-inoculation of the plant growth-promoting rhizobacteria (PGPR) *S. liquefaciens* with *Bradyrhizobium* showed the increase in legume nodulation and nitrogen fixation at optimal soil temperatures and the ability to reduce the negative effects of low root zone temperature (RZT) on soybean [*Glycine max* (L.) Merr.] nodulation and nitrogen fixation (Zhang et al. 1996). However, in contrast, Pan et al. (2002) investigated that the use of the PGPR *S. liquefaciens* with genistein (one of the major isoflavonoid compounds in soybean seed and root) gives no additional improvement in nodulation and nitrogen fixation in the field-grown soybean plant, but the improvement is seen when it is treated individually with PGPR or genistein. *S. liquefaciens* identified by 16S rRNA with analysis of gene *gyrB* and *gyrA* showed the enhancement rate of PGPR colonization that improved grain yield and zinc content of wheat as compared to chemical zinc fertilizers (Abaid-Ullah et al. 2015). In addition, Radwan et al. (2005) investigated that *S. liquefaciens* shows effective and better phytoremediation potential of broad bean plants grown in oily sand. Pan et al. (1999) reported that exogenous application of 0.2 μg/ml K in maize seeds with *S. liquefaciens* inoculation resulted in increased root size and weight. Exploiting the potential of *S. liquefaciens* to act as crop protectants (biological control agent) has been the focus of many research groups. Their biocontrol capabilities result largely from their ability to produce a battery of antifungal metabolites which can also affect beneficial fungal-root symbiosis (Raajimakers et al. 2009). Varma et al. (2012) found that *S. liquefaciens* MG1 clearly inhibited the root growth stimulation of *Piriformospora indica*; however, when *Piriformospora indica* is applied in the barley roots in an axenic system, root development is already seen enhanced in the seedling stage. Barriuso et al. (2008) suggested that the molecules C6-HSL and OC6-HSL of AHLs detected in the supernatants of cultures of plant growth-promoting rhizobacterium may be produced in situ and in the rhizosphere. The molecule OC-HSL indicates an oxo substitution at the third carbon atom, and HC-HSL indicates a hydroxy substitution at the third carbon atom. Moreover, the sensor strain *S. liquefaciens* MG44 (pJBA132) detected the production of short-chain AHLs.

6.4.4 *Serratia proteamaculans*

Plant-associated *S. proteamaculans* have considerable agricultural interest, and several strains of *S. proteamaculans* have recently been studied in relation to their possible use as biocontrol agents in agriculture. It is a Gram-negative, rod-shaped, non-sporulating, and motile bacterium. It is a diverse and widely dispersed group that have beneficial effects on ecologically and economically important plants, and others are known as opportunistic pathogens in humans and other organisms. Neupane et al. (2013) demonstrated that *S. proteamaculans* isolated from the rhizosphere of wild *Equisetum* sp. has the ability to stimulate plant growth and to suppress the growth of several soilborne fungal pathogens like *Verticillium dahliae* and *Rhizoctonia solani*, which are economically important crops. Plant pathogens are also capable of exploiting a wide array of mechanisms in order to counteract and compete against antagonism from both microbial antagonists and other pathogens. *S. proteamaculans* S4 have been sequenced and have revealed genetic traits that may explain the diverse plant growth-promoting activities and antagonistic interactions with *Rhizoctonia solani*. Changes in the plant pathogenic fungus *R. solani* AG-3 in response to the antagonistic bacteria *S. proteamaculans* by transcriptome analysis revealed that approximately 10% of the fungal transcriptome was differentially expressed during challenge with *S. proteamaculans* (Gkarmiri et al. 2015). Alstrom (2001) reported that the strain of soil bacteria *S. proteamaculans* isolated from oilseed rape roots suppressed the pathogen *V. dahliae*. Zahir et al. (2009) reported that the potential strain *S. proteomaculans* showed promising performance under axenic conditions. Inoculation with this strain showed effective and significantly increased plant height, root length, grain yield, and straw yield up to 60%; chlorophyll content and K^+/Na^+ of leaves also increased. Under salinity stress, the 1-aminocyclopropane-1-carboxylic acid-deaminase activity of this microbial strain might have caused a reduction in the synthesis of stress (salt)-induced inhibitory levels of ethylene. Therefore, *S. proteomaculans* employed for salinity tolerance in wheat. Berg et al. (2002) demonstrated that *S. proteamaculans* showed chitinolytic activity and antagonistic activity toward *Verticillium*, *Rhizoctonia solani*, *S. sclerotiorum*, and *P. cactorum*. The enhancement in the biofertilization with the co-inoculation of the PGPR (*S. proteamaculans* with others) at their optimal dose increased nodule number, plant dry weight, and nitrogen fixation of the plant soybean in the field and greenhouse condition, based on the inducible activator-like lipo-chitooligosaccharide (LCO) analog which stimulated root nodule formation. Thus, the addition of PGPR supernatant to *Bradyrhizobium japonicum* inoculant increased nodule weight by 53.7% and plant weight by 31.2% under 25 °C root zone temperature, which was at essentially the same level as the co-inoculation of *B. japonicum* with the *S. proteamaculans* 1–102 culture (Bai et al. 2002a, b). Similarly, Zhang et al. (1996) also reported that *S. proteamaculans* 1–102 has potential to increase legume nodulation and nitrogen fixation at optimal soil temperatures and also has the capability to decrease the negative effects of low root zone temperature (RZT) on soybean nodulation and fixed nitrogen. Dashti et al. (2000) studied the survival of promising strain *S. proteamaculans* 1–102 under the

field conditions in methyl bromide fumigated and non-fumigated soils; however, *S. proteamaculans* 1–102 is colonized best at a low RZT (15 °C), and the population of *S. proteamaculans* 1–102 applied to the rhizosphere increased over time in fumigated soil over the non-fumigated soil indicating that the PGPR survive and proliferate better under fumigated conditions. Alstrom (2001) showed the potential of bacteria that are adapted to the oilseed rape root environment for use in the biological control of *Verticillium dahliae* by suppressing all the pathogens not only directly but also indirectly in in vitro assays. The majority of the strains possessed the ability to produce cellulases, proteases, and phosphatases, and some even produced chitinases and induced hypersensitive responses, indicating the potential for nutrient acquisition as well as colonization capacity and active recognition by the plant cells. Ryan et al. (2008) revealed that, to date, few endophytic bacterial genome sequences have been published; however, genome sequencing of a number of endophytes including *S. proteamaculans* 568 is under way at the US Department of Energy Joint Genome Institute (www.jgi.doe.gov). According to the production of bacteriocin and sensitivity, BiOLOG and API 20E strip profiles, and 16S rRNA sequence analysis of the *S. proteamaculans* subsp. *quinovora*, Grimont et al. (1983) formed a cohesive group at the species level as also confirmed by DNA-DNA cross-hybridization and phenotypic characterization (Ashelford et al. 2002).

6.4.5 *Serratia fonticola*

S. fonticola isolated from the rhizosphere of pea roots is known to confer activity against *Rhizoctonia* species by suppressing the plant disease and also to directly improve plant health by improving the availability of nutrients and by providing phytostimulants. It also has phosphate solubilization, indole-3-acetic acid production, ammonia production, hydrogen cyanide (HCN) production, and siderophore production abilities (Devi et al. 2013). Plant growth-promoting rhizobacteria strain *S. fonticola* containing ACC deaminase showed a significant increase in the growth, nodulation, and yield of lentil by decreasing the ethylene concentration. Similarly, combined inoculation with *S. fonticola* with other PGPR increased N concentration of grains under both pot and field conditions (Zahir et al. 2011). Saidi et al. (2015) studied the efficacy of bacteria that includes *S. fonticola* isolated from the fresh Tuber of aestivum fruits ascocarps as biocontrol agents against the bacteria and fungi responsible for spoiling truffle fruits. These bacteria showed a high rate of antifungal activity which indicates that truffle may be a common source for selection of microorganisms with biotechnological potential and may be useful for biocontrol of food, plant, and soilborne pathogenic bacteria and fungi. Interestingly, biological control agent *S. fonticola* was able to produce lytic enzymes such as chitinases, glucanases, proteases, and biologically active substances in vitro against the *Rhizoctonia solani* in lettuce and sugar beet plants (Faltin et al. 2004). The efficacy of bacterial antagonists *S. fonticola* against *Botrytis cinerea* causing gray mold was determined in various parts of strawberry plants where *B. cinerea* are known to cause major diseases of strawberries (Ilhan and Karabulut 2013). The component of

bacterial communities including *S. fonticola* found in the end root and exoroot and associated exoroot (root zone soil) in potatoes (*Solanum tuberosum* L.) has functional versatility and ability of antibiosis against *Phytophthora erythroseptica* Pethyb (causal agent of pink rot of potatoes), *Streptomyces scabies* (Thaxt.) Waksm. and Henrici (causal agent of potato common scab), and *Fusarium oxysporum* Schlecht Emend. Snyder and Hansen (causal agent of *Fusarium* potato wilt). Sturz et al. (2005) postulated the difference in the frequency of *S. fonticola* isolates with antibiosis ability among endoroot versus exoroot populations points to the adaptation of endophytic bacterial communities that favor host defense against pathogens that attack the host systematically (Sturz et al. 2005). The *S. fonticola* that have activity against *Verticillium dahliae* with the production of siderophores and antibiotics metabolites interacted with three bryophytes species, *Totula ruralis*, *Aulacomnium palustre*, and *Sphagnum rubellum*, which represent typical moss species of three nutrient-poor plant communities at the southern Baltic Sea coast, Germany. The high recovery of antagonistic isolates strongly suggests that bryophytes represent an ecological niche which harbors a diverse and hitherto largely uncharacterized microbial population with yet unknown and untapped potential biotechnological applications like for biological control of plant pathogens (Opelt and Berg 2004). Li et al. (2015) witnessed that the plant-associated *S. fonticola* RB-25 isolated from the waste landfill exhibited plant growth-promoting activities and also reported that an additional ChiC was found in *S. fonticola* RB-25 genomes but ChiB was not present in *S. fonticola* RB-25, which suggested that this bacterium may not degrade chitin efficiently or some other novel ChiB functioning genes may exist. *S. fonticola* RB-25 was found to have homologs of tellurium resistance genes. The presence of prodigiosin, bacteriocins, multidrug-resistant proteins, and chitinases indicates its antagonistic potential that can suppress the growth of the vital plant pathogenic bacterium *Ralstonia solanacearum* and fungi *Fusarium oxysporum* and *Sclerotinia sclerotiorum* in vitro.

6.4.6 *Serratia odorifera*

Serratia odorifera is an antagonistic rhizobacterium emitting a diverse complex bouquet of volatiles. Vespermann et al. (2007) and Kai et al. (2007 and 2008) conducted a comprehensive investigation including *S. odorifera* against pathogenic fungi, including *Aspergillus niger*, *Fusarium culmorum*, *Fusarium solani*, *Microdochium bolleyi*, *Paecilomyces carneus*, *Penicillium waksmanii*, *Phoma betae*, *Phoma eupyrena*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Trichoderma strictipile*, and *Verticillium dahliae*. The rhizobacteria *S. odorifera* inhibited the mycelial growth of most fungi. The extent of inhibition depended on the individual bacteria-fungi combination. Noticeably, *Fusarium solani* turned out to be resistant against the bacterial volatiles. The spectra of bacterial volatiles produced included many unknown components; however, 2-phenylethanol, 1-undecene, dodecanal, dimethyl disulfide (DMDS), and dimethyl trisulfide (DMTS) could be identified (Kai et al. 2007). DMDS and 1-undecene indeed inhibited the growth of

F. culmorum when applied as individual compounds in dual-culture tests (Kai et al. 2009). Effmert et al. (2012) studied that soil is one of the major habitats of bacteria and fungi. The interactions are part of a communication network that keeps microhabitats in balance. Prominent mediator molecules of these inter- and intraorganismic relationships are inorganic microbial volatile compounds (mVOCs). A growing body of evidence indicates that mVOCs are eco-friendly and can be exploited as a cost-effective sustainable strategy for use in agricultural practice as agents that enhance plant growth, productivity, and disease resistance. As naturally occurring chemicals, mVOCs have potential as possible alternatives to harmful pesticides, fungicides, and bactericides as well as genetic modification. Kanchiswamy et al. (2015) demonstrated that efficiently adopting mVOCs may contribute to sustainable crop protection and production by discussing the mVOCs role in inducing phenotypic plant responses and their potential physiological effects on crops. They also analyzed the potential and actual limitations for mVOCs use and deployment in field conditions as a sustainable strategy for improving productivity and reducing pesticide use. Furthermore, *S. odorifera* emits NH_3 (NH_4^+) and sodorifen which is then used by aerobic and anaerobic microorganisms and many microorganisms like ammonia-oxidizing bacteria (*Nitrosomonas*, *Nitrosospira*, and *Nitrosococcus*) for a carbon source, nitrogen source, olfaction, antibiotic resistance, a toxic compound, and an electron donor as well. Sturz et al. (2004) studied under the field conditions that soil acidification with $+SO_4$ treatments stimulated the development of rhizobacterial communities including *S. odorifera* that generated secondary metabolites with (in vitro) antibiosis against *Streptomyces scabies* which is commonly found in the potato plant (*Solanum tuberosum* L.) and occurred both in liquid and vapor phases (volatile gases). Antibiosis against the *S. scabies* mediated by $+SO_4$ treatments, and the competitive communities that engender, would be less effective in controlling potato common scab, as the active moieties would be rapidly volatilized into the atmosphere, whereas in biocontrol process, it would be more effective in wetter seasons, and the active moieties retained in and around the potato root zone for greater biologically significant periods of time. Some strains isolated from the root nodules of *Vicia faba* have the ability to solubilize phosphorus, siderophore production, the presence of symbiotic genes, and other growth-promoting traits. The prevalence of non-nodulating isolates in nodule extracts may be explained by different alternatives, including loss of symbiotic genes, opportunistic colonization by rhizospheric bacteria, or surface contamination of nodules. However, since they survived after chemical surface sterilization of nodules and represented the major phenotype recovered from each nodule, they were considered as putative endophytes. Saïdi et al. (2013) found that 55% of nodule isolates were putative endophytes, and high frequency of endophytes in *V. faba* root nodules prompted the study of their effect on plant growth. However, the strain related to the species *S. odorifera*, causing serious diseases including pneumonia and bacteremia, is also known (Lee et al. 2006). In this case, it would be necessary to check the pathogenicity of strain prior to recommending their future use. The application of bacteria *S. odorifera* with potential for using biocontrol agents for parasitic weeds offers an additional approach for biocontrol of *Orobanche* sp. that can supplement current methods of control in an

integrated weed management strategy. It is mainly isolated from the faba bean (*Viciafaba*) as well as from diseased *Orobanche* underground structures and an *Orobanche*-suppressive soil from three districts of Northern Tunisia (Zermane et al. 2007). Faltin et al. (2004) reported the potential of plant-associated antagonists *S. odorifera* isolated from diverse plant species, and microenvironments have potential for biocontrol and plant growth by a hierarchical combination of assays toward different *Rhizoctonia solani* Kühn in lettuce and sugar beet plants. *S. odorifera* was able to produce urease and utilized urea and L(+) sorbose as substrate, representing a novel, non-pigmented subgroups of *S. marcescens* also confirmed by using SDS-PAGE of whole-cell protein patterns, DNA-DNA hybridization, and 16S rDNA sequencing (Tan et al. 2001).

6.4.7 *Serratia grimesii*

S. grimesii is one of the common rhizobacteria as already mentioned. It can lead to natural suppressiveness against take-all disease, and their association has been extensively studied. *S. grimesii* is known for its antagonistic property to the soil-borne fungal pathogen in potato rhizosphere soil against *Pectobacterium carotovorum* (formerly *Erminia carotovora*) and *Verticillium dahlia.* In the field release experiment, rifampicin-resistant mutants of a plant-associated *S. grimesii* were used for the seed tuber inoculation of transgenic T4 lysozyme-expressing potatoes, transgenic control potatoes, and non-transgenic parental potatoes. The T4 lysozyme-sensitive *S. grimesii* L16-3-3, originally isolated from the rhizosphere of parental potatoes, showed in vitro antagonism toward the plant pathogenic fungus *Verticillium dahliae*. It was able to colonize the rhizo- and geocaulosphere of transgenic plants and non-transgenic parental plants established in the rhizosphere. The effects of the inoculants on the indigenous microbial community were monitored by analysis of PCR-amplified fragments of the 16S rRNA genes of the whole bacterial community after separation by denaturing gradient gel electrophoresis (DGGE) (Lottmann et al. 1999; Lottmann et al. 2000). P-solubilizing bacteria *S. grimesii* of wheat rhizosphere was found to produce IAA, fix N_2, solubilize zinc, and also showed EPS-, ACC deaminase-, and biocontrol activities (Abaid-Ullah et al. 2015). In addition, the use of *S. grimesii* from exoroot (root zone soil) of the potato plant into the soil has shown some efficiency of antibiosis against *Phytophthora erythroseptica*, Pethyb. (causal agent of pink rot of potatoes), *Streptomyces scabies* (Thaxt.) Waksm. and Henrici (causal agent of potato common scab), and *Fusarium oxysporum* Schlecht. Emend. Snyder and Hansen (causal agent of *Fusarium* potato wilt) (Sturz et al. 2005). *S. grimesii* was commonly associated with maize roots. This species has been previously isolated from the rhizosphere of several plant species (Grimmont et al. 1981), including strawberry, oilseed rape, and non-transgenic potato (Berg et al. 2002). Prischmann et al. (2008) demonstrated *Serratia* species including *S. grimesii* from bulk soil and roots of field-grown maize genotypes, half of which infested with rootworm eggs from a reared colony and non-diseased, larval rootworms from the same colony. Larval corn rootworms (Coleoptera: Chrysomelidae)

are soil-dwelling insect pests that damage maize (*Zea mays* L.) by consuming root tissues, thus lowering grain yield. There is no much literature about interactions between rootworms and soil bacteria, including potential impacts of maize rhizobacteria, such as entomopathogenic *Serratia* sp., on subterranean rootworm pests. The strain associated with diseased rootworms may have potential as biological control agents, and based on literature, additional *Serratia* biotypes associated with the maize rhizosphere may function as plant growth-promoting agents via antagonistic action against plant pathogenic fungi.

In an extensive study, plant-associated bacteria and their structure and functions are important not only for understanding their ecological role and the interaction with plants and plant pathogens but also for any biotechnological application. In biotechnology, rhizosphere-associated bacteria can be applied directly for biological control of plant pathogens as biological control agents (BCAs), for growth promotion as PGPR, or as biofertilizers and rhizoremediators. Rhizosphere-associated bacteria with a high capacity for biocontrol and plant growth promotion can be potentially dangerous for human health. Therefore, it is important to understand the mode of action and specific properties of the PGPR. It is well known that antagonistic properties and underlying mechanisms are highly strain-specific, but identification of bacteria is based mainly on 16S rRNA sequencing.

6.5 Role of *Serratia* sp in Induced Systemic Resistance in Crop Plants Against Diseases and Pests

Application of mixtures of different PGPR strains to the seeds or seedlings of certain plants has resulted in increased efficiency of induced systemic resistance (ISR) against several pathogens (Ramamoorthy et al. 2001). Various non-pathogenic PGPR strains have the ability to induce systemic disease resistance in plants against broad-spectrum phytopathogens (Kloepper et al. 2004; Elbadry et al. 2006). The induction of systemic resistance by rhizobacteria, which are non-pathogenic, is referred to as ISR (van Loon et al. 1998). The expression of induced resistance can be local or systemic when it is expressed at sites not directly exposed to the inducer agents (Stadnik 2000). The use of PGPR for inducing systemic resistance against diseases has been demonstrated in field conditions. This type of induced resistance shows advantages such as effectiveness against various pathogens, stability due to the action of different mechanisms of resistance, systemic, energy economy, and metabolic utilization of genetic potential for resistance in all susceptible plants (Bonaldo et al. 2005). ISR is quite similar to systemic acquired resistance (SAR), making the plant resistant to subsequent attacks of pathogenic organisms, such as viruses, bacteria, and fungi (Bakker et al. 2007). SAR or ISR do not provide complete resistance to any particular pathogen but provide substantial protection to plants for a long time to a broad range of pathogens. The activation of defense mechanisms induced by fungi, bacteria, viruses, and nematodes can be achieved by different routes, which may occur alone or concomitantly (Bonaldo et al. 2005). The induction of resistance to disease is an added advantage to the promotion of plant

growth and yield by the application of PGPR. The presence of the PGPR in the rhizosphere makes the entire plant, including the shoot, more resistant to pathogens (Figueiredo et al. 2010). Induction of systemic resistance by strain *S. marcescens* 90–166 against *Fusarium* wilt of cucumber incited by *Fusarium oxysporum* f. sp. *cucumerinum* has been investigated by Liu et al. (1995). Kloepper et al. (1993) treated cucumber seeds with rhizobacterial strain *S. marcescens* 90–166 and recorded a significant decrease in the incidence of bacterial wilt. Similar investigations on the treatment of cucumber seeds against angular leaf spot disease caused by *S. marcescens* 90–166 have been made by Wei et al. (1996). They observed more systemic protection in the plants (reduction of total lesion diameter can be seen) whose seeds are inoculated with the strains of PGPR as compared to the uninoculated plants. Raupach et al. (1996) demonstrated induced systemic resistance in cucumber against some fungal and bacterial diseases by *S. marcescens* and have the capacity to protect *Cucumis sativus* L. cv. Seed treatment with *S. marcescens* consistently reduced mean numbers of symptomatic plants when cucumber mosaic cucumovirus (CMV) was inoculated onto cotyledons and delayed the development of symptoms in cucumber and tomato.

Several studies have been carried out to elicit ISR by PGPR in plants: incarnation, application of *S. marcescens* which have the potential for ACC deaminase activity, phosphate solubilization, production of siderophore, indole acetic acid production, nitrogen fixation, and ammonia production. It showed growth at an increased salt (NaCl) concentration of up to 6%, indicating its potential to survive and associate with plants under salinity stress (150–200 mM). It significantly reduced the inhibition of plant growth (15–85%) caused by salt stressors. In addition, inoculation with *S. marcescens* also reduced the disease severity caused by fungal infection, which illustrated its ability to confer induced systemic resistance ISR in host plants. Treatment of wheat plants with the test organism caused an alteration in anti-oxidative enzymes activities (superoxide dismutase, catalase, and peroxidase) under various salinity levels and therefore minimizes the salinity-induced oxidative damages to the plants (Singh and Jha 2016). Similarly, Chakraborty et al. (2010) evidenced the increased emergence of new leaves and branches and leaf biomass in the tea plant (*Camellia sinensis*) with the application of prepared bioformulations of *S. marcescens* (TRS-1) with sawdust, rice husk, and tea waste. *S. marcescens* (TRS-1) acts as an antagonist to a number of fungal pathogens in vitro, including brown root rot of tea caused by *Fomes lamaoensis*. There was an increase in phenolics, as well as peroxidase, chitinase, β-1,3-glucanase, and phenylalanine ammonia-lyase, in the tea plant with the inoculation of *S. marcescens* (TRS-1). In a previous study, *S. marcescens* NBR11213 has been reported to induce plant growth promotion and, also biological control of foot and root rot disease of betel vine caused by *Phytophthora nicotine*. The enhancement in the accumulation of phenolics and defense enzymes in betel vine, which was treated with *S. marcescens*, reduced the root rot disease caused by *P. nicotineae* (Lavania et al. 2006). Jeun et al. (2004) reported that the inoculation of plant growth-promoting strain *S. marcescens* (90–166) induced systemic protection against anthracnose pathogen. Similarly, the accumulation of phenolics is also highly significant in ISR. Both POX

and CHT are PR proteins, and their increased activity indicated the activation of such PR proteins during the defense. In addition, POX plays a key role in the biosynthesis of lignin which limits the extent of pathogen spread because of the antifungal activity (Bruce and West 1989). Similar findings of accumulation of PR proteins such as POX, CHT, GLU, and PAL after application with biocontrol agents and their involvement in inducing systemic resistance against the pathogen have been reported by several workers in different crops (Meena et al. 2000; Oostendorp et al. 2001; Bargabus et al. 2004; Bharati et al. 2004). The previous study has demonstrated that plant growth-promoting rhizobacteria *S. marcescens* 90–166 mediates the induced systemic resistance to *Fusarium* wilt disease of cucumber (*Cucumis sativus* L.) caused by *Fusarium oxysporum* (Kloepper et al. 2001a, b). Therefore, the higher accumulation of defense-related enzymes and phenolics occurred when resistance is induced in the plants.

6.6 Mechanism of ISR-Mediated Plant Stress Management by *Serratia* Species

To bring about the ISR in the host plant, PGPR plays several mechanisms through strengthening or fortifying the physical and mechanical properties of the cell wall as well as changing the physiological and biochemical reaction of the host, leading to the synthesis of defense against the invasion of disease-causing agents. Some of these mechanisms identified in *Serratia* sp. is summarized below.

6.6.1 Modification of the Structural Cell Wall in Host Plants

The accomplishment of protecting the plant from invading pathogens relies primarily on its ability to build a line of defense rapidly for protecting cell walls against the spread of a pathogen (Benhamou et al. 1996a). Plant growth-promoting rhizobacteria induce structural modification of the cell wall in response to a pathogenic attack (Benhamou et al. 1996b; M'Piga et al. 1997). Seed treatment of cucumber and tomato with *S. marcescens* induced strengthening of cell walls in cucumber and tomato, in response to fungal and bacterial diseases, and also protected the plants against cucumber mosaic cucumovirus (CMV) (Raupach et al. 1996). This type of rapid defense reaction does not allow the pathogen to invade and also offers the host plant sufficient time to employ other defense mechanisms to fight the pathogens.

Research over the past years has demonstrated that induced systemic resistance (ISR) can be a potential mechanism by which PGPR demonstrates biological disease control (Kloepper et al. 1996). ISR is dependent on colonization of the root system by sufficient numbers of PGPR. Previous studies have shown that *S. proteamaculans* 1–102 promotes soybean-bradyrhizobia nodulation and growth (Bai et al. 2002a). Some biocontrol PGPB strains protect plants by activating gene encoding defense enzymes such as peroxidase, chitinase, phenylalanine ammonia-lyase, β-1,3-glucanase, and others involved in the synthesis of phytoalexin. In tomato, cell wall

thickening, deposition of phenolic compounds, and formation of callose restricted the growth of *F. oxysporum* f. sp. *radical-lycopersici* to the epidermal cell and outer cortex in the root system which were observed in *Serratia*-treated plants (M'Piga et al. 1997). *S. marcescens* 90–166 has been implicated in the elicitation of ISR on tobacco against the wildfire disease caused by *Pseudomonas syringae* pv. *tabaci* (Press et al. 1997). Such a rapid defense reaction at sites of fungal entry delays the infection process and allows sufficient time for the host to build up other defense reactions to restrict pathogen growth to the outermost layer of the root tissue.

6.6.1.1 Serratia-Mediated Biochemical/Physiological Changes in the Host Plants

Effects of ISR by PGPR are presented due to accumulation of pathogenesis-related (PR) proteins (M'Piga et al. 1997) and synthesis of phytoalexins and other secondary metabolites (Zdor and Anderson 1992). ISR by plant growth-promoting rhizobacteria antagonist *S. marcescens* 90–166 was confirmed by microtiter plate and a detached leaf assay against tobacco blue mold disease caused by *Peronospora tabacina*. It was indicated by the reduction in sporulation of *P. tabacina* in the pot trial (Zhang et al. 2002). Involvement of the lytic enzyme production in the induction of resistance by *S. plymuthica* C48 inhibited spore germination and germ-tube elongation in *Botrytis cinerea* (Frankowski et al. 2001). The ability to produce extracellular chitinases is considered crucial for *S. marcescens* to act as an antagonist against *Sclerotium rolfsii* by accumulating lytic enzymes at the site of penetration of the fungus, resulting in the degradation of the fungal cell wall. Pathogenesis-related peroxidase and chitinase proteins have been found to induce systemic resistance. In tomato roots, *Serratia*-mediated ISR against the root-knot nematode *Meloidogyne incognita* enhanced the levels of enzymatic activity in tomato roots and specific polyphenol oxidase (PPO), and β-1,2-glucanase (GLUC) activities detected in the roots were found when inoculated with *S. marcescens* (Abd-Elgawad and Kabeil 2012). ISR has been correlated with a twofold increase in the activity of pathogenesis-related peroxidase and chitinases proteins. Two peroxidases and one chitinase (35 kDa) isoforms have been induced in the PGPR-treated plant inoculated with rice sheath blight pathogen, *Rhizoctonia solani* (Nandakumar 1998). Similarly, in cucumber, the increase in the levels of chitinase and peroxidase enzyme was noticed when PGPR-mediated ISR *S. marcescens* 90–166 was inoculated against cucumber mosaic cucumovirus (Raupach et al. 1996).

Defense mechanisms by chemicals other than PR proteins are also induced in ISR by *Serratia* sp. in certain plants. ISR leads to the enhanced PR gene transcript and defense-related substances. Many of these substances are known to involve in defense reaction against plant pathogens. These include oxidative enzymes such as peroxidase (PO), which are implicated in the formation of phenols contributing to the synthesis of defense barriers for the cells (Avdiushko et al. 1993). Enzymes such as phenylalanine ammonia-lyase (PAL) mediate phenolic compound biosynthesis. The plants produce other enzymes of the defense including peroxidases, phenylalanine ammonia-lyase (PAL), and polyphenol oxidase (PPO) that act as catalysts for the formation of lignin. PAL and other enzymes are also involved in the formation

of phytoalexins (Figueiredo et al. 2010). In other PGPRs, ISR induced protection in both wild-type *Arabidopsis* and transgenic *Arabidopsis* with NahG-gene (coding for salicylic hydrolase) without activating PR gene expression (Van Wees et al. 1997). This clearly indicates that the PR protein accumulation is not the only defense compounds involved in the induction of systemic resistance in these hosts, and there is the possibility of involvement of other defense compounds in ISR. Moreover, Press et al. (1997) found that the biological control strain *S. marcescens* 90–166 was able to induce protection in both wild-type and transgenic NahG tobacco plants against *P. syringae* pv. *tabaci*, indicating that the ability to trigger an SA-independent pathway controlling systemic resistance is not uncommon among ISR-inducing rhizobacteria. However, not all resistance-inducing rhizobacteria trigger an SA-independent resistance. Zdor and Anderson (1992) evidenced increased peroxidase activity as well as an increase in the level of mRNAs encoding for phenylalanine ammonia-lyase (PAL) and chalcone synthase in the early stages of interaction between bean roots and various bacterial endophytes. The enzymes produced by antagonistic strains have a crucial role to play in disease resistance. The production of enzymes related to pathogenesis (PR proteins) by strains of rhizobacteria is considered as one of the most important properties of the antagonistic strains (Saikia et al. 2004). These enzymes such as chitinase, glucanase, peroxidase, and polyphenol oxidase are expressed during the interaction between the pathogen and host, which catalyze the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure (Avdiushko et al. 1993). Similarly, other enzymes such as phenylalanine ammonia-lyase and lipoxygenase also contribute to the defense reactions including inhibition of growth of the pathogen and induction of phytoalexins (Li et al. 1991). The extent of activity and accumulation of these enzymes depend mainly on the inducing agent, besides the genotype of the plant, physiological conditions, and pathogens (Tuzun 2001). The induced resistance system in some plants is very complex. There are three generally recognized pathways of induced resistance wherein two of these are involved in the direct production of pathogenesis-related (PR) proteins; in one pathway, the production of PR proteins is generally the result of attack by pathogenic microorganisms, whereas in the other, PR proteins are generally produced as a result of wounding, or necrosis-inducing plant pathogens; both pathways, however, have alternative mechanisms for induction. Typically, the pathogen-induced pathway relies on salicylic acid (SA) that is produced by the plant as a signaling molecule, whereas the wounding pathway relies on jasmonic acid (JA) as the signaling molecule. These compounds and their analogs induce similar responses when they are applied exogenously, and no doubt, there is considerable cross talk between the pathways (Pieterse et al. 2001). The JA-induced pathways have been designated to induce systemic resistance (ISR), and this has been also used to refer to quite different processes that are initiated by rhizobacteria (Fig. 6.2). The rhizobacterial strain *S. marcescens* 90–166 mediates induced systemic resistance (ISR) to fungal, bacterial, and viral pathogens. This strain (*S. marcescens*) produced salicylic acid (SA) using the salicylate-responsive reporter plasmid pUTK21 by analyzing culture extracts for the production of SA in broth culture with the help of high-pressure

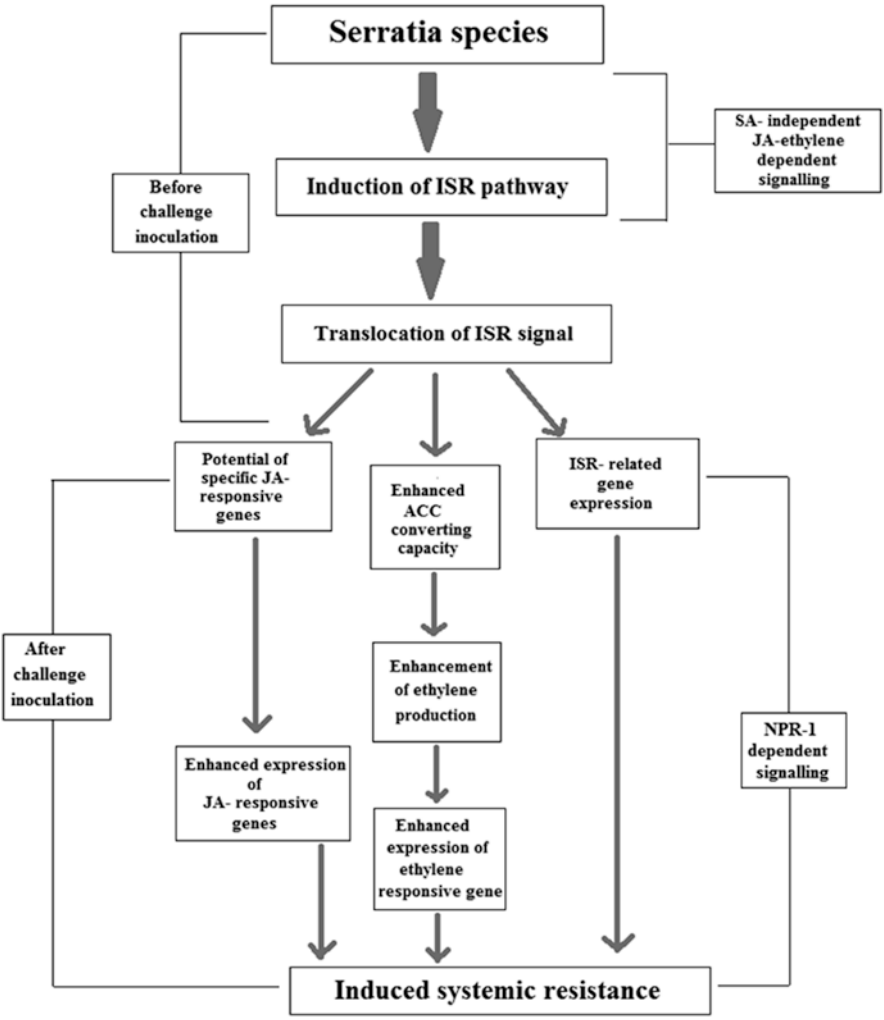

Fig. 6.2 Mechanism of induced systemic resistance with the involvement of jasmonic acid and ethylene in *Serratia* species

liquid chromatography (Press et al. 1997). Ferraz et al. (2015) also confirmed that the antagonists *S. marcescens* (UFV252) with hormone jasmonic acid (JA) reduced the bacterial spot symptoms in the tomato plants caused by the most destructive *Xanthomonas gardneri* and potentiate defense enzymes in the leaves of tomato plant infected by *X. gardneri*. The salicylate- and jasmonate-induced pathways are characterized by the production of a cascade of PR proteins which include antifungals (chitinases, glucanases, and thaumatins) and oxidative enzymes (viz., peroxidases, polyphenol oxidases, and lipooxygenases). Low-molecular-weight compounds with antimicrobial properties (phytoalexins) can also accumulate. The

third type of induced resistance is the one which is provoked by non-pathogenic root-associated bacteria and is referred to as rhizobacteria-induced systemic resistance (RISR), which led to the development of systemic resistance to plant diseases. However, it is functionally very different, as the PR proteins and phytoalexins are not induced by root colonization by the rhizobacteria in the absence of attack by plant pathogenic microorganisms. Once pathogen attack is increased, disease is reduced. Thus, RISR results in potentiation of plant defense responses in the absence of cascade of proteins that is typical for SA-induced system. *S. marcescens* induced PR-1a gene promoter with systemic resistance against wildfire disease of tobacco. Induction PR-1a gene activity assessed using transgenic tobacco plants expressing the β-glucuronidase (GUS) gene fused to the PR-1a gene promoter. Treatment of tobacco with SA and *S. marcescens* enhanced GUS activity in the wildfire disease plant caused by *Pseudomonas syringae* pv. *tabaci* (Park and Kloepper 2000). Similarly, Someya et al. (2002) recorded an antagonist bacterium *S. marcescens* strain B2 able to control rice blast after being sprayed onto rice phylloplane by pouring the bacterial suspension into the rhizosphere soil of rice plants. The reduction in infection was accompanied by an enhanced level of lipooxygenase of defense-related enzyme. Defense mechanisms induced by PGPR against insect pests are different. PGPR does not kill insects, but the application of PGPR brings about some physiological changes in the host plant that prevents the insects from feeding as has been demonstrated in cucumber against cucumber beetles (Zehnder et al. 1997). In the case of *Serratia* species, there is a negative effect on the *Acalymma vittatum* and *Diabrotica undecimpunctata* herbivore insects in cucumber plants. Normally, *Diabrotica* beetles are attracted to volatiles and cucurbitacins (triterpenoids occur mainly in cucurbitaceae), coming from cucurbit blossoms, and probably use these olfactory clues in long-range host finding. The cucurbitacin causes locomotory arrest and compulsive feeding of *Diabrotica* beetles. Due to the PGPR treatment, there is a shift in the metabolic pathway in cucumber plant away from the cucurbitacin synthesis and toward that of other plant defense compounds, resulting in fewer beetles being attracted (Zehnder et al. 1997).

The defense mechanism induced by PGPR against nematodes is by altering root exudates or inducing the host to produce repellents that affect nematode attraction or recognition of the host (Oostendorp and Sikora 1989) and altering the syncytial development or sex ratio in the root tissue (Wyss 1989). The root-knot nematode *Meloidogyne incognita* was controlled by induced systemic elicitor *S. marcescens* enhancing its polyphenol oxidase (PPO) and β-1,3-glucanase (GLUC) activities inside the tomato roots, and this is correlated with the reduced nematode infestation (Abd-Elgawad and Kabeil 2012).

6.7 Conclusions

According to PGPR, their modes of action are biocontrol, biofertilizers, phytostimulators, and biopesticides with certain bacteria having overlapping application. The beneficial effects of plant-associated bacterium *Serratia* species include inducing

systemic resistance in host plants. Most of the *Serratia* species are known to produce ISR against multiple pathogens attacking the same crop. In addition, the application of strain *Serratia* also reduces insect and nematode damage by suppressing the disease in host plants. The broad spectrum of control using *Serratia* strains can provide an effective, economical, and practical way of plant protection since some *Serratia* strains are endophytic in nature which makes them suitable for use in vegetatively propagated crops because of their capability to colonize and persist in the intercellular space of epidermal cells, thereby reducing the need for further application if the same vegetative parts are used as propagative material. Thus it is becoming more apparent that most of the *Serratia* strains can promote plant growth by several mechanisms. However, carefully controlled field trials of crop plants inoculated along with *Serratia* are necessary for maximum commercial exploitation of *Serratia* strains for broad-spectrum activity against multiple pathogens and pests.

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

7

Rahul Singh Rajput, Prachi Singh, Jyoti Singh, Shatrupa Ray, Anukool Vaishnav, and Harikesh Bahadur Singh

Abstract

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

Keywords

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

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7.1 Introduction

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

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

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

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

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

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

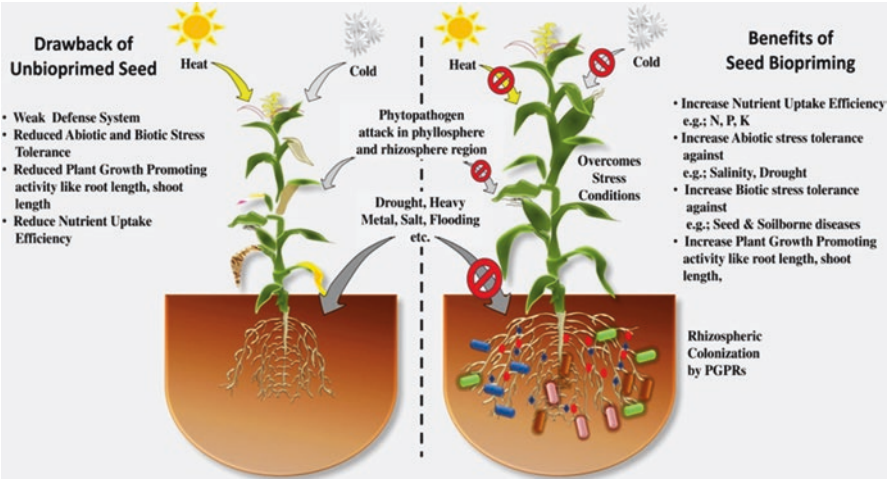

Fig. 7.1 Pictorial representation of seed biopriming effect on the crop

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

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

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

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

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

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

7.4 Action Mechanism of PGPRs

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

7.4.1 Bioprotectant

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

7.4.1.1 Production of Antibiotics

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

Table 7.2 Biocontrol agents used against various seed-borne pathogens

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

(continued)

Table 7.2 (continued)

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

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

7.4.1.2 Production of Siderophore

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

7.4.1.3 Production of Hydrolytic Enzymes

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

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

7.4.1.4 Induction of ISR

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

7.4.2 Plant Growth Promotion

7.4.2.1 Modulation of Phytohormone Production

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

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

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

7.4.3 Increase Nutrition Uptake

7.4.3.1 Nitrogen Fixation

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

7.4.3.2 Phosphate Solubilization

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

7.4.3.3 Potassium Solubilization

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

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

7.5 Future Prospects of PGPR Incorporation in Seed and Soil

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

7.6 Conclusion

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

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

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Plant Small RNAs: Big Players in Biotic Stress Responses

8

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Abstract

A myriad of small RNAs (18–25 nt in length) undergo heterogeneous modifications to inflect RNA stability and other complex physiological processes like stress responses, metabolism, immunity, and epigenetic inheritance of environmentally acquired traits. Such small RNAs include microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), and tRNA-derived small RNAs (tsRNAs). Worldwide crop production and human health are affected when plants are attacked by pathogens and pests. Therefore, a large collection of genes get up- or down regulated to mediate the defense responses in plants against pathogens (bacteria, fungi, oomycetes, and viruses). Host endogenous small RNAs, thus, come into play to counter biotic stress where RNA silencing machinery is utilized to facilitate pathogen-associated molecular pattern-triggered immunity and effector-triggered immunity. RNA interference (RNAi) pathways trigger gene silencing in interacting species from even different kingdoms (cross-kingdom RNAi). Diverse pathways are involved in regulating the defense mechanism including Dicer-like proteins (DCLs), double-stranded RNA (dsRNA) binding protein, RNA-dependent RNA polymerases (RDRs),

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RNA polymerase IV and V, small RNA methyltransferase HEN1, and Argonaute (AGO) proteins showcasing their functional specificities as well as verbosity. Transgenic plants are newly emerging players that help in solving the problem of pathogen attack in fields. In this chapter, the recent breakthrough on the function of sRNAs in response to biotic stress, mainly in plant-pathogen interaction, and its application in disease control is discussed.

Keywords
Biotic stress · Small RNA · Cross-kingdom RNAi · Argonaute · Gene silencing

8.1 Introduction

8.1.1 Zigzag Model

World population is increasing at a constant rate leading to agricultural land loss. This problem caters for diverse means to improve global food production. Another problem accounted for is the loss in crop productivity and grain quality due to bacteria, fungi, oomycetes, viruses, and insects. Therefore, it is required to unleash the biotic stress responses in plants and develop innovative tools using traditional and modern breeding approaches for crop protection against pathogens and pests (Bebber and Gurr 2015). On the contrary, pathogens have acquired the ability to counter such barriers to access nutrients and flourish inside plants thereby provoking their immune system. Nevertheless, plants have derived a defense mechanism to overcome pathogen infection by activating or suppressing a large array of genes (Jones and Dangl 2006). The "zigzag model" is proposed which explains in an easier way the different layers of innate immunity when plants are infected with pathogens (Jones-Rhoades et al. 2006). To avoid spreading infection by pathogen, the very first means of defense against them is pattern recognition receptors (PRRs). These receptors are cell surface-localized, transmembrane proteins and can detect conserved pathogenic patterns known as microbe−/pathogen−/host danger-associated molecular patterns (MAMPs or PAMPs or DAMPs) and hence shoot up the MAMP-/PAMP-triggered immunity (MTI/PTI) to limit the spread of pathogen (Jones and Dangl 2006). Flagellin peptide, elongation factor Tu protein (EF-Tu), and chitin are the best-studied MAMPs that form a major component of fungal cell walls and lipopolysaccharides (LPS). The perception of MAMPs relies on PRRs where FLS2 and EFR recognizing flagellin and EF-Tu possess to have a same structural construction formed by extracellular leucine-rich repeats (LRR) and a cytoplasmic kinase domain. On the contrary, CERK1, an *Arabidopsis* PRR, recognizes chitin containing three extracellular LysM domains and a cytoplasmic kinase domain. This recognition helps in inducing callose deposition, producing reactive oxygen species, accumulating salicylic acid (SA), and expressing pathogenesis-related (PR) genes (Yang and Huang 2014). Pathogens, on the other hand, have developed schemes to outpower MTI by sending effector proteins inside plant cells

that abolish early recognition and downstream signaling events of MTI, therefore, resulting in effector-triggered susceptibility (ETS) (Feng and Zhou 2012). But plants too have emerged to protect themselves from this infection by using their resistance (R) proteins that recognize the specific effectors and activate effector-triggered immunity (ETI). This immune response is more sturdy and speedy (Chisholm et al. 2006). There is another hypersensitive response (HR), which causes cell death at the site of infection to restrain the growth of the pathogen. The effector proteins that are produced are called Avr factors. In the latter case, the R proteins [nucleotide binding site (NBS) and an LRR domain] guard the Avr factors and detect their modification caused by the effector proteins (Mackey et al. 2002). MAP kinase gets activated when pathogen's molecules are perceived by PRRs or R proteins leading to a reprogramming in host's gene expression along with the activation of genes with antimicrobial function (PR, pathogenesis related) (Tsuda and Katagiri 2010).

The war of defense and counter-defense between pathogens and plants has resulted in distinct collection of pathogen effectors and resistance genes.

8.2 Role of RNA

Posttranscriptional modifications are found extensively in stable and structured RNAs (tRNA and rRNA, mRNAs, and an expanding catalog of small and large noncoding RNAs) (Li and Mason 2014). Recent discovery of reversible 6-methyladenosine (m^6A) modifications in mRNAs (Dominissini et al. 2012) as well as key enzymes for their dynamic regulation is observed. Other studies have documented pseudouridine (Li et al. 2015), 5-methylcytidine (m^5C) (Hussain et al. 2013), and most recently, 1-methyladenosine (m^1A) (Dominissini et al. 2016) in mRNAs. RNA modifications are also observed in small RNAs to perform various cellular functions that include development in plants, metabolic study, maintenance of genome integrity, immunity against pathogens, and abiotic stress responses. Regulation of gene expression is performed by small RNA in a sequence-specific manner either transcriptionally or posttranscriptionally (Chapman and Carrington 2007).

Eukaryotic organisms possess 20–40-nucleotide (nt)-long noncoding RNA molecules called small RNAs, and depending on their biogenesis and precursor structure, small RNAs are placed in two discrete groups: microRNAs (miRNAs) and small interfering RNAs (siRNAs) (Yang and Huang 2014).

8.2.1 MicroRNA

Small noncoding RNA generated from an imperfectly base-paired hairpin structure with 21–24 nt is called miRNA (Chen 2009). MicroRNAs (miRNAs) negatively regulate gene expression at the posttranscriptional level through mRNA degradation or translation repression (Iwakawa and Tomari 2013). Plant miRNAs are derived

from the distinct noncoding transcripts of miRNA genes which are transcribed by enzyme RNA polymerase II. The primary miRNAs (pri-miRNAs) form a secondary fold-back structure and thereupon get processed by the RNase III-type enzyme Dicer-Like1 (DCL1) to create the precursor miRNAs (pre-miRNAs) (Rogers and Chen 2013). The miRNA duplexes once formed from pre-miRNA are stabilized by 2°-O-methylation and catalyzed by Hua Enhancer 1 (Yang et al. 2006) and transported to the cytoplasm by HASTY (Bollman et al. 2003). The passenger strand of the miRNA duplexes is often removed by unwinding or cleavage (Kawamata and Tomari 2010), and the guide strand is maintained in the RNA-induced silencing complex (RISC) that defines target recognition. Plant miRNAs exert a considerable effect on gene expression and mediate the cleavage of target mRNAs with near-perfect complementarity (Voinnet 2009).

8.2.2 SiRNA

Small interfering RNAs (siRNAs) are formed from near-perfect complementarity long double-stranded RNAs (dsRNAs) and are generated either from antisense transcription or by the action of RNA-dependent RNA polymerases (RDRs) (Katiyar-Agarwal and Jin 2010). There are many subclasses of siRNA present in plants depending on origin and biogenesis: trans-acting siRNAs (ta-siRNAs), heterochromatic siRNAs (hc-siRNAs), natural antisense transcript-derived siRNAs (nat-siRNAs), and long siRNAs (lsiRNAs).

8.3 RNA Silencing

Communication taking place between organisms whether pathogenic, parasitic, or symbiotic mediates the transport of regulatory molecules across the cellular boundaries between the host and its interacting pathogens/pests/parasites or symbionts. This triggers gene silencing in trans in the non-related species, a mechanism called cross-kingdom or cross-organism RNAi (Knip et al. 2014).

RNA interference (RNAi) is a gene silencing event that regulates sequence-specific gene and gets induced by double-stranded RNA (dsRNA). This results in inhibition of translation or transcription. Gene regulation is initiated by sRNAs in hosts or pathogens by posttranscriptional gene silencing (PTGS) or transcriptional gene silencing (TGS). PTGS is induced by miRNAs and siRNAs through messenger RNA (mRNA) cleavage/degradation or translational inhibition with the help of an RNA-induced silencing complex (RISC), while TGS is induced by siRNAs and some specific miRNAs. TGS is responsible for DNA methylation, histone modification, or chromatin modification (Cui and Cao 2014). A number of pathways are involved in producing regulatory small RNAs using various conserved protein families like the RNA-dependent RNA polymerases (RDRs), the double-stranded RNA-binding proteins (DRBPs), the Dicer-like proteins (DCLs), the small RNA methyltransferase (HEN1), and the Argonaute (AGO) proteins. Plant sRNAs and

RNA interference (RNAi) pathway components are major regulatory players in providing immunity to plants against viruses, bacteria, fungi, oomycetes, and pests (Seo et al. 2013). Transposable element (TE) regions transcribe sRNAs in filamentous plant pathogens, and silencing this TE can help in fighting infection (Chang et al. 2012).

8.4 RNA Silencing Suppressors of Pathogens

8.4.1 Viral Suppressors of RNA Silencing

Many viruses cipher specific proteins to suppress the host antiviral silencing response and to cause infection in them. These viral suppressors of RNA silencing (VSRs) perform at three different levels, i.e., they can (a) inhibit generation of viRNAs, (b) inhibit loading of viRNAs in RISC by binding to the viRNA, and (c) inhibit components of RISC. Table 8.1 discusses the mode of action of VSRs in plants.

8.4.2 Bacteria-Encoded Suppressors of RNA Silencing

Bacterial pathogens too have developed similar silencing suppressors to combat antibacterial defense responses in plants as in viruses. Navarro et al. (2008) identified several *Pst* type III secretion effectors that enhance the disease susceptibility by suppressing host RNA silencing machinery. Effectors include AvrPtoB which represses transcription of miRNA genes and lowers the level of pri-miR393, AvrPto which interferes with miRNA precursor processing and downregulates mature miR393 level, and HopT1 which inhibits the action of the AGO1 protein in the RISC complex. Likewise, fungi and oomycetes too have developed RNAi suppressors to counteract host antipathogen RNA silencing mechanisms.

8.5 Host Endogenous Small RNAs in Plant-Microbe Interactions

When pathogen interacts with its host at first, it triggers the immunity response in plants known as pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI). Now, bacteria too rectify PTI by secreting and injecting effector proteins into plant cells leading to PTI suppression. Finally, host plant releases resistance components such as resistance (R) proteins that can recognize effectors and elicit effector-triggered immunity (ETI) (Chisholm et al. 2006). Unlike viruses that replicate inside the host cell, bacteria, fungi, and other microbes interact with plants without undergoing DNA or RNA replication and transcription inside the plant cell. In such interactions, host endogenous small RNAs play a pivotal role in counteracting these pathogens.

Table 8.1 Mode of action of viral silencing suppressors in plants

Suppressor	Source	Mode of action	Reference
AC4	Geminivirus	Competes with AGOs by binding to single-stranded siRNA and thereby preventing RISC assembly	Chellappan et al. (2005)
AC2	Begomovirus	Transcriptional activator. Induces expression of any gene, which might be a silencing suppressor	Trinks et al. (2005)
HcPro	Potyvirus	Mimics *hen1* mutations. viRNAs are oligo-uridylated and partially degraded due to lack of 2-*O*-methylation	Wu et al. (2010a, b)
		Interacts with a calmodulin-related protein, overexpression of which suppresses silencing	
		Amino acids 180, 205, and 396 of HcPro are critical for suppression of miRNA, ta-siRNA, and VIGS pathway but not for sense PTGS	
P6	Cauliflower	Is imported in the nucleus and binds to DRB4 protein. Suppresses RNA silencing	Haas et al. (2008)
	Mosaic virus	Pathway, possibly by inactivating DRB4, which is an essential component required for DCL4 action	
2b	Cucumber	Interacts physically with siRNA-loaded RISC and inhibits its slicing action	Goto et al. (2007)
	Mosaic virus	In vitro assays suggest that 2b binds to siRNAs to a lesser extent than to long dsRNAs	
		2b inhibits the production of RDR1-dependent viral siRNAs	
P0	Polerovirus	Promotes ubiquitin-dependent proteolysis of AGO1	Pazhouhandeh et al. (2006)
P69	Tymovirus	Inhibits viRNA amplification	Chen et al. (2004)
AL2	Curtovirus	Interacts with adenosine kinase, whose inhibition possibly prevents methylation of viral DNA	Wang et al. (2005)
p126	TMV	Encodes methyltransferase and helicase. Binds duplex siRNA and inhibits HEN1-dependent methylation and degradation	Blevins et al. (2006)
RNAse III	*Closteroviridae*	In vitro assays suggest that RNAse III suppresses siRNA silencing by cleaving 21-, 22-, and 24-bp siRNAs into 14-bp fragments	Cuellar et al. (2009)

8.5.1 Noncoding Small RNAs

Small noncoding RNAs (sncRNAs), discovered in eukaryotes, are 18–30-nt-long molecules which perform numerous functions such as gene expression control, defense against other parasitic nucleic acids, epigenetic modification, and heterochromatin regulation (van der Krol et al. 1990). There are ample functions and beneficial applications reported so far. Few of them are encompassing cell-to-cell signaling and communication in multicellular organisms (Mittelbrunn and Sanchez-Madrid 2012), trans-generational RNAi (Bond and Baulcombe 2014) and memorization (Rasmann et al. 2012), cell fate differentiation and vascular formation (Benkovics and Timmermans 2014), systemic antiviral immunity (Saleh et al. 2009), environmental RNAi (Zhuang and Hunter 2012), cancer prevention and diagnosis (Salido-Guadarrama et al. 2014), and intercellular immune activation (Robbins and Morelli 2014). MicroRNAs (miRNAs) and small interference RNAs (siRNAs) are the best-studied sncRNAs.

In response to different pathogen stressors, various targets and functions of sRNAs are summarized in Table 8.2.

Table 8.2 Response of sRNA to different pathogen stressors

Small RNA	Small RNA source	Host/pathogen	Target genes	Expression of genes upon infection	Roles in plant pathogen infection
miR159	Plant	*Arabidopsis*/ bacterium *P. syringae*	MYB33, MYB65, MYB101	UP	Regulates gibberellin and ABA signaling pathways
miR160	Plant	*Arabidopsis*/ bacterium *P. syringae*	ARF10, ARF16, ARF17	UP	Increases PAMP-induced callose deposition
	Plant	*M. esculenta*/ fungus *C. gloeosporioides*	ARF10	UP	Regulates plant auxin and enhances plant defense response
miR167	Plant	*Arabidopsis*/ bacterium *P. syringae*	ARF8 and ARF6	UP	Regulates auxin signaling pathways and enhances plant defense responses
miR390	Plant	*Arabidopsis*/ bacterium *P. syringae*	TAS3	DOWN	Triggers the accumulation of ta-siRNAs that regulate arf3 and arf4 for auxin signaling

(continued)

Table 8.2 (continued)

Small RNA	Small RNA source	Host/pathogen	Target genes	Expression of genes upon infection	Roles in plant pathogen infection
miR398	Plant	*O. sativa*/fungus *M. oryzae*	SOD2	UP	Overexpression of miR398 increases the accumulation of hydrogen peroxide and defense-related genes and decreases fungal growth
miR399	Plant	*Citrus*/ bacterium Ca. *L. asiaticus*	PHO2	UP	Contributes to HLB symptoms and phosphorus homeostatis and signaling
miR408	Plant	Wheat/fungus *Puccinia striiformis f.* sp. *tritici*	TACLP1, a type of plantacyanin protein	UP/DOWN	Negatively regulates wheat resistance to stripe rust
miR1885	Plant	*Brassica napus*/ virus TuMV	TIR-NBS-LRR	UP	Represses ETI
nat-SiRNAATGB2	Plant	*Arabidopsis*/ bacterium *P. syringae*	PPRL	UP	Contributes to plant immunity by suppressing a negative regulator of the RPS2 pathway
AtlsiRNA-1	Plant	*Arabidopsis*/ bacterium *P. syringae*	AtRAP	UP	Contributes to plant immunity by silencing a negative regulator

8.6 Components of the Small RNA Biogenesis Pathway Play an Important Role in Plant Defense

Many plant genomes possess multiple components such as DCLs, RDRs, and AGOs in the RNAi silencing machinery. *Arabidopsis* has four DCLs, six RDRs, and ten AGOs, many of which are involved in plant defense signaling pathway.

8.6.1 Dicer-Like Proteins and Their Associated Proteins

Four DCLs present in *Arabidopsis* process dsRNA or fold-back RNA precursors to generate siRNAs and miRNAs, respectively. The role of DCLs and their compensatory functions in the production of virus-derived small RNAs (viRNAs) is well

understood using single, double, or triple mutants of DCLs in genetic experiments. A loss of function mutation in both DCL4 and DCL2 is enough to cause viral susceptibility (+ssRNA) in plants (Diaz-Pendon et al. 2007).

Qu et al. (2008) observed that all four DCL proteins, key components of RNA silencing pathway, are involved in providing an antiviral defense in plants with functional hierarchy as (DCL4>DCL2>DCL3>DCL1) in processing viral RNAs into viRNAs (Deleris et al. 2006). Other important cofactors like small dsRNA-binding proteins (DRBs) of DCL proteins are known, but these do not show hierarchical redundancy as do DCLs (Curtin et al. 2008). DRB4 when interacting with DCL4 confers resistance against viruses (Qu et al. 2008). On the contrary, DCL2 and DCL3 do not need interaction with DRB for production of viRNAs (Curtin et al. 2008). Another protein HEN1 containing dsRNA binding domain plays an important role in viral resistance (Park et al. 2002). When mutation was done in *hen1* of *Arabidopsis*, hyper-susceptibility to cauliflower mosaic virus (CMV) was observed in the plant as compared to wild type suggesting that HEN1 contributes to resistance against the virus (Boutet et al. 2003). Along with the abovementioned, DCL proteins are also involved in the production of small RNAs thereby giving antibacterial immunity in plants. The *dcl1* mutant showed heightened susceptibility to *Pst* DC3000 *hrcC*$^{-}$, a nonpathogenic strain that can evoke PTI (Navarro et al. 2008). HYL1, the dsRNA-binding protein associated with DCL1, is also involved in bacterial infection resistance as the *hyl1* mutant was susceptible to *Pst (avrRpt2)*.

8.6.2 RNA-Dependent RNA Polymerases

Elaborated studies have stated RDRs to be induced by antiviral defense as well as in the presence of defense signaling compounds such as salicylic acid (SA) (Xie et al. 2001). It was observed that when the expression levels of RDR1 are lowered in transgenic antisense *Arabidopsis* plants, viral RNAs get piled up and susceptibility to TMV and potato virus X (PVX) infection is increased. NtRDR1 is also involved in fighting against potato virus Y (PVY) infection and its ortholog AtRDR1 transmits defense against tobamovirus and tobravirus because *Arabidopsis rdr1* mutant plants had enhanced levels of viral RNAs (Yu et al. 2003). A functional homolog of AtRDR6, NbRDR6, provides resistance against viruses (Qu et al. 2005) as down-regulation of NbRDR6 increased the susceptibility to many different viruses at high temperatures.

8.6.3 Argonautes

Silencing of target genes is activated by AGOs as these are associated with small RNAs and form RISC complexes (Hannon 2002). In *Arabidopsis*, 10 AGOs are found to take part in plant immunity. hc-siRNAs promote transcriptional gene silencing (TGS) by guiding RNA-directed DNA methylation (RdDM) and histone modification in plants (Vaistij et al. 2002). AGO4 is a leading nuclear RNAi effector

associated with hc-siRNAs or ra-siRNAs that allows DNA methylation (Li et al. 2008) which links DNA methylation and plant defense together. Using both cytosine and histone methyltransferases, *Arabidopsis* plants silence viral chromatin of cabbage leaf curl virus (CaLCuV) and beet curly top virus (BCTV) (Raja et al. 2008). Viral suppressors AL2 and L2 stop adenosine kinase (ADK) activity which otherwise generates S-adenosylmethionine (a methyltransferase cofactor). Therefore, plants infected with virus in the absence of L2 had hypermethylation of viral DNA, and to recover from viral infection, AGO4 is needed (Raja et al. 2008). AGO4 also helps in antibacterial defenses. In addition to AGO4, AGO1 and AGO7 play a pivotal role in slicing viral RNAs (Qu et al. 2008). AGO1 is the primary slicer because it targets viral RNAs with more compact structures, but AGO7 is an alternate slicer which targets RNAs with less complexity. The biogenesis of AtlsiRNA-1 involved AGO7, as *ago7* mutant that does not accumulate AtlsiRNA-1 (Katiyar-Agarwal et al. 2006). However, other *ago* mutant plants, including *ago3*, *ago4*, and *ago9*, showed no significant change in the level of AtlsiRNA-1 as compared with wild type. AGO7 is also associated with TAS3 ta-siRNA (Fahlgren et al. 2006). AGO7 accumulates bacteria-induced AtlsiRNA-1 hence suggesting its role in antibacterial defense.

8.7 Cross-Kingdom RNAi and sRNA Trafficking

When two unrelated interacting organisms communicate with each other, it is called cross-kingdom RNAi. This process is observed in both animal and plant systems. Plants transfer RNAi signals into interacting organisms, such as filamentous fungi, oomycetes, nematodes, parasitic plants, and pests, to restrain their growth. This process is known as HIGS, the most noticeable example of cross-kingdom RNAi in plants (Koch et al. 2013). In order to develop pest- and pathogen-resistant crops, scientists have engineered diverse plant species, from model plants to commercial crops, so as to express exogenous artificial RNAi signals that suppress the gene of parasitic nematodes, herbivores, and fungal and oomycete pathogens by targeting their mRNAs (Koch and Kogel 2014). HIGS is functional and successfully used against parasitic plants such as *Orobanche* and *Cuscuta* spp. and in model plants such as *Arabidopsis thaliana* and tobacco *Nicotiana benthamiana* as well as in important crops, including wheat, barley, *Medicago*, and banana, to efficiently work against a variety of fungal and oomycete pathogens, such as *Blumeria graminis*, *Puccinia tritici*, *Fusarium* spp., and *Phytophthora capsici* (Koch and Kogel 2014). Basic mechanism of HIGS is that it alters the fungal morphology and growth inhibition in plants, thereby reducing virulence. Additionally, HIGS is also used to study gene function in non-transformable species (Yin et al. 2014). A HIGS approach was carried out on *Glomus* spp. to study gene function of the monosaccharide transporter 2 (Helber et al. 2011), showing that HIGS is functional on arbuscular mycorrhiza, which forms symbiotic relationship with hosts. Successfully applying HIGS helps plants to deliver mobile gene silencing signals for communication and manipulating diverse interacting organisms.

There are evidences of RNAi signaling taking place in the opposite direction. Advanced pathogens and parasites use cross-kingdom RNAi to suppress host

immunity for infection (Weiberg et al. 2015). Three Bc-sRNAs in *Botrytis*-host interaction suppress *Arabidopsis* and tomato immunity genes in vivo (Mayoral et al. 2014). It is also estimated that sRNAs are also likely to be exchanged between the parasitic plant and its host, but the study still awaits the research output. Secretion and uptake of protein and other macromolecules participate in providing barrier against pathogens and parasites (Huckelhoven 2007) and in pathogenesis and effector-triggered suppression of host plant immunity (Kale and Tyler 2011).

8.8 Small RNA Biogenesis Pathways in Plants

Arabidopsis is taken as a model plant to study small RNA pathways in plants. Generative work involves both forward and reverse genetic screens to study the cellular proteins participating in biogenesis and function of miRNAs and siRNAs. A brief review of different kinds of small RNA pathways known in *Arabidopsis* is discussed below.

8.8.1 Biogenesis and Mechanism of miRNAs in Plants

The very first observation of microRNAs (miRNAs) took place in a nematode *Caenorhabditis elegans* (Lee et al. 1993). These are also known as short temporal RNAs (stRNAs) because they were expressed temporally in a mutant nematode. These endogenous noncoding small RNAs accelerate the growth, development, and survivability of plants. Transcription of miRNA gene is carried out by RNA polymerase II forming primary transcripts (pri-miRNAs) as a stem-loop structure of 1000-bp-long nucleotides (Chen 2005). Two processing steps are involved in the formation of mature miRNAs. The first step is carried out inside the nucleus where the microprocessor complex acts on pri-miRNAs to pre-miRNAs (precursor miRNAs) of 60–70 nt long. Two proteins, Drosha (169 kDa, RNAse III protein) and Pasha (dsRNA binding protein/DGCR8), constitute the microprocessor complex (Creelman and Mullet 1997). Two orthologs of Drosha and Pasha, namely, Dicer-like 1 (DCL-1) and Hyponastic Leaves 1 (HYL-1), are engaged in preliminary processing step of miRNA biogenesis pathway in plants (Schauer et al. 2002). To allow second processing step occuring in the cytoplasm, HASTY transport protein (ortholog of exportin-5) is required to transport pre-miRNAs from nucleus to cytoplasm. In subsequent step, ATP-dependent RNAse III protein (Dicer) converts hairpin dsRNA (pre-miRNA) into 21–24-nt-long mature miRNA-miRNA∗ duplex with 2-nt 3′ overhangs. This enzyme recognizes 2-nt 3′ overhangs and eliminates about ~21-nt sequence from its ends (Du et al. 2011). Out of two strands in miRNA duplex, one is called as antisense miRNA (miRNA) which has G:U base pairs, mismatches, and unpaired base pairs at its 5′ end, while the other strand is known as sense strand (miRNA∗). A complex is formed between Argonaute 1 (AGO1) protein and one strand of miRNA to guide miRNA to target its complementary mRNA sequence. The destiny of target mRNA depends on the degree of its complementarity with associated miRNA sequence. Complete

degradation occurs from near-perfect complementarity, while repression of protein translation occurs from partial complementarity. This miRNA biogenesis pathway is under the feedback regulation by two principal miRNAs, miR162 and miR168, causing cleavage of DCL1 mRNA and AGO1 mRNA (Zhang et al. 2011), respectively.

The ability of miRNAs in crop improvement can be well documented as transgenic plants harbor miRNAs under constitutive and inducible promoters that can specifically downregulate target genes of interest with limited non-autonomous effect.

8.8.2 siRNA

Antisense transcription or cellular RNA-dependent RNA polymerase (RDR) is used to derive siRNAs. In plants, there are four discrete siRNAs present: trans-acting siRNAs (ta-siRNAs), natural antisense transcripts (NATs)-derived siRNAs (nat-siRNAs), heterochromatic siRNAs (hc-siRNAs) or repeat-associated siRNAs (ra-siRNAs), and long siRNAs (lsiRNAs). For the initiation of ta-siRNA formation, RNA Pol II transcribes noncoding TAS genes where long primary transcript products upon cleavage by miRNAs and RNA-induced silencing complexes (RISCs) produce a 5′ fragment or a 3′ fragment which acts as a template for complementary strand synthesis, also coordinated by RDR6 and SGS3 (Vazquez 2006). DCL4 and DRB4 act consecutively on dsRNA molecule to form ta-siRNAs (Gasciolli et al. 2005). Intersecting regions of sense and antisense transcripts of *cis*-NATs give rise to nat-siRNAs. RNA interference is exploited in order to accomplish desirable traits in crops by operating the gene expression (Table 8.3). After the identification of the target genes, RNAi construct with hairpin cassette was created. Plant transformation and later screening and traits evaluation take place.

8.8.3 miRNA vs. siRNA

The most important regulators of gene expression are microRNAs (miRNAs) and short-interfering RNAs (Vazquez 2006) having size of 20–24 nt long. The difference between the two lies in precursor structures, pathway of biogenesis, and modes of action (Axtell 2013) (Table 8.4). Both are processed from long RNA precursors by Dicer-like ribonucleases (Bernstein et al. 2001) and regulate the target gene repression (Hammond et al. 2000).

8.8.4 Transposon-Associated sRNAs in Eukaryotic Plant

Eukaryotic pathogens are capable of silencing TEs by producing transposable element (TE)-associated sRNAs. The transcription of sRNA effectors in *Botrytis cinerea* takes place via TEs to suppress host immunity-related genes. In return, host plant resistance (*R*) genes get clustered in genomic loci embellished with TEs. TEs

Table 8.3 Traits improved by targeting the specific genes in plants

	Traits improvement Biotic stress	Targeted gene	Plant	Reference
Virus resistance	*Bean golden mosaic virus* (BGMV)	*AC1*	Bean	Bonfim et al. (2007)
	Barley yellow dwarf virus (BYDV)	*BYDV-PAV*	Barley	Wang et al. (2000)
	Rice dwarf virus (RDV)	*PNS12*	Rice	Shimizu et al. (2009)
	Turnip yellow mosaic virus (TYMV)	*P69*	Tobacco	Niu et al. (2006)
	Turnip mosaic virus (TuMV)	*HC-Pro*	Tobacco	Niu et al. (2006)
Insect resistance	*Helicoverpa armigera*	*CYPAE14*	Cotton	Mao et al. (2007)
	Corn rootworm	*V-ATPase A*	Maize	Baum et al. (2007)
Nematode resistance	*Meloidogyne incognita*	Splicing factor and integrase	Tobacco	Yadav et al. (2006)
	Meloidogyne incognita	*16D10*	*Arabidopsis*	Huang et al. (2006)
Bacterial resistance	*Xanthomonas citri* subsp. *citri* (Xcc)	*PDS* and *CalS1*	Lemon	Enrique et al. (2011)
	Agrobacterium tumefaciens	*iaaM* and *ipt*	*Arabidopsis*	Escobar et al. (2001), Dunoyer et al. (2006)
Fungal resistance	*Magnaporthe grisea Xanthomonas oryzae*	*OsSSI2*	Rice	Jiang et al. (2009)
	Magnaporthe grisea	*OsFAD7* and *OsFAD8*	Rice	Yara et al. (2007)
	Phytophthora infestans	*SYR1*	Potato	Eschen-Lippold et al. (2012)
	Blumeria graminis f. sp. *tritici*	*MLO*	Wheat	Riechen (2007)
Enhanced drought tolerance		Farnesyl transferase	Canola	Wang et al. (2009)
		C-kinase 1 (*RACK1*)	Rice	Li et al. (2009)
		OsDSG1	Rice	Park et al. (2010)
		OsDIS1	Rice	Wang et al. (2011a, b)

show epigenetic control of *R*-gene expression by *R*-gene sRNAs. Likewise, pathogen protein effector genes occur as clusters and scatter with TEs. In another example, protein effector gene-derived sRNAs in *Phytophthora* spp. control the expression levels of effector. For both pathogen protein effector genes and host plant *R* genes, sRNAs play the crucial regulators assisted with TE transposition. TEs are a core source of sRNA production where pathogens allow regulation of TEs and TE-associated protein effector gene expression by sRNAs, delivering sRNA effectors into host cells to change host defense gene expression. In plants, the advent of

Table 8.4 A comparison of the types of sncRNAs

	siRNA	miRNA	ta-siRNA	nat-siRNAs
Derived from	Invasive nucleic acids (virus, transgenes, heterochromatin)	Noncoding regions. Distinct genomic loci. Encoded by own genes	Noncoding regions	Antisense genes
Transcribed by	Depends on origin	RNA pol II	RNA pol II	RNA pol II
Processed by	DCL, RDR, SDE, NRPD	DCL1, HYL1, HEN1	RDR6/ SGS3, DCL, miRNAs	DCL1, HYL1
Targets transcripts in	Cis	Trans	Trans	Cis
Binds to	AGO1, AGO2	AGO1	AGO1, AGO7	AGO1

sRNAs upon infection epigenetically controls *R*-gene expression thereby activating defense genes. There are chances that plants may deliver their own RNA or protein molecules into pathogen cells. These events affect plant-pathogen interaction to provide host resistance, pathogen virulence, and host adaptation.

8.9 sncRNAs and Viruses: New Frontiers of Defense

For universal gene expression changes, current studies affirm the use of sncRNAs in plant-virus interactions. It has been proposed that plant miRNA expression that targets plant transcripts changes its response virus recognition affecting both viral replication and spreading. Numerous plant miRNAs after viral infection get either up- or downregulated (Pacheco et al. 2012). For example, when turnip mosaic virus infects *Brassica rapa*, miR1885 is induced in its response and targets a TIR-NBS-LRR (TNL) disease resistance gene (He et al. 2008).

8.10 Biotic Stress Resistance

Ample economic loss is posed by plant pathogens due to depletion in crop production. Therefore, several RNAi strategies are on the board to provide improvement in crop defense mechanisms against various biotic stresses (viruses, bacteria, fungi, nematodes, and insects).

8.10.1 Virus Resistance

Virus-induced gene silencing (VIGS) is an RNA-mediated PTGS mechanism that allows plants to protect themselves from foreign gene invasion (Ding 2010).

Pathogen-derived resistance (PDR) provides plants resistance against virus through genetic engineering (Simon-Mateo and García 2011). This PDR is either protein mediated where transgene encodes the protein or RNA mediated where transgene forms the transcript. To attain PDR, hairpin dsRNAs including small hairpin RNA (shRNA), self-complementary hpRNA, and intron-spliced hpRNA are produced in vivo using inverse repeat sequences from viral genomes. This approach was used successfully to anchor resistance in cassava plants against African cassava mosaic virus (ACMV) (Vanderschuren et al. 2009). Another means of providing resistance against viruses is targeting the coat protein (CP) gene through RNAi. This strategy was shown by Powell-Abel et al. (2006) in transgenic tobacco expressing the CP gene of tobacco mosaic virus (TMV) thus giving resistance to TMV. This method was further utilized to generate resistance against many different viruses such as potato resistant to potato virus Y (PVY) (Missiou et al. 2004), tobacco resistant to beet necrotic yellow vein virus (BNYVV) (Andika et al. 2005), *Cucumis melo* resistant to papaya ring spot virus type W (PRSV-W) (Krubphachaya et al. 2007), *N. benthamiana* resistant to cucumber green mottle mosaic virus (CGMMV) (Kamachi et al. 2007), and *N. benthamiana* and *Prunus domestica* resistant to plum pox virus (PPV) (Hily et al. 2007). RNA silencing approach is not restricted to RNA viruses alone but also seen in DNA viruses. For example, following infection with geminivirus *Vigna mungo* yellow mosaic virus (VMYMV), blackgram plant recovers back when inoculated with hpRNA construct containing the promoter sequence of VMYMV under the control of the 35S promoter (Pooggin et al. 2003). On the advent of infection by turnip mosaic virus (TuMV) in *Brassica rapa*, two miRNAs, bra-miR158 and bra-miR1885, were greatly upregulated (He et al. 2008), the condition only seen in this particular interaction.

8.10.2 Bacterial Resistance

Bacteria spread at a speedy rate and therefore it is tough to control diseases caused by them. Supression of two genes of *Agrobacterium tumefaciens* carried out by RNAi involved in crown gall tumor formation (iaaM and ipt) also helps in reducing the production of tumors in *Arabidopsis* (Dunoyer et al. 2006). This approach could be further spread out to other plants. Resistance to plants from bacterial disease is negatively regulated by fatty acids and their derivatives (Jiang et al. 2009). Multiple pathogens can be resisted in *Arabidopsis* and soybean plants by RNAi-mediated suppression of *SACPD* gene that encodes for fatty acid desaturase (Jiang et al. 2009). In *Arabidopsis*, miR393 is said to repress auxin signaling by negatively regulating the F-box auxin receptors like *TIR1*, hence restricting the infection by bacteria *Pseudomonas syringae* (Navarro et al. 2006). Thus, transgenic *Arabidopsis* plants where miR393 is overexpressed have enhanced bacterial resistance with some developmental alterations (Navarro et al. 2006). But two different miRNAs, miR398 (Jagadeeswaran et al. 2009) and miR825 (Fahlgren et al. 2007), are said to be downregulated by bacterial infections. miR398 expression targets coding for two Cu/Zn superoxide dismutases that are CSD1 and CSD2 were analyzed, and it was observed

that CSD1 was upregulated on the outburst of bacterial infection in accordance with the downregulation of miR398 under biotic stress (Jagadeeswaran et al. 2009).

MiR482/2118 family of miRNAs were shown to target a number of NBS-LRR mRNAs encoding disease resistance proteins in tomato (*Solanum lycopersicum*) and other members of Solanaceae (Shivaprasad et al. 2012). MiR482-mediated silencing of *R* genes gets affected by viral and bacterial invasion. These miRNAs are either upregulated or downregulated and affect gene expression by either suppressing negative regulators or inducing positive regulators of immune responses.

8.10.3 Fungal Resistance

Fungal resistance is regulated by posttranscriptional gene silencing (PTGS). In *Arabidopsis* RNA silencing mutants *sgs2*, *sgs3*, *ago7*, *dcl4*, *nrpd1a*, and *rdr2* displayed exhibited heightened susceptibility to *Verticillium* strains (Ellendorff et al. 2009). In another example, RNAi-mediated suppression of a rice gene *OsSSI2* embellished resistance to blast fungus *Magnaporthe grisea* and leaf blight bacterium *Xanthomonas oryzae* (Jiang et al. 2009) by suppressing two genes, namely, *OsFAD7* and *OsFAD8* (omega-3 fatty acid desaturases) (Yara et al. 2007). Similarly, RNAi-mediated targeting of genes for lignin production led to enhanced resistance in soybean against phytopathogen *Sclerotinia sclerotiorum* due to reduced lignin content (Peltier et al. 2009). However, in case of wheat, 24 miRNAs are known to get affected by the fungus *Blumeria graminis f.* sp. *tritici* (Bgt) which is causing the deadly disease of wheat powdery mildew (Xin et al. 2010). On the other hand, rice miRNA osa-miR7695 negatively regulates a natural resistance-associated macrophage protein 6 (OsNramp6) against the blast fungus *Magnaporthe oryzae*. To overcome this disease, overexpression of Osa-miR7696 was carried out (Campo et al. 2013).

8.11 Biotechnological Use of Mobile sRNAS in Plants

Plant defenses against pathogens and pests get accelerated by the discovery of sRNAs as mobile gene regulators thereby providing alluring and new strategies for crop improvement (Koch and Kogel 2014). HIGS, too, has played a great role in efficiently providing resistance against distinct plant herbivores, nematodes, and filamentous pathogens, when targeting important virulence genes. HIGS is a well-known tool under controlled lab conditions when applied to specific host and definite pathogen, but in field conditions, their suitability is compromised due to fluctuating environmental stresses and humungous variation in genes of pathogen and pest populations. Thus, more advanced studies and experimentation are needed to carry forward. Transportation of sRNA in different interactions such as plant-pathogen, plant-parasite, or plant-symbiont has made it feasible to construct the beneficial fungi or disarmed pathogens (with essential virulence genes deleted) and alter plant physiology via trans-kingdom gene silencing. Moreover, when the target

pathogen mRNAs are emphasized, a broad range of pathogens and pests can be controlled in a transgene-free plant framework via RNAi signals. RNA silencing-based technique can be further strengthened when a decent knowledge on molecular mechanisms of RNA communications and transport between plants and interacting organisms is attained. While genetically engineered crops have always been under domain of public eye, an understanding of cross-kingdom RNAi may help relieve public concerns. Some more applications of mobile sRNAs in plants are in metabolic engineering and systemic-induced resistance (Saurabh et al. 2014). Even food RNAi might become an important part of plant food-based technologies in the future (Hirschi 2012). Feeding studies stated that oral uptake of sRNA-containing nutrients led to accumulation of food-borne sRNAs in body fluids and organs, indicating their partial survival inside the intestinal tract (Liang et al. 2014). Research is ongoing to see if food-borne sRNAs have any negative or positive impacts on the physiology of the individual who consumes foods with plentiful sRNAs (Dickinson et al. 2013).

8.12 Conclusion

Research in sncRNAs is ultimately one of the most effective and encouraging fields in plant defense biology, and many more advances are waiting to be explored in this area of research. A large number of studies discussed here emphasize on the significance of sncRNAs in gene regulation in response of plants to pathogens (viruses, bacteria, and fungi). The induction and repression of sncRNAs in plants toward pathogens depend upon the incompatible and compatible interactions indicating that these RNAs can both act as positive and negative regulators of plant immunity. Biotechnological tools and strategies need to be implemented to speed up the resistance studies in plants against various pathogens. During symbiotic interactions, relevance of repression of R genes provides a bridge between pathogenic and beneficial interactions. When effectors interact with the plant silencing machinery, pathogens can surpass the plant immunity mechanisms. Since the complete annotation of sequence of miRNAs involved in biotic stresses still needs to be carried out in crop plants like rice, maize, soybean, mustard, *Jatropha*, barrelclover, etc., genes of small RNAs (miRNAs) can be used for analysis of stress tolerance in biotic conditions. Computational methods and high-throughput techniques like miRNA microarray, real-time PCR, or northern blot are utilized to identify expressed miRNAs and their target(s) which provide plant defense against various biotic stresses. Studying the complexity of regulation these proteins had to undergo in order to provide crops resistance against pathogens is required. Comparing the antiviral and the antibacterial roles of the small RNA biogenesis factors may shed light on the complex modes of regulation these proteins have to undergo to confer plants' disease resistance. The study of VSRs and BSRs along with their targets may help to solve redundancy in the activity of several RNA silencing components during plant-microbe interactions. An insight into plant defense mechanisms will help to improvise crops of economic importance which should be pathogen-free too.

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9 Interaction of Rhizobacteria with Soil Microorganisms: An Agro-Beneficiary Aspect

Anita Surendra Patil, Surendra Rajaram Patil, and R. Z. Sayyed

Abstract

The plant growth-promoting rhizobacteria (PGPR) have been extensively used for the plant growth enhancement in agriculture and horticulture in several ways. Several PGPR formulations with modified technology are nowadays available for increasing agriculture production. Microbial communities are associated with plants that are highly diverse and specifically associated with types of plant species, which controls plant health via several mechanisms. Recent evidence supports the fact that plants under attack recruit beneficial microbes into their rhizosphere, which supports plant growth via various direct and indirect mechanisms. It is essential to develop proper understanding of interactions between host plants and associated microbial community to elucidate their role in crop improvement. The research is being focused on establishing the facts about their mutualistic interactions and diversity, so they can be exploited for biocontrol and growth promoters. Efforts are still needed to know more about plant microbiomes as a system that can further help in analyzing the complex interactions.

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9.1 Introduction

Due to the imbalance in nitrogen N_2 cycle, poor nutritional status, biophysical properties of soil, and extreme climatic factors, the incidence of pests and various diseases as biotic stress and abiotic stress are the interlinked factors for the reduction in agriculture production (Gopalakrishnan et al. 2015). Soil health is a critical factor in agriculture sustainability, foods security, and energy renewability. As per the IFRI, 2012, up to 2030, the population needs to increase food by 50%, energy by 45%, and water by 30%. Although the serious efforts with perticular requirements have been done, but it is impossible to retain the soil fertility and enhancment in production in case of degraded land.

About 78% of atmospheric nitrogen is required for the synthesis of nucleic acid, enzyme, proteins, and chlorophyll. It is a vital element for the plant growth, but in gaseous form, it is unavailable for direct assimilation by plants. Biological nitrogen fixation (BNF) is a process of converting atmospheric N into plant assailable N such as ammonia through a cascade of reactions between prokaryotes and plant with the use of a complex enzyme system, as up to 65% of N currently used in agriculture is by BNF. Also, a variety of industrial N fertilizers is used to enhance biological productivity. A reversal decline or restoration in soil health is only possible with the use of manures, vermin-composts, biopesticides, biofertilizers, etc., also with the management of agricultural practices such as natural fellow, intercropping, relay cropping, cover cropping, crop rotation, and dual-purpose legumes. Rhizobia can be used as predominant inoculants for enhancing nitrogen fixation for 5–20 years and are effective colonizers persisting in soil (Sanginga et al. 1994).

9.2 The Rhizosphere Microbiome

The rhizosphere, which is the narrow zone of soil that is influenced by root secretions, can contain up to 1011 microbial cells per gram root (Egamberdieva et al. 2008) and more than 30,000 prokaryotic species (Mendes et al. 2011) and fungal and various other beneficial microorganisms (Glick 2012). The soil bacteria and plant interaction in the rhizosphere are the determinants of plant health and soil fertility. Also soil bacteria are key determinants in biogeochemical cycles for micro−/macroelemental circulations, which have been used for enhancing crop productions for decades.

The accumulation of simple and complex natural matter results in enrichment of soil (10–100-fold). Microbial flora includes bacteria, fungus, and algae along with protozoa, among which rhizospheric bacteria significantly influenced the plant

growth. Rhizospheric bacteria can be further categorized according to their proximity and association with roots: (1) bacteria, which live near root surfaces (rhizosphere); (2) group of bacteria colonizing the root surfaces (rhizoplane); (3) group of bacteria entering inside and residing in root tissues, inhabiting spaces between cortical cells known as root nodules (endophytes); and (4) group of bacteria living inside cells in specialized root structures.

The type of microbiome associated with plants is affected by soil conditions including temperature, moisture, salinity, and chemicals and also types of plants found in those soils (Glick et al. 1999). As concentration of bacteria found around the roots of plants (i.e., in the rhizosphere) is typically much greater than in the rest of the soil, they are attracted for nutrients including sugars, amino acids, organic acids, and other small molecules from plant root exudates that may account for up to a third of the carbon that is fixed by a plant (Whipps 1990; Glick 2012). The associated population of bacteria may affect plants in one of three ways. The interaction between soil bacteria and plants may be beneficial, harmful, or neutral for the plant (Lynch 1990).

The group of pathogenic bacteria can have a severe impact on plant health due to adverse interactions between plants and pathogens, ignoring the importance of additional microbial flora that can significantly affect the infection process (Berendsen et al. 2012). Usually, plants are in close association with the microbes that inhabit the soil in which they grow. Due to the huge diversity of soil microbial communities, they represent the greatest reservoir of biological diversity known in the world (Gams 2007; Buee et al. 2009).

Soilborne pathogens usually grow saprophytically in the rhizosphere to adhere to the host or to increase in sufficient numbers so that they can easily infect host tissue and effectively escape the rhizosphere battle zone. It has been confirmed that the success of a pathogen in terms of infection is influenced by the type of microbial community present in rhizoplane. Natural soil has the ability to combat a pathogen to a certain extent. This can be estimated from the disease severity following pathogen inoculation in sterile soil in comparison to non-sterile soils in experimental design. This phenomenon is known as the "general disease suppression" and is totally confined to total microbial bioactivity. Organic additive from natural resources can enhance microbial populations in soil, which resulted in enhanced general disease suppressiveness (Hoitink and Boehm 1999).

Free-living soil bacteria beneficial to plant growth, commonly known as the plant growth-promoting rhizobacteria (PGPR), promote plant growth by colonizing them. Bacteria belonging to PGPR are further classified as extracellular plant growth-promoting rhizobacteria (ePGPR) and category 4 as intracellular PGPR (iPGPR). The ePGPR include the genera *Bacillus*, *Pseudomonas*, *Enterobacter*, *Klebsiella*, *Azospirillum*, *Erwinia*, *Caulobacter*, *Serratia,* *Arthrobacter*, *Micrococcus*, *Flavobacterium*, *Chromobacterium*, *Agrobacterium*, and *Hyphomicrobium*, whereas iPGPR include the genera *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Azorhizobium*, *Mesorhizobium*, and *Allorhizobium* (Gopalakrishnan et al. 2015; Patil et al. 2017).

9.3 Plant Growth Promotion by Rhizobacteria

It has been seen that plant root offers a niche for the proliferation of soil bacteria that thrive on root exudates and lysates. The rhizosphere usually passes 100-fold higher densities of bacteria than in bulk soil and up to 15% of root surface covered by microcolonies of various strains of bacteria. During existence, they utilize the nutrients released from host plant for their growth, and they, in turn, produced agro-beneficial secreted metabolites into the rhizosphere. These compounds can act as signaling molecules for neighboring cells or root cells of the host plant (Kiely et al. 2006; Van Loon 2007).

Rhizobium-legume symbiosis has enough signal exchange in which the host plant releases flavonoid metabolites that act as a signal for the bacterium to secrete Nod factors. Nod factors are perceived by root hairs and function as a hormone to induce root nodule formation in which *Rhizobium* can fix atmospheric N_2. The bacterium grows at the expense of carbohydrates from the host but provides fixed N_2 for amino acids biosynthesis in return (Gray and Smith 2005). This example illustrates the concept of plant growth-promoting *Rhizobium* (PGPR) in the nitrogen-poor environment; they promote legume plant growth by providing a limiting nutrient.

Growth promotion by soil microorganisms can be considered as continuing interaction between plant and microorganism that ranges from deleterious (pathogen) to beneficial (PGPR) (Glick et al. 1999; Ryu et al. 2003). About 85 years ago in the Netherlands, Prof. Johanna Westerdijk of the phytopathological laboratory "Willie Commelin Scholten" in Baarn reported observations about the recovery from damping off of turfgrass. Several pathogenic *Pythium* spp. were responsible for the disease; also it is noted that grass seeds germinated to a higher percentage in non-sterile than in sterilized soil (Van Luijk 1938). This is the first example that soil microrganisms can promote plant growth. As this is due to stimulatory effects of metabolites present in raw soil, it can turn out that nonpathogenic *Pythium* spp. that were also present were taken over by the antagonistic mechanism and counteracted the actions of the pathogenic *Pythium* spp. and other pathogenic soil microorganisms.

The enhancement of seed germination and recovery from damping off of the turfgrass that was caused by the nonpathogenic *Pythium* sp. were shown to be as the promotion of plant growth. However, this property is due to mechanisms adapted for the disease suppression, which is possessed by many of the bacteria present in soil (Compant et al. 2005; Haas and Defago 2005) along with rhizobacteria which can enhance plant growth in the absence of potential pathogenic microorganism in gnotobiotic system (Van Loon and Bakker 2003).

The various microbial entities colonize the rhizosphere, viz., bacteria, fungi, actinomycetes, protozoa, and algae, of which bacteria are the most abundant (Kaymak 2010). The term "plant growth-promoting rhizobacteria (PGPR)" for such associated beneficial microbes was introduced by Kloepper and Schroth (1978). Although PGPR are associated with the roots to exert beneficial effects on plant growth and development, they can also exert biocontrol effects like phytopathogenic microorganisms (Son et al. 2014). Based on the various interactions with host

plants, PGPR can also be classified into symbiotic bacteria, as they live inside plants and exchange bio-metabolites directly, and the free-living rhizobacteria, which live in the rhizosphere (Gray and Smith 2005).

Several rhizobacterial growth promotion mechanisms have been enlisted as direct and indirect ways. The direct mechanisms are the use of PGPR as biofertilizers, stimulation of root growth, rhizoremediation, and plant stress elimination. The plant growth promotion indirectly depends on the use of rhizobacteria as biological control agents via reduction of the impact of diseases by secretion of antibiosis, induction of systemic resistance, and competition for nutrients and biological niches (Egamberdieva and Lugtenberg 2014). Symbiotic rhizobacteria usually reside in the intercellular spaces of the host plant, but few of them are able to form mutualistic interactions with their hosts and penetrate inside plant cells. Along with this, a few associated ones are able to integrate their physiology with the host plant, leading to the formation of specialized structures, i.e., specific root structures known as nodules by *Rhizobia* (Fig. 9.1).

PGPR colonize the root system which results in a positive impact on the plant's physiological process (Wu et al. 2005) and are involved in plant resistance due to biotic or abiotic stress (Abeles et al. 1992). Recently PGPR are classified into four groups based on their mechanisms:

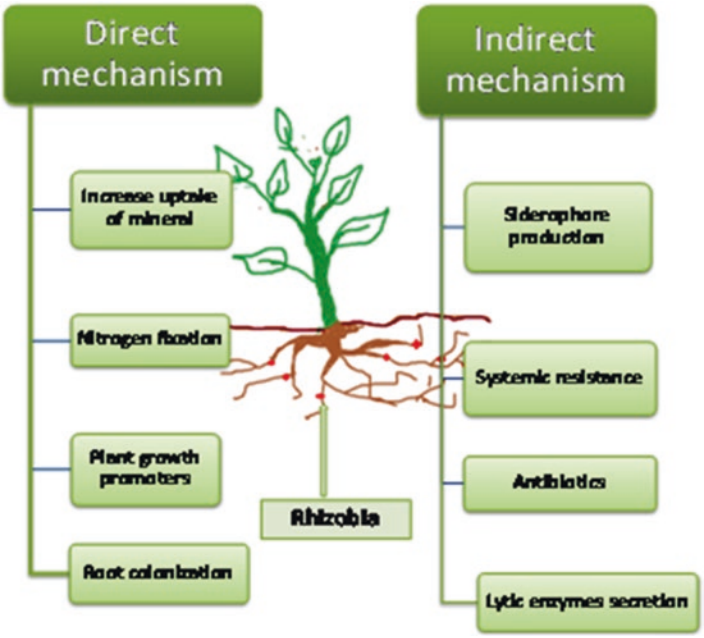

Fig. 9.1 Schematic representation showing direct and indirect mechanisms adapted by PGPR for plant growth promotion

1. Biofertilizers – due to their ability to fix atmospheric nitrogen and solubilize mineral phosphate (Salantur et al. 2006).
2. Phytostimulation – due to their ability to produce plant hormones (Egamberdiyeva 2007), IAA, cytokinins, and gibberellins (Glick 1995; Marques et al. 2010).
3. Rhizoremediation – due to their ability to degrade and recycle organic pollutants (Somers et al. 2004).
4. Biopesticides – due to their ability to produce siderophores (iron chelating) because of synthesis of antibiotics, enzymes, and various fungicidal compounds (Dey et al. 2004; Ahmed et al. 2008).

9.4 Role of Rhizobacteria for Plant Growth Enhancement

PGPR are reported to have a positive role in enhancing plant growth promotion through various mechanisms. The mechanisms of PGPR that promote plant growth include: (i) abiotic stress tolerance, (ii) nutrient fixation for easy uptake and utilization by plant, (iii) production of plant growth regulators/promoters, (iv) production of siderophores, (v) production of antibiotics, and (vi) production of lytic enzyme such as chitinase, glucanase, and ACC deaminase for the prevention of plant diseases (Garcia-Fraile et al. 2015; Vejan et al. 2016). However, the mode of action of different PGPR varies depending on the type of host plant they are associated with and environmental stresses as biotic and abiotic types. Biotic refers to the stresses due to plant pathogens and pests such as viruses, fungi, bacteria, nematodes, insects, etc., while abiotic refers to stresses due to the high concentration of heavy metal, nutrient deficiency, salinity, temperature of the soil, and drought conditions. Abiotic stresses are considered to have a negative impact on agricultural yield and primarily depends on the type of soils and plant factors (Nadeem et al. 2010).

Nutrient Availability PGPR has the ability to enhance the availability of a nutrient in the rhizosphere by fixing nutrients in bioforms, thus preventing them from leaching out (Choudhary et al. 2011). Usually, free-living nitrogen-fixing bacteria, such as *Azospirillum*, are often associated with cereals in temperate zones and are also reported to be able to improve rice crop yields (Tejera et al. 2005). Few PGPR have the ability to solubilize phosphate (Wani et al. 2007), resulting in increased availability of phosphate ions in the soil, which can be easily taken up by the plants. The effect of PGPR on nutrient uptake by rice was reported in which PGPR strains such as *Pseudomonas fluorescens* and *Pseudomonas putida* are used (Lavakush et al. 2014).

Production of phytohormone influences physiological processes at low concentrations. The influenced processes include growth, differentiation, and development and other processes, such as the effect on stomatal movement (Davies 2013). Rhizospheric microorganisms may also produce or modulate phytohormones under in vitro conditions so that many PGPB can alter phytohormone levels and thereby affect the plant's hormonal balance and its response to stress (Glick et al. 2007). IAA released by rhizobacteria is shown to interfere with plant developmental

processes because the endogenous pool of plant IAA may be altered by the acquisition of IAA that has been secreted by soil bacteria (Glick 2012). Variation of IAA production among the ten PGPR strains is shown by Prakash and Karthikeyan (2013). The IAA production was shown to be highest in *Pseudomonas* sp. (94%), *Azospirillum* sp. (80%), *Azotobacter* sp. (65%), and *Bacillus* sp. (40%). Similarly, production of IAA by *Bacillus* is a general characteristic of rhizobacterial isolates (Agrawal and Agrawal 2013).

Ethylene influences many aspects of plant growth and development and is reported to be produced in severe abiotic stress conditions which adversely affect root growth (Kundan et al. 2015). 1-Aminocyclopropane-1-carboxylate (ACC) deaminase is a vital enzyme present in plant growth-promoting rhizobacteria (PGPR), which regulates ethylene production by metabolizing ACC (an immediate precursor of ethylene biosynthesis in higher plants) into alpha-ketobutyrate and ammonia. Inoculation with PGPR combined with ACC deaminase activity could be quite helpful in promoting plant growth and development under stress conditions by reducing stress-induced ethylene production. By lowering the abundance of the ethylene precursor ACC, the PGPR ACC activity is thought to decrease root ethylene production, which in turn can alleviate the repressing effect of ethylene on root growth (Glick 2005).

PGPR which possesses the ability to degrade ACC in the rhizosphere could shorten the deteriorating cycle and reconstruct a healthy root system in plants that would certainly help them to withstand environmental stress. Furthermore, PGPR that produces ACC deaminase and synthesizes IAA may facilitate plant growth. Enzyme ACC deaminase involved in the primary mechanism rhizobacteria is utilized to degrade ethylene (Glick 2014). Ahmad et al. (2013) proved that *Rhizobium* and *Pseudomonas* ACC deaminase-producing strains can improve the growth and physiology of the plant.

Gibberellin (GA) involves seed germination and emergence, floral induction, flower and fruit development, and stem and predominately shoot elongation (Spaepen and Vanderleyden 2011; Vejan et al. 2016). Cytokinins stimulate a plant's cell division, vascular cambium sensitivity, and vascular differentiation and induce the proliferation of root hairs but inhibit lateral root formation and primary root elongation (Riefler et al. 2006). Several studies revealed that many soil bacteria, in general, and PGPB, in particular, can produce either cytokinins or gibberellins or both (Nieto and Frankenberger Jr. 1989).

9.5 Production of Siderophores

Several reports have shown that PGPR secretes siderophores. Siderophores are low molecular weight iron-binding protein compounds involved in the process of chelating ferric iron (Fe (iii)) from the environment. When Fe is limited, microbial siderophores provide plants with Fe, enhancing their growth. Flores-Felix et al. (2015) showed that *Phyllobacterium* strain which secretes siderophore promotes the growth and quality of strawberries.

9.6 Production of Antibiotics

Antibiotics of volatile organic compounds (VOCs) produced by plant growth-promoting rhizobacteria (PGPR) are heavily involved in improving plant growth and induce systemic resistance (ISR) toward pathogens. Several bacterial species, from diverse genera including *Bacillus*, *Pseudomonas*, *Serratia*, *Arthrobacter*, *and Stenotrophomonas*, produce VOCs that influence plant growth. Acetoin and 2,3-butanediol synthesized by *Bacillus* are the best known of these compounds and are responsible for significant improvements in plant growth (Ryu et al. 2003). VOCs are reported to directly and/or indirectly mediate plant biomass enhancement, disease resistance, and the ability for abiotic stress tolerance. The production of one or more antibiotics is the mechanism most commonly associated with the ability of plant growth-promoting bacteria to act as antagonistic agents against phytopathogens (Glick et al. 2007). The mechanism of antibiosis is to produce low molecular weight compounds which disturb the major enzymes and metabolism of other microorganisms and thus retard growth (Kundan et al. 2015).

9.7 Production of Lytic Enzymes

PGPR produces metabolites contributing to the antibiosis and antifungal properties used as defense systems against phytopathogenic entities. The mechanism would involve the production of hydrolytic enzymes, viz., chitinase and glucanase. Major fungal cell wall components are made up of chitin and beta-glucan, thus chitinase- and beta-glucanase-producing bacteria would inhibit fungal growth. It has been reported that *Sinorhizobium fredii* KCC5 and *Pseudomonas fluorescens* LPK2 produce chitinase and beta-glucanases and control *Fusarium* wilt produced by *Fusarium udum* (Kumar et al. 2010). PGPB that synthesize one or more of these enzymes have been found to have biocontrol activity against a range of pathogenic fungi including *Botrytis cinerea*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Phytophthora* sp., *Rhizoctonia solan*, and *Pythium ultimum* (Glick 2012).

9.8 Microbial Mutual Interactions in Agroecosystem (AES)

The physicochemical and structural properties of soil including their development have been greatly influenced by rhizospheric metabolic activities (Choudhary et al. 2011). Usually, agricultural sustainability requires optimal use and management of soil fertility and soil physical properties and supposed to be based on soil microbial diversity as an indicator of soil quantity (Tilak et al. 2005).

It has been reported that soil microbial diversity and soil function are related to soil type and type of soil management (Reeve et al. 2010). The rhizosphere environment is influenced by roots and nutrient availability which provide niches for microbial community. Among these, various *Bacillus* and fluorescent *Pseudomonas* spp. protect plants from infectious agents by approaches such as nutrient competition,

production of antibiotics and lytic enzymes, and induction of plant defense mechanisms (Bakker et al. 2007; Weller 2007).

The rhizosphere due to microbial populations forms stable soil aggregates of 2–20u m diameter, which are held together by various bacterial production and hyphae of saprophytic and arbuscular mycorrhiza (AM). Thus, due to the increase in N_2, content increases nodulation and N_2-fixing ability due to the symbiosis between *Rhizobium* and AM fungi (Barea et al. 2005). Recently, PGPR as an inducer of systemic resistance in crop against pathogen has been demonstrated in field conditions (Wei et al. 1996). PGPR increase plant resistance to fungal, bacterial, and viral diseases (Maurhofer et al. 1998), insects (Zehnder et al. 1997), and nematodes (Sikora 1992), by producing bacterial metabolites to reduce the population or activities of pathogens or deleterious microbes present in the rhizosphere (Glick 1995; Kloepper 1996).

Several reports have shown the production of volatile metabolites, i.e., antibiotics (e.g., pyrrolnitrin, phycocyanin, 2–4-diacetyl-phloroglucinol) which suppress the pathogens (Subba Rao 1993; Glick 1995). Siderophores play an important role in the biocontrol of soilborne plant diseases and in plant iron nutrition (Loper and Buyer 1991). Under aerobic environment, iron exists as insoluble hydroxides and oxyhydroxides, which are not accessible to both plants and microbes (Rajkumar et al. 2010). Generally, bacteria synthesizing low molecular weight compounds termed as siderophores are capable of sequestering Fe^{3+}, and due to high affinity for Fe^{3+}, making the iron available for plants. The siderophores are low molecular weight compounds of two types, viz., extracellular and intracellular, or which can form a stable complex with heavy metals such as Al, Cd, Cu, and U and also with NP (radionucleotides (Neubauer et al. 2000). The siderophores produced by various rhizobial species such as *R. meliloti*, *R. tropici*, *R. leguminosarum*, *S. meliloti*, and *Bradyrhizobium* sp. (Carson et al. 2000; Arora et al. 2001) are supposed to release plant stress against heavy metal stress.

Subsequently, siderophores have been shown to suppress *Fusarium oxysporum* (Baker et al. 1986). Because siderophores sequester the limited supply of iron (III) in the rhizosphere, they limit its availability to pathogens and suppress their growth (Kloepper et al. 1980). *R. meliloti* was reported producing siderophores in iron stress condition which exclude the pathogen *Macrophomina phaseolina* causing charcoal rot of groundnut (Arora et al. 2001). In acid soils, rhizobia producing siderophores in the rhizosphere can inhibit the growth of fungal pathogen (Schippers et al. 1987).

Numerous plants are capable of using bacterial Fe-siderophore complexes as a means of obtaining Fe from soil (Wang et al. 1993). This is supported by findings of Hughes et al. (1992), in which enhanced Fe uptake in oat is due to siderophore production. The role of siderophore in enhancing Fe uptake has been reported by Loper and Schroth (1986) and Biswas et al. (2000).

Nonpathogenic rhizosphere microorganisms can be shown to be detrimental to plant growth. The reports which highlighted the importance of bacteria responsible for the inhibition of root growth, known as a deleterious microorganism or DRMO, which also included nonpathogenic fungi detrimental to the growth of root

(Cherrington and Elliott 1987). In various ways, DRMOs that adversely affect plant include a high level of IAA, siderophore production, ethylene, HCN, and various unidentified phytotoxin formation. Phytotoxin promotes the effect of bacterial secretion on root growth of young lettuce (*Lactuca sativa*) under axenic condition (Barazani and Friedman 1999). The high concentration of IAA released by DRB accounted for the suspension of root growth. Schippers (1988) reported that model in which DRMO's use glycine and proline in potato root exudates to synthesis HCN, which was taken by roots and those also require Fe^{2+}. At this stage PGPR can introduce in rhizosphere, werethey deprive the DRMO's of Fe^{2+} by producing siderophores with stronger iron chelating power than that produced by DRMO's.

9.9 Effect of Diazotrophs in Rhizosphere Environment

The rhizosphere is the narrow zone of the soil that surrounds the root which is rich in nutrient due to the accumulation of a variety of organic compound released from roots (Curl and Truelove 1986). This accumulated nutrient is used as carbon and energy source by microorganisms; thus, their activity is intense at this region, i.e., 10–100 times higher than in bulk soil (Weller and Thomashow 1994).

Plants accumulate bacteria called rhizobacteria; they colonize the roots and are claimed as a beneficial, deleterious, and neutral group based on their effect on plant growth which led them to being referred to as PGPR (Kloepper et al. 1989), a group that includes different bacterial species, viz., *Bacillus*, *Azotobacter*, *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Burkholderia*, and *Herbaspirillum* (Weller and Thomashow 1994; Probanza et al. 1996).

A group of bacteria known as diazotrophs which do have the ability to convert N_2 into ammonia, which can be used by the plant, also belong to the PGPR group. These bacteria, due to their properties of competitive survival in carbon-rich and nitrogen-poor habitat, are highly enriched in the rhizosphere (Dobereiner and Pedrosa 1987).

This group of bacteria belongs to the genus *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, and *Azorhizobium* fixed with nitrogen in symbiotic with legume roots and thus are not considered as PGPR in a highly specific symbiotic interaction. However, the members of *Rhizobiaceae* are able to form nonspecific interaction with roots of other plants without forming a nodule which stimulates growth and production (Biswas et al. 2000; Yanni et al. 2001), and thus they are considered as PGPR (Dobbelaere et al. 2003). The diazotrophs which are loosely or more intimately (endophytes) associated with plants, viz., *Azotobacter*, *Azoarcus*, *Azospirillum*, *Azotobacter*, *Herbaspirillum*, *Klebsiella*, *Paenibacillus*, and *Pseudomonas*, are assumed to be responsible for nitrogen contribution to their host plant.

The experiments have been carried out to see N_2 fixed by assumed diazotrophs, the plant experiments inoculated with non-nitrogen fixing (nif) mutants, coupled with careful ^{15}N-based balance studies. The study showed that in most of the cases, BNF is not involved in plant proliferation. Nif mutants *Azospirillum*, *Azoarcus* sp.

strain BH72, and *P. putida* GR 12-2 are still capable of stimulating plant growth (Bashan and Levanony 1989; Hurek et al. 1994). The experiment for interpretation of direct N_2 transfer by *Acetobacter diazotrophicus* is associated with sugar cane (Sevilla et al. 2001). The wild-type strain and nif D mutant of *A. diazotrophicus* unable to fix N (Nif^-) were used to inoculate sterile sugar plantlets prepared from meristem tissue culture, while sugarcane plants inoculated with wild-type strain grew better, and high N content (Sevilla et al. 2001) indicates that *A. diazotrophicus* transfers fixed N_2 to sugarcane as plant growth promotion trail along with IAA and gibberellins (Fuentes-Ramirez et al. 2001; Bastián et al. 1998). It has been confirmed that the amount of fixed N_2 supplied to host plant is low (Rao et al. 1998). A free-living diazotroph does not excrete N from their cells, but in case of associative diazotrophs, the fixed N_2 remains mainly in bacterial cells and released to host at a later stage of plant growth after death and decay of bacterial biomass (Rao et al. 1998).

9.10 Interaction of Soil Microorganisms

The enhanced additive and/or synergistic effect was demonstrated by dual inoculation. It has been reported that combined inoculation of *Rhizobium* with *Azospirillum* or with *Azotobacter* shows increased matter production, grain yield, and N_2 content of several legumes as compared to *Rhizobium* alone (Burdman et al. 2000; Burns et al. 1981). This can be analyzed by early nodulation, increase in number of nodules, high N_2 fixation, and improvement in the root (Volpin and Kapulink 1994; Okon and Itzigsohn 1995). Mixed inoculation of *Vicia faba* L. with four different *Rhizobium/Azospirillum* and *Rhizobium/Azotobacter* combinations led to changes in total content, concentration, and distribution of macro−/micronutrients K, P, Ca, Mg, Fe, B, Mn, Zn, and Cu as compared to *Rhizobium* only (Rodelas et al. 1999).

Rhizobium act synergistically with *Arbuscular mycorrhizal* fungi (AMF) to increase lettuce biomass production (Galleguillos et al. 2000). AM improve plant growth through increased uptake of P and other mineral nutrients in the soil of low fertility (Smith et al. 2001). The increase in early and final rhizobial root population reported to occur when co-inoculated with *P. polymyxa* and *R. elite* on *Phaseolus vulgaris* (Petersen et al. 1996). Pandey et al. (1998) reported on the strain of *Azotobacter chroococcum* and *Azospirillum brasilense* on maize; they stimulate population of actinomycetes which can produce antibiotic and group of bacteria which are able to grow on N_2-free medium. After co-inoculation with diazotrophic bacteria, multiple interactions with endogenous populations may take place, i.e., synergistic, competitive, or antagonistic, which exist in order to enable the bacteria to survive and compete in complex microbial communities.

Interactions within the microbial population and their host are dependent on the appropriate expression of specific genes involved in their interactions. Recently the role of N-acyl-L-homoserine lactone (AHL)-mediated gene expression received attention. AHL-mediated gene regulation is a bacterial signaling system that controls gene expression in a population density-dependent manner (Fuqua et al. 1994; Eberl 1999). A large number of plant-associated bacteria produce AHL signal

molecules (Steidle et al. 2001) as population density sensors in one sp. and for communication between cells of different species. Steidle et al. (2001) have shown that AHL serves as a universal language for cross communications between different bacterial populations and their hosts.

It has been known that rhizospheric bacteria play a crucial role in maintaining soil fertility and upgrading plant growth via various direct and indirect mechanisms (Patil et al. 2017). There are few reports indicated in which cyanide is produced as peculiar growth characteristic of certain *Pseudomonas* species, having growth promotion as well as a growth inhibition characteristic. It has been seen that cyanide can act as a biocontrol agent against certain plant pathogens (Martinez-Viveros et al. 2010) and does possess the deleterious effects on plant growth (Bakker and Schippers 1987). Auxin production by PGPR can also cause positive as well as negative effects on plant growth depending upon its concentration. At low concentrations, it enhances plant growth, whereas at a high level it inhibits plant root growth and development (Xie et al. 1996).

The production of rhizobitoxine by *Bradyrhizobium elkanii* does possess dual effect. It acts as an inhibitor of ethylene synthesis, and it also alleviates the negative effect of stress-induced ethylene production on nodulation (Vijayan et al. 2013). Rhizobitoxine is also considered as a toxin which induces foliar chlorosis in soybeans (Xiong and Fuhrmann 1996). Thus, the selection of PGPR is a very crucial and effective step to use them for obtaining maximum benefits in terms of improved plant growth and development.

9.11 Synergistic Effects of Rhizobial Co-inoculations

The synergistic effects of certain specific co-inoculations cause synergy by adding help to improve the performances of other bacteria. In such situation, PGP rhizobia and the host genotype have to be selected after careful examinations (Remans et al. 2008), which supports increase growth, yield, and cost-effectiveness. It has been reported that *Azospirillum* can increase infection site providing a space for *Rhizobium* to enhance nodule formation (Tchebotar et al. 1998); increase phytohormone, vitamin, and siderophore production (Cassan et al. 2009); and enhance fixed nitrogen quantity (Remans et al. 2008). *Azotobacter* when co-inoculated with *Rhizobium* has shown enhancement in phytohormones and vitamins and nodulation (Gopalakrishnan et al. 2015).

There is an enhancement in weight of root and seed yield of chickpea when co-inoculated with *Rhizobium* together with *B. subtilis* OSU-142 and *B. megaterium* M-3 (Elkoca et al. 2008). Increased nodulation was found when *A. lipoferum* and *R. leguminosarum* by *Trifolii* were co-inoculated in white clovers (Tchebotar et al. 1998). The antagonistic activities were shown to be against *F. oxysporum* and *R. solani* on chickpea by coculture inoculation of *Mesorhizobium*, *Azotobacter chroococcum*, *P. aeruginosa*, and *T. harzanium* (Verma et al. 2013).

The association of endophytic actinomycetes confers many advantages to host plants such as the production of indole-3-acetic acid (IAA) that helps in the growth of roots or the production of siderophore that binds iron (Fe^{3+}) from the environment and subsequently helps to improve nutrient uptake (Merckx et al. 1987; Leong 1996), as well as the protection against plant pathogens by producing antibiotics or extracellular enzymes (Clegg and Murray 2002; Bailey et al. 2006). It has also been reported that presowing of mungbean seeds with different inoculants (*Rhizobium*, PGPR, and PSB) alone or with various combinations shows significant enhancement in nodulation and yield. The combined inoculums of the above three in the experiment showed the highest number of nodules/plant (21.0), dry weight of nodules/plant (87.66 mg), and grain yield (12.94 q/ha) (Bansal 2009).

9.12 Application of Nano-biotechnology in Agriculture

Nanotechnology in agriculture has gained tremendous momentum in the last decade with an abundance of research in a particular field, but the pace of development is modest, even though many disciplines come under the umbrella of agriculture. Nano-agriculture, which currently focuses on target farming that involves the use of nano-sized particles such as nano-fertilizer, offers exclusive tools for improving the productivity of the crop plants through efficient nutrients uptake by the plants (Tarafdar et al. 2013). The unique properties of nano-sized particles with respect to their physical, chemical, and biological properties compared to those at a larger scale provide the potential to protect plants, detect plant diseases, monitor plant growth, enhance food quality, increase food production, and reduce waste. Nano-based devices and tools, like nano-capsules, nano-particles, and even viral capsids, are examples of uses for the detection and treatment of diseases, the enhancement of nutrients absorption by plants, and the delivery of active ingredients to specific sites. The vast efficiency of nano-fertilizers compared to ordinary fertilizers has been proven as they reduce nitrogen loss due to leeching, emissions, and long-term incorporation by soil microorganisms (Liu et al. 2006). Nano-encapsulation technique could be used as a versatile tool to protect PGPR, enhancing their service life and dispersion in fertilizer formulation and allowing the controlled release of the PGPR (Fig. 9.1).

The nano-sensors have also relevant implications for application in agriculture, in particular for soil analysis, easy biochemical sensing and control, pesticide, and nutrient delivery. Intervention in farming has a bright prospect for improving the efficiency of nutrient use through nano-formulations of fertilizers, breaking yield barriers through bio-nanotechnology, surveillance, and control of pests and diseases, understanding mechanisms of host-parasite interactions at the molecular level, and developing new-generation pesticides.

9.13 Conclusion

Pathogens that severely affect plant health are a chronic threat to food production and ecosystem stability worldwide. The increasing use of chemical inputs causes several negative effects such as pathogen resistance to the applied agents and their non-targeted environmental detrimental effects. Furthermore, the growing cost of pesticides along with world consumer demand for pesticide-free food has led to a search for substitutes for growth and yield production. Biological agents are considered as an alternative or a supplemental way of reducing the use of chemicals in agriculture and growth promotion. Similarly, modulation of the rhizosphere bacteria consortia and identifying different mechanisms of action facilitate the combination of strains to hit pathogens with a broader spectrum of microbial weapons.

Several studies have been clearly reported that various groups of bacteria other than rhizobia are able to colonize inside the legume nodules. Research has been focused on the fact that coexistence of rhizobia and non-rhizobia in the rhizosphere can increase the growth and production of legumes. Various endophytic root colonizers also play an important role in root odulation along with mutual coordination for the benefits of both parts. Although much studies have been focused on the cordial relationship of rhizospheric bacteria, still their interrelating mechanisms are to be explored. Thus, the recent techniques in biotechnology can be applied to further improve strains that have prized qualities (e.g., formulation ease, stability, or otherwise exceptionally suited to plant colonization) by creating transgenic strains that combine multiple mechanisms of action.

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10 Role of Indigenous Technology Knowledge in Biological Control of Crop Diseases Under Organic Agriculture in India: An Overview

Shamarao Jahagirdar, Gududatta Hegade, S. A. Astaputre, and D. N. Kambrekar

Abstract

Crop diseases take a heavy toll in agriculture. The estimated loss due to diseases alone ranges between 15% and 20% annually. The loss is more in perishable and storage losses. The contamination by several storage fungi has often led to aflatoxin production and food spoilage. This has led to increased concern over food safety and security in India. The recent thrust on organic agriculture will answer all these questions. Disease management strategies in organic agriculture aim at long-term sustainable management strategies in a holistic approach. Under organic agriculture, traditional methods form the basis of management of plant diseases in low input situations. The ancient Indian literature documents use of plant products, animal products, and wastes for curing diseases of human beings and plants. The research efforts made on managing the diseases of banana, black pepper, tobacco, and soybean are discussed in this book chapter.

Keywords

Organic agriculture · Plant disease management · Traditional knowledge · Seed treatment

10.1 Introduction

More than 200 million farmers and farm workers are the backbones of Indian agriculture. The establishment of an agrarian economy is the mainstay of reform process in Indian agriculture sector which provides food security to more than one billion people and also provides raw materials for industry and exports. Organic

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agriculture is gaining importance due to increased awareness about ill effects of pesticides on plants, animals, and human beings. The history and traditional knowledge of agriculture dates back to Vrikshayurveda wherein there was mention of ailment curing in plants by using locally available resources. This gives hint on the management of crop diseases by using indigenous technology knowledge. India has a very strong base in organic agriculture and management of crop diseases. The major thrust of organic agriculture is on pooling, distilling, evaluating traditional wisdom, and harnessing it for sustainable growth. In this chapter, all such traditional knowledge will be explained with a scientific background and research findings in some selected crop diseases.

10.2 Renewed Interest Toward Organic Agriculture in India

The scientific cultivation methods in organic agriculture started with the environment and health-conscious people of the developed world. However, organic agriculture is for securing a place on international markets, export promotion, economic self-reliance, finding alternatives to decreased access to agricultural inputs, natural resource conservation, food self-sufficiency, and rural and wider social development.

However, a large number of small-scale subsistence farmers in India produce simply for consumption, do not participate in the market, and are left behind due to globalization. The major challenge is to establish organic agricultural policies that combine income generation and improved domestic food production. The latter involves raising the productivity of poorly endowed areas by maximizing the use of local resources. Such policies would better respond to self-reliance, local food needs, and health of resource-poor farmers. In India, the interest in organic agriculture is growing because it requires less financial inputs and places more reliance on the available natural and human resources. Studies undertaken to date seem to indicate that organic agriculture offers a comparative advantage in areas with less rainfall and relatively low natural soil fertility levels. In fact, agricultural labor realizes a good return, which is very important where paid labor is almost nonexistent.

Organic agriculture does not need costly investments in irrigation, energy, and external inputs but rather substantial investments in capacity building through research and training. Pro-poor organic agricultural policies have the potential to improve local food security, especially in marginal areas.

10.3 Plant Disease Management in Organic Agriculture

Plant diseases are the major constraints of yield reduction in various crop plants. There are about 1000 diseases which affect economic crop plants causing significant damages. Majority of plant diseases are caused by fungi followed by viruses,

bacteria, nematodes, phytoplasmas, and a number of other plant pathogens. Imbalance of nutrients and extreme variation in environmental factors also lead to serious plant diseases. Integrated management of these diseases needs a synergy with the natural environment. Traditional agricultural practices were tailor-made to maintain this synergy and, thus, form the basis for plant disease management. Organic agriculture is usually associated with pre-industrial peasant agriculture. Indigenous knowledge is the largest single knowledge source not yet fully exploited.

10.4 Traditional Knowledge in the Management of Plant Diseases

India has a treasure of indigenous knowledge concerning plant health developed and documented several centuries ago. The three major ancient texts inter alia are:

1. Varahamihira, 505 AD, *Brihat Samhita*, Vrikshayurveda Part 1, Chapter 55 (edited by M Ramakrishna Bhat, Motilal Banarasidas, Bangalore, India, 1950).
2. Chavundaraya, 1025 A.D *Lokopakara*, Vrikshayurveda, Chapter 6 (edited by H. Shesha Iyenger, Government Central manuscripts Library, Madras, India, 1950).
3. Sarangahara, 1363 A.D. Vrikshayurveda (edited by S. K. Ramachnadra Rao, Kalpatharu Research Academy, PO Box, 1857, Bangalore, 1993, India).

The most detailed of all the Vrikshayurveda work seems to be that of Chavundaraya, 1025 A.D. It is, however, to be noted with caution that development of this body of knowledge needs to be viewed in pest disease scenario prevalent ten to fifteen centuries ago, during which many of the current pests/diseases may not have existed. However, the methods employed have characteristics such as (1) multi-pronged attack on the pest/disease, (2) improving plant health thereby increasing resistance capability, (3) enriching soil with buffering of useful microbial activity, and (4) broad-spectrum effects on pest/disease which are desirable.

(1) a few indigenous methods of plant protection as outlined in the ancient text, (2) the documentation of research endeavor in a few indigenous knowledge practices, (3) local practices adopted for plant disease management, (4) policy implications.

10.4.1 A Few Indigenous Methods of Plant Protection in Ancient Texts

Esteemed authors of ancient texts make a mention of their painstaking efforts while observing and researching on indigenous methods that have been documented. In this paper mainly methods of disease control are highlighted.

The Following Methods Are Drawn from Lokopakara, chapter VI-Vrikshayurveda

(a) *Disease avoidance* (as in 12th stanza):
The incense of *Embelia ribes* Burm (Vayuvilanga; fam: Primulaceae), *Commiphora*w*ightii* (Mahishakshi; fam: Burseraceae), fish meat, and flowers of *Terminalia tomentosa* (methi; fam: Combretaceae) will provide resistance to plant diseases. If growing tips of plants dry up or set broken or get whitened, such plants are inferred to have been affected by the disease. In the affected plant, warm ghee has to be smeared and black soil applied to combat the disease.

The Following Methods Are Drawn from *Brihat Samhita*, Part 55, Chap. VI, Vrikshayuverda

(b) *Seed treatment*
The seeds need to be soaked in cow's milk for 10 days. Later, they are rolled in cow dung mixed with the flesh of deer and hog. Thus treated soils are planted in soil treated with sesamum. The seeds sown are to be sprinkled with milk and water.
(c) *Disease treatment*
The ulcers of affected trees are removed with a knife. A paste made using *Embelia ribes* (Vayuvilanga; fam: Primulaceae), cow's ghee, and silt is applied to the affected parts and later be sprinkled with water and milk.

The Following Methods Are Drawn from Vrikshayurveda of Sarangahara
The pathological conditions of wind (vata), bile (pitta), and Phlera (kapha) which are responsible for diseases in human beings are causes of diseases in plants also. When trees are affected by pests, the affected parts are to be removed. The diseases due to vata (wind) are overcome by application of clarified butter and fresh juice, and that due to bile is overcome with substances that are cold mixed with water, and that due to kapha (phlera) are cured with substances which are acidic mixed with hot water or pungent and bitter substances. Plants attacked by pests are treated with a mixture of fresh cow urine, clarified butter, *Embelia ribes* Burm, mustard, and sesamum applied to trunk and then are to be watered with milk and water. Trees which are attacked by any type of pests are to be treated with the paste from bark of Cassia fistula (Kakkagida) (fam: Fabaceae), *Sapindus laurifolius* (arishta) (fam: Sapindaceae), Alsto*n*ia scholaris (Saptaparni) (fam: Apocynaceae), *Embelia ribes* Burm (Vayuvilanga) (fam: Primulaceae), and Cyperus esculentus (fam: Cyperaceae) and Cyperus esculentus *tengamurte* (fam: Cyperaceae) in cow's urine. Exudation from trees can be stopped with the application of bark paste of Setaria italica (Priyangu/ Navane) (fam: Poaceace) and *Terminalia arjuna* (arjuna) (fam: Combretaceae) in boiled milk. Surapala in Vrikshayurveda suggested measures of treatment of plant diseases. Trees that grow too close and touch each other do not yield adequate fruits

owing to their roots entwining and injuring each other, so the recommended appropriate plant-to-plant distance as applicable to individual species should be followed. In scoring of leaves, arrest of the growth of leaves, drying up of branches, and excessive exudation of sap, remedies like the clearing of the affected part and then pestering them with sriangdhara and ghee and nurturing the well mixed with milk have been recommended. Sterility by application of kulattha, masammurdga sesamum along with honey. Traditional farming practices in the past have provided effective and sustainable management of crop diseases. Most of present day susceptibility of cultivars have been avoided from right technique and planting materials. They practiced carefully seed soaking and crop rotation and avoided planting at full moon or when sun had a halo. Most of the traditional farmers strictly adhere to plant and crop architecture. Cultural management practices such as fresh burning, adjusting crop sowing depth or time of planting, fallowing, flooding, mulching, cropping with zero tillage, planting in raised beds, sanitation, and tillage.

10.5 Our Research Efforts in Managing Crop Diseases by ITK Measures

Invalidation of Indigenous Technology Knowledge, UAS, Bangalore, is the pioneer research institute in the country which for the first time took research efforts in plant protection. In general management of soilborne plant pathogens are very difficult particularly *Fusarium* group which is soil inhabitant and remains in the soil for more than 50 years. Hence, it is difficult to manage this pathogen by any methods including chemical methods.

The cow milk, curd, ghee, cow dung, and cow urine have been used individually for curing many ailments as described in the ancient text. It is known that cow ghee and curd contain certain living entities and antimicrobial substances. The Panchagavya is the product of five cow products such as milk, curd, ghee, dung, and urine. In traditional Hindu family, it is also taken as Panchamrutha in little quantity for purification of both external and internal environment of the system. The innovative research on the use of Modified Panchagavya Mixture (MPG-3) was carried out on three soilborne diseases like fusarium wilt of tomato and banana and also foot rot of black pepper. The traditional panchagavya was modified by adding yeast and common salt and three formulations were tested. The component three MPG-3 was most effective in managing all these plant diseases which include 2 ml of ghee, 5 ml of curds, 5 ml of milk, 40 g of dung, and 48 ml of urine mixed with 2 g yeast and 2 g salt for 100 ml preparation. These components were mixed by adding one after the other in a plastic container and kept for fermentation for seven to 10 days by closing the plastic container. The addition of salt was to reflect Jim Martin's living water promoting microbial activity which was further augmented with the addition of yeast. The fermented preparation was diluted ten times with water and filtered through two layers of muslin cloth to obtain a clear filtrate. The filtrate was used in different delivery methods of seedling dip for 30 min and soil drenching for the pre-infested soil with the pathogen in the investigations.

10.5.1 Management of Panama Disease of Banana

In the case of Panama disease of banana, MPG-3 was used at 10^1 dilutions along with different bioagents like *Trichoderma viride* (0.25%), Pseudomonas fluorescens (1 hour dip, 10^8 cells/ml), and *Bacillus subtilis* (1 hour dip, 10^6 cells/ml). The MPG-3 gave better influence on plant height, number of leaves, maximum root length, pseudostem girth, etc. There was a reduction in Fusarium population in MPG-3 provided encouraging results compared with seedling dip. The population of *Fusarium oxysporum* f.sp. *cubense* declined significantly to $11.8x10^4$ cfu/g after 150 days of planting. These results indicate the promise shown by MPG-3 in eco-friendly and cost-effective management of Fusarium wilt (Shamarao et al. 2001).

10.5.2 Management of Foot rot of Black Pepper

Developed and standardized effective IDM package: Soil application of T. viride (75 g/pt) + spraying with metalaxyl (1.25 g/lt) + Akomin (4 ml/lt) or MPG 3 (10^1) for the management of foot rot of black pepper (Shamarao 1998; Shamarao et al. 2000a, b).

10.5.3 Management of Damping off of Tomato in the Nursery and Main Field

The research work carried out at UAS, Bangalore, clearly demonstrated the role of MPG-3 as PGPR component and ISR activity against fusarium wilt of tomato (Bhaskar 1994).

10.5.4 Management of Tobacco Mosaic Virus Through Organics

Tobacco Mosaic Virus (TMV) is the major stumbling block for successful cultivation of bidi tobacco in Nipani area. Identification of resistant source against such systemic biotic infection is a challenging task for plant pathologists and plant breeders. In order to give a boost to ruling cultivators which are susceptible for TMV, Viroson at 2% (27.7% disease incidence) followed by *Bougainvillea* leaf extract 5% (30.2% disease incidence) and neem 1500 ppm (31.8% disease incidence) were applied. Among ITK measures, Panchagavya at 5% (37.7%) followed by cow urine 10% (37.8% disease incidence) were applied. The untreated check recorded maximum incidence of 56.5%. There was no significant difference between the treatments with respect to growth parameters. However, increased plant height, leaf length, and leaf breadth were recorded in Viroson, neem 1500 ppm, and cow urine application indicating role induced systemic resistance. Maximum cured leaf yield

(1206 kg/ha) was recorded in cow urine at 10% followed by Viroson 2% (1157 Kg/ha). Among quality parameters, nicotine % ranged from 2.66% to 4.16% with maximum (4.16%) in neem leaf extract followed by 3.77% in buttermilk at 5%. The reducing sugar ranged from 5.63% to 10.14% with maximum (10.14%) in neem at 1500 ppm followed by 9.78% in cow urine at 10%. The chloride % was within the limit of <1 except buttermilk (1.07%). Thus, the investigations opened a new window of opportunity in managing TMV infections through ITK measures enhancing both leaf yield and quality parameters in bidi tobacco (Shamarao et al. 2008).

10.5.5 Management of Asian Soybean Rust in India

The Asian soybean rust, *Phakopsora pachyrhizi* Syd, causes significant yield loss in India. Lack of resistant cultivars, growing concern over the use of chemical pesticides, and increasing area under organic soybean cultivation have led to exploitation of indigenous technology knowledge in the management of Asian soybean rust. The pooled analysis over 2 years revealed that among the ITK measures, the application of cow urine at 10% + Pongamia pinnata oil at 0.5% recorded minimum Percent Disease Index (PDI) of 37.9 followed by cow urine at 10% alone (40.3). The chemical elicitors like $MnSO_4$, Multi-K, or plant-based extracts like *A. vesica*. Pongamia pinnata oil and bioagent like *Trichoderma harzianum* along with cow urine be used in developing integrated disease management strategies against Asian soybean rust in India which will help in reducing the chemical pesticides in long-term sustainable management. The present findings drew the first line of research in utilization of indigenous technology knowledge in managing rust and enhancing both yield and quality parameters of soybean in India (Shamarao et al. 1997; Shamarao et al. 2009).

10.6 Eco-Friendly Strategies in the Integrated Management of Root-Knot Nematode in Bidi Tobacco-2003 to 2007

10.6.1 Preamble

Different types of tobacco are being cultivated in India under different agro-climatic conditions. Among these, the Nipani area of Belgaum District of Karnataka in India is known for the production of quality bidi tobacco. Monocropping of tobacco in this region has resulted in building up populations of *Meloidogyne incognita* (Kofoid and White) Chitwood. The disease has become a constraint for tobacco cultivation in the area. It infects at any stage of the crop and causes considerable loss in quality and yield of tobacco. Bidi tobacco is bread and butter of Nipani farmers of Northern Karnataka. Our 4-year research efforts finally gave a solid recommendation as poultry manure (1 t/ha) mixed with carbofuran 3G (5 kg/ha) was the most effective, suitable, eco-friendly, and economically viable strategy for the management practice of root-knot disease of bidi tobacco. This has significantly reduced the use of carbofuron in the area (Shamarao and Hundekar 2007a, b, 2008).

Some of the Classical Examples of Local Practices Adopted for Plant Disease Management Are

- Shade regulation for coffee leaf rust and blister light of tea.
- Growing windbreakers like silver oak, casurina, jack, etc. to avoid sun scorching of young shoots of plantation crops.
- Tying of areca nut seedlings with coconut and areca nut fronds to protect them from Western sun scorching.
- Pasting of lime on areca trees to avoid ill effects due to sun scorching.
- Watering nursery beds in the early morning for higher seedling vigor and stand particularly followed in chilli and brinjal.
- Burning nursery with leaf litter and farm waste to overcome certain soilborne pathogens.
- Raised beds, fields, and ridges used to manage some soilborne pathogens. In Mexico, raised beds known as chinampas or floating garden which were used to control *Pythium*, *Phytophthora,* and other soilborne pathogens.
- Collection and burning of stubbles in the field to overcome the problem of soilborne pathogens.
- Flooding with water to overcome the problems of soilborne pathogens by creating anaerobic conditions. In our studies flooding for 85–100 days brought down significantly the *Fusarium oxysporum* f.sp. *cubense* population causing panama disease of banana.
- Earthing up to overcome the problem of *Pythium* damping off in a nursery in brinjal and tomato.
- Kotte tying for areca bunches to overcome the problem of kolerogam of areca nut.
- Planting across the wind direction helps to manage some airborne diseases.
- Mixed cropping of jowar with tur to prevent the movement of mites which transmit sterility mosaic of pigeon pea and to minimize tur wilt.
- Manipulation of planting time/sowing to overcome problems of foliar diseases e.g., Tikka disease of groundnut and anthracnose of chili.
- Summer ploughing to reduce the problem of nematode infestation and soilborne pathogens.
- Magi cultivation: Crop rotation with legumes, cereals, and millets to overcome the problem of soilborne plant pathogens.
- Mulching with green manure in paddy to overcome the problem of soilborne pathogens.
- Salt water treatment for wheat to overcome the problem of seed-borne diseases e.g., Bunt and seed gall in wheat.
- In North Costa Rica, traditional farmers in many areas used a system called tapaga, meaning covered beans and combination of mulch and beans which effectively prevented bean blight.
- The benching of maize earheads in Mexico to overcome the seed-borne infestation due to fungal pathogens.
- Tying of paddy thread prepared out of hay near the crown region of coconut and placement of 1 kg of salt to overcome the problem of stem bleeding in areacnut (Shamarao et al. 2003).

10.7 Role of Biological Control in Organic Agriculture

Plant diseases are managed through different practices employing different agents but management through bioagents is called biological control of plant diseases. Although Plant pathologists had used the term biological control agent in the early 1900s, there was no attempt to formally define the term biological control. Baker and Cook (1974) defined biological control as "reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant stage by one or more organisms accomplished naturally or through manipulation of the environment, host or antagonist, or mass introduction of one or more antagonists." Further, Cook and Baker shortened this definition to "Biological control is the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man."

Mukhopadhyay (1987) highlighted that "Biological control of soil-borne plant pathogens by *Trichoderma* sp. and other bioagents as a vital area of plant pathological research all over the world these days." Biological plant protection is a too important component in the eco-friendly management of plant diseases all over the globe. It is now widely accepted that biological control of crop diseases is a distinct possibility for the future and can successfully be exploited within the framework of Integrated Pest/Disease Management System.

10.8 Features of Biological Control of Plant Diseases

Following are some of the points presently supporting management of plant diseases through bioagents.

1. It avoids environmental pollution of soil, air, and water as is being experienced in chemical control.
2. It avoids the residue toxicity of crop products when consumed but it is very much experienced in fungicidal control.
3. It avoids adverse effect on beneficial microorganisms including antagonist in the soil whereas chemical is lethal.
4. It is less expensive compared to chemical control as there are more expensive.
5. Continuous use of bioagents avoid development of resistant strains while continuous use of systemic and specific fungicides has resulted in development of new resistant strains thus making chemical ineffective.
6. Bioagents application is usually once and does not need repeated applications whereas chemicals need to be repeatedly applied as they lose effectiveness after some time.
7. Bioagents are more effective especially for soilborne diseases, as fungicides may not reach pathogen site.
8. In the absence of satisfactory control measures in virus diseases, biological control is presently satisfactory.

9. It aims at risk-free management of plant diseases wherein chemical control are risky including phytotoxicity.
10. It becomes part of modern large-scale agriculture and helps in increasing crop production within existing resources maintaining biological balance.

10.9 Advances in the Approaches to Biological Control

There are four approaches: biological control of inoculum, which includes (a) destruction of propagules or biomass of pathogen by hyperparasites, hyperpathogens, or predators, (b) prevention of inoculum formation, (c) weakening or displacement of the pathogen in infested residues (food base) by antagonists, and (d) reduction of the vigor of virulence or pathogen by agents such as mycoviruses or hypovirulence and 2. biological protection agents infection: it is achieved by (i) protection of planting material, (ii) protection of roots with a biological seed treatment, (iii) biological protection of foliage and flowers, and (iv) inoculation of pruning wounds with antagonists.

10.10 Mechanisms of Biological Control

Antagonism includes antibiosis, competition, and mycoparasitism, and the mechanism of biological control of plant diseases operates through one or both or all of these together or singly. In addition, the mycorrhizae, plant growth-promoting rhizobacteria (PGPR), cross-protection, and induced resistance are also operating during the biological control process.

10.10.1 Antibiosis

Antibiosis is the inhibition of pathogen by the metabolic (antibiotic) product or products of the antagonist. The antagonist releases antibiotics or other metabolic products (enzymes) which are harmful to the pathogen and inhibit its growth.

Antagonist	Antibiotic produced
Agrobacterium radiobacter	Agrocin 84
Glicladium virens	Gliotoxin, viridin, gliovirin
Trichoderma viride	Gliotoxins, dermadine, viridin, trichodermin
Bacillus subtilis	Bulbiformin
Pseudomonas fluorescence	Phenazines, pyoluteorin, pyrrolnitrin
Streptomycin	Aureofungin, kasugamycin, streptomycin, cycloheximide
Penicillin spp.	Griseofulvin, penicillin

(Srikant Kulkarni 2004)

10.10.2 Competition

It is the endeavor of two or more microorganisms to gain the measure each wants from supply of substrate in the specific form and under specific conditions in which that substrate supply is not sufficient for both. In essence the competition is for nutrients (high energy carbohydrates and nitrogen) and also for space and oxygen but not for temperature, pH, and water potential. The antagonists grow very fast and utilize all the food and occupy the space and thus make the pathogen weak. Heterotrophic rhizobacteria like fluorescent pseudomonas compete for iron with plant pathogens, use iron very efficiently, and produce siderophores (microbial iron transport agents) which complex with the iron and thus adversely affects them.

10.10.3 Mycoparasitism and Predation

Mycoparasitism (= hyperparasitism) is defined as parasitism of one fungus by another. Several necrotrophic mycoparasitism have potential biocontrol agents. The mechanism of mycoparasitism includes different kinds of interactions like coiling of hyphae around the pathogen, penetration, production of haustoria, and lyses of hyphae. Recently, it is also postulated that necrotrophic parasites kill susceptible by the action of toxins, antibiotics, and/or enzymes.

10.10.4 Induced Systemic Resistance

In the later part of the 1990s, the research on plant growth-promoting rhizobacteria (PGPR) and induced systemic resistance (ISR) clearly gave a hint on the role of useful microbes in imparting resistance to plants for a specific group of the pathogen. Free-living root-colonizing bacteria (rhizobacteria) have been studied for the past century as possible inoculants for increasing plant productivity and controlling microbial pathogens. Soil or seed applications with plant growth-promoting rhizobacteria (PGPR) have been used to enhance the growth of several crops (Glick 1995), as well as to suppress the growth of plant pathogens. PGPR that colonizes root systems through seed applications and protects plants from foliar diseases include *Pseudomonas* sp., *Bacillus* sp., *Paenibacillus* sp., and *Serratia* sp. The mechanisms for plant growth promotion and induced systemic resistance (ISR) by PGPR have been extensively studied in the past decade. There are several determinants for mechanisms of growth promotion that include bacterial synthesis of the plant hormones (indole-3- acetic acid (IAA), cytokinin, and gibberellin), the breakdown of plant-produced ethylene by bacterial production of 1- aminocyclopropane-1-carboxylate (ACC) deaminase, and increased mineral and N availability in the soil. Recently, the phenomenon that PGPR elicit plant defense has also been found to lead to a state of ISR in the treated plant. ISR occurs when the plant's defense mechanisms are stimulated and primed to resist infection by pathogens. ISR is different from systemic acquired resistance (SAR) that triggers systemically plant

defense response following hypersensitive response after inoculation of plant pathogens. Previous works demonstrated that several bacterial determinants such as siderophores, salicylic acid (SA), and lipopolysaccharides (LPS) contributed to ISR (Ryu et al. 2005).

10.11 Role of Biological Control in the Integrated Disease Management (IDM)

As biocontrol agents alone may not completely and effectively manage the disease, it can be done by the components in the integrated management of diseases. Also under high disease pressure or pathogen population pressure, the biocontrol may be less effective and needs other practices also to completely manage the disease. Shamarao et al. (2001) reported the effectiveness of seed treatment with biocontrol agent if integrated with other management practices i.e., the use of moderately resistant cultivar + biocontrol + FYM helped in managing the disease.

Keys to the Success of Biological Agent

- Selection of virulent strain of antagonist.
- There should be sufficient growth and sporulation on the mass culture media in case of facultative before applying.
- Advance application to provide enough time for interaction between antagonist and target pathogen.
- The soil temperature and moisture should be optimum for establishment and growth of antagonist.
- Sufficient organic matter (food base) should be present in the soil for multiplication, survival, and activity of the antagonist.
- Monitoring the population of the target pathogen and antagonist from time to time on selective media.
- Handing, production, and storage should be easy, and also product should be cheap and available.
- Integration of biocontrol with tolerant/resistant varieties and/or chemical control may be more feasible.

A Future Line of Research Work with Respect to Biological Control

1. Isolation and evaluation of native antagonists, their multiplication, and preparation of seed treatment formulations.
2. Mapping up of population dynamics of the pathogen and biocontrol agents in various geographic areas of production systems.
3. Application of biotechnological techniques, genetic engineering, protoplast fusion technique, etc. may be employed in developing efficient strains of antagonists of site-specific, area-specific, and crop-specific would further pave the way for faster developments in biological control.

10.12 Implications

Plant protection experts all over the world are beginning to evince keen interest in indigenous methods of plant protection. Of late, this knowledge is being made available and research endeavors may be reoriented toward validation of indigenous methods encouraging integrated disease management (IDM) practices. Indigenous knowledge by itself cannot be a sole method of management, but integration with other methods would surely pave way for effective management of diseases without affecting natural ecosystem adversely.

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Free-Living PGPRs in Biotic Stress Management

11

Ashwini Marotirao Charpe

Abstract

Plant growth-promoting rhizobacteria (PGPR) is a heterogeneous group of microorganisms found in the rhizosphere. They live in association with roots and stimulate the plant growth and/or reduce the incidence of plant disease. The term PGPR is used to describe soil bacteria that colonize the rhizosphere of plants, growing in, on, or around plant tissues that stimulate plant growth by several mechanisms. The PGPRs are involved in various biotic activities of the soil ecosystem to make it dynamic for nutrient turnover and sustainable crop production by affecting plant growth. Generally, PGPR promotes plant growth directly due to their ability for nutrient supply (nitrogen, phosphorus, potassium, and essential minerals) or modulating plant hormone levels or indirectly by decreasing the inhibitory effect of various pathogens on plant growth and development in the form of biocontrol agents, root colonizers, and environment protectors. PGPRs can protect plants from diseases by a wide variety of mechanisms like antibiosis, induction of systemic resistance, siderophore production, production of 1-amino cyclopropane-1-carboxylate deaminase (ACC), signal interference while quorum sensing (QS) and inhibition of biofilm formation, production of lytic enzymes, production of volatile organic compounds (VOCs), promoting beneficial plant–microbe symbioses by competition for nutrients and niches, interference with pathogen toxin production, etc. A particular PGPR may affect plant diseases by using any one, or more, of these mechanisms. Bacteria of diverse genera have been identified as PGPRs, of which *Bacillus* and *Pseudomonas* spp. are predominant and have been implied in biocontrol due to their effective competitive interactions with bacteria, fungi, oomycetes, protozoa, viruses, and nematodes attacking a variety of crops.

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11.1 Introduction

The narrow zone of soil surrounding living roots is called the rhizosphere (Hiltner 1904) which is characterized by increased microbial activity and by a specific microbial community structure (Duineveld et al. 2001; Kim et al. 2006). Beneficial free-living soil bacteria present in the rhizosphere are referred to as plant growth-promoting rhizobacteria (PGPR) (Kloepper and Schroth 1978). PGPRs colonize the rhizosphere, the rhizoplane, or the root itself (Patten and Glick 2002). A successful plant–microbe interaction is a result of effective colonization of microbes (Lugtenberg et al. 2002). Steps of colonization include attraction, recognition, adherence, invasion (in case of endophytes and pathogens), colonization, and growth. Plant roots send signals in the form of root exudates which are recognized by microbes (Berg 2009). PGPRs reach root surfaces by active motility guided by chemotactic responses to these root exudates (Pinton et al. 2007). Generally, PGPRs function in three different ways: synthesizing particular compounds for the plants, facilitating the uptake of certain nutrients from the soil, and preventing the plants from diseases. Indirect plant growth promotion includes the prevention of the deleterious effects of phytopathogenic organisms (Rawat and Mushtaq 2015). Some common examples of genera exhibiting plant growth-promoting activity are *Azospirillum*, *Azotobacter*, *Bacillus*, *Bradyrhizobium*, *Burkholderia*, *Corynebacterium*, *Pseudomonas*, *Rhizobium*, *Serratia*, etc. (Rawat and Mushtaq 2015). The application of rhizosphere microorganisms as biocontrol agents may be a promising alternative to decrease use of chemical pesticides as they can guard the health of plants in an eco-friendly manner (Akhtar et al. 2012). In recent years, considerable attention has been paid to PGPR to replace agrochemicals (fertilizers and pesticides) for the plant growth promotion by a variety of mechanisms that involve biocontrol of soil- and seedborne plant pathogens and in promoting changes in vegetation (Sivasakhti et al. 2014). Regarding the use of rhizobacteria as biocontrol 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).

Plant growth-promoting rhizobacteria can be classified into extracellular plant growth-promoting rhizobacteria (ePGPR) and intracellular plant growth-promoting rhizobacteria (iPGPR) (Viveros et al. 2010). The ePGPRs may exist in the rhizosphere, on the rhizoplane, or in the spaces between the cells of root cortex, while iPGPRs locate generally inside the specialized nodular structures of root cells. The bacterial genera such as *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*,

Micrococcus, *Pseudomonas*, and *Serratia* belong to ePGPR (Ahemad and Kibret 2014). The iPGPR belongs to the family of Rhizobiaceae includes *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium*, endophytes and *Frankia* species both of which can symbiotically fix atmospheric nitrogen with the higher plants (Bhattacharyya and Jha 2012). PGPRs play a significant role in management of biotic stresses through various mechanisms like antibiosis, induction of systemic resistance, siderophore production, production of 1-aminocyclopropane-1-carboxylate deaminase (ACC), signal interference while quorum sensing (QS) and inhibition of biofilm formation, production of lytic enzymes, production of volatile organic compounds (VOCs), promoting beneficial plant–microbe symbioses by competition for nutrients and niches, interference with pathogen toxin production, etc. which have been discussed elaborately in this chapter along with the examples of various crops where application of PGPRs has effectively managed the various biotic stresses.

11.2 Mechanisms Involved in Biocontrol

Plant growth promotion by PGPR can be achieved indirectly through biocontrol activity against plant pathogens (Fig. 11.1). Several ways of controlling fungal and bacterial pathogens have been described in PGPR. Mechanisms of biological control by which rhizobacteria can promote plant growth indirectly by reducing the level of disease include antibiosis (Lugtenberg and Kamilova 2009), production of bacteriocins (Riley and Wertz 2002), production of lytic enzymes (Neeraja et al. 2010; Maksimov et al. 2011), induction of systemic response (Naznin et al. 2012), interference with quorum sensing system (Perez-Montano et al. 2013), competition for iron uptake (Mehnaz 2013), production of stress controllers (Glick et al. 2007), competition for nutrients and niches (Kamilova et al. 2005), hyperparasitism (Harman et al. 2004; Kamilova et al. 2008), production of volatile organic compounds (VOCs), and rhizospheric competence (Perez-Montano et al. 2014). A particular PGPR uses all or few of these mechanisms for controlling biotic stresses.

11.2.1 Antagonism

Microorganisms are related to the synthesis of antimicrobial compounds, thus providing protection against pathogens (Davidson 1988; Laslo et al. 2012). Mechanisms responsible for antagonistic activity include inhibition of the pathogen by antibiotics, toxins, and surface active compounds (biosurfactants) and a mechanism that develops production of extracellular cell wall degrading enzymes such as chitinase and 1,3-glucanase (Whipps 2001; Compant et al. 2005a; Haas and Défago 2005). Members of the bacterial genera *Bacillus*, *Pseudomonas*, *Serratia*, *Stenotrophomonas*, and *Streptomyces* and the fungal genera *Ampelomyces*, *Coniothyrium*, and *Trichoderma* are well-studied microorganisms with proven microbial influence on plant health. When testing microbial isolates

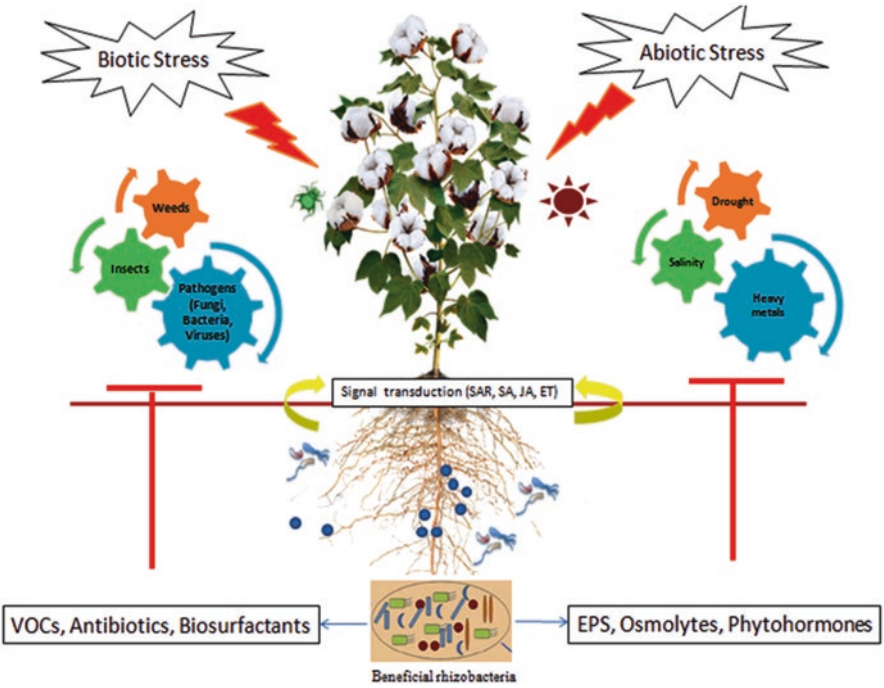

Fig. 11.1 Role of PGPRs in plant disease suppression

from plant-associated habitats, between 1% and 35% showed the antagonistic capacity to inhibit the growth of pathogens in vitro (Berg 2009). PGPRs have also been shown to produce various antagonistic metabolites that are involved in direct inhibition of plant pathogens (Shoda 2000; Raaijmakers et al. 2010) including the inhibition of microbial growth by diffusible antibiotics, volatile organic compounds, biosurfactants, toxins, and enzymes.

11.2.2 Production of Antibiotics

Indirect mechanisms of plant growth promotion by PGPRs include killing or reducing the growth of one or more phytopathogens, by the production of antibiotics (Bevivino et al. 1994; Rodríguez and Fraga 1999; Richardson et al. 2009). Antibiotics encompass a heterogeneous group of organic, low-molecular-weight compounds that are deleterious to the growth or metabolic activities of other microorganisms (Duffy et al. 2003). The phenomenon of antibiosis by PGPR is deciphered in detail in the past two decades (Lugtenberg and Kamilova 2009; Shilev 2013).

The production of antibiotics zwittermicin A (aminopolyol) and kanosamine (aminoglycoside) by the *Bacillus cereus* UW85 strain has been demonstrated by Silo-Suh et al. (1994) and He et al. (1994), which suppresses oomycete pathogens

and contributes to the biocontrol of damping-off disease of alfalfa. *Bacillus subtilis* strains produce a variety of powerful antifungal metabolites, e.g., zwittermicin A, kanosamine, and lipopeptides from the surfactin, iturin, and fengycin families (Emmert and Handelsman 1999; Ongena and Thonart 2006). A variety of antibiotics have been discussed, including compounds such as oligomycin A, kanosamine, zwittermicin A, and xanthopuccine produced by *Bacillus*, *Streptomyces*, and *Stenotrophomonas* sp. to prevent the proliferation of plant pathogens (Generally fungi) by Compant et al. (2005a). Antibiotics more recently discovered in biocontrol strains are d-gluconic acid (Kaur et al. 2006) and 2-hexyl-5-propyl resorcinol (Cazorla et al. 2006). *Bacillus amyloliquefaciens* is known for lipopeptide and polyketide production for biological control activity against soilborne pathogens (Ongena and Jacques 2008). Antibiotics, such as polymyxin, circulin, and colistin, produced by the majority of *Bacillus* spp. are active against Gram-positive and Gram-negative bacteria, as well as many pathogenic fungi (Maksimov et al. 2011).

Similarly, Hill et al. (1994) demonstrated the production of antibiotic Pyrrolnitrin by the *Pseudomonas fluorescens* BL915 strain which is able to prevent the damage of *Rhizoctonia solani* during damping-off of cotton plants. Most of the identified *Pseudomonas* biocontrol strains produce antifungal metabolites (AFMs), of which phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol (DAPG), and pyoluteorin are the most frequently detected classes. However, new AFMs belonging to the class of cyclic lipopeptides, such as viscosinamide (Nielsen et al. 1999) and tensin (Nielsen et al. 2000), have been discovered. Viscosinamide prevents the infection of sugarbeet by *Pythium ultimum* (Thrane et al. 2000). The genetic basis of the biosynthesis of the more frequently detected AFMs in *Pseudomonas* has been elucidated. More recently, new information has become available on the biosynthesis of pyoluteorin in *P. fluorescens* Pf-5 (Nowak-Thompson et al. 1999) and of DAPG in *P. fluorescens* Q2-87 (Bangera and Thomashow 1999; Delany et al. 2000). The causal agent of tomato foot and root rot (TFRR) an important disease of tomato is restricted by *P. fluorescence* by competing for nutrients from the root and for niches on the root by delivering the antibiotic along with the whole root system (Chin-A-Woeng et al. 2000). The DAPG produced by pseudomonads is an effective and extensively studied antibiotic that causes membrane damage to *Pythium* spp. and is particularly inhibitory to zoospores of this oomycete (De Souza et al. 2003). Phenazine, also produced by pseudomonads, possesses redox activity and can suppress pathogens of plants such as *Fusarium oxysporum* and *Gaeumannomyces graminis* (Chin-A-Woeng et al. 2003). The biocontrol abilities of pseudomonad strains essentially depend on the production of antifungal antibiotics along with other mechanisms (Haas and Keel 2003). The *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, raising the possibility that phenazines might contribute to iron mobilization in soils (Hernandez et al. 2004; Haas and Defago 2005). According to Haas and Defago (2005), six classes of antibiotic compounds (for which their modes of action are partly understood) are better related to the biocontrol of root diseases: phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides like viscosinamide (all of which are diffusible), and

hydrogen cyanide (HCN, which is volatile). Production of amphisin, DAPG, oomycin A, phenazine (phenazine-1-carboxylic acid and phenazine-1-carboxamide), pyoluteorin, pyrrolnitrin, tensin, tropolone, *N*-acyl homoserine lactone, and cyclic lipopeptides by pseudomonads have also been demonstrated (Thomashow and Weller 1996; Dunne et al. 1998, Loper and Gross 2007). In soils, antibiotic DAPG producing *Pseudomonas* sp. was reported for biocontrol of disease in wheat caused by the fungus *Gaeumannomyces graminis* var. *tritici* (De Souza et al. 2003). Bacterization of wheat seeds with *P. fluorescens* strains to produce the antibiotic phenazine-1-carboxylic acid (PCA) resulted in significant suppression of take-all in about 60% of field trials (Weller 2007).

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). One problem with depending too much on antibiotic-producing PGPR as biocontrol agents is some phytopathogens may develop resistance to specific antibiotics due to increased use of these strains. To prevent this from happening, some researchers have utilized biocontrol strains that synthesize one or more antibiotics (Glick 2012).

11.2.3 Production of Volatile Organic Compounds (VOCs)

Apart from the production of antibiotic, some rhizobacteria are also capable of producing a volatile compound known as hydrogen cyanide (HCN) for biocontrol of black root rot of tobacco, caused by *Thielaviopsis basicola* (Sacherer et al. 1994). Lanteigne et al. (2012) also reported the production of DAPG and HCN by *Pseudomonas* contributing to the biological control of bacterial canker of tomato. Volatiles other than HCN such as acetoin, 2,3-butanediol, or blends of volatiles produced by *Bacillus* spp. (Ryu et al. 2003) or by fungi (Strobel 2006) are also involved in plant protection.

11.2.4 Production of Biosurfactants

Lipopeptide biosurfactants produced by *B. subtilis* (Ongena et al. 2007) and by pseudomonads (De Bruijn et al. 2007) have also been implied in biocontrol due to their potential positive effect on competitive interactions with organisms including bacteria, fungi, oomycetes, protozoa, nematodes, and plants. Rhamnolipid and phenazine have been demonstrated to act synergistically against soilborne diseases caused by *Pythium* spp. (Perneel et al. 2008).

11.2.5 Production of Bacteriocins

Other molecules used in microbial defense systems are bacteriocins. 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 1993). Bacteria can produce a wide variety of compounds with antimicrobial activity used as defense systems including bacteriocins, which also have a bactericidal mode of action (Riley and Wertz 2002). According to a review by Riley and Wertz (2002), bacteriocins differ from traditional antibiotics in one critical way: they commonly have a relatively narrow killing spectrum and are only toxic to bacteria closely related to the producing strain. An acyl-homoserine lactone (AHL) from biocontrol strain *P. fluorescens* F113 was elucidated and surprisingly identified as the rhizobial small bacteriocin *N*-(3-hydroxy-7-cis-tetradecanoyl) homoserine lactone (Schripsema et al. 1996). The production of this bacteriocin and two more common AHLs is directed by the *hdtS* gene product, which belongs to a novel class of acyl synthases (Laue et al. 2000). 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. Like colicin, a name derived from *E. coli*, other bacteriocins have been thus defined and named, such as pyocins from *P. pyogenes* strains, cloacins from *Enterobacter cloacae*, marcescins from *Serratia marcescens,* and megacins from *B. megaterium* (Cascales et al. 2007). Interestingly, bacteriocins from *Bacillus* spp. are increasingly becoming more important due to their sometimes broader spectra of inhibition (as compared with most lactic bacterial bacteriocins), 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).

11.2.6 Production of Lytic Enzymes

Growth enhancement through enzymatic activity is another mechanism used by PGPR. Indirect growth promotion occurs via the biocontrol of pathogens conferred by PGPR by the production of secondary metabolites such as fungal cell wall degrading hydrolytic enzymes or lysozymes, e.g., chitinase and β-1,3-glucanase, proteases, lipases, dehydrogenase, phosphatases, proteases, etc. (Glick 2001; Cattelan et al. 1999; Pal et al. 2001; Riley and Wertz 2002; Compant et al. 2005a; Haas and Defago 2005; Hayat et al. 2010; Neeraja et al. 2010; Maksimov et al. 2011; Joshi et al. 2012). Through the activity of these enzymes, PGPRs play a very significant role in plant growth promotion particularly to protect them from biotic stresses by suppression of pathogenic fungi including *Botrytis cinerea*, *Sclerotium rolfsii*, *Fusarium oxysporum*, *Phytophthora* sp., *Rhizoctonia solani*, and *Pythium ultimum* (Upadhyay et al. 2012; Nadeem et al. 2013).

11.2.7 Induction of Systemic Responses: ISR and SAR

Induced systemic resistance (ISR) was discovered by Van Peer et al. (1991) in carnation plants that were systemically protected by the *P. fluorescens* strain WCS417r against *Fusarium* wilt *caused by F. oxysporum* f. sp. *dianthi* and by Wei et al. (1991) in cucumber plants, where rhizobacterial strains protected the leaves against anthracnose caused by *Colletotrichum orbiculare*. They inoculated nonpathogenic *Pseudomonas* spp. on roots and observed the trigger of a plant-mediated resistance response in aboveground plant parts. The inducing rhizobacteria and the pathogens were inoculated and remained confined and spatially separated on the same plant so that microbial antagonism was excluded and the protective effect was plant-mediated. A similar phenomenon was observed when certain strains of *B. cereus*, which are poor colonizers, showed to be good biocontrol agents (Gilbert et al. 1994).

ISR differs from SAR (systemic acquired resistance). ISR is the response of the plant to the stimulation received from nonpathogenic PGPR (Van Loon et al. 1998), whereas SAR is the response of the plant to the stimulation received from a plant pathogen. In ISR, infected plants increased their levels of jasmonic acid (JA) and ethylene (ET) as a sign of active defense (De Laat and Van Loon 1982; Gundlach et al. 1992; Mauch et al. 1994). In SAR treatment of tobacco roots with *P. fluorescens,* CHA0 triggers the accumulation of salicylic acid (SA)-inducible pathogenesis-related (PR) proteins in the leaves (Maurhofer et al. 1994). Induced resistance is the state of an enhanced defensive ability developed by plants when appropriately stimulated (Van Loon et al. 1998). These accumulating signaling molecules coordinate the defense responses and, when applied exogenously, are sufficient to induce resistance (Ryals et al. 1996).

Rhizobacteria-mediated ISR resembles pathogen-induced SAR in that both types of induced resistance render uninfected plant parts more resistant to plant pathogens (Van Wees et al. 1997; Van Loon et al. 1998), including fungal, bacterial, and viral pathogens, as well as nematodes and insects (Zehnder et al. 1997; Van Loon et al. 1998; Bent 2006; Pozo and Azcon-Aguilar 2007). The ability to develop ISR in response to certain rhizobacteria has been demonstrated in several species of plants such as bean, tomato, tobacco, radish, cucumber, and carnation (Van Loon et al. 1998) and appears to depend on the specificity of the interaction between rhizobacteria and plants (Van Loon 2007). It is observed that the same strain induces resistance against several pathogens in the same plant (Somers et al. 2004). 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). ISR is characterized by a specific relationship between plant and PGPR species. In fact, a PGPR that produces ISR in one plant species may not do it in another (Vleesschauwer and Höfte 2009).

In contrast to many biocontrol mechanisms, extensive colonization of the root system is not required for ISR, as shown by the *P. fluorescens* WCS365 (Dekkers et al. 2000) using root colonization mutants. It is unlikely that a poor colonizer acts through antibiosis since colonization is the delivery system for antifungal

components along the root system (Chin-A-Woeng et al. 2000). The dependency of ISR on JA and ET is based on enhanced sensitivity to these hormones rather than on an increase in their production (Pieterse et al. 2000, 2001). The protection mediated by ISR is significantly less than that obtained by SAR (Van Loon 2000), and a degree of dependence on plant genotype is observed in the generation of ISR (Bloemberg and Lugtenberg 2001). However, ISR and SAR together provide better protection than each of them alone, indicating that they can act additively in inducing resistance to pathogens (Van Wees et al. 2000). ISR has been reported as one of the mechanisms by which PGPRs reduce plant disease – modulating the physical and biochemical properties of host plants (Pieterse et al. 2002).

Specifically, *Pseudomonas* and *Bacillus* spp. are the rhizobacteria most studied that trigger ISR (Kloepper et al. 2004; Van Wees et al. 2008). Vleesschauwer and Hofte (2009) proposed the terminology ISR to depict induced systemic resistance promoted by nonpathogenic rhizobacteria or PGPR, irrespective of the signaling pathway involved in this process, while the term SAR is used to describe salicylic acid-dependent induced resistance triggered by a localized infection by necrotic pathogenic bacteria. Several strains from *Pseudomonas*, *Bacillus*, and *Azospirillum* genera are the major group of PGPR that has been described eliciting ISR response. There are other species included in the symbiotic group of rhizobacteria that are used in coinoculations with different PGPR and can be involved in ISR (Elbadry et al. 2006).

Induced resistance may be defined as a physiological state of enhanced defensive capacity elicited in response to specific environmental stimuli, and consequently, the plant's innate defenses are potentiated against subsequent biotic challenges (Avis et al. 2008). One or more bacterial determinant must be recognized by specific plant receptors so that resistance is induced. ISR and SAR, which are part of plants systemic resistance responses, are activated by certain microorganism molecules referred to as elicitors. Elicitors are the molecules that induce the ISR defense responses. Cell wall polysaccharides (lipopolysaccharides (LPS) and exopolysaccharides (EPS)) are the most described biotic elicitors, along with flagella, salicylic acid, cyclic lipopeptides, antifungal factor Phl, siderophores, antibiotics like 2,4-diacetylphloroglucinol, the signal molecule AHLs, biosurfactants, N-alkylated benzylamines, and volatile blends produced by *B. subtilis* GB03 and, to a lesser extent, the individual volatiles acetoin and 2,3-butanediol (Ryu et al. 2003; Iavicoli et al. 2003; Shuhegge et al. 2006; Ongena et al. 2007; Van Loon 2007; Ramos et al. 2008; Berg 2009; Vleesschauwer and Hofte 2009; Doornbos et al. 2012).

Rudrappa et al. (2008) reported that infection of leaves of *A. thaliana* seedlings with the foliar pathogen *P. syringae* pv. *tomato* Pst DC3000 results in enhanced secretion of l-malic acid by the roots, and that the enhanced level of l-malic acid selectively signals and recruits the beneficial rhizobacterium *B. subtilis* FB17, which is a biocontrol bacterium that protects the plant through ISR. Previously, De Weert et al. (2002) reported that another biocontrol bacterium, *P. fluorescens* WCS365, which also acts through ISR (Kamilova et al. 2005), shows strong chemotaxis toward the major tomato root exudate component, citric acid.

The ISR is the phenomenon in which the interaction of some bacteria with the plant root results in plant resistance to some pathogenic bacteria, viruses, and fungi (Lugtenberg and Kamilova 2009). Both ISR and SAR can overlap in some cases. In many cases, SAR can also be triggered without tissue necrosis as demonstrated in *Arabidopsis thaliana* (Mishina and Zeier 2007). ISR shares many properties with innate immunity in humans (Lugtenberg and Leveau 2007). ISR offered by *Pseudomonas* is discussed in depth by Kloepper et al. (2004) and that is offered by *Bacillus* is discussed by Van Loon (2007). However, the observation that certain antifungal metabolites (AFMs) can induce ISR explains this phenomenon. Therefore, this is speculated that many of the *Bacillus* strains that can act as biocontrol agent act through ISR rather than antibiosis. Biopriming plants with some PGPRs can also provide systemic resistance against a broad spectrum of plant pathogens. Diseases of fungal, bacterial, and viral origin and in some instances even damage caused by insects and nematodes can be reduced after application of PGPR (Naznin et al. 2012).

11.2.8 Interference with Quorum Sensing (QS) System

Quorum sensing is a phenomenon by which many bacteria regulate their gene expression in response to changes in their population density, which involves communication between cells mediated by small diffusible signal molecules called as autoinducers (Fuqua et al. 1994). Many bacteria only express pathogenicity/virulence factors at a high bacterial cell density, sensed when the level of QS molecules such as homoserine lactones (AHLs) accumulate in the medium (Bassler 1999). Bacteria able to interfere in the QS systems may be potentially used against bacterial pathogens. In fact, the virulence of *Erwinia carotovora*, whose virulence factors are regulated by QS, is attenuated in the presence of the lactonase enzyme produced by *Bacillus* (Dong et al. 2002). Several bacteria produce acylase (*Ralstonia*) or lactonase (*Bacillus*) enzymes that degrade the AHL molecules (Dong et al. 2002; Lin et al. 2003). AHLs are required, for example, for the synthesis of cell-wall-degrading enzymes of the pathogen *Erwinia carotovora*. Signal interference is a biocontrol mechanism based on the degradation of the AHL (Lin et al. 2003). AHLs are the most common autoinducer molecules; they regulate the expression of genes implied in the production of the virulence factor or biofilm formation in several plant pathogens (Quinones et al. 2005). QS is also interrupted by AHL lactonases of *B. thuringiensis* strains that hydrolyze the lactone ring or by AHL acylases that break the amide link. Recently, it was shown that AHL acylase plays a role in the formation of biofilms (Shephard and Lindow 2008). Lack of biofilm formation probably makes biocontrol easier. Many plants are able to produce molecules which specifically interfere in the QS systems of plant-associated bacteria and, in any case, depending on the bacterium being detected as a pathogen or as a beneficial microorganism the molecule enhances or inhibits the phenotypes regulated by QS (Perez-Montano et al. 2013).

11.2.9 Competition for Iron Uptake by Siderophore Production

Siderophores are low-molecular-weight iron-binding proteins having a binding affinity with ferric ions. They improve plant growth and development by increasing the accessibility of iron in the rhizospheric soil (Kloepper et al. 1989). Among most of the bacterial siderophores studied, bacteria those produce 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. 1980a). A pseudobactin siderophore produced by *P. putida* B10 strain was also able to suppress *Fusarium 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. 1980b). When antibiosis is carried out on a test plate containing a medium with a low ferric iron concentration, and when the test strain inhibits fungal growth in the absence but not in the presence of added Fe3+ ions, the bacterial strain likely produces a siderophore, i.e., a Fe3+ ion-chelating molecule. Upon binding the ion, the formed siderophore-Fe3+ complex is subsequently bound by iron-limitation-dependent receptors at the bacterial cell surface, and the Fe3+ ion is subsequently released and become active in the cytoplasm as Fe2+ ions. Bacteria producing high concentrations of high-affinity siderophores in the rhizosphere can inhibit the growth of fungal pathogens when the Fe3+ concentration is low, e.g., in acid soils (Schippers et al. 1987). Studies have demonstrated the suppression of soilborne fungal pathogens through the release of iron-chelating siderophores by fluorescent pseudomonads, rendering it unavailable to other organisms (Loper 1988; Paulitz and Loper 1991; Dwivedi and Johri 2003). Further, PGPR has been demonstrated as enhancing the plant growth by the production of very efficient extracellular siderophores that allow control of several plant diseases by depriving them of iron nutrition, resulting in increased crop yield (O'Sullivan and O'Gara 1992). Iron is one of the bulk minerals present on the surface of the earth, yet it is unavailable in the soil for plants. This is because iron is commonly present in the form of Fe3+ in nature which is highly insoluble (Hofte 1993). To overcome this problem, PGPR secretes siderophores. Plants sequester iron by utilizing siderophores secreted by PGPR (Marschner and Rohmeld 1994). Siderophores produced by PGPR have a high affinity with Fe3+ from the rhizosphere and, consequently, retain a most of the iron available, inhibiting the proliferation of phytopathogenic fungi and enhancing iron uptake by plants (Bevivino et al. 1998; Masalha et al. 2000; Katiyar and Goel 2004; Dimkpa et al. 2009; Laslo et al. 2012).

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 Fe3+ (Crosa and Walsh 2002). These molecules act as solubilizing agents for iron from minerals or organic compounds under conditions of iron limitation. Siderophores, generally form 1:1 complex with Fe3+, which are then taken up by the cell membrane of bacteria, where the Fe3+ is reduced to Fe2+ and released from the siderophore into the cell (Boukhalfa and Crumbliss 2002). The uptake of Fe3+ via siderophore is largely used by pathogenic and nonpathogenic microorganisms from the soil, human body, and marine environments. The importance of

siderophore is closely related to iron, which is an essential element for different biological processes (Crosa and Walsh 2002). The active transport system through the membrane begins with the recognition of the ferric-siderophore by specific membrane receptors of Gram-negative and Gram-positive bacteria that can move by diffusion and be returned to the cell surface (Boukhalfa and Crumbliss 2002; Andrews et al. 2003).

However, it is still unclear if bacterial siderophore complexes can significantly contribute to the iron requirements of the plant. Siderophores can chelate Fe3+ with high affinity, allowing its solubilization and extraction from most mineral or organic complexes (Wandersman and Delepelaire 2004). Siderophore production confers competitive advantages to PGPR that can colonize roots and exclude other microorganisms from this ecological niche (Haas and Defago 2005). Under highly competitive conditions, the ability to acquire iron via siderophores may determine the outcome of competition for different carbon sources that are available as a result of root exudation or rhizodeposition (Crowley 2006). Iron is an essential nutrient for virtually all forms of life. However, in most aerobic microbial habitats, Fe2+ is oxidized to Fe3+, forming insoluble compounds that are unavailable to microorganisms. In those circumstances, some bacteria and arbuscular mycorrhizal fungi (AMF) produce siderophores (Miethke and Marahiel 2007; Machuca et al. 2007). In aerobic conditions at physiological pH, the reduced Fe^{2+} form is unstable and is readily oxidized to the Fe3+ form, which normally occurs as a poorly soluble iron hydroxide basically unavailable to biological systems (Krewulak and Vogel 2008; Osorio et al. 2008). Many plants can use various bacterial siderophores as iron sources, although the total concentrations are probably too low to contribute substantially to plant iron uptake. Various studies showed the isolation of siderophore-producing bacteria belonging to the *Bradyrhizobium*, *Pseudomonas*, *Rhizobium*, *Serratia*, and *Streptomyces* genera from the rhizosphere (Kuffner et al. 2008). *Bacillus* and *Klebsiella* are some other genera that produce siderophores. Microorganisms capable of producing siderophores are beneficial to plants because they increase iron availability to the plant (Lugtenberg and Kamilova 2009; Ahmed and Holmstrom 2014).

The ability of rhizobacteria to produce siderophores and metabolites contributing to antibiosis has been the focus of many studies dedicated to investigating PGPR (Maksimov et al. 2011). Iron is an essential growth cofactor for living organisms. For the soil microorganisms, availability of solubilized Fe3+ in soils is limited at neutral and alkaline pH (Mehnaz 2013). Indirect mechanisms include PGPR decreasing or preventing the destructive effects of one or more phytopathogens, by the production of siderophores (Reed et al. 2015). 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 (Crowley 2006). Hundreds of siderophores have been identified and reported for cultivable microorganisms, some of which are widely recognized and used by different microorganisms, while others are species-specific (Crowley 2006; Sandy and Butler 2009).

11.2.10 Production of Stress Controllers

Plant-growth-promoting bacteria that contain the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase facilitate plant growth and development by decreasing plant ethylene levels. Such bacteria take up the ethylene precursor ACC and convert it into 2-oxobutanoate and NH_3. Several forms of stress are relieved by ACC deaminase producers, including the effects of phytopathogenic bacteria (Glick and Bashan 1997; Glick et al. 2007; Van Loon 2007). Bacterial strains exhibiting ACC deaminase activity have been identified in a wide range of genera the fungus *Thielaviopsis basicola* (Voisard et al. 1989), *Pseudomonas putida* against *Macrophomina phaseolina* in chickpea, and *Azotobacter chroococcum* against *Fusarium oxysporum* in *Sesamum indicum*, respectively, in field condition (Maheshwari et al. 2012).

11.2.11 Competition for Nutrients and Niches (CNN)

Competition of biocontrol bacteria with the pathogen for nutrients and niches in the rhizosphere (CNN) has been suggested for decades as a possible mechanism of biocontrol (Stephens et al. 1993), but the experimental proof was lacking. To this end, Simons et al. (1996) applied a mixture of rhizosphere strains onto surface-sterilized seeds, which were subsequently allowed to germinate in a gnotobiotic system. After 1 week, the root tip, which contained the best competitive root colonizers, was removed from the seedling and the bacterial content was briefly allowed to multiply and subsequently applied onto fresh seeds for a new enrichment cycle. After three of such cycles, the isolated bacteria were as good as, or even better, in competitive root tip colonization than their model colonizer, *P. fluorescens* WCS365. The isolated bacteria also grow efficiently on root exudate. Kamilova et al. (2005) and Validov (2007) argued that if this mechanism exists, such biocontrol strains can be selected. Most of the PGPR isolates collected by them, including *Pseudomonas* strains PCL1751 and PCL1760, controlled TFRR, and the proposed mechanism were confirmed by mutant studies. Kamilova et al. (2005) observed that one of the best competitive root-tip-colonizing strains did not control TFRR. It was concluded that an efficient overall colonization of the root is not sufficient for biocontrol (Kamilova et al. 2005). An explanation for this phenomenon came from the work of Pliego et al. (2008), who isolated two similar enhanced root colonizers, of which only one showed control of white root rot in avocado. It appeared that the strains colonized different sites on the root. Apparently, an exact mini niche on the root has to be colonized to protect the plant against the pathogen. A study on biocontrol of TFRR in stonewool showed that after 3 weeks, more cells of the CNN strain *P. putida* PCL1760, which was selected for biocontrol in the stone wool substrate (Validov 2007), are present on the root compared with all other culturable bacteria combined. This illustrates the enormous protective capacity of this CNN strain.

11.2.12 Hyperparasitism

The major biocontrol mechanism used by *Trichoderma* species is based on predation and parasitism by enzymatic destruction of the cell wall of fungal pathogens (Harman et al. 2004). This colonization makes the fungus less virulent.

Fusaric acid secreted by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Forl*) hyphae serves as a chemoattractant for *P. fluorescens* WCS365 cells (De Weert et al. 2003). During biocontrol of TFRR by *P. fluorescens* WCS365, it forms microcolonies on the hyphae of the pathogen *Forl* (Bolwerk et al. 2003). Scanning electron microscopy has shown that *P. fluorescens* WCS365 also colonizes *Forl* hyphae when incubated in root exudate. Testing on different media showed that on the poorer growth medium, hyphae are colonized more extensively (Kamilova et al. 2008). This observation supports an earlier suggestion (Kamilova et al. 2007) that to use them as a food source bacteria colonize hyphae. When incubated in root exudate, microconidia of *Forl* germinate. The presence of *P. fluorescens* WCS365 inhibits spore germination, presumably due to nutrient deprivation. After growth of *Forl* hyphae in exudate, the hyphae develop microconidia that can spread the pathogen through the environment. The presence of *P. fluorescens* WCS365 also leads to a reduction of this spore formation process and therefore reduces pathogen spread. In conclusion, *P. fluorescens* WCS365 inhibits activity, survival, and germination of the pathogen, colonizes its hyphae, and inhibits the formation of new spores (Kamilova et al. 2008; Validov et al. 2009).

11.2.13 Rhizospheric Competence

In addition to above traits, PGPR must be rhizospheric competent, i.e., able to survive in the rhizospheric soil where the microbial communities can be affected by a wide range of factors, such as soil characteristics, plant type, or agronomic practices which determine the presence or predominance of determined types of bacteria. Rovira (1956) reported that only a small part of the root surface is covered by bacteria. Successful biological control on the basis of plant-associated antagonists not only requires better knowledge of the complex regulation of disease suppression by antagonists in response to biotic and abiotic factors but also knowledge of the dynamics and composition of plant-associated microbial communities and what triggers plant colonization (Normander and Prosser 2000). The most popular sites for bacterial growth are junctions between epidermal cells and areas where side roots appear. Poor rhizoplane colonization is a factor that can limit biocontrol efficacy (Schippers et al. 1987; Weller 1988). In recent years, it has been proven that root colonization indeed is required for some biocontrol mechanisms, such as antibiosis (Chin-A-Woeng et al. 2000) and CNN (Kamilova et al. 2005; Validov 2007).

PGPRs colonize the rhizosphere, the rhizoplane (root surface), or the root itself (within radicular tissues) (Gray and Smith 2005). To act efficiently, the biocontrol agent should remain active under a large range of conditions, such as varying pH, temperature, and concentrations of different ions. These requirements are not easy

to fulfill. Therefore, it is not surprising that the efficacy of several first-generation commercial biocontrol products (Kamilova et al. 2005; Validov 2007) is not always sufficient. For a bacterium to be suitable for biocontrol, it must not only synthesize and release the antibiotic but also compete successfully. Also, the bacterium should escape in sufficient numbers from predators feeding on rhizosphere bacteria like protozoan grazers (Jousset et al. 2006). Furthermore, the bacterium should produce the antibiotic in the right microniche on the root surface (Pliego et al. 2008). It would be very useful to match correctly the appropriate PGPR with the right plant and environmental condition to achieve the best results on plant growth. In this sense, more effort should be done on the development of good inoculant delivery systems that facilitate the environmental persistence of the PGPR and this fact would allow diminishing the number of chemical pesticides used for enhanced crop productivity (Perez-Montano et al. 2014).

To increase the efficacy of disease control, seeds were inoculated with two strains that use different mechanisms of biocontrol, but such cocktails did not result in better disease control. An explanation to this is that the cell numbers of each of the two bacteria on the root are reduced below the threshold level required for control. Bacteria indigenous to soil compete with biocontrol strains for root colonization and produce various factors that can decrease the beneficial effect of a biocontrol strain. Because new stonewool is practically free from living microbes, it has the disadvantage that incoming pathogens destroy many plants in a greenhouse but the advantage that such a system can easily be buffered with biocontrol bacteria. For example, *P. putida* PCL1760 remains the dominant microbe on the root for at least 3 weeks and has a high affinity for stonewool (Validov 2007). A similar effect was found in salinated desert soil in Uzbekistan, which is poor in organic matter and therefore in indigenous microflora. The indigenous microflora is rich in plant pathogens as well as potential human pathogens (Berg et al. 2005; Egamberdiyeva et al. 2008). Under these circumstances, seed inoculated with biocontrol bacteria adapted to these stress conditions strongly decreases the level of plant diseases and may help to protect field-workers from exposure to pathogens (Egamberdiyeva et al. 2008). It is well understood that as our understanding of mechanisms of biocontrol and selection procedures for active strains increases, biocontrol products will improve and therefore biocontrol has a good future (Copping 2004). A summary of biomolecules involved in various mechanisms of biocontrol offered by PGPRs is presented in Table 11.1.

11.3 Molecular Basis of Biocontrol by Rhizobacteria

PGPRs are able to control fungal and bacterial plant diseases in suppressive soils. The mechanisms responsible for this biocontrol activity include competition for nutrients, niche exclusion, ISR, and the production of antifungal metabolites (AFMs), etc. The biocontrol agents belonging to the genus *Pseudomonas* are best characterized at the molecular level. The molecular basis of various biocontrol mechanisms is discussed here.

Table 11.1 Summary of biomolecules involved in various mechanisms of biocontrol offered by PGPRs

Mechanism of biocontrol	PGPR	Molecule involved
Antibiotics	*Bacillus* sp.	Zwittermicin A (aminopolyol) Kanosamine (aminoglycoside) Lipopeptides (surfactin, iturin, and fengycin) d-Gluconic acid 2-Hexyl-5-propyl resorcinol Polyketide Polymyxin Circulin Colistin
	Pseudomonas sp.	Pyrrolnitrin Phenazines (phenazine-1-carboxamide and phenazine-1-carboxylic acid) 2-4-Diacetylphloroglucinol (DAPG) Pyoluteorin Cyclic lipopeptides (viscosinamide and tensin) Amphisin Oomycin A Tropolone N-acyl-homoserine lactone
Volatile organic compounds	*Bacillus* spp.	Acetoin, 2,3-butanediol
	Pseudomonas sp.	Hydrogen cyanide (HCN)
Biosurfactants	*Bacillus* spp.	Lipopeptides
	Pseudomonas sp.	Lipopeptides (rhamnolipid)
Bacteriocins	*Pseudomonas* sp.	N-acyl-homoserine lactone N-(3-hydroxy-7-cis-tetradecanoyl)-homoserine lactone
	P. pyogenes	Pyocins
	B. megaterium	Megacins
Lytic enzymes	*Bacillus* sp. *Pseudomonas* sp.	Lysozymes (chitinase, β 1,3 – glucanase, proteases, lipases, dehydrogenase, phosphatases, proteases)
Elicitors of induced systemic resistance	*P. fluorescence*	Jasmonic acid (JA) Ethylene (ET) Citric acid
	B. cereus	Jasmonic acid (JA) Ethylene (ET)
	B. subtilis	Cell wall polysaccharides (lipopolysaccharides, i.e., LPS, and exopolysaccharides, i.e., EPS)
	Azospirillum	Jasmonic acid (JA) Ethylene (ET)

(continued)

Table 11.1 (continued)

Mechanism of biocontrol	PGPR	Molecule involved
Elicitors of systemic acquired resistance	*P. fluorescence*	Salicylic acid (SA)
Interference with quorum sensing due to degradation of autoinducers, homoserine lactones (AHLs)	*B. thuringiensis*	Acyl-homoserine lactonase
	Ralstonia	Acyl-homoserine acylase
Siderophores	*P. fluorescence* *P. putida* *Bradyrhizobium* *Rhizobium* *Serratia* *Streptomyces* *Bacillus* spp. *Klebsiella*	Pyoverdine Pseudobactin Carboxylate Hydroxamate Phenol catecholate
Stress controllers	*Thielaviopsis basicola* *P. putida* *Azotobacter chroococcum*	1-aminocyclopropane-1-carboxylate (ACC)
Chemoattractant of hyperparasitism	*P. fluorescence*	Fusaric acid

11.3.1 Regulation of Antibiotic Production

Individual genes have been discovered that are responsible for the presence of functional groups of phenazine compounds. For example, *phzO* is required for the 2-hydroxy group (Delaney et al. 2001) and *phzH* is responsible for the 1-carboxamide group (Chin-A-Woeng et al. 2001). On the basis of homology and mutant studies, functions have been proposed for many other identified biosynthetic genes. However, biochemical studies are required to prove their functions. *Streptomyces* and *Bacillus* species also provide biocontrol, which is being characterized at the molecular level. In *B. cereus*, the biosynthetic gene cluster responsible for the production of the antibiotic zwittermicin A has been identified (Stohl et al. 1999).

11.3.2 Regulation of Antifungal Metabolite Production

The production of AFMs in *Pseudomonas* subjects to complex regulation. In *P. fluorescens*, key factors in the regulation of the biosynthesis of most AFMs are global regulation and QS. Global regulation is directed by the *gacS*/*gacA* genes, which encode a two-component regulatory system that senses a yet unknown signal. QS involve the production of AHL signal molecules by an AHL synthase gene LuxI (Bassler 1999). AHL sufficiently binds to and activates a transcriptional regulator LuxR at a threshold concentration of AHL, which is reached only when a certain density of bacterial cells is present. The activated form of the

transcriptional regulator then stimulates gene expression. Spontaneous *gacS* or *gacA* mutants of *P. fluorescens* strain CHA0 have a substantial selective advantage over the wild-type strain when grown in a liquid medium. It has been demonstrated in a nutrient broth medium containing yeast extract by mineral amendments or by diluting the medium that partially explains the observed stimulation of AFM production in *P. fluorescens* strain CHA0 by various minerals and carbon sources (Duffy and Defago 1999; Duffy and Defago 2000). The involvement of GacS/GacA in the regulation of extracellular products, such as protease, HCN, and other AFMs, has been established firmly. It is shown that GacA indirectly controls the HCN synthase genes *hcnABC* and the protease gene *aprA* in *P. fluorescens* CHA0 by a posttranscriptional mechanism involving a distinct recognition site that overlaps the ribosomal binding site (Blumer et al. 1999). It is also demonstrated that the global translational repressor RsmA acts downstream of the GacA-dependent pathway (Blumer et al. 1999), but some products of the *infC* operon compete with RsmA and stimulate production (Blumer and Haas 2000a). At the transcriptional level, the *hcnABC* genes are regulated by the anaerobic regulator ANR. An AHL from biocontrol strain *P. fluorescens* F113 was elucidated and surprisingly identified as the rhizobial small bacteriocin *N*-(3-hydroxy-7-cis-tetradecanoyl) homoserine lactone (Schripsema et al. 1996). The production of this bacteriocin and two more common AHLs is directed by the *hdtS* gene product, which belongs to a novel class of acyl synthases (Laue et al. 2000). Other forms of regulation of AFM production include a global regulation of AFM production by the Lon protease in *P. fluorescens* Pf-5 (Whistler et al. 2000), regulation of DAPG production by the transcriptional repressor PhlF (Delany et al. 2000; Delaney et al. 2001), and the positive effect of PrrB RNA on secondary metabolite production in *P. fluorescens* F113 (Aarons et al. 2000). Another finding in *P. fluorescens* CHA0 is the regulation of DAPG production by autoinduction and its repression by salicylate and pyoluteorin produced by the same cells, as is the repression of DAPG production by the fungal metabolite fusaric acid (Schnider-Keel et al. 2000). In contrast, phenazine-1-carboxamide production in strain PCL1391 is not subjected to autoinduction (Chin-A-Woeng et al. 2001).

In *P. aeruginosa*, the activity of anaerobic regulator ANR has also been indicated to respond to iron availability (Blumer and Haas 2000b) and to AHL signal molecules (Pessi and Haas 2000). It was shown that a mutation in the *lexA* gene of *P. chlororaphis* PCL1391 resulted in a ten-fold increase in phenazine-1-carboxamide production, which can be explained by the fact that the *lexA* mutant produces elevated levels of AHL (Chin-A-Woeng 2000). An exciting report on transgenic plants that produce various AHLs (Fray et al. 1999) presented a new perspective on biocontrol and on optimizing the application of biocontrol strains. It was suggested that plants can produce and secrete substances that mimic AHL activity and could, therefore, influence the density-dependent behavior of rhizobacteria (Teplitski et al. 2000).

11.3.3 Regulation of Quorum Sensing

The GacS/GacA regulatory system also controls QS, as was shown in *P. aureofaciens* 30-84 (Chancey et al. 1999), illustrating the enormous complexity of the regulation of secondary metabolite production in *Pseudomonas*. In *Pseudomonas* biocontrol agents, various quorum systems have been identified that are involved in the regulation of AFMs. A complex regulation by QS has also been identified in *Rhizobium leguminosarum*, which contains multiple QS systems that form a regulatory cascade (Lithgow et al. 2000).

11.3.4 Regulation of ISR and SAR

ISR and SAR act through different signaling pathways. Obviously, the easy handling of the *Arabidopsis thaliana* plant is being the main model for PGPR-elicited ISR studies (Ruy et al. 2004). Induction of SAR is through salicylic acid (SA), and ISR requires jasmonic acid (JA) and ethylene (ET) signaling pathways (Van Loon et al. 1998; Van Loon 2007; Glick 2012). ISR is triggered by nonpathogenic microorganisms and starts in the root, extending to the shoot. However, the metabolic pathway involved in this process is poorly studied (Ramos et al. 2008). *Arabidopsis* plants inoculated with the pathogen *P. syringae* pv. tomato or sprayed with SA developed SAR and accumulated PR-1, PR-2, and PR-5 mRNAs (Pieterse et al. 1996) and inoculated with *P. fluorescens* WCS417r or *P. putida* WCS358 developed ISR, but PR gene expression or accumulation of PRs was not detected (Van Wees et al. 1997). ISR can be triggered in NahG mutant plants that are unable to accumulate SA. Based on this, one can conclude that PRs are induced concomitantly with SAR, whereas SA and the activation of PR genes are not a part of the pathway leading to ISR in *Arabidopsis* (Pieterse et al. 1996). These signaling molecules coordinate the activation of a large set of defense responses and when applied exogenously, can induce resistance themselves (Pieterse et al. 1998). The *Arabidopsis* JA response mutant *jar1* and the ET response mutant *etr1* were tested for the development of ISR. Both mutants were unable to develop ISR against *P. syringae* pv. tomato upon colonization of the roots by WCS417r bacteria (Pieterse et al. 1998), illustrating the dependency of ISR signaling on these phytohormones. Methyl jasmonate (MeJA) and the ET precursor 1-aminocyclopropane-1-carboxylate (ACC) also promote resistance against *P. syringae* pv. tomato DC3000 in SA-non-accumulating NahG plants. Besides that, MeJA-induced protection is blocked in *jar1*-1, *etr1*-1 and *npr1*-1 plants, whereas 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 ET response pathways are successively engaged to trigger a defense reaction that, like SAR, is regulated by NPR1 (Pieterse et al. 1998). The evidence shows that for induction of resistance, it is an essential specific recognition between the plant and the rhizobacteria. For instance,

Pseudomonas putida WCS358r and *P. fluorescens* WCS374r act in different ways depending on the plant species. In *Arabidopsis*, WCS358r elicits ISR, but not in radish and carnation plants (Van Peer et al. 1991; Van Peer and Schippers 1992; Leeman et al. 1995; Van Wees et al. 1997). In contrast, radish plants are responsive to WCS374r, while *Arabidopsis* is not (Leeman et al. 1995; Van Wees et al. 1997). SA accumulation occurs both locally and, at lower levels, systemically, in line with the development of SAR. Application of exogenous SA also induces SAR in many plant species (Van Loon et al. 1998). 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). Some of these pathogenesis-related proteins (PRs) are 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 *Arabidopsis* mutant *npr1* does not express PR genes and does not exhibit SAR. Since rhizobacteria-mediated ISR is independent of SA and not associated with PRs, it is expected that ISR would still be expressed in this mutant. However, the *npr1* mutant of *Arabidopsis* does not display *P. fluorescens* WCS417r-mediated ISR. This implies that 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). Reports of Pieterse et al. (1996, 1998, 2000) with the reference rhizobacterial strain *P. fluorescens* WCS417r demonstrated that, at least in *Arabidopsis*, WCS417r-mediated ISR functions independently of SA, depended on NPR1, but required components of the JA and ET response pathways.

In SAR, the first infection predisposes the plant to resist further attacks. SA activates specific sets of defense-related genes called PRs. Generally, ISR is not accompanied by the activation of PR genes. The enhanced defensive capacity characteristic of SAR is always associated with the accumulation of PRs (Van Loon 2007). 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; Vleesschauwer and Hofte 2009). In noninduced plants, NPR1 is present as a multimer, and during SAR induction, SA triggers the conversion of NPR1 into a monomeric form (Verhagen et al. 2006). These monomers are translocated to the nucleus (Kinkema et al. 2000), where 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 (Despres 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).

In fact, Lopez-Baena et al. (2009) showed that the absence of "nodulation outer proteins" from *Sinorhizobium fredii* HH103, secreted across the type III secretion system, provoked a higher induction of SA-dependent PR1 gene with respect to the wild type despite this microorganism being a soybean symbiotic bacterium, that is,

a non-pathogenic bacteria. Elucidation of the plant factors involved in the pathways leading to ISR and SAR has shown that induced disease resistance can be enhanced by the simultaneous activation of ISR and SAR pathways (Van Wees et al. 2000).

11.3.5 Regulation of Rhizosphere Competence

Inoculant bacteria are often applied in seed coatings. After sowing, the inoculant bacteria should be able to establish itself in the rhizosphere at population densities sufficient to produce a beneficial effect. Therefore, efficient inoculant bacteria must survive in the rhizosphere, should use the nutrients exuded by the plant root, should proliferate, should be able to efficiently colonize the entire root system, and should be able to compete with indigenous microorganisms. In field experiments, poor root colonization has often been found to be correlated with inadequate biocontrol. Identification of the genes and traits involved in the processes of inoculation and root colonization gives a more detailed insight into plant–bacterial interactions and leads to the more efficient application of inoculant strains (Bloemberg and Lugtenberg 2001).

P. fluorescens genes that are specifically expressed in the rhizosphere (i.e., *rhi* genes) have been identified using in vivo expression technology (IVET) (Rainey 1999). More than 20 *rhi* genes have been identified, of which 14 showed significant homology to genes that are involved in nutrient acquisition, stress response, or secretion. Another six *rhi* genes show no homology to genes with identified functions. Many root colonization genes and traits from *Pseudomonas* biocontrol species have been identified (Lugtenberg and Dekkers 1999; Lugtenberg et al. 2001) and have even been used to improve colonization of wild-type strains of *Pseudomonas* (Dekkers et al. 2000).

The first step in the inoculation process is the attachment of the bacterial cells to the seed. A screen for mutants of the rhizobacterial strain *P. putida* KT2440 resulted in the identification of a set of putative surface and membrane proteins involved in attachment to corn seeds. Among these proteins are homologs of a calcium-binding protein, of hemolysin, and of a potential multidrug efflux pump (Espinosa-Urgel et al. 2000).

Efficient scavenging for iron using siderophores makes *Pseudomonas* strains more competitive. On the basis of identified colonization genes and traits, colonization mutants of *P. chlororaphis* strain PCL1391 have been constructed. These mutants have lost their ability to control TFRR, showing for the first time that root colonization is an essential trait for biocontrol (Chin-A-Woeng et al. 2000). To come to a better understanding of how bacteria function in the rhizosphere, the plant should also be taken into account. Therefore, the effect of the rhizosphere of different recombinant inbred tomato lines on the ability of *B. cereus* to control *Pythium torulosum* was analyzed (Smith et al. 1999). The results indicated a genetic basis from the plant side for the efficient growth and performance of biocontrol agents in the rhizosphere. Interestingly, a follow-up study showed a clear and consistent growth difference for *B. cereus* in the spermosphere of the different inbred tomato

lines, whereas this difference was not observed for two *Pseudomonas* biocontrol species (Simon et al. 2001).

In the rhizosphere, inoculant bacteria compete for nutrients and niches with endogenous microorganisms, such as other bacteria and fungi. Studies have shown that organic acids form the nutritional basis of rhizosphere colonization. A defect in the utilization of organic acids, which form the major group of tomato exudates, results in decreased competitive colonization of the tomato rhizosphere, whereas a defect in the use of sugars does not result in a colonization defect (Lugtenberg et al. 1999). Genes of *Pseudomonas* biocontrol strains have been identified that can be induced or repressed by the presence of phytopathogenic fungi. IVET technology has been used to show that the presence of *Phytophthora parasitica* can induce various genes in *P. putida*, including genes encoding diacylglycerol kinase, ABC transporters, and outer membrane porins (Lee and Cooksey 2000). In contrast, two ribosomal RNA operons of *P. fluorescens* were found to be repressed by *Pythium ultimum* (Smith et al. 1999).

11.3.6 Improvement of PGPRs by Genetic Modification

Identification of genes responsible for the ability of rhizobacterial strains to improve plant growth creates the potential to genetically modify biocontrol strains for improved performance or to construct novel biocontrol strains. For example, complete operons, as well as single genes under the control of their own regulatory genes or regulated by the constitutive expression of the *tac* or *lac* promoters, have been transferred to rhizobacterial strains (Bloemberg and Lugtenberg 2001). *P. fluorescens* strains carrying a mini-Tn5 vector that included the complete biosynthetic operon for the antifungal metabolite phenazine-1-carboxylic acid (PCA) were enhanced in their rhizospheric competence, as well as in their ability to suppress fungal diseases (Timms-Wilson et al. 2000). There have been other studies in which the introduction of genes into rhizobacterial strains has enhanced biocontrol potential. These genes include the Cry-toxin-encoding *cry1Ac7* gene of *B. thuringiensis* (Downing et al. 2000), the chitinase-encoding *chiA* gene of *Serratia marcescens* (Downing et al. 2000; Downing and Thomson 2000), and the 1-aminocyclop ropane-1-carboxylic acid (ACC) deaminase gene from *Enterobacter cloacae* (Wang et al. 2000). In addition, Dekkers et al. (2000) showed that the transfer of the *sss* gene of *P. fluorescens* WCS365 can enhance the competitive colonization ability of other *P. fluorescens* strains. Similarly, the biocontrol potential of *Pseudomonas* strains producing PCA was extended after the introduction of the *phzH* gene from *P. chlororaphis* PCL1391. The introduction of this gene resulted in the production of phenazine-1-carboxamide (PCN) by these strains and improved their ability to control TFRR disease (Chin-A-Woeng et al. 2001).

11.3.7 Whole-Genome Analysis

The revolutionary technological advancements in high throughput DNA sequencing have resulted in the publication of many whole-genome sequences. The sequencing of approximately 35 microbial genomes has been completed (http://www.tigr.org/tdb/mdb/mdbcomplete.html), and those of another 150 were in progress (http://www.tigr.org/tdb/mdb/mdbiprogress.html). Among these are several rhizosphere-inhabiting bacteria such as *P. aeruginosa* (Stover et al. 2000), *P. putida*, *P. fluorescens*, *P. syringae* pathovar *tomato*, *Sinorhizobium meliloti*, *Mesorhizobium loti* (Kaneko et al. 2000), *Bacillus subtilis* (Kunst et al. 1997), and *Streptomyces coelicolor*. In addition, a genomic encyclopedia of the rhizobacterial strain *P. fluorescens* SBW25 (PfSBW25) has been built on the basis of short-run noncontiguous sequence data at Oxford University (URL http://www.plants.ox.ac.uk/sbw25/) (Spiers et al. 2001). The obtained sequence data, by comparative and functional genomics, facilitated the identification of genes that are specifically present in PGPR that are specifically expressed on the seed or in the rhizosphere, that are involved in the regulation and production of secondary metabolites (e.g., antifungal metabolites) or whose expression is influenced by other rhizosphere organisms, such as fungi. The construction of bacterial artificial chromosome (BAC) libraries for the study of gene expression and to identify genes of interest is of great value, especially in the study of bacteria whose genome has not been sequenced, as has been shown for *B. cereus* (Rondon et al. 1999).

11.3.8 Visualization of Bacteria and Expression of the Gene in the Rhizosphere

After inoculation, PGPR must establish in the rhizosphere where they interact with the root and with the indigenous population of microorganisms, which include phytopathogenic fungi and mycorrhiza as well. To visualize and monitor bacterial populations in the rhizosphere, progress has been made during the past years by using confocal laser scanning microscopy (CLSM) in combination with various fluorescent markers (Bloemberg and Lugtenberg 2001). The results of these studies demonstrate that *Pseudomonas* biocontrol strains colonize the seed and root surface at the same locations as do the plant pathogenic fungi that they control (Lugtenberg et al. 1999; Tombolini et al. 1999). A combination of immunofluorescence and an rRNA-targeting probe that monitors the presence and metabolic activity of *P. fluorescence* DR54 inoculant cells in the sugar beet rhizosphere (Lubeck et al. 2000) showed that bacteria at the root tip are metabolically most active and that indigenous bacteria enter the rhizosphere 2 days after inoculation. Visualization of interactions among the carrot roots, mycorrhizal mycelium, and *P. fluorescens* CHA0 bacteria showed that mucoid mutant strains of CHA0 adhere much better to the root,

indicating that acidic extracellular polysaccharides can enhance root colonization (Bianciotto et al. 2000). The study of microbial communities has been facilitated by the use of combinations of the green fluorescent protein (GFP), its color variants cyan fluorescent protein (CFP) and a yellow fluorescent protein (YFP), and DsRed as markers. Broad host range plasmids expressing the *e-GFP*, *e-yfp*, *e-cfp*, or the *rfp* (*red fluorescent protein*) have been constructed using rhizosphere stable vectors (Bloemberg et al. 2000; Heeb et al. 2000; Stuurman et al. 2000). Using these plasmids, up to three differently marked bacterial populations could be studied on the root. Such experiments have indicated that *Pseudomonas* microcolonies on the root surface are initiated by one bacterial cell and that bacteria from outside the growing colony can join, as indicated by the frequent presence of mixed-color colonies on the older part of the root (Dekkers et al. 2000; Bloemberg et al. 2000).

The construction of unstable variants of the GFP (Andersen et al. 1999) made it possible to study transient gene expression in the rhizosphere, as has been shown for ribosomal activity in *P. putida* cells by Ramos et al. (2000). A gfp-based system for the detection of AHLs is demonstrated by Andersen et al. (2001) that allow the visualization of QS and cross-talk among rhizospheric bacteria. Visualization of gene expression in the rhizosphere provides detailed information on the functioning of bacterial cells in a specific environment. Spatiotemporal analysis of gene expression in the rhizosphere is made possible by visualizing bacterial cells that harbor an unstable *gfp* variant under control of the promoter to be analyzed and the constitutive expression of *rfp* (Bloemberg and Lugtenberg 2001).

11.4 Application of PGPRs on Crop Diseases

Due to the ever-growing human population, it has become imperative to maintain high productivity, but it has also become essential to alter the environment as little as possible, to achieve this. It is an utmost necessity to work for more environmentally sustainable agriculture by maintaining ecosystems and biodiversity. One promising way to decrease negative environmental impact resulting from the continuous use of chemical pesticides is the use of PGPR (Perez-Montano et al. 2014). Their beneficial effects on plants can be achieved by the direct interaction with their host plant and also indirectly due to their antagonistic activity against plant pathogens. Different PGPRs can be administered to crops in some formulations that are commercially available (Lucy et al. 2004), and recently, the popularity of microbial inoculants has substantially increased, due to extensive and systematic research that has enhanced effectiveness and consistency of PGPRs (Thakore 2006; Berg 2009). Moreover, numerous studies are being conducted to evaluate plant growth effects by applying different microbial consortia or combinations, like AMF–PGPR, symbiotic-nitrogen-fixing rhizobia-PGPR, or different PGPR (Singh and Kapoor 1999; Swarnalakshmi et al. 2013). The progress in using PGPRs on some crop plants is discussed here.

11.4.1 Cereals

11.4.1.1 Maize (*Zea mays*)

The toxigenic fungus Fusarium is one of the major genera associated with maize. Some PGPRs such as *B. amyloliquefaciens* and *Microbacterium oleovorans* were able to protect maize against *Fusarium verticillioides* when applied in the form of seed coatings (Pereira et al. 2011). Interestingly, some PGPR species have appeared to promote plant growth by acting both as biofertilizers and as biocontrol agents. Strains of *B. cepacia* have been recorded with biocontrol characteristics against *Fusarium* spp., along with growth stimulation of maize under iron-poor conditions by siderophore production (Bevivino et al. 1998). *Burkholderia* sp. has been reported to suppress Maize rot (Singh 2013).

11.4.1.2 Rice (*Oryza sativa*)

More than 70 diseases affecting rice crops have been reported as causing estimated yield losses of 5–30%, depending on the year, zone, rice cultivar, pathogen, etc. The three main rice pathogens *Xanthomonas oryzae* pv. *oryzae*, *Rhizoctonia solani*, and *Magnaporthe oryzae* are responsible for bacterial leaf blight, sheath blight, and blast on rice plants, respectively. Most of the studies on the use of PGPR in rice biocontrol are focused on the treatment and prevention of these diseases (Han et al. 2005). *Bacillus* and *Pseudomonas* are the predominant PGPR genera used against these pathogens, due to their antagonism against the growth of several fungal and bacterial microorganisms. This PGPR usually produces siderophores, antibiotics, chitinases, and proteases, which could be responsible for the antagonism against pathogens. Satisfactory yield due to disease control by these PGPRs is observed in the greenhouse and in field experiments by reducing the severity of diseases up to 90% depending on the PGPR, pathogen, and rice cultivar (Filippi et al. 2011). *P. fluorescens* has been found effective against Sheath blight disease of rice (Singh 2013). *Bacillus* sp. and *Azospirillum* can control rice blast disease (Singh 2013). Fluorescent *Pseudomonas* spp. is reported to control rice sheath rot caused by *Sarocladium oryzae* (Singh 2013). Seed and foliar application of *P. fluorescens* have been found to reduce sheath blight of rice (Singh 2013).

11.4.1.3 Wheat (*Triticum aestivum*)

Different wheat pathogens play a direct role in the destruction of natural resources in agriculture. Mavrodi et al. (2012) have isolated new strains of *Pseudomonas* from agricultural soils, river silt, and soils from herbarium specimens that show the ability to reduce disease symptoms of both *Rhizoctonia solani* and *Pythium ultimum*, two wheat soilborne fungal pathogens, correlated with growth promotion of wheat seedlings at the same time.

11.4.1.4 Pearl Millet or Bajra (*Pennisetum glaucum*)

B. subtilis and *B. pumilus* have been demonstrated to control Downy mildew disease of pearl millet (Singh 2013).

11.4.2 Pulses

11.4.2.1 Chickpea (*Cicer arietinum*)

The use of multistrain inoculants is also a good strategy that enables organisms to successfully survive and maintain themselves in communities. Singh et al. (2013) showed the synergistic effect of antagonistic fungi *Trichoderma* with combined application of *Pseudomonas* and demonstrated that rhizobial strains can protect chickpea from infection by the collar rot pathogen *Sclerotium rolfsii*.

11.4.2.2 Black Gram or Urdbean (*Vigna mungo*)

Siddiqui et al. (2001) demonstrated the use of PGPR for the management of root diseases complex of urdbean. They showed the efficacy of two strains of *P. aeruginosa* (Pa-5 and IE-2) and a *B. subtilis* isolate for the management of three soilborne root-infecting fungi including *Macrophomina phaseolina*, *F. solani*, and *Rhizoctonia solani* and the root-knot nematode, *Meloidogyne javanica* on urdbean. The seed treatment and soil application of *P. fluorescens* reduced root rot of black gram caused by *M. phaseolina* (Singh 2013).

11.4.2.3 Pigeon Pea (*Cajanus cajan*)

B. subtilis, *P. chlororaphis,* and endophytic *P. fluorescens* inhibit the growth of stem blight pathogen *Corynespora capicola*. *B. subtilis* in peat supplemented with chitin-containing materials showed better control of *F. udum* in pigeon pea. Strains of *Burkholderia cepacia* have also been shown to have biocontrol of *Fusarium* spp. (Singh 2013).

11.4.3 Oilseeds

11.4.3.1 Mustard (*Brassica* sp.)

P. fluorescens has been reported as a potential biological control agent due to its ability as a good rhizospheric colonizer and protecting plants against root rot of mustard in field condition (Fenton et al. 1992; Kumar et al. 2002; Arora et al. 2008).

11.4.3.2 Soybean (*Glycine max*)

Nonrhizobial PGPR species, most of them are endophytes, were isolated from root nodules, leaves and stems and are ubiquitous in plant tissues, have been studied to restrict plant pathogens on soybean. Some genera like *Bacillus*, *Paenibacillus*, and *Pseudomonas* are actively being used for this purpose against plant pathogens like *R. solani*, *R. bataticola*, and *Colletotrichum* (Senthilkumar et al. 2009). However, it has been argued whether these biocontrol agents act as antagonists in the process, or they act as producers of ISR in the soybean.

11.4.3.3 Peanuts (*Arachis hypogea*)

Inoculation with *Trichoderma* sp. has been the preferred choice for novel biocontrol agents against *Aspergillus niger*, the causal agent of collar rot of peanut (Gajera and

Vakharia 2012). *B. subtilis* in peat supplemented with chitin showed better control of *A. niger* and *F. udum* in groundnut (Singh 2013).

11.4.4 Vegetables

11.4.4.1 Common Beans (*Phaseolus vulgaris*)

There are PGPRs of the genera *Bacillus* or *Pseudomonas* with an important role in biocontrol of bean diseases such as bacterial wilt caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* or root rot caused by *R. solani*, respectively (Neeraj 2011; Martins et al. 2013). Some PGPR shows different abilities, for instance, biocontrol and biofertilization, at the same time. Thus, inoculation with *B. cepacia* SAOCV2 is found to promote the growth of common beans by several mechanisms such as P mobilization, increasing 44% the plant P content and promoting also antagonism toward the pathogenic species of *Fusarium*. Moreover, this result is correlated with a larger number of nodules leading to an increase in N_2 fixation and indicates that the inhibition of fungal growth enhances the bacterial community in the plant rhizosphere, including rhizobia (Peix et al. 2001). Van Loon (2006) and Jourdan et al. (2007) reported that bacterial compounds as elicitors like lipopeptides especially surfactin and fengycin produced by *B. subtilis* strains were able to stimulate ISR in the bean plants and also decreased the impact of subsequent pathogen infection. They have also reported the cellular changes taken place in the plants as one of the major phenomena of restricting fungal development.

11.4.4.2 Tomato (*Lycopersicon esculentum*)

Cho and Chung-Soon (1998) investigated the effects of rhizobacteria on the early growth of tomato seedlings and reported that *Azospirillum* spp. and *Pseudomonas* spp. inhibited the growth of *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. Khan and Akram (2000) evaluated the effect of soil application of *Paecilomyces lilacinus*, *Gliocladium virens*, *P. fluorescens* PRS-9, *B. polymyxa*, and pesticides (aldicarb + thiram) against a wilt disease complex of tomato caused by *M. incognita* and *F. oxysporum* f. sp. *lycopersici* and on growth and yield of tomato in field plots. The nematode and fungus acting alone caused characteristic root galling and shoot wilting, respectively, and significantly reduced plant growth and yield. In concomitant inoculation, severity of fusarial wilt was significantly increased and plant growth and yield reductions were also considerably greater compared to the sum of individual effects of the pathogens. On such plants, pathogenesis and reproduction of the nematode were, however, suppressed. Application of *P. lilacinus* and *G. virens* significantly enhanced the plant growth and yield of nematode and fungus-inoculated plants, respectively. Greatest enhancement of plant growth and yield of nematode fungus-infected plants occurred with *P. fluorescens*, followed by the pesticides and *G. virens* or *P. lilacinus*. Hatching of larvae, gall formation, egg mass production, fecundity, and soil population of *M. incognita* and wilting index and rhizospheric population of *F. oxysporum* f. sp. *lycopersici* were decreased due to application of the control agents except for *B. polymyxa*, which caused increase in

the dry matter production and yield of uninfected tomato plants and soil population and fecundity of the nematode and rhizosphere population of the fungus (Khan and Akram 2000). Similarly, Hanafi and Fellah (2006) reported inoculation of tomato plants with *B. subtilis* effective against root fungal pathogen *Pythium* spp. Van Loon (2006) and Jourdan et al. (2007) have investigated the role of bacterial compounds as elicitors of the ISR. They reported that the lipopeptides, especially surfactin and fengycin, produced by *B. subtilis* strains are able to stimulate tomato plants and also decreased the impact of subsequent pathogen infection. Cellular changes have also been reported as one of the major phenomenon of restricting fungal development in the plants. Many rhizospheric isolates belonging to *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp., and *Pichia* spp. collected from tomato growing areas showed antagonistic activity against *Botrytis cinerea*, *F. graminearum*, *Colletotrichum capsici*, *Alternaria solani*, *P. capsici*, and *Mycosphaerella melonis* (Guo-Jian et al. 2002; Rahman and Khan 2002; Yuan and Zhou 2006; Sadfi et al. 2007; Nguyen and Ranamukhaarachchi 2010; Lin et al. 2010). Sivakumar et al. (2008) evaluated the efficacy of antagonistic microorganisms against *F. oxysporum f.sp lycopersici* and reported minimum disease incidence with antagonistic microorganisms. Out of the 47 PGPRs isolated from a range of monocots, several were found to have highly inhibitory effects against the tomato disease TFRR caused by fungal pathogen *F. oxysporum radicis-lycopersici* and other pathogens *Sclerotium bataticola*, *Pythium ultimum*, and *F. graminearum* (Lugtenberg and Kamilova 2009; Laslo et al. 2012). *B. cereus* is found effective against foliar diseases of tomato (Singh 2013).

PGPR all over the world has been reported to reduce the virus attack on the crops. Murphy et al. (2000) studied the effect of PGPR strains for ISR against cucumber mosaic virus on tomato. Kandan et al. (2005) investigated the biocontrol efficacy of strains of *P. fluorescens* against tomato spotted wilt virus (TSWV) in tomato and reported a significant reduction in TSWV incidence with a concomitant increase in growth promotion and yields in both the glasshouse and field conditions. Hanafi and Fellah (2006) studied their effect on whitefly transmitted tomato mottle virus in tomato plants. They all concluded that tomatoes treated with PGPR demonstrated a reduction in the incidence of viral infection and a significant increase in tomato yields. *B. pumilus*, *Kluyvera cryocrescens*, *B. amyloliquefaciens*, and *B. subtilis* are found effective against cucumber mosaic cucumovirus (CMV) of tomato (Singh 2013). *B. amyloliquefaciens*, *B. subtilis*, and *B. pumilus* are found effective against tomato mottle virus (Singh 2013).

11.4.4.3 Brinjal or Eggplant (*Solanum melongena*)

The influence of some bacterial isolates of *Mycobacterium* spp., *Micrococcus* spp., *Escherichia coli*, *B. subtilis*, *S. marcescens*, *P. aeruginosa*, and *Sarcina* spp. as biocontrol agents was evaluated by Shalaby and Sedik (2008) against the root-knot nematode *M. incognita* infecting eggplant, under greenhouse conditions, and they reported that most of the tested bacterial isolates significantly reduced numbers of galls, their developmental stages, and egg masses in roots. Ramesh et al. (2009) characterized and identified 28 antagonistic endophytic bacterial isolates associated

with eggplant against *Ralstonia solanacearum* causing bacterial wilt and observed that plants treated with *Pseudomonas* isolates (EB9, EB67), *Enterobacter* isolates (EB44, EB89), and *Bacillus* isolates (EC4, EC13) reported reduced wilt incidence by more than 70%.

11.4.4.4 Cucumber (*Cucumis sativus*)

PGPR strains INR7 *(B. pumilus)*, GB03 *(B. subtilis)*, and ME1 *(Curtobacterium flaccumfaciens)* were tested singly and in combinations for biocontrol against multiple cucumber pathogens. Investigations under greenhouse conditions were conducted with three cucumber pathogens *Colletotrichum orbiculare* (causing anthracnose), *Pseudomonas syringae* pv. *Lachrymans* (causing angular leaf spot), and *Erwinia tracheiphila* (causing cucurbit wilt disease) inoculated singly and in all possible combinations. There was a general trend across all experiments toward greater suppression and enhanced consistency against multiple cucumber pathogens using strain mixtures. The same three PGPR strains were evaluated as seed treatments in two field trials over two seasons, and two strains, *IN26 (Burkholderia gladioli)* and INR7, also were tested as foliar sprays in one of the trials. In the field trials, the efficacy of ISR activity was determined against introduced cucumber pathogens naturally spread within plots through the placement of infected plants into the field to provide the pathogen inoculum. PGPR-mediated disease suppression was observed against angular leaf spot in 1996 and against a mixed infection of angular leaf spot and anthracnose in 1997. The three-way mixture of PGPR strains (INR7 plus ME1 plus GB03) as a seed treatment showed intensive plant growth promotion and disease reduction to a level statistically equivalent to the synthetic elicitor Actigard applied as a spray (Raupach and Kloepper 1998). Cho and Chung-Soon (1998) investigated the effects of rhizobacteria on early growth of cucumber seedlings and reported that *Azospirillum* spp. and *Pseudomonas* spp. inhibited the growth of *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp. *B. pumilus* is found effective against bacterial wilt disease in cucumber (Singh 2013). Singh (2013) has also demonstrated the utility of *B. subtilis* in controlling CMV in cucumber. Similarly, *B. amyloliquefaciens* has been found to suppress wilt caused by *F. oxysporum* in cucumber (Yuan et al. 2013).

11.4.4.5 Squash (*Cucurbita* spp.)

Bacillus spp. is reported to suppress blight of squash (Singh 2013).

11.4.5 Fruits

11.4.5.1 Papaya (*Carica papaya*)

Crop management systems influence plant productivity and nutrient use efficiency, as well as PGPR, which is known to influence the growth of plants via phytohormone production, phosphate solubilization, nitrogen (N) fixation, and antimicrobial activity. Melo et al. (2016) compared the influence of two crop management systems on microbial PGPR features. PGPR isolated from the rhizospheres of papaya

grown under two distinct management systems (conventional and organic) were identified and characterized. The 12 strains most efficient in solubilizing inorganic phosphate belonged to the genera *Burkholderia*, *Klebsiella,* and *Leclercia.* Nitrogen fixation was observed in the strains *B. vietnamiensis* from the conventional farming system and *B. vietnamiensis*, *B. cepacia* and *Leclercia* sp. from the organic farming system. The *B. vietnamiensis*, *B. cepacia,* and *Klebsiella* sp. isolates showed antifungal activity, while *Leclercia* sp. did not. The strains *B. vietnamiensis* and *Enterobacter* sp. (isolated from the conventional farming system) and *Klebsiella* sp. (isolated from the organic farming system) were efficient at solubilizing phosphate, producing phytohormones and siderophores, and inhibiting the mycelial growth of various phytopathogenic fungi (*Botrytis cinerea*, *Pestalotia* sp., *Alternaria* sp., *Phoma* sp., *F. culmorum*, and *Geotrichum candidum*). *F. culmorum*, *Phoma* sp., and *Alternaria* sp. induce peduncular rot in papaya (Suzuki et al. 2007; Nery-Silva et al. 2007) seriously affecting papaya export and farmers' income. Physiological differences between the isolates from the two crop management regimes were distinguishable after 10 years of distinct management (Melo et al. 2016). The most effective isolates tested were *Klebsiella* sp. C1 and *B. vietnamiensis* C, which inhibited mycelial growth by 78% and 76%, respectively. *B. vietnamiensis* O inhibited mycelial growth of *G. candidum* by 81%. Inhibition of mycelial growth of *F. culmorum* by the isolates was over 30%, the greatest inhibition being by *B. vietnamiensis* C (67%), *Klebsiella* sp. O1 (68%), *B. vietnamiensis* O (66%), and *Klebsiella* sp. O3 (63%). The greatest inhibition of mycelial growth of the fungus *Pestalotia* sp. was by the isolates *B. vietnamiensis* C (68%) and *Klebsiella* sp. C1 (62%). The phytopathogenic fungus *Alternaria* sp. was inhibited by more than 30%. The greatest inhibition was by *Klebsiella* sp. C1 (45%), *B. vietnamiensis* O (58%), and *Klebsiella* sp. O2 (44%). *Phoma* sp. was inhibited by up to 40%, the greatest inhibition being by *Klebsiella* sp. C3 (40%) and *B. cepacia* O (37%). All the bacterial isolates inhibited the phytopathogenic fungus *Botrytis cinerea* by less than 44% and the greatest inhibition was that by *B. vietnamiensis* C, *B. cepacia* O, and *B. vietnamiensis* O (Melo et al. 2016).

11.4.5.2 Stone Fruits (Apple, Peach, Pear, etc.)

Biological control has been successfully applied using the nonpathogenic strain *Agrobacterium rhizogenes* K84 for almost 30 years. It was the first example of biocontrol against pathogenic strains (mainly biovar 2) of *Agrobacterium* in different hosts and countries all over the world (Lopez et al. 1987, 1989; Farrand 1990; Moore and Canfield 1996; Rhouma et al. 2004). Strain K84 is supplied commercially on agar plates or in a peat substrate and is used by suspending the bacterial cells in water, then dipping seeds, seedlings, or cuttings in this suspension before planting. In spite of the success of K84, some potential problems arise from its application (Moore and Canfield 1996). The principal cause of failure of efficacy of K84 was related to pAg84 transfer, following which the genes controlling agrocin 84 productions and resistance can be acquired from strain K84 by conjugal transfer, resulting in a breakdown of the biocontrol capacity (Lu 1994; Stockwell et al. 1996; Penalver and Lopez 1999) because the recipient becomes resistant to agrocin 84

thus remaining pathogenic. In order to avoid this transfer and safeguard crown gall biocontrol ability, the recombinant strain K1026 was engineered (Jones et al. 1988). The plasmid pAgK1026 is incapable of conjugal transfer to a detectable frequency in the laboratory (Jones et al. 1988). However, due to restrictions on the use of genetically modified organisms, K1026 is currently not utilized in many countries. Moreover, crown gall biocontrol using K1026 can break down via transfer of the Ti plasmid from a pathogenic *Agrobacterium* donor to K1026, which becomes pathogenic. Therefore, a search for other antagonists for controlling crown gall is currently underway all over the world.

Rhizobacteria are known as a good colonizer of the rhizosphere and include interesting antagonists to several soilborne plant pathogens (Rangajaran et al. 2003). Thus, in the work done by Rhouma et al. (2008), they explored the efficacy of 12 rhizobacterial strains screened from a collection of antagonists for their potential to control *A. tumefaciens* in vitro and in *planta*. Crown gall disease caused by *A. tumefaciens* is considered as the main bacterial disease of stone fruit rootstocks all over the world against which only prophylactic control measures are available. Therefore, 12 rhizobacterial strains and 2 reference antagonists *A. rhizogenes* K84 and K1026 were investigated for their efficacy against the causal agent of the disease in vitro, in pots, and in the field. In vitro and in pot experiments were carried out with three strains of *A. tumefaciens* (C58, B6, and AR125), whereas field trials were conducted in a nursery with a soil naturally contaminated by the bacterium. In vitro experiments revealed that *A. rhizogenes* (K84 and K1026) and *A. radiobacter* (O33, O34, MM8.2, and MM8.1) were effective only against nopaline-producing strains C58 and AR125. However, *B. subtilis* and *P. fluorescens* strains were effective also against nopaline- and octopine-producing agrobacteria. On the basis of pot tests, five rhizobacterial strains (O33, O34, BSCH14, BSCH15, BSCH16) and the two reference strains (K84, K1026), that significantly reduced the percentage of galled plants, were selected for field testing in a nursery. Apart from the reference antagonists, only rhizobacterial strains BSCH14 and O33 significantly reduced the percentage of galled plants to 3.85% and 5.19%, respectively. The preliminary characterization of the antibacterial compounds of *B. subtilis* BSCH14 showed that they were of proteinaceous nature, stable at 100°C for 60 min, and resistant to pH variation. The study has shown that effective biological control using rhizobacterial strains can be obtained, thus suggesting their possible use in crown gall disease management (Rhouma et al. 2008).

11.4.5.3 Banana (*Musa paradisiaca*)

Banana bunchy top virus (BBTV) is one of the deadly viruses which severely affects the yield of banana crop in Western Ghats, Tamil Nadu, India. It has been demonstrated that application of *P. fluorescens* strain significantly reduced the BBTV incidence in hill banana under greenhouse and field conditions (Singh 2013). Similarly, *B. amyloliquefaciens* has been found to suppress wilt caused by *F. oxysporum* in banana (Xu et al. 2014).

11.4.5.4 Grapes (*Vitis vinifera*)

Burkholderia sp. strain PsJN has been reported to endophytically colonize the grapes by Compant et al. (2005b).

11.4.6 Flowers

11.4.6.1 Cyclamen (*Cyclamen* sp.)

S. marcescens has been reported to have the ability to produce chitinolytic enzymes and antibiotics and has been found to suppress damping-off, caused by *R. solani* and *Fusarium* wilt caused by *F. oxysporum* (Someya et al. 2000) and gray mold caused by *Botrytis cinerea* in cyclamen (Someya et al. 2001).

11.4.7 Spices

11.4.7.1 Red Chilli or Bell Pepper (*Capsicum annuum*)

108 endophytic strains were isolated from capsicum plants by Hong et al. (2002). They reported strains BS-1 and BS-2 (identified as *B. subtilis*) as most effective as they exhibited 57.34–94.08% control against anthracnose disease caused by *Colletotrichum gloeosporioides*. Many rhizospheric isolates belonging to *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp., and *Pichia* spp. collected from pepper growing areas showed antagonistic activity against *Botrytis cinerea*, *F. graminearum*, *C. capsici*, *Alternaria solani*, *P. capsici*, and *Mycosphaerella melonis* (Guo-Jian et al. 2002; Rahman and Khan 2002; Yuan and Zhou 2006; Sadfi et al. 2007; Nguyen and Ranamukhaarachchi 2010; Lin et al. 2010). Suryanto et al. (2010) examined the ability of chitinolytic bacteria as a biocontrol agent of *Fusarium* wilt of red chilli seedlings and observed relative reduction in disease incidence ranged from 28.57% to 60.71% and further compared different biocontrol agents and ranked BK08 as the most potential candidate for biological control agent of *Fusarium* wilt. Quyet-Tien et al. (2010) found that *P. polymyxa* increased plant growth of pepper by decreasing the severity of *Xanthomonas axonopodis* pv. *Vesicatoria*. *Bacillus* spp. can suppress Blight of pepper (Singh 2013). Recently, Son et al. (2014) found that among selected PGPR isolates, four significantly decreased gray leaf spot disease severity with PGPR *Brevibacterium iodinum* KUDC1716 providing the highest disease suppression in pepper.

11.4.7.2 Black Pepper (*Piper nigrum*)

It is reported that *B. subtilis* controls soilborne pathogen of black pepper (Singh 2013). In another greenhouse study, Dinesh et al. (2014) found lowest root rot and taller plants by the application of *Burkholderia cepacia* BRB 21 in black pepper.

11.4.7.3 Ginger (*Zingiber officinale*)

In a study, 5 of the 100 isolates (*B. cepacia* GRB25, *B. amyloliquefaciens* GRB35, *S. marcescens* GRB58, *S. marcescens* GRB68, and *P. aeruginosa* GRB91) exhibited

>70% inhibition of *Pythium myriotylum* causing soft rot in ginger and were used for further growth promotion and biocontrol studies in the greenhouse and field. The greenhouse study revealed that GRB35 (*B. amyloliquefaciens*) and GRB68 (*S. marcescens*) registered markedly higher sprouting (96.3%) and lower disease incidence (48.1%) and greater rhizome yield (365.6 g pot^{-1} and 384.4 g pot^{-1}, respectively), while control registered the lowest sprouting (66%), maximum soft rot incidence (100%), and lowest rhizome yield (134.4 g pot^{-1}). In the field experiments also, *S. marcescens* GRB68 and *B. amyloliquefaciens* GRB35 registered the greatest sprouting (80% each), markedly lower soft rot incidence (5.2% and 7.3%, respectively) and higher yield (5.0 and 4.3 kg^3m^{-2}, respectively) compared to chemicals like bactericide Streptomycin sulfate (73.0%, 18.5% and 2.3 kg^3m^{-2}, respectively), fungicide combination Metalaxyl–Mancozeb (73.0%, 14.0% and 3.8 kg^3m^{-2}, respectively), and control (73.0%, 25.1% and 2.2 kg 3 m^{-2}, respectively). Overall, the results suggested that for growth promotion and management of soft rot disease in ginger, *B. amyloliquefaciens* GRB35 and *S. marcescens* GRB68 could be good alternatives to chemical measures. Since *S. marcescens* has been reported to be an opportunistic human pathogen, the use of *B. amyloliquefaciens* was recommended for integration into nutrient and disease management schedules for ginger cultivation. Based on the partial 16SrDNA analysis, GRB35 was found to be closely related to other PGPR strains like *B. pumilus* ST277 (EU350371) and *B. amyloliquefaciens* EML-CAP3 (DI338931). Similarly, GRB68 was closely related to PGPR like *S. marcescens* JASM1 (KF528829) (Raghavan et al. 2015).

11.4.8 Plantation Crops

11.4.8.1 Tobacco (*Nicotiana tabacum*)

P. fluorescens has been suggested as potential biological control agent due to its ability to colonize rhizosphere and protect plants against a wide range of important agronomic fungal diseases such as black root rot of tobacco in field condition (Fenton et al. 1992; Kumar et al. 2002; Arora et al. 2008). *B. pumilus* is reported to be effective against blue mold disease of tobacco (Singh 2013).

11.4.8.2 Sugarbeet (*Beta vulgaris*)

Due to its ability to colonize rhizosphere, *P. fluorescens* is found capable of protecting plants against damping-off of sugar beet in field condition (Fenton et al. 1992; Kumar et al. 2002; Arora et al. 2008).

11.5 Future Prospects and Strategies

Many bacterial strains are found beneficial in laboratory culture, only some of them perform successfully in a laboratory greenhouse, and an even lesser number are found functional under practical conditions of commercial greenhouse and field. An understanding of possible reasons for the failures in greenhouses and in the field

may equip the researchers to isolate improved strains. It is easy to isolate the strains used in antibiosis, and therefore, they are most commonly used. Sometimes, these strains fail to perform for a number of reasons:

1. Regulation of the synthesis of secondary metabolites by these strains is complicated and is not yet understood clearly (Duffy and Defago 1999; Haas and Keel 2003). It is found that the synthesis of secondary metabolites is affected by growth temperature, salinity, and concentrations of ferric, phosphate, sulfate, and ammonia ions, for instance, Van Rij et al. (2004) have demonstrated that these factors show a strong influence on the level of phenazine-1-carboxamide production.
2. Van den Broek et al. (2005) have shown that many biocontrol traits, such as root colonization, motility, and the production of AFMs, biosurfactants, chitinases, lipases, and proteases, are subject to phase variation. Phase variation is a process of reversible, high-frequency phenotypic switching mediated by mutation, reorganization, or modification of DNA.
3. It is observed that sometimes produced antibiotics are degraded. For instance, the Phl-producing strain *P. fluorescens* CHAO itself produces an enzyme that removes acetate group from Phl and hence results in a less active derivative of Phl as demonstrated by Bottiglieri and Keel (2006).
4. It is noticed that signal molecules AHLs required for synthesis of several AFMs and exoenzymes are degraded by enzymes from competing bacteria. Van Rij et al. (2005) have shown that the *Fusarium* metabolite fusaric acid inhibits phenazine synthesis of *P. chlororaphis* PCL1391 and therefore its biocontrol activity. In fact, fusaric acid interferes before or at the level of AHL synthesis, which is required for phenazine synthesis.
5. Not all fungal pathogens are simply affected by antagonistic organisms. Some pathogens defend themselves and develop resistance. In principle, they can utilize a range of possible mechanisms to defend themselves, such as enzymatic inactivation of the antifungal toxin by chemical modification, repression of biosynthetic toxin genes, modification of the target of the antifungal toxin, and secretion of the antifungal toxin (Duffy et al. 2003).

Due to aforesaid reasons, registration of antibiotic-producing products is discouraged as they also have possible cross-resistance with antibiotics applied for human and animal use. It suggests that biocontrol strains based on mechanisms other than antibiosis might have a better future for surviving the registration procedure and therefore becoming a product. Till now, no strain has been selected that utilize ISR. Similarly, bacteria having CNN property can easily be selected for the environmental conditions in which they are supposed to be applied. At the same time, the need of today's world is high productivity and enhanced production of the crop by maintaining the fertility of the soil in an eco-friendly manner. Hence, the research has to be focused on:

1. The new concept of bioengineering that can favorably partition the exotic biomolecules to create a unique set of interaction between plant and microbes should be applied to develop new strains (Tewari and Arora 2013).
2. Future research in rhizosphere biology should rely on the development of molecular and biotechnological approaches to increase our knowledge of rhizosphere biology and to achieve integrated management of soil microbial populations.
3. It is required to explore fresh alternatives of bioinoculants for other high-value crops such as vegetables, fruits, and flowers.
4. Instead of single strains, multistrain bacterial consortium should be developed that could effectively reduce the harmful impact of biotic stress on plant growth.
5. Marketing of bioinoculant products and release of these transgenics into the environment as eco-friendly alternatives to agrochemicals will depend on the generation of biosafety data required for the registration of PGPR agents.
6. Apart from this, future research in optimizing growth condition and increased shelf life of PGPR products, non-phytotoxic to crop plants, tolerate adverse environmental condition, provide higher yield, and cost-effective PGPR products for use of agricultural farmer will also be helpful.

11.6 Conclusion

PGPRs are an environmentally friendly alternative to chemical pesticides due to which the scope and demand for bioinoculants are continuously expanding. *Rhizobium* and *Bradyrhizobium* inoculants have been successfully marketed for over a century. A comprehensive screening followed by field testing helps in identifying rhizobacterial strains adaptable to a diverse environment and soil conditions. Many strains of PGPRs have shown immense biocontrol potential against a variety of crops. Apparently, these PGPRs hold great promise as a viable alternative to chemical pesticides and can be integrated into appropriate nutrient management and disease management schedules for various crops. However, caution is advisable while deploying PGPRs, for instance, *Serratia marcescens* has been implicated to be an opportunistic human pathogen. Many bacterial biocontrol products based on *Pseudomonas*, *Bacillus*, *Streptomyces*, and *Agrobacterium* strains and fungal biocontrol products based on *Trichoderma* spp. are available in the market. However, it is required to further optimize the efficacy of these products. The molecular discovery of many traits and genes that are involved in the beneficial effects of PGPRs has resulted in a better understanding of the performance of bioinoculants in the field and hence provides the platform to enhance the beneficial effects of PGPR strains by genetic modification.

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Biotic and Abiotic Stress Management by AM-Mediated PGPRs

12

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Abstract

Arbuscular mycorrhizal fungi or AM fungi improve mineral and water nutrition of most of the land plants by developing a mutualistic symbiosis with the plants and thus increase the resistance of plants to biotic and abiotic stress. The intraradical proliferation of soilborne plant pathogens is greatly affected by root colonization by AM fungi. Specifically, the rhizobacteria associated with the AM extraradical network and the mycorrhizosphere are attributed to the biocontrol exerted by the AM fungi. Mycorrhizosphere is the soil zone under the influence of the root and AM association with some particular characteristics. Mycorrhizosphere provides a conducive environment for proliferation of antagonistic microorganisms that suppresses the growth of phytopathogens. Rhizobacteria associated with AM structures and mycorrhizosphere are found to have strong antagonistic potential against various soilborne phytopathogens. The phenomenon is attributed to the capacity of AM fungi to stimulate the establishment of antagonistic rhizobacteria in mycorrhizosphere ahead of the infection by root pathogens and triggering the localized and systemic defense mechanisms of the crop plants. Mechanisms of biocontrol, biocontrol of many diseases of various crop plants, and abiotic stress management under water and salt stress conditions of various crop plants by AM-mediated rhizobacteria have also been discussed in this chapter.

Keywords

Rhizobacteria · AM fungi · Mutualism · Disease Resistance · Antagonism

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12.1 Introduction

Rhodes and Gerdemann (1975) and Smith and Read (2008) explained root colonization by external mycelium of AM fungi provides increased surface area for nutrient and water absorption by the plants from the soil leading to symbiotic association with a large number of land plants.

Kabir et al. (1997) and Olsson et al. (1999) have described the external mycelium of AM fungi as the largest component of the soil microbial biomass. External mycelium of AM fungi develops an extensive mycelial network in the soil and the hyphae provide sites of interaction with soil inhabitant microbes. As explained by Linderman (1988), Marschner et al. (2001), Marschner and Timonen (2005), Kim et al. (2006) and Lioussanne et al. (2009a), association of AM fungi with plant roots greatly affects the biomass and community structure of soil inhabitant microbes defining the uniqueness of mycorrhizosphere. In the soil the zone affected only by AM fungi is termed as mycosphere. Various AM-associated rhizobacteria (AMB) have been isolated from mycorrhizosphere and AM structures. *Paenibacillus*, *Bacillus*, and *Pseudomonas* species are mainly isolated from mycorrhizosphere and AM structures by Andrade et al. (1997), Budi et al. (1999), Mansfeld-Giese et al. (2002), Lioussanne (2007), and Bharadwaj et al. (2008b) by isolation on culture media; Rillig et al. (2005) have adopted phospholipid fatty acid analysis (PLFA); and Xavier and Germida (2003), Roesti et al. (2005), and Lioussanne (2007) have used polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) technique. Duineveld et al. (2001) analysed bacterial communities in the rhizosphere of Chrysanthemum via denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA as well as DNA fragments coding for 16S rRNA.

They have further confirmed that the AM structures are important nutrient-rich niches for soil inhabitant microbes. Bianciotto et al. (1996b) and Bianciotto and Bonfante (2002) have proposed a new taxon of *Burkholderiaceae Candidatus glomeribacter gigasporarum* which is obligate bacterial endosymbiont of spore vacuoles, mycelium, and intraradical hyphae of *Gigaspora margarita*-colonizing clover plants. This endobacterium was phenotypically described in detail by Jargeat et al. (2004). Bonfante (2003) has demonstrated that this endobacterium is widespread within *Gigasporaceae* family of AM fungi, and Bianciotto et al. (2004) have demonstrated that it is transmitted vertically. Minerdi et al. (2001) have shown that instead of *G. margarita*, these endobacteria contain nitrogen fixation genes that fixes nitrogen that is later delivered to symbiont plant by AM fungi. This indicates that the benefits of symbiosis delivered by AM fungi to symbiont plant are partially due to the activity of AM-associated rhizobacteria. St-Arnaud and Vujanovic (2007), Lioussanne et al. (2009b), and Lioussanne (2010) have explained that the beneficial effects of AM fungi on the host-plant physiology and decline in the intraradical or mycorrhizosphere population of soilborne phytopathogens are possibly due to the synergistic mechanism of AM fungi and rhizobacteria associated with it. Due to the health and environmental risks imposed by chemical pesticides, it has become imperative to employ eco-friendly biocontrol agents like free-living plant growth-promoting rhizobacteria and AM-mediated rhizobacteria for sustainable agriculture. Gosling et al. (2006) have

explained that improving the understanding of mechanisms of biocontrol by AM-mediated rhizobacteria will help in improving the performance of AM fungi under natural field condition to attain maximum benefit for the crop.

In this chapter, mechanisms of biocontrol by AM-mediated rhizobacteria have been discussed that will help in managing the biological control of diseases induced by soilborne plant pathogens, due to the root colonization by AM fungi, which is specifically because of the rhizobacteria associated with the AM fungi.

12.2 Mechanisms of Biocontrol by the AM-Mediated Rhizobacteria

The phenomenon of biocontrol of soilborne phytopathogens by AM fungi was first described by Gerdemann (1968, 1974). Whipps (2004) has described the effect of AM fungi on various fungi, stramenopiles, nematodes, and bacteria. Carlsen et al. (2008) have demonstrated that the infection of *Pythium ultimum* was totally prevented by the symbiosis of *Glomus mosseae* with cv. Sonja of clover plant. Reviews by Harrier and Watson (2004), Whipps (2004), St-Arnaud and Vujanovic (2007), Avis et al. (2008), Vierheilig et al. (2008), Akhtar and Siddiqui (2009), and Lioussanne et al. (2009b, c) have summarized the characteristics of this biocontrol with respect to the extent of suppression of phytopathogens, involvement of AM fungi, association of plant taxa, culture condition, time of inoculation of AM fungi, time of inoculation of pathogen, level of root colonization, time of harvesting, and mechanisms of biocontrol involved in this interaction. The mechanisms of biocontrol by AM-mediated rhizobacteria are discussed next.

12.2.1 Induction of Systemic Resistance (ISR)

Van Loon et al. (1998) has reported for the first time the induction of systemic resistance by rhizosphere bacteria. Induction of systemic resistance due to colonization by AM fungi is demonstrated by Pozo et al. (2002), Zhu and Zao (2004), and Khaosaad et al. (2007); they observed reduction in disease symptoms on non-mycorrhizal roots of plants grown by split root systems where one of the split was inoculated with AM fungi. Pozo and Azcon-Aguilar (2007) have also confirmed the fact that induction of systemic resistance due to colonization by AM fungi is responsible for the reduction of pathogen-induced disease symptoms. The presence of ISR-related compounds has been detected in plants showing biocontrol activities due to colonization with AM fungi. Pozo et al. (2002) and Garmendia et al. (2006) have identified new isoforms of superoxide dismutases and peroxidases. Cordier et al. (1998) have identified the type 1 pathogenesis-related proteins (PR-1 proteins). Singh et al. (2004) and Zhu and Zao (2004) have observed increased concentrations of phenolic acids. Similarly, Pozo et al. (2004), Hause et al. (2002, 2007), and Isayenkov et al. (2005) detected the accumulation of jasmonic acid in mycorrhizal roots as seen in case of free-living PGPRs.

In an experiment done by Liu et al. (2007), transcript profiling and real-time quantitative PCR were used to explore the transcriptional changes triggered by AM colonization. It has shown a complex pattern of local and systemic changes in gene expression in roots of *Medicago truncatula*. Although in this experiment, transcripts for defense-related proteins were found to be only locally expressed.

A contrasting result was obtained by Toussaint et al. (2008) where they did not notice the increase in concentrations of defense-related compounds rosmarinic and caffeic acids, phenolics, and essential oils when basil plants were inoculated with AM fungi *G. mosseae* to protect them from phytopathogen *Fusarium oxysporum* f. sp. *basilica*. This indicates that some other mechanisms than the systemic or localized plant defenses are involved in the resistance imparted by AM-mediated rhizobacteria (Lioussanne (2010).

12.2.2 Cell Wall Modifications

In tomato roots, a local cell wall modification such as accumulation of callose around arbuscule-containing cortical cells is reported by Cordier et al. (1998).

12.2.3 Defense-Related Enzymes

Pozo et al. (1996, 1998, 1999) have detected the constitutive and additional isoforms of defense-related enzymes chitinases, chitosanases, β-1,3-glucanases, peroxidases, and superoxide dismutases that were locally expressed in the roots colonized by AM fungi. Contradictory evidence is presented by Pozo et al. (2002) and Carlsen et al. (2008) that these enzymes and flavonoids are not related to the capacity of AM-mediated biocontrol. Whereas, Budi et al. (2000) and Selim et al. (2005) have demonstrated cellulolytic, proteolytic, chitinolytic, and pectinolytic activities of the isolate *Paenibacillus* sp. B2.

12.2.4 Antibiotic Production

Budi et al. (2000) and Selim et al. (2005) have shown that the isolate *Paenibacillus* sp. B2 also liberates the antibiotic polymyxin B1 and two other polymyxin-like compounds antagonistic against *F. solani* and *F. acuminatum*.

12.2.5 Improved Phosphorus Nutrition

Previously it was hypothesized that the improvement in phosphorus nutrition of host plants due to AM colonization is responsible for improving the disease resistance supported by the vigorous growth of inoculated plants. This hypothesis was disproved by Trotta et al. (1996), Yao et al. (2002), St-Arnaud and Elsen (2005), and

Toussaint et al. (2008) who have demonstrated that the AM-mediated biocontrol is not affected by phosphorus status of the soil as well as the plant. It is rather governed by some other mechanisms.

12.2.6 Competition for Nutrients and Niches

In mycorrhizosphere as well as on the host roots, AM-mediated rhizobacteria and AM fungi itself compete for space and nutrients with soilborne pathogens (Lioussanne, 2010). Biomass and energy reserves of both AM fungi *G. mosseae* and phytopathogen *Aphanomyces euteiches* co-occupying pea roots are shown to be decreased through their signature fatty acid profiles generated by Larsen and Bodker (2001) indicating competition for nutrients among them. Similarly, *Phytophthora nicotianae* and *G. mosseae* do not occupy the same tomato root tissues simultaneously demonstrated Cordier et al. (1996). Davis and Menge (1980), Baath and Hayman (1983), and Krishna and Bagyaraj (1983) have demonstrated the competition between plant pathogens and AM fungi by the reduction in the extent of mycorrhizal colonization in the presence of different plant pathogens. Due to such type of competition faced by AM-mediated biocontrol, it is necessary to inoculate the host plants with AM fungi in advance of infection by soilborne pathogens to make the biocontrol effective. On the basis of quantification of genomic DNA by quantitative real-time PCR, Filion et al. (2003) have documented the significant reduction in *F. solani* f. sp. *phaseoli* population in the mycorrhizosphere, mycosphere, and the bulk soil of a compartmentalized soil-root system inoculated with *G. intraradices.* At the same time, genomic DNA of AM fungi was not found to be significantly modified due to the influence of pathogen. This indicates that in this case, biotic and abiotic characteristics of the mycorrhizosphere were more responsible for pathogen suppression as compared to competition for nutrients and niches among AM fungi and the pathogen.

St-Arnaud et al. (1995) and Elsen et al. (2001, 2003) have recorded direct reduction in the population of nematodes *Radopholus similis* and *Pratylenchus coffeae* due to an extraradical network of *G. intraradices*. Similarly, it has also shown a reduction in the number of conidia formed by root rot pathogen *F. oxysporum* f. sp. *chrysanthemi (Foc).* At the same time, Elsen et al. (2001, 2003) observed that this suppression was not uniform for all the developmental stages of the nematode. Similarly, nematode suppression has not shown a direct correlation with the mycelial and spore densities of *G. intraradices.* St-Arnaud et al. (1995) have also observed that despite suppression of conidia formation, spore germination and hyphal growth of *F. oxysporum* f. sp. *chrysanthemi* were significantly increased in the presence of *G. intraradices*. This indicates that the direct inhibition of pathogen development by AM structures is a weak component of biocontrol exerted by *G. intraradices*.

12.3 Antimicrobial Properties of Root Exudation of AM-Colonized Plants

Filion et al. (1999) have demonstrated that the crude extract from *G. intraradices* extraradical network suppresses conidial germination of *Foc*. Antisporulent properties of exudates of strawberry roots colonized by *G. etunicatum* and *G. monosporum* on the pathogen *Phytophthora fragariae* have been reported by Norman and Hooker (2000). In contrast to these findings, Lioussanne et al. (2008) have demonstrated that the exudates of AM-inoculated roots of tomato attracted more zoospores of pathogen *P. nicotianae* as compared to non-AM-inoculated plants in vitro condition when tomato seedlings were inoculated with *G. intraradices*, although this was reported to be dependent on the harvest time. This finding is in line with the report of Scheffknecht et al. (2006) who have found doubling of germination of microconidia of *F. oxysporum* f. sp. *lycopersici (Fol)* due to root exudates of tomato plants colonized by *G. mosseae* as compared to exudates collected from non-mycorrhizal roots. Lioussanne et al. (2009d) had directly quantified the root infection by the pathogen in soil condition to assess the role of root exudates of mycorrhizal plants in AM-mediated biocontrol, and they have found that the exudates from tomato roots colonized with *G. intraradices* or *G. mosseae* had no impact on *P. nicotianae* intraradical growth. At the same time, direct application of these AM fungi had a significant inhibitory effect on the pathogen colonization. It suggests that the exudates from mycorrhizal plants neither restrict the growth of pathogen directly nor do they promote any beneficial organism to perform the task indirectly.

On the basis of above mentioned facts, we can summarize that the mechanism of biocontrol imparted by AM fungi is case specific and only one mechanism is partially effective. Hence, biocontrol imparted by AM fungi seems to be an outcome of a combination of more than one mechanisms from the above mentioned types that work synergistically in displaying the biocontrol potential exerted by AM fungi and that is further dependent on physicochemical properties of soil, plant genotype, the strain of AM fungi, and the capacity of AM fungi to interact with soilborne plant pathogens in mycorrhizosphere.

12.4 Mycorrhizosphere

Mycorrhizosphere is the rhizosphere zone around AM-colonized roots which is conducive to the growth of microorganisms that are antagonistic to the growth of soilborne plant pathogens. This can be better understood by growing mycorrhizal plant *Tagetes patula* together with non-mycorrhizal plant *Dianthus caryophyllus* inoculated with *G. intraradices* which shows a reduction in disease caused by *F. oxysporum dianthi* in *D. caryophyllus* as well along with *T. patula.* St-Arnaud et al. (1997) have explained this to happen due to the mycorrhizosphere developed around *T. patula* roots by AM fungi overlapping the plain rhizosphere of *D. caryophyllus* roots. Vigo et al. (2000) have explained on the basis of a reduction in the number of infection sites on the tomato roots pre-colonized with *G. mosseae* after inoculation

of *P. nicotianae* zoospores that the antagonistic effect of mycorrhizosphere on pathogen proliferation starts even before the infection by pathogens. Virtually mycorrhizosphere is the resultant of mutual interaction of plants, AM fungi, and AM-associated rhizobacteria synergistically affecting each other's growth. Rillig and Mummey (2006) have shown the release of glomalin a glycoprotein by AM fungi resulting in the formation of aggregates that act as microsites for the establishment of root and microbes. Another microsite is an extraradical network of AM fungi that favor the growth of certain bacteria. Plant growth-promoting rhizobacteria (PGPRs) like P-solubilizing bacteria and N-fixing bacteria interact synergistically with AM fungi and increase the availability of P and N to the plant thus promoting plant growth leading to improved resistance towards pathogen attack (Bowen and Rovira, 1999; Johansson et al. 2004; Barea et al. 2005; Artursson et al. 2006 and Lioussanne et al. 2009b). As per research conducted by Nehl et al. (1997) and Bowen and Rovira (1999), biocontrol potential of AM-mediated PGPRs can be attributed to release of toxic compounds, competition for nutrients and niches, by reducing the availability of Fe and Mn to pathogenic organisms, by modifying the hormone balance of plant, and by triggering defense mechanism of plants. Barea et al. (2005) have reported that the synergistic effect of AM colonization with rhizobacteria depends on the combination of species of PGPR and AM fungi, nutritional status of soil, and other environmental conditions. Furthermore, Akhtar and Siddiqui (2008) have reported the synergistic effect of co-inoculation of *G. intraradices* with the biocontrol agents *Pseudomonas striata* and *Rhizobium* sp., and Siddiqui and Mahmood (1998) demonstrated the improved biocontrol potential due to dual inoculation of *G. mosseae* with *Pseudomonas fluorescens* against root rot of chickpea caused due to nematode infestation. Species-specific nature of the interaction of AM fungi with biocontrol-related bacteria has been shown by Järderlund et al. (2008) during the interaction of two PGPRs *P. fluorescens* SBW25 and *Paenibacillus brasilensis* PB177 with *G. mosseae* and *G. intraradices* on winter wheat infested with *Microdochium nivale.* Barea et al. (2005) have reported several studies showing no adverse effect of antagonistic fungi as well as PGPRs on AM fungi. Mycorrhization helper bacteria (MHB), defined by Garbaye (1994) as bacteria which consistently promote mycorrhizal development and even increases AM impact on pathogens, seem to be the PGPRs with antagonistic potential against soilborne plant pathogens. Symbiotic association of AM fungi and rhizobacteria has also been highlighted by Frey-Klett et al. (2007).

12.5 Mechanisms of Interaction Between AM Fungi and AM-Mediated PGPRs

Associations of PGPRs with mycorrhizal fungi are sometimes specific to one species of AM fungi or sometimes they are common to several species of AM fungi (Rillig et al. 2005). Bharadwaj et al. (2008b) have described that the association of bacteria present on the surface of spores of *G. mosseae* and *G. intraradices* is affected by spore type of AM fungi and the species of host plant with which AM

fungi is associated. This specificity is supposed to be mainly related to spore size and rough surface of spores. Similarly, Bianciotto et al. (1996a), Artursson and Jansson (2003), and Toljander et al. (2006) have also demonstrated the specificity of adherence of PGPRs with the spores as well as hyphae of AM fungi and found that the fungal vitality plays a significant role in maintaining this specificity. Rudrappa et al. (2008) described the capacity to prepare biofilms to improve adherence of PGPRs to AM surfaces thus strengthening their association. One such example cited by Bianciotto et al. (2001b) found that the non-mucoid strain of PGPR with biocontrol potential *P. fluorescens* CHAO could establish a limited number of sites, whereas mucoid mutants of this bacteria having improved production of acidic extracellular polysaccharides (EPS) required for developing biofilms were able to establish at a large number of sites called clusters on non-mycorrhizal carrot roots, roots colonized with *G. margarita*, and extraradical hyphae of this AM fungus, proving that EPS has a significant role in improving association of PGPRs with AM fungi and their host plant. This fact is further proven by Bianciotto et al. (2001a) when they observed that the EPS-deficient mutants of *Azospirillum brasilense* and *Rhizobium leguminosarum* had greatly lost the capacity to adhere to mycorrhizal root and structures of AM fungi. Levy et al. (2003) have demonstrated that the adherence of PGPRs to AM fungi is not limited to surfaces due to biofilm formation rather they may colonize even inside spores as they observed with various strains of *Burkholderia* inoculated on germinating spores of *Gigaspora decipiens.* Surface association of rhizobacteria with AM fungi has been further proved by Roesti et al. (2005) by scanning electron microscopy of spores of *G. geosporum*. Eroded surface of spores and presence of mucilaginous products suggest that the rhizobacteria directly consume AM fungi. Filion et al. (1999) had observed stimulation of the growth of *Pseudomonas chlororaphis* in the presence of crude extracts collected from an extraradical network of in vitro grown *G. intraradices*. This finding has strengthened the fact that AM fungi stimulate the growth of rhizobacteria by providing nutrients not only through their exudates but also through the mycelial constituents that can be obtained by rhizobacteria after feeding on them directly. Similarly, the stronger attraction of rhizobacteria *Azotobacter chrococcum* and *P. fluorescens* has been observed by Sood (2003) due to the exudates collected from tomato roots colonized by *G. fasciculatum* as compared to the exudates collected from non-colonized roots.

Few studies also present contradictory evidence proving independence of rhizobacteria on exudates of AM fungi. Toljander et al. (2007) have found the inhibitory effect of AM exudates on rhizobacterial colonization. Similarly, Vestergard et al. (2008) recorded no difference in PCR-DGGE profile of rhizobacteria even after reduction of exudation in pea plants due to defoliation, whereas missing and additional bands were observed from the rhizosphere of plants pre-colonized with *G. intraradices*. PCR-DGGE analysis done by Lioussanne et al. (2009a) has revealed that application of root exudates collected from roots colonized with *G. intraradices* or *G. mosseae* does not affect PGPR population in tomato rhizosphere, whereas direct root colonization by *G. intraradices* or *G. mosseae* significantly improves rhizobacterial colonization as compared to non-mycorrhizal plants. This indicates

that the rhizobacterial community structure is not affected by mycorrhizal exudates rather it is supported by physical presence and specific interaction of AM fungi. Christensen and Jakobsen (1993) and Raiesi and Ghollarata (2006) argued that the decrease in microbial activity in mycorrhizosphere is due to competition for substrates. Similarly, Ravnskov et al. (1999) had demonstrated a reduction in the population of rhizobacterial strain *P. fluorescens* DF57 antagonistic to *Pythium* sp. in mycorrhizosphere of *G. intraradices* associated with cucumber roots.

12.6 Applications of AM-Mediated Rhizobacteria on Crop Plants

12.6.1 Biotic Stress Management by AM-Mediated PGPRs

Most AM-mediated PGPRs described so far in detail showed antagonistic characteristics toward soilborne pathogens or behaved as mycorrhiza helper bacteria (MHB, Xavier and Germida, 2003).

12.6.1.1 Tomato

Root rot caused by *Phytophthora nicotianae* var. *parasitica*, *P. aphanidermatum*, *Rhizoctonia solani*, and *Ralstonia solanacearum* and wilts caused by *Verticillium* sp. *F. solani*, *F. acuminatum*, and *F. oxysporum* are economically important diseases of tomato. AM-mediated PGPRs are found to effectively control these diseases as well as root-knot nematode *Meloidogyne* species.

Cordier et al. (1996) demonstrated a reduction of disease due to *Phytophthora nicotianae* var. *parasitica* in mycorrhizal tomato by affecting the colonization patterns of root tissues by the pathogen. Similarly, Pozo et al. (1996) demonstrated the induction of new chitinase isoforms in tomato roots during interactions with *Glomus mosseae* and/or *P. nicotianae* var. *parasitica*, thus imparting resistance to the host plant. Trotta et al. (1996) have also demonstrated the antagonistic potential of arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants against soilborne root pathogen *P. nicotianae* var. *parasitica*. Cordier et al. (1998) reported induction of cell defense responses associated with localized and systemic resistance to *P. parasitica* in tomato by an arbuscular mycorrhizal fungus. Pozo et al. (1998, 1999) have demonstrated triggering of chitosanase, chitinase, and β-1,3-glucanase activities as well as compared localized versus systemic effect in tomato roots due to the effect of arbuscular mycorrhizal fungi on defense responses during *P. parasitica* infection in tomato plants. Lioussanne et al. (2008) reported that mycorrhizal colonization with *Glomus intraradices* and development stage of transformed tomato roots significantly modify the chemotactic response of zoospores of the pathogen *P. nicotianae.* Lioussanne et al. (2009c) have also reviewed the role of root exudates and rhizosphere microflora in the arbuscular mycorrhizal fungi-mediated biocontrol of *P. nicotianae* in tomato. Further, Lioussanne et al. (2009d) have reported a reduction in the growth of the soilborne pathogen *P. nicotianae* in tomato roots colonized with

arbuscular mycorrhizal fungi but found to be unaffected by the application of root exudates collected from corresponding mycorrhizal plants.

Studies performed in parallel and aiming to identify AM-mediated PGPRs isolated *Paenibacillus* spp. with biocontrol activities. Budi et al. (1999) isolated *Paenibacillus* sp. B2 from the mycorrhizosphere of *G. mosseae* and identified by phylogenetic comparison of the 16S rRNA gene sequence and analytical profile index (API) that was found antagonistic against various soilborne pathogens in vitro and reduced tomato root necrosis caused by *P. nicotianae*. Budi et al. (2000) and Selim et al. (2005) have demonstrated cellulolytic, proteolytic, chitinolytic, and pectinolytic activities of this isolate *Paenibacillus* sp. B2 and shown that the isolate also liberates the antibiotic polymyxin B1 and two other polymyxin-like compounds antagonistic against *F. solani* and *F. acuminatum*. On the basis of electron microscopy, it is evident that the presence of *Paenibacillus* sp. B2 results in disorganization of cell walls and/or cell contents of *P. nicotianae* and *F. oxysporum*. Budi et al. (1999) have also observed increased root and shoot fresh weights of mycorrhizal tomato plants colonized by *G. mosseae*. Mansfeld-Giese et al. (2002) have identified *Paenibacillus polymyxa* and *P. macerans* from the mycorrhizosphere, the hyphosphere (root-free soil and sand compartments) and from a rooftree sand compartment abundantly washed to collect bacterial isolates closely associated with *G. intraradices* mycelium (called mycosphere) through the use of a compartmentalized growth system. All *Paenibacillus* strains tested from these AM-influenced soil zones were demonstrated by Li et al. (2007) to prevent pre-emergence damping-off caused by *Py. aphanidermatum*. Out of 18 cultivable isolates from surface-disinfected spores of *G. mosseae*, 14 (especially isolates identified as *Bacillus simplex*, *B. niacini*, *B. drententis*, *Paenibacillus* spp., and *Methylobacterium* sp.) showed antagonism against various soilborne pathogens particularly *P. nicotianae* and also *F. solani* and three strains of *F. oxysporum* (Lioussanne 2007). Bharadwaj et al. (2008b) classified bacteria isolated from surface-decontaminated spores of *G. intraradices* and *G. mosseae* extracted from field rhizospheres of *Festuca ovina* and *Leucanthemum vulgare* within two phylogenic clusters: A corresponding to *Proteobacteria* and B corresponding to *Actinobacteria* and *Firmicutes*. Bacteria from both clusters have displayed antagonistic properties against *Rhizoctonia solani* in vitro dual culture assays.

Among other soilborne pathogens of tomato, Baath and Hayman (1983) reported suppression of *Verticillium* wilt on tomato plants by the use of vesicular-arbuscular mycorrhizae. Siddiqui and Mahmood (1998) have demonstrated the antagonistic effect of plant growth-promoting bacterium, an AM fungus, and soil types on the morphometrics and reproduction of *Meloidogyne javanica* on tomato. Zhu and Zao (2004) observed a localized and systemic increase of phenols in tomato roots induced by *Glomus versiforme* that inhibits *Ralstonia solanacearum*. Scheffknecht et al. (2006) demonstrated the inhibitory effect of root exudates of mycorrhizal tomato plants on microconidia germination of *Fusarium oxysporum* f. sp. *lycopersici* than root exudates from non-mycorrhizal tomato plants.

12.6.1.2 Potato

Bharadwaj et al. (2008a) demonstrated that arbuscular mycorrhizal fungal spore-associated bacteria having two isolates of *Stenotrophomonas maltophilia*, three isolates of *Pseudomonas* spp., one isolate of *B. subtilis*, and one isolate of *Arthrobacter ilicis* affect mycorrhizal colonization and plant growth and were all antagonistic against potato pathogens *Erwinia carotovora* var. *carotovora* (Ecc), *Verticillium dahliae*, *Phytophthora infestans*, and *R. solani* in vitro that produced siderophores and proteases and decreased the weight of rotten potato tissues caused by Ecc. Yao et al. (2002) demonstrated the antagonistic effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by *Rhizoctonia solani*.

12.6.1.3 Chickpea

Akhtar and Siddiqui (2008) demonstrated biocontrol of a root rot disease complex of chickpea by *Glomus intraradices*, *Rhizobium* sp., and *Pseudomonas striata*.

12.6.1.4 Carrot

Bianciotto et al. (2001b) demonstrated mucoid mutants of the biocontrol strain *P. fluorescens* CHA0 show increased ability in biofilm formation on mycorrhizal and non-mycorrhizal carrot roots resulting into improved biocontrol potential. St-Arnaud et al. (1995) demonstrated an alteration in the growth of *Fusarium oxysporum* f. sp. *chrysanthemi* in an in vitro dual culture system with the vesicular-arbuscular mycorrhizal fungus *Glomus intraradices* growing on *Daucus carota* transformed roots.

12.6.1.5 Sorghum

Budi et al. (1999) isolated a bacterium compatible with arbuscular mycorrhiza development and antagonistic toward soilborne fungal pathogens from the *Sorghum bicolor* mycorrhizosphere. Selim et al. (2005) isolated and partially characterized antagonistic peptides produced by *Paenibacillus* sp. strain B2 isolated from the sorghum mycorrhizosphere.

12.6.1.6 Clover

Carlsen et al. (2008) demonstrated triggering of plant defense mechanism by an increase in the level of flavonoids in roots of white clover due to the interaction of arbuscular mycorrhizal fungi and a pathogenic fungus.

12.6.1.7 Cucumber

Christensen and Jakobsen (1993) reported a reduction in bacterial growth by a vesicular-arbuscular mycorrhizal fungus in the rhizosphere of cucumber (*Cucumis sativus* L). Li et al. (2007) have demonstrated biocontrol of *Pythium* damping-off in cucumber by arbuscular mycorrhiza-associated bacteria from the genus *Paenibacillus*.

12.6.1.8 Citrus

Davis and Menge (1980) explained the influence of *Glomus fasciculatus* and soil phosphorus on *Phytophthora* root rot of citrus.

12.6.1.9 Beans

Filion et al. (2003) have demonstrated a reduction in the population of *Fusarium solani* f. sp. *phaseoli* in mycorrhizal bean plants and surrounding mycorrhizosphere soil using real-time polymerase chain reaction and direct isolations on selective media.

12.6.1.10 Pepper

Garmendia et al. (2006) have demonstrated an increase in the levels of defense-related enzymes in pepper roots during interactions with arbuscular mycorrhizal fungi and/or the pathogen *Verticillium dahliae*.

12.6.1.11 Barley

Khaosaad et al. (2007) have demonstrated a systematic reduction of take-all disease in roots of mycorrhizal barley plants.

12.6.1.12 Groundnut

Krishna and Bagyaraj (1983) demonstrated the antagonistic potential of *Glomus fasciculatum* against *Sclerotium rolfsii* in peanut.

12.6.1.13 Basil

Toussaint et al. (2008) demonstrated the antagonistic effect of *Glomus mosseae* on *Fusarium oxysporum* f. sp. *basilici* through increased concentrations of rosmarinic and caffeic acids and essential oil compounds in basil.

12.6.1.14 Pea

Singh et al. (2004) demonstrated the antagonistic potential of AM fungi against powdery mildew (*Erysiphe pisi*) disease due to biochemical changes induced in pea (*Pisum sativum*).

12.6.1.15 Marigold

St-Arnaud et al. (1997) have explained the inhibition of *Fusarium oxysporum* f. sp. *dianthi* in *Tagetes patula* plants colonized by *Glomus intraradices*.

12.6.2 Abiotic Stress Management by AM-Mediated PGPRs

Vesicular-arbuscular mycorrhizal symbiosis is reported to improve water relations and drought tolerance in plants (Augé 2001). Chang (2007) has studied the use of PGPRs and an AM fungus to improve plant growth in saline soils for phytoremediation. Several studies demonstrated improvement in abiotic stress tolerance of crop plants by the application of AM-mediated PGPRs and are discussed next.

12.6.2.1 Citrus

Wu et al. (2006) studied effects of water stress and arbuscular mycorrhizal fungi on reactive oxygen metabolism and antioxidant production by citrus (*Citrus tangerine*) roots and reported that inoculation with AM fungi improves tolerance of citrus plants to water stress.

12.6.2.2 Strawberry

Aysen et al. (2016) recorded influence of arbuscular mycorrhizae and PGPRs on proline content, membrane permeability, and growth of strawberry (*Fragaria* × *ananassa* Duch.) under salt stress. They reported that though the salinity is one of the most important factors negatively affecting the yield in crop species, use of PGPRs and AM fungi improves proline and anthocynin levels, membrane permeability, and growth of strawberry cv. 'San Andreas' under different salt treatments (0, 30, and 60 mM/L NaCl). The leaf area was measured 0, 15, 30, 45, and 60 days after saline solution application on the plants. The results showed that increasing concentrations of NaCl decreased all growth parameters. Increased salt concentration led to increased proline level compared to the control. Bacterial application at 60 mM/L NaCl concentration provided the highest ameliorative effect and therefore determined the most effective protection of the plant against salt stress. It was observed that the anthocyanin content increased in line with the increasing salt concentration. In general, the salt applied on the plants causes an increase in membrane permeability and thus disrupts membrane stability and becomes a significant factor damaging the plant. Membrane permeability increased at applications with 30 mM/L and 60 mM/L NaCl. Their results revealed that bacterial application can have an ameliorative effect that helps the plant to tolerate the negative effects of salt stress by increasing proline and anthocyanin levels.

12.6.2.3 Wheat

Miransari et al. (2008) used arbuscular mycorrhiza to alleviate the stress of soil compaction on wheat (*Triticum aestivum* L.) growth. Roesti et al. (2006) demonstrated that survival of wheat crop under drought condition is dependent on plant growth stage, fertilizer management, and bioinoculation of arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria that positively affect the rhizobacterial community structure in rainfed wheat fields, thus strengthening the plants to survive under moisture stress condition.

12.6.2.4 Soybean

Nogueira et al. (2007) demonstrated the positive influence of mycorrhiza and associated rhizobacteria to improve the availability of extractable iron and manganese in soil and their uptake by soybean.

12.6.2.5 Maize

Azaizeh et al. (1995) demonstrated the effects of vesicular-arbuscular mycorrhizal fungus and other soil microorganisms of growth, mineral nutrient acquisition and root exudation of soil-grown maize plants.

12.7 Conclusion

The capacity of AM fungi to control disease symptoms and the intraradical and rhizospheric proliferation of soilborne pathogens is complex and influenced by various mechanisms acting in synergy with each other. Among these mechanisms, the capacity of the AM extraradical network to stimulate beneficial microorganisms is strongly involved. Various bacteria with high capacities of antagonism against several soilborne pathogens have been identified within AM extraradical structures or in the mycorrhizosphere of several AM species. The AM-mediated biocontrol is not the fruit of the AM fungus function only but is strongly related to the capacity of the AM fungus to constitute an environment favorable to the establishment of rhizobacteria with biocontrol abilities and ability to support phytoremediation under water and salt stress conditions. Ongoing studies on the specificity of AM fungi with other beneficial microorganism interactions related to the bacterial capacity of attachment to the AM structures permit to improve the understanding of the biocontrol by AM-mediated PGPRs. Further identification of PGPRs allows not only effective inoculations of new biocontrol agents easier to grow in artificial conditions than AM fungi but will also provide powerful synergistic controls of soilborne pathogens and abiotic stresses by dual inoculation of AM fungi with other biocontrol agents that establish preferentially in the mycorrhizosphere. This will provide a tool for an environmentally friendly, profitable, and sustainable agriculture.

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Plant Growth-Promoting Rhizobacteria: An Overview in Agricultural Perspectives

13

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Abstract

Soil microbiology is a millennium dollar important field in the agriculture sector in terms of growth, development, and high yield. Earlier efforts were in the direction of use of chemical fertilizers to get fast and quick results. But during the last decades, some harmful effects of these seem to be showing discouraging results for rhizospheric microflora. Plant growth-promoting rhizobacterial world is an amazing and magical invisible world with promising results. Commonly available PGPR genera include bacterial strains such as *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Caulobacter*, *Chromobacterium*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, etc. If their colonization is encouraged by creating favorable conditions for their growth, then the cost of an external phytohormone, growth enhancers, and nitrogen fixers can be minimized. This review focuses on some of the significant characteristics of direct mechanism of action of PGPR which should be focused more and should be implemented successfully under various agricultural lands where cultivation practices are literally difficult.

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Rhizobacteria · Abiotic and biotic stress · Sustainable agriculture

13.1 Introduction

Due to the vast increase in population, there is tremendous pressure on the agricultural section to improve agricultural practices and generate more yield within a short period of time and with limited cultivable land. Use of chemical fertilizers was considered as a quick solution for these problems such agricultural practices are normally undergoing from generations to generations in farmers family. Negative influences of use of such chemical fertilizers led to pollution of air, water, and soil. Due to excessive applications of chemical fertilizers, they get accumulated in soil year after year making the land unproductive and fertile agricultural area into non-fertile. The second main disadvantage of such chemical fertilizers is that these are expensive enough and led to financial imbalances in farmers' life. So it became difficult for farmers to cope with such uncontrolled situations. Soil microbiology is a science which deals with the study of various microorganisms including bacteria, fungi, protozoa, actinomycetes, etc. Fertility of soil is not only dependent upon the chemical composition of soil but also on microorganisms inhabiting in it. Several factors like pH, temperature, moisture, organic carbon contents, and enzymes determine the activity of rhizosphere.

It is observed that the rhizomicrobiome zone can promote plant growth and with the help of several direct or indirect mechanisms contribute to better plant health (Richardson et al. 2009). There may be a beneficial plant-microbe interaction named as symbiotic associations (Bulgaelli et al. 2013). The symbiotic association is mostly an obligatory interaction and between rhizobia and legume plants and between members of family Fabaceae and arbuscles in endomycorrhizael (Parniske 2008). The second type of association is generally non-obligatory called cooperation or associative symbiosis which involves colonization of bacteria on the root surface and root tissue. These bacteria are known to stimulate growth and health of plant and are referred as plant growth-promoting rhizobacteria (PGPR) (Barea et al. 2005).

13.2 Forms of Plant Growth-Promoting Rhizobacteria

Viceros et al. (2010) have explained two forms of PGPR. One is extracellular PGPR and the another is intracellular PGPR. Extracellular PGPR strains include *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Caulobacter*, *Chromobacterium*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, and *Serratia* sp. which reside in rhizosphere itself (Ahemad and Kibret 2014). Intracellular PGPR includes Rhizobiaceae family members like *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and rhizobium endophytes with *Frankia* sp. All intracellular

PGPR fix atmospheric nitrogen with the higher plants (Wani et al. 2013; Bhattacharya and Jha 2012).

Most common PGPR belong to genera *Acetobacter*, *Acintobacter*, *Alcaligenes*, *Arthrobacter*, *Azocarpus*, *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Derxia*, *Enterobacter*, *Gluconacetobacter*, *Herbaspirillum*, *Klebsiella*, *Pseudomonas*, *Rhodococcus*, *Serratia*, *Stenotrophomonas*, and *Zoogloea* (Sophareth et al. 2013; Babalola 2010).

13.3 Mechanism of Action of PGPR

Literature survey shows that the mode of action of PGPR is due to their direct and indirect mechanisms. The direct mechanism involves the synthesis of certain plant growth-promoting substances or nutrients from the environment and makes it available to plant. The indirect mechanism involved prevention of deleterious effect of one or more phytopathogens by PGPR by one or several mechanisms.

The direct mechanism includes nitrogen fixation, phytohormone production, phosphate solubilization, and increasing iron availability. Organic substances like plant growth regulators that influence various physiological and morphological processes at very low concentration (Arshad and Frankenberger 1998).

13.4 Direct Mechanism of Action of PGPR

Here due to the direct action of PGPR, improvement of plant growth and yield is possible. PGPR provides solubilization of certain minerals like phosphorus, potash, and fix atmospheric nitrogen and supplies it to plant with the production of various phytohormones including auxin, gibberellic acid, cytokinin, etc. (Tables 13.1 and 13.2).

13.4.1 PGPR in Nitrogen Fixation

Nitrogen is available in the atmosphere, sea, and rocks. Conversion of atmospheric nitrogen into ammonia with the help of microorganism is called biological nitrogen fixation. Nitrogen fixation process can be followed by free-living microorganisms

Table 13.1 Mechanism of action of PGPR

Direct action	Indirect action
Nitrogen fixation	Antibiosis
Phytohormone production	HCN production
Phosphate solubilization	Lytic enzymes
Potash solubilization	Siderophore production
Phytopathogen production	Induced systemic resistance
	Nutrient and niche competition

Table 13.2 Direct mechanisms adopted by PGPR for plant growth promotion

Microbial strain	Mechanism	Reference
Aneurinibacillus aneurinilyticus CKMV1	**Nitrogen fixation**	Chauhan et al. (2017)
Burkholderia sp.		Govindrajan et al. (2007)
Gluconacetobacter sp.		Munoz Rojas and Caballero Mellado (2003)
Herbaspirillum sp.		Elbeltagy et al. (2001)
Azospirillum sp.		De Felipe and Fijaction (2006)
Phytohormone		
Pseudomonas putida	Indoleacetic acid	Ahemad and Khan (2012a)
Klebsiella sp.	Indoleacetic acid	Ahemad and Khan (2011)
Enterobacter asburiae	Indoleacetic acid	Ahemad and Khan (2010)
Rhizobium	Indoleacetic acid	Ahemad and Khan (2012a, b)
Mesorhizobium	Indoleacetic acid	Ahemad and Khan (2012a, b)
Acinetobacter	Indoleacetic acid	Rokhbaksh-Zamin et al. (2011)
Aspergillus niger sp.	IAA, gibberellic acid	Bilkay et al. (2010)
Bacillus sp.	Auxin synthesis	Ahemad and Khan (2010)
	Cytokinin synthesis	Sokolova et al. (2011)
Paenibacillus sp.	Indoleacetic acid	Bent et al. (2001)
Sphingomonas sp.	Gibberellic acid	Khan and Kang (2014)
Aneurinibacillus aneurinilyticus CKMV1	IAA	Chauhan et al. (2017)
Streptomyces sp.	IAA	Jog et al. (2014)
Pseudomonas fluorescence	IAA	Ramyasmruthi et al. (2012)
Aneurinibacillus aneurinilyticus CKMV1	**Phosphate solubilization**	Chauhan et al. (2017)
Pseudomonas fluorescence		Ramyasmruthi et al. (2012)
Streptomyces sp.		Rahul jog et al. (2014)
Kurthia and rhizospheric bacteria		Sharma et al. (2013)
Rhizospheric strain		Dagnow et al. (2015)

in the rhizosphere and symbiotically living microbial strains. But the rate of nitrogen fixation by the free-living microbial group is comparatively lower than the symbiotic ones. Free-living nitrogen-fixing microorganism includes *Azotobacter*, *Rhodospirillum*, *Klebsiella*, *Clostridium*, and various cyanobacteria. Symbiotic nitrogen-fixing microbial strains include *Rhizobium* sp. which forms root nodules on roots of leguminous plants. There is host specificity observed during *Rhizobium* sp. *Rhizobium meliloti* shows specificity to alfalfa plants. *R. trifolii* is specific for clovers, and *R. leguminosarum* is specific to pea, lentil, and vetch plants. Mycorrhizae fungi and symbiotic nitrogen-fixing fungi *Frankia* may reside in inter- or intracellular spaces of the roots. *Beijerinckia* with rice and *Digitaria decumbens* with *Spirillum lipoferum* are examples of associative symbiosis (Powar and Daginawala 2010).

Nitrogen-fixing bacterial species belongs to genus *Azospirillum*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Enterobacter*, *Flavobacterium*, *Pseudomonas*, *Rhizobium*, and *Serratia* sp. which exerts beneficial influences on rhizosphere and nitrogen fixation (Tilak et al. 2005). *Azospirillum* sp. is one of the most studied and commercially used on large scale in Argentina, Mexico, Europe, South Africa, and India for nitrogen fixation in plants like rice, maize, wheat, sorghum, and millet (Bashan et al. 2011; Stella and Sivasakthivelan 2009).

13.4.2 PGPR in Phytohormone Production

PGPR produces various phytohormones like auxin, gibberellic acid, cytokinin, and ethylene oxide which can proliferate root foundation by advancement in nutrient, water, and mineral uptake (Arora et al. 2013). Such hormones are effective even at a very low concentration and microbial cells produce these hormones and release them in rhizosphere at a very slow rate.

Tryptophan acts as a precursor compound for the production of auxin in microbial genera like *Pseudomonas*, *Rhizobium*, *Bradyrhizobium*, *Agrobacterium*, *Enterobacter*, *Klebsiella*, etc. (Shilev 2013). Microbial genera including *Rhizobacteria*, *Azotobacter*, *Pantoea agglomerans*, *Rhodospirillum rubrum*, *Pseudomonas fluorescence*, *Bacillus subtilis*, and *Paenibacillus polymyxa* are either cytokinin or gibberellic acid producers or both (Kang et al. 2010).

Cytokinin is a phytohormone that stimulates cell division, root meristem differentiation, and proliferation of root hairs. It is mainly produced by the PGPR community like *Arthrobacter giacomelloi*, *Azospirillum brasilense*, *Bradyrhizobium japonicum*, *Bacillus licheniformis*, etc. (Hussain and Hasnain 2009). Arkhipova et al. (2007) reported that shoot growth can be enhanced by inoculation of bacteria having cytokinin production capacity with plants.

Ethylene oxide is a phytohormone which is mainly involved in fruit ripening and abscission of various plant parts, and mainly it participates for senescence (Glick et al. 2007). A small amount of methionine is needed for the production of this hormone by PGPR *Azospirillum brasilense* (Perrig et al. 2007).

Abscisic acid is an important next phytohormone that is responsible for the closure of stomata to limit water loss during stress conditions. It is also involved in lateral root formation (Bauer et al. 2013).

Gibberellic acid has been documented by Dodd et al. (2010) from PGPR community like *Achromobacter xylosoxidans*, *Acinetobacter calcoaceticus*, *Azospirillum* sp., *Bacillus* sp., *Herbaspirillum* sp., and *Gluconacetobacter* sp. This hormone is mainly responsible for the formation of primary root elongation and lateral root extension (Yaxley et al. 2001). All these hormones are involved in making plant defense strong by stimulating jasmonate and salicylic acid pathways (Pieterse et al. 2000).

13.4.3 Influence of PGPR Hormones on Plant Defensive Metabolites

13.4.3.1 Jasmonates

This compound is mainly involved in reproductive processes, flowering, fruit ripening, sensesense, and direct and indirect defense mechanism (Sea et al. 2001). It plays an important role in environmental stress. It is mainly studied from the dicotyledonous plant *Arabidopsis*, tobacco, and tomato. Monocotyledonous plants have the capacity to produce jasmonate in trace amount (Yan et al. 2012).

13.4.3.2 Brassinoides

These are produced by the plant in response to phytohormones for combating with high temperature (Janeiczko et al. 2011) and soil salinity (Abbas et al. 2013).

13.4.3.3 PGPR in Phosphate Solubilization

Phosphorus is available in the soil in the form of phosphates. It accounts approximately 2% of dry body weight of plants. It is insoluble in nature, and generally, plants cannot utilize it directly from the soil. It may be in the form of tricalcium phosphate, dicalcium phosphate, and rock phosphate. Here is microflora actually works to transform phosphorus from insoluble form to soluble form. The principal mechanism for phosphorus solubilization includes the production of organic acids which plays an important role in solubilization of available phosphorus source into utilizable phosphorus source. Phosphate-solubilizing microorganism is a heterogeneous type of microorganism including bacteria and fungi. Efficiency and economics of phosphate fertilizer utilization depend upon microbial population present in the soil for phosphate solubilization.

Phosphate-solubilizing PGPR belongs to genera *Bacillus*, *Pseudomonas*, *Mesorhizobium ciceri*, and *Mesorhizobium mediterraneum* (Rivas et al. 2006). *Pseudomonas* sp., *Erwinia herbicola*, and *Burkholderia cepacia* produce gluconic acid during phosphate solubilization, whereas *R. leguminosarum* and *Bacillus firmus* produce 2-keto-D-gluconic acid during phosphate solubilization.

13.4.4 PGPR in Potash Solubilization

Potash is available in the soil in the form of microcline, muscovite, biotite, and field spars which are silicate minerals. These minerals are slowly mineralized in nature. Potash is the second most important component needed for growth of plant other than nitrogen and phosphates. Potash is involved mainly in cell enlargement and improving water status of plants. It is also considered as an important factor for adding sugary nature to fruits and improvement in withstanding stress condition of plant, and it maintains the quality of fruits and vegetables. Unlike phosphates, it cannot be directly utilized by plants.

Potash-solubilizing PGPR is a group of microorganisms which includes *Acidithiobacillus ferrooxidans*, *Bacillus edaphicus*, *Bacillus mucilaginous*,

Burkholderia, *Paenibacillus* sp., and *Pseudomonas* sp. are reported as releasing potassium in easily accessible form (Liu et al. 2012).

13.5 Indirect Mechanism of Action by PGPR

PGPR plays a significant role in controlling disease-causing microflora in the rhizosphere and thus provides a stress-free environment for the growth and development of the plant. PGPR strain like *Acinetobacter* (Jog et al. 2014), *Bacillus*, *Brevibacillus*, *Lysinibacillus*, *Paenibacillus*, *Terribacillus*, *Bacillus amyloliquefaciens* (Herman et al. 2008), *Azospirillum* sp. (Rye et al. 2006), and *P. polymyxa and P. fluorescens* (Senthil et al. 2011; Reman et al. 2007) have been reported to control *A. niger*, *Penicillium* sp., *Micromonospora*, *Corynespora cassiicola*, *Myrus price*, Tomato mottle virus, rhizosphere fungi, and fungal E681 disease. It is reported that PGPR species, *Bacillus*, *Pseudomonas*, *Streptomyces*, *Coniothyrium*, *Gliocladium*, and *Trichoderma*, controls fungal diseases created by oomycetes (Cook and Baker 1983). Various mechanisms adopted by PGPR that indirectly supports plant growth promotion through biocontrol of phytopathogens are summarized in Table 13.3.

Table 13.3 Indirect mechanisms adopted by PGPR for plant growth promotion

PGPR Strain	Mechanism	Reference
Antibiotic production		
Bacillus sp.	Bacillomycin	Moyne et al. (2001)
Bacillus sp.	Zwittermicin A	Zhang and Fernando (2004)
Bacillus subtilis	Iturin	Constantinescu (2001), De Weert (2007)
Pseudomonas sp.	2,4 Diacetylphloroglucinol	Velusamy et al. (2006)
Pseudomonas fluorescens sp.	Pyrrolnitrin	Tazawa et al. (2000)
Pseudomonas fluorescens sp.	Viscosamide	Nielson et al. (2002)
Pseudomonas aureofaciens sp.	Phenazine 1 carboxylate	Powell et al. (2000)
Pseudomonas sp.	2,4 Diacetylphloroglucinol	De souza et al. (2003)
Pseudomonas sp.	Phenazine	Chin-A-Woeng et al. (2003)
P. fluorescence	**HCN production**	Ramyasmruthi et al. (2012)
Aneurinibacillus aneurinilyticus CKMV**1**		Chauhan et al. (2017)
Pseudomonas and *Bacillus* sp.		Ajay kumar et al. (2016)
Enzyme enzymes production		
Actinoplanes philippinensis	Glucanase	El-Tarabily and Sivasithamparam (2006)
Bacillus circulans and *Serratia marcescens*	Chitinase	Kishore et al. (2005)
Paenibacillus illinoisensis		Jung et al. (2003)
Serratia plymuthica		Kamensky et al. (2003)
Pseudomonas fluorescens	Lytic enzyme	Vivekananthan et al. (2004)
Streptomyces sp.	Phytase	Jog et al. (2014)

13.5.1 Antibiosis

It is well known that there is a struggle for existence in nature. In soil rhizosphere, competition for nutrition and survival exists among the bacterial and fungal rhizoflora. This term is generally referred to as antagonism. In nature, one microorganism may injure or kill the other microorganisms. This antagonistic relationship between the microflora can be useful for plant growers if plants are heavily infected with pathogenic strains. The most widely studied PGPR for antibiotic production is *Pseudomonas fluorescens*, which was first reported to produce phenazine-like antibiotic compound. It is reported that bacterial microflora produces numerous antifungal metabolites which includes ammonia, butyrolactone, 2,4 diacetylphloroglucinol, HCN, kanosamine, oligomycin A, oomycin A, phenazine-1-carboxylic acid, protein, visconamide, xanthobaccin, and zwittermicin (Kabir et al. 2013; Kaki et al. 2013; Milner et al. 1996; Nielson et al. 1998; Kang et al. 1999; Nakayama et al. 1999). It is reported that *B. subtilis* produce antifungal metabolite zwittermicin A and kanasamine (Pcypoux et al. 1999) and *Pseudomonas* sp. produce antifungal metabolites like viscosamide, pyoluteorin, 2,4 diacetylphloroglucinol, pyrrolnitrin, phenazine, and butyrolactone as biocontrol agents (Hass and Defago 2005).

13.5.2 PGPR in HCN Production

Hydrogen cyanide is a gas which is produced as secondary plant metabolite that affects negatively on root growth and root metabolism. It has the potential to act as a chemical player in biological control of pathogens (Heydari et al. 2008).

Hydrogen cyanide is a dreadful toxic metabolic inhibitor synthesized, extracted by PGPR in predation and competition type of association in the rhizosphere. It is observed that the host plant is not affected due to this cyanide, but it is effective against weeds around the plant rhizosphere (Zeller et al. 2007).

13.6 PGPR as Producer of Lytic Enzymes

PGPR strains can produce certain enzymes such as chitinase, dehydrogenase, lipase, phosphatase, and protease that exhibit their mode of action against phytopathogens (Senthilraja et al. 2013; Hayat et al. 2010). It is observed that PGPR residing in rhizosphere named as *Bacillus licheniformis*, *B. cereus*, *B. circulans*, *B. thuringiensis*, and *Serratia marcescens* have found to produce chitinolytic enzymes and have phytopathogenic potential against *Rhizoctonia solani* and *Fusarium oxysporum* sp. (Someya et al. 2007).

13.7 PGPR in Siderophore Production

Siderophores are small high-affinity iron-chelating molecules synthesized by microbial cells present in the rhizosphere. Earth crust has ample supply of iron-containing compounds, but these are not in easily utilizable forms in the rhizosphere. These are in the form of iron oxides and iron hydroxides. Due to their presence soil color becomes red and yellow in color (Kraemer 2005). Microbial cells in rhizosphere have the capacity to produce siderophores to chelate iron molecules insoluble Fe^{+3} complex forms. Major groups of siderophores include catecholates, hydroxamates, and carboxylates. Fungal siderophores include hydroxamates belonging to ferrichrome family which is further divided into five groups depending upon the side chain of hydroxamate functional group (Winkelmann 2007). These siderophores have an antagonistic effect by preventing the growth of other harmful bacteria and fungi in the rhizosphere (Sayyed et al. 2019; Crowley 2006).

13.7.1 PGPR in Inducing Systemic Resistance

Induced systemic resistance is the first mechanism of saving from various phytopathogens by plants. This mechanism in the plant should be boosted up because it is one of the most eco-friendly and cost-effective mechanisms for disease management and crop improvements (Edreva 2004). Plant defenses are preconditioned prior infection or treatment involves systemic acquired resistance (SAR) and induced systemic resistance (ISR). Both SAR and ISR are mediated by PGPR in the rhizosphere. It can be done by triggering salicylic acid-dependent SAR pathway producing salicylic acid (Choudhary et al. 2007). When any part of the plant is affected by phytopathogen infection, plant activates its ISR mechanism that is linked hypersensitive response which works in tissue enforcement and antibiotic production at the damaged site (Patel et al. 2016). The second set of the mechanism of protection involves the role of PGPR present in the rhizosphere. The induced systemic residence is observed in PGPR group especially in *Pseudomonas putida*, *S. marcesens*, *Flavimonas oryzihabitans*, *Bacillus pumilus*, etc. when plant faces some environmental challenges as well as for the protection of plant from pathogens. There is secretion of certain compounds by PGPR in induced systemic resistance in plants that includes synthesis of siderophores, pyoverdine, salicylic acid, fructose, rhamnose, flagellin, etc.

Jasmonic acid is involved as an important signaling molecule in plant defensive mechanism against pest and plant pathogens (Patel et al. 2016). It leads to the induction of PR proteins, chitinase which is a family of peptides involved with protection mechanism (Van Loon and Van Strien 1999). Pathak et al. (2017) reported the role of jasmonic acid and ethylene in induced systemic resistance (ISR) of *P. fluorescens* in *Arabidopsis*.

13.7.2 Nutrient and Niche Competition

As soil is a common culture medium for all microbial strains, there is always competition for nutrients and space in the rhizospheric area. Depending upon the dominance of microbial species in that area, the severity of disease is dependent (Kamilova et al. 2006). PGPR is always in competition in the rhizosphere. The presence of flagellar lipopolysaccharide, chemotaxis, and utilization power of chemicals of root exudate makes their survival in the rhizosphere (Lungtenberg and Kamilova 2009). Iron is a component needed for ATP synthesis, heme formation, and reduction of ribonucleotide precursors of DNA (Saraf et al. 2011). *Pseudomonas fluorescens* has a versatile requirement for nutrients and mainly dominates around the root surface in rhizosphere for utilizing chemicals around root exudates (Sorensen 1997).

13.8 Effect of PGPR on Soil Properties

Plants and animals are living constituents of soil. The soil is a dynamic living system containing a diverse microbial population. Soil under the agricultural sector is rich in organic matter which with other constituents maintains soil fertility. There is a natural ecosystem that maintains the balance of minerals in the soil.

Das and Singh (2014) carried out experimentation on the effect of PGPR on soil properties. They combined farmyard manure, compost, and PGPR at various concentrations and studies various parameters like pH, electric conductivity, organic carbon, organic sulfur, and NPK to the soil. They concluded that when farmyard manure and compost are combined with PGPR, there is a significant increase in all these parameters which positively affects plant growth and development. Parallel experimentation carried by Shinde et al. (2008) reported that upon application of PGPR, available nitrogen, phosphate, and potash were increased from 199.0 to 282.0, 14.77 to 27.52, and 366.7 to 448.75 kg per hectare, respectively.

13.9 PGPR with New Technologies

Farmers are now applying various biofertilizers and biopesticides in agriculture. Biofertilizers mainly used in fields are generally nitrogen fixers and those microbial cells which produces plant growth-promoting compounds. There is a rapid increase in research and development sections of industrial areas for development and implementation of microbial cells which can be used as biofertilizers. As mentioned earlier, PGPR can be useful in many ways to crops like nitrogen fixation, phytohormone production, improvement of soil quality, etc. The main thing is that efforts have been made to use a consortium of PGPR rather than using a single cell formulation which will be helpful for improving quality as well as maximum agriculture yield.

Nanotechnology is an emerging field in agriculture with use of new nanosized particles which can be used as nanofertilizers. These can be used as a new tool in agriculture for increasing crop yield in new ways (Liu et al. 2016). These nanoparticles have to be developed and implemented in such a way that they can protect the plant, detect various plants diseases, monitor plant growth, and enhance food quality and production with a reduction in waste (Suman et al. 2010). These nanosized nanoparticles are less toxic than chemical fertilizers and they can be integrated with biofertilizers.

Rhizoengineering is another field in rhizosphere and PGPR research which is based on the partitioning of the exotic biomolecules which create a unique channel for interaction between plant and microbes (Tiwari and Arora 2013). Much focus is needed on molecular and biotechnological aspects for increasing rhizospheric knowledge. Phytohormone engineering is another promising approach to deal with extreme environmental factors. But the development of stable phytohormone engineered plant having specific genes which could help the plant to overcome difficulties during stressful conditions (Sakamoto et al. 2003).

Under extreme conditions like extreme cold, cold-tolerating genetically engineered genes in PGPR rhizospheric zone might be future technology for selective encouragement of only expected growth thereby minimizing phytopathogenic microbes and adverse environmental conditions (Nadeem et al. 2013).

13.10 Conclusion

Impact of globalization has been creating a significant impact on agriculture. Uncertainty in agricultural climatic conditions, low or moderate monsoon, and lack of proper management during cropping have raised demand for food production. In such a scenario, biofertilizer technology has opened a new horizon for improvement in agricultural practices.

Microbiology in agriculture welfare made improvement of plants in terms of growth, health, yield, and commercialization of biofertilizer and biopesticide divisions in industrial sectors. PGPR works in terms of direct and indirect mechanisms. These all mechanisms work consistently for the growth and development of plants. No doubt huge numbers of genera in PGPR group are still acting as magical players in physiology and metabolism of plant cells. But efforts should be directed in right direction to design a consortium of PGPR group collectively in such a form that they can withstand extreme drought, high salt concentration, extreme cold, or any other extreme environmental conditions. Although PGPR group is efficient for plant-microbe rhizosphere health interaction, expression of some genes in PGPR and plant itself are needed. Further research in the development of biofertilizer efficiency, rhizosphere engineering, and nanotechnology is needed in this direction for improving the functioning of PGPR in the right perspective in human as well as agriculture welfare.

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Actinobacteria for Biotic Stress Management

14

Sunita Sakure and Sarika Bhosale

Abstract

Actinobacteria are one of the active members of soil micro flora, and they play a key role in soil nutrients cycling and crop yield. *Actinobacteria* in rhizosphere of different plants produce various growth-promoting substances that stimulate growth of plants even under unfavorable environmental conditions such as drought, heavy metal-polluted soils, salinity, and nutrient deficiencies. Several *Actinobacteria* are involved in the solubilization of phosphate and zinc in soil which play significant role in number of metabolic pathways. They also produce plant hormones such as auxins and gibberellins which promote plant growth by increasing seed germination, root elongation, and dry weight of the root. Production of lytic enzymes such as amylase, protease, cellulase, chitinase, and glucanase plays an important role in plant disease control and in turn improves soil health. Various *Actinobacteria* are found to produce different types of siderophores which starve plant pathogens for iron and inhibit their growth. These multifaceted plant growth-promoting activities of *Actinobacteria* make them an agriculturally important organism. One of the most important members of this group known as *Streptomyces* species strain 5406 has also been practiced in China for biological control of pathogens of cotton plant. Actinobacterial role as PGP has been investigated in wheat, rice, and beans. *Actinobacteria* are also found to produce ACC (1-aminocyclopropane-1-carboxylate) deaminase which protects the plants under environmentally stressful conditions. This chapter

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summarizes the efforts of researchers to demonstrate the beneficial role of *Actinobacteria* on plant health and agricultural productivity.

Keywords
Actinobacteria · PGP · Biocontrol · Stress management · Trehalose · ACC deaminase

14.1 Introduction

There are a number of different species of bacteria which grow in, on, or around plant tissue and around rhizospheric soil of bacteria and stimulate plant growth by a variety of mechanisms. All these bacteria are collectively called as plant growth-promoting rhizobacteria (PGPR). Due to the hazardous effect of chemical fertilizers and pesticides on human life, it is necessary to get attention on search for alternatives like PGPR. Recent research data on application of PGPR to soil reflects a significant increase in overall growth parameters including plant height, root length, and dry matter production of shoot and root of plants. Investigation of mechanism of mode of action of PGPR is increasing at a rapid rate so as to develop them commercially as biofertilizer. PGPR are currently commercialized as novel inoculum for plant growth promotion through direct and indirect mechanisms. The direct mechanisms of plant growth promotion may involve the synthesis of substances or facilitation of the uptake of nutrients from the environment (Verma et al. 2010). The direct growth-promoting mechanisms are nitrogen fixation, increasing the availability of nutrients in the rhizosphere, and production of phytohormones such as auxins, cytokinins, and gibberellins (Sevilla et al. 2001; Vessey 2003). The indirect mechanisms of plant growth promotion include the production of antimicrobial substances to lessen or prevent the deleterious effects of phytopathogens on plants or increasing the natural resistance of the host (Verma et al. 2010; Cartieaux et al. 2003). The indirect mechanisms of plant growth promotion are biocontrol agent, competition for sites on roots and displacement of pathogens, induced systemic resistance, tolerance under stress conditions (Dunne et al. 1998; Kloepper and Beauchamp 1992; Lorito et al. 1998).

14.2 *Actinobacteria* in Plant Growth Promotion

The phylum *Actinobacteria* includes a considerably high number of plant growth-promoting genera than bacteria (Hamedi et al. 2011). Plant growth-promoting *Actinobacteria* emit a vast collection of chemical modulators, which directly stimulate plant growth and act indirectly by supporting other plant advantageous microbes. Soil-dwelling *Actinobacteria* either kill or inhibit the growth of plant pathogens via antibiotic production, thereby ensuring the good health of plants (Shivlata and Satyanarayana 2017). *Actinobacteria* are a diverse group of Gram-positive,

filamentous, spore-forming, free-living bacteria predominant inhabitant of soil. The unique characteristics of bacteria make them a medicinally and agriculturally important organism. They are among the dominant soil microflora and rhizosphere and can colonize tissues of plants without causing any impairment to the plants. Therefore, *Actinobacteria* hold an outstanding position due to their diversity. Due to their typical unicellular and filamentous morphology, their survival in the soil or any unfriendly environment becomes long-lasting. It was widely thought that *Actinobacteria* are only soil inhabitants; however, genomic studies revealed that they are present in both freshwater and extreme environments such as thermal hot springs and Antarctic caves (Bentley et al. 2004). Genome size of *Actinobacteria* is in the range of 0.93 Mb (*Tropheryma whipplei*) and 11.9 Mb (*Streptomyces bingchenggensis*) (Verma et al. 2010). Some *Actinobacteria* harbor circular (*Nocardia*), linear (*Streptomyces*) plasmids. *Actinobacteria* have been considered as a transitional group between bacteria and fungi.. The phylum *Actinobacteria* is one of the most dominant taxonomic units of the domain Bacteria (Ventura et al. 2007) that constitutes six major classes (*Actinobacteria*, *Acidimicrobiia*, *Coriobacteriia*, *Nitriliruptoria*, *Rubrobacteria*, and *Thermoleophilia*).

The rhizosphere, defined as the zone of soil directly influenced by plant roots, represents a unique biological niche within the soil environment (Lechevalier 1989). The rhizosphere supports an abundance of diverse saprophytic microorganisms able to decompose polymeric organic matter such as lignocelluloses and chitin in the soil (Whips 2001), thereby making important contributions to nutrient cycling and the formation of humic substances (Trigo and Ball 1994). Mundt and Hinkle were able to isolate different species of *Streptomyces* and *Nocardia* from 27 different plant species, finding these *Actinobacteria* present endophytes in different plant tissues such as seeds and ovules. Sardi et al. isolated and observed through direct microscope examination the endophytic *Actinobacteria* from the roots of 28 plant species from Northwestern Italy, finding *Actinobacteria* belong to the genus Streptomyces and other common genera, namely, *Streptoverticillium*, *Nocardia*, *Micromonospora*, and *Streptosporangium* (Vurukonda et al. 2018). *Frankia* is known to form effective symbiosis with the species of *Alnus* and *Casuarina.* Survival and establishment of PGPR in the rhizosphere is a major apprehension of agricultural microbiologists. A chief source of concern is reproducibility in the field due to the composite interaction between the plants, microbes, and the environment (soil fertility and moisture, day length, light intensity, length of growing season and temperature)

14.3 Mechanisms of Plant Growth Promotion

14.3.1 Phytohormone Production

One of the direct mechanisms by which PGPR promote plant growth is by the production of plant growth regulators or phytohormones (Glick 1995). Botanists recognize five major groups of hormones: auxins, cytokinins, gibberellins, ethylene, and abscisic acid (Saharan and Nehra 2011). Indole 3-acetic acid (IAA) is the member

of the group of phytohormones and is generally considered the most important native auxin (Ashrafuzzaman et al. 2009). IAA functions as a significant signal molecule in the regulation of plant development including organogenesis; tropic responses; cellular responses such as cell expansion, division, and differentiation; and gene regulation (Ryu and Patten 2008). IAA is a natural auxin involved in cellular development and physiological processes in plants. Different soil microorganisms including bacteria (Stein et al. 1990), fungi (Finnie and Van Staden 1985), and algae (Rifat et al. 2010) are capable of producing physiologically active quantities of auxins. IAA is naturally stirring in plants, and it controls many physiological processes like cell enlargement and tissue differentiation and responses to light and gravity; similarly it stimulates spore germination and mycelia elongation in the *Streptomyces* sp. (Matsukawa et al. 2007). Several *Streptomyces* sp. such as *S. olivaceoviridi*, *S. rimosus*, and *S. rochei* from the tomato rhizosphere have the ability to produce IAA and improve plant growth by increased seed germination, root elongation, and root dry weight (El-Tarabily 2008). *Actinobacteria* facilitate the production of plant hormones such as IAA and cytokinins that are closely associated with plant growth (Ghosh et al. 2011).

Production of IAA in *Streptomyces* is tryptophan dependent and it follows the route of indole acetamide (Lin and Xu 2013). *Streptomyces filipinensis* No. 26 isolate promoted the growth of tomato grown under greenhouse conditions by stimulating the root and shoot length and produced IAA at a concentration of 77.43 μg/100 g of dry weights on the roots (Khamna et al. 2009). A significant quantity of IAA (52.3 μg/ml) was secreted by *Streptomyces* sp. isolated from the rhizosphere region of medicinal plants (Khamna et al. 2009). Maximal IAA secretion of 143 μg/ml was also observed for *Streptomyces* sp. isolated from the rhizosphere region of medicinal plants (Manulis et al. 1994). It is reported that 80% of microorganisms isolated from the rhizosphere of crops possess the ability to synthesize and release auxins as secondary metabolites which are known to promote root elongation and plant growth (Patten and Glick 2002). *Streptomyces* genus has been reported to produce high amount of growth-regulating hormone IAA in vitro. Similarly, many *Actinobacteria* are known to produce IAA and reported to increase plant shoot and root lengths. Although above-reported cultures are known to produce only IAA, an interesting fact of three Actinobacterial species, namely, *Streptomyces olivaceoviridis*, *S. rimosus*, and *S. rochei* cultures, was that they produced all three growth hormones, viz., auxin-, gibberellin-, and cytokinin-like substances, and enhanced the growth of wheat plants (Aldesuquy et al. 1998).

Cytokinins are a class of phytohormones which are known to encourage cell divisions, cell enlargement, and tissue development in certain plant parts. Gibberellins (GA) are a class of phytohormones most commonly associated with modifying plant morphology by the extension of plant tissue, particularly stem tissue (Salisbury 1994). Gibberellic acid (GA) is an important member of gibberellins family and acts as a natural plant growth hormone, controlling many developmental processes such as the induction of hydrolytic enzyme activity during seed germination, stem elongation, induction of flowering, improvement of crop yield,

overcoming dwarfism, elimination of dormancy, sex expression, enzyme induction and leaf and fruit senescence, etc. (Rangaswamy 2012; Rios-Iribe et al. 2010; Burckner and Blechschmidt 1991; Kumar and Lonsane 1989).

14.3.2 Solubilization of Minerals

Phosphorus is an essential macroelement necessary for the growth and development of living organisms. It is a primary part of various biological molecules such as nucleic acids, phospholipids, and energy-rich compounds (ATP, NADH, and NADPH). It has a vital role in several metabolic pathways such as cell division, signal transduction, macromolecular biosynthesis, and photosynthesis (Shenoy and Kalagudi 2005) and constitutes approximately 3% of total dry cell weight (Bhardwaj et al. 2014). In general, the available form of P is present in very low concentration (less than 1 mg/kg) as a result of the formation of metal complexes with Fe, Al, and Si (Hamdali et al. 2008). Promod and Dhevendaran (1987) observed maximum solubilization of insoluble phosphate by *Pseudomonas* and *Vibrio* in 3 days. Zone of solubilization around the colony on media containing solid agar and release of phosphate in the medium could be recognized to the release of organic acids, viz., citric, glyoxalic, malic, ketobutyric, succinic, fumaric, and tartaric by the microbes. Phosphate solubilization is most frequent among *Actinobacteria* such as *Streptomyces*, *Micrococcus*, *Micromonospora*, *Kitasatospora*, and *Thermobifida*.

Actinobacteria are of unique interest since these filamentous bacteria are often able to colonize plant tissue and to produce spores, a resistant form important for survival in agricultural soil (Hamdali et al. 2008). These interesting characteristics of *Actinobacteria* were mainly established under laboratory conditions (Hamdali et al. 2008) with green house by using phosphate-solubilizing *Actinobacteria*. The P-solubilizing ability of *Actinobacteria* has attracted interest in recent years because this group of soil organisms is not only capable of surviving in extreme environments (e.g., drought, fire.) but also possess other potential benefits (e.g., production of antibiotics and phytohormones-like compounds) that could simultaneously benefit plant growth (Hamdali et al. 2008). A study by Hamdali et al. (2008) has indicated that approximately 20% of *Actinobacteria* can solubilize P, including those in the common genera *Streptomyces* and *Micromonospora*. Rock phosphate-solubilizing *Actinobacteria* were reported to promote the growth of wheat plants in vitro as well as in vivo (Hamdali et al. 2008). The primary mechanism of P solubilization by PGPA is due to the production of organic acid and acidification of rhizosphere, thereby solubilization of unavailable to available form of P (Palaniyandi et al. 2011). Further, phosphorus availability enhancement is attributed to the chelation of cations such as Fe^{+2}, Al^{+3}, or Ca^{+2} which form insoluble phosphates and thereby help in the solubilization of insoluble phosphate. *Actinobacteria* can hydrolyze phytate (which constitutes up to 60% of soil organic phosphorus) by secreting phosphates such as phytases and acidic/alkaline phosphatases (Palaniyandi et al. 2011).

Dastager and Damare (2013) isolated *Actinobacteria* from the sediments of Chorão Island, Goa province, India. Out of 200 isolates, 30 isolates were prominent in the phosphate solubilization activity, and maximum solubilization was recorded to be 89.3 ± 3.1 to 164.1 ± 4.1 μgm/L after 6 days of incubation in six of all isolates.

Zinc is also one of the preliminary requirements for plants in tissue development and reproduction. Reported concentration of zinc in plants is around 5–100 mg/kg. It is involved in tissues of growing plant for proper development and reproduction. Any zinc deficiency leads to reduced synthesis of carbohydrates, nucleotides, auxins, cytochromes, chlorophyll, and membrane integrity which ultimately develops susceptibility to heat stress (Singh et al. 2015).

14.3.3 Siderophores Production

Siderophores are the low molecular weight (200–2000 Da) compounds secreted by bacteria to chelate the iron which is present in the environment in the insoluble form. Availability of iron in the ocean water limits marine production. As a strategy to cope up this iron-deficient condition, marine bacteria found to have the ability to produce amphiphilic siderophores like ferrioxamines (Boiteau et al. 2016). *Actinobacteria* has been reported to have the ability to produce ferrioxamine-type siderophores which are reported in eastern South Pacific Ocean remains (Table 14.1). Siderophores are mainly classified as catecholate, hydroxamate, and carboxylate type, and some bacteria have the ability to produce mixed carboxylate-hydroxamate type. Production of siderophores is extracellular or intracellular. Synthesis of siderophores occurred by non-ribosomal peptide synthetase (NRPS) or NRPS independent pathways (Oves-Costales et al. 2009). Siderophores produced by one *Actinobacteria* helps the development of other *Actinobacteria*. According to the report available, siderophore desferrioxamine E produced by *S. griseus* stimulated the growth and development of *S. tanashiensis* (Yamanaka et al. 2005). Patzer and Braun (2010) found that DNA of *Streptomyces* comprises siderophore biosynthetic

Table 14.1 List of *Actinobacteria* producing siderophore

Name of the *Actinobacteria*	Name of the siderophore	References
Streptomyces griseus	Desferrioxamin (Nocardamine)	Imbert et al. (1995); Meiwes et al. (1990); Yamanaka et al. (2005)
Streptomyces tendae	Enterobactin	Fiedler et al. (2001)
Streptomyces coelicolor	Coelichelin	Challis and Ravel (2000); Lautru et al. (2005)
Streptomyces ATCC 700974	Griseobactin	Patzer and Braun (2010)
Streptomyces pilosus	DesferrioxamineB (trade name Desferal)	Müller et al. (1984)

gene cluster encoding proteins similar to DhbABCEFG which is involved in the incorporation of DHBA into siderophores via a non-ribosomal peptide synthetase, and this gene cluster also contains genes which encode proteins for the siderophore secretion, uptake, as well as its degradation. Siderophores produced by *Actinobacteria* have shown to maintain the ecology and productivity of soil and water. Microbial siderophores may stimulate plant growth directly by increasing the availability of iron in the soil surrounding the roots (Kloepper and Beauchamp 1992). Marschner and Römheld (1994) reported that plants may also utilize siderophores synthesized by microorganisms colonizing the rhizosphere; this would be a source of soluble iron for the host plant. Plants such as sorghum, oats, peanut, cotton, cucumber, and sunflower demonstrated the ability to use radio-labelled microbial siderophores as a sole source of iron (Wang et al. 1993).

There is also a positive correlation between siderophore production and observed enhancement of plant growth (Becker 1988). There are multiple proposed mechanisms by which this may occur. First, sequestration of iron in the rhizosphere by PGPR renders the iron less available to potential pathogens in the rhizosphere (Kloepper and Beauchamp 1992) The *Actinomycete*-specific association had a positive influence on the physiology of the host plant.

14.4 Enhancers of Soil Fertility

Composting is microbial degradation of complex organic matter into nutrient-rich humus that nurtures plants and helps in restoration of productivity of eroded soil. *Actinobacteria* secrete various types of peroxidases among which lignin peroxidases facilitate humification and composting via hydrolysis of lignin into humic acid-like complexes. The composition of Actinobacterial community changes during various stages of composting, for example, the presence of both mesophilic (*Streptomyces*) and thermo-tolerant species (*Saccharomonospora viridis*, *Thermobifida fusca*, and *Thermobispora bispora*) has been recorded at different phases of compost formation (Steger et al. 2007). Secretion of different enzymes by *Actinobacteria* like amylases, chitinases, cellulases, and peroxidases makes the *Actinobacteria* suitable for soil fertility as these enzymes help to mineralize the complex organic material into simpler forms which can be assimilated by plants. *Actinobacteria*, such as *Streptomyces* spp., influence soil fertility through the involvement of many components and serve as nutrient enhancers. Besides producing siderophores and solubilizing phosphate, they are known to produce various enzymes including amylase, chitinase, cellulase, invertase, lipase, keratinase, peroxidase, pectinase, protease, phytase, and xylanase which make the complex nutrients into simple mineral forms. This nutrient cycling capacity makes them ideal candidates for natural fertilizers (Jog et al. 2016).

14.5 Stress Tolerance

Biotic stress to the plants is caused by different plant pathogens. Fungi, bacteria, viruses, weeds, insects, and other living organisms damage the plants in a variety of ways. The main abiotic stress to the plants is caused by fungi. There are various reports reflecting the importance of actinobacteria for antiphytopathogenic activity. Kanini et al. (2013) isolated and identified potential antifungal streptomycetes from rhizosphere and nonrhizosphere soil and carried out in vivo experiments on beans. Srividya et al. (2012) evaluated *Streptomyces* sp. 9p as effective biocontrol against chilli soil-borne fungal phytopathogens.

Application of *Actinobacteria* for biotic stress management can be achieved by use of three main strategies. Actinobacterial species which are effective colonizers of plant systems with the production of antiphytopathogenic compounds and plant growth-promoting activities will be the best solution for biotic stress management. Various mechanisms of disease control by *Actinobacteria* have already been discussed in this topic under the heading of *Actinobacteria* as biocontrol agents.

Plants have to face biotic and abiotic stress mainly. Abiotic stress includes different environmental conditions. Abiotic stresses like drought, flooding, salinity, and extreme temperatures can be managed by using the bacterial strains which can produce cytokinins, ACC deaminases, abscisic acid, trehalose, exopolysaccharides, and volatile organic acids. Abiotic stress results in the production of stress ethylene. So the main strategy for management of this stress is by lowering the amount of ethylene. This can be achieved by ACC deaminase-producing bacteria. Management of abiotic stresses can be achieved by three main strategies using *Actinobacteria*.

14.5.1 Management of Abiotic and Biotic Stress

14.5.1.1 Trehalose Production

Trehalose is stable non-reducing sugar. High levels of trehalose protects the plants from stresses like extreme temperature, drought, and salinity. Trehalose is resistant to acid and temperature. It forms gel phase and prevents degradation of proteins protecting plants from both high and low temperatures. There are reports of trehalose-producing *Streptomyces* spp.

14.5.1.2 ACC Deaminase Production

Glick et al. (1998) put forward the theory that the mode of action of some PGPR was the production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase, an enzyme which could cleave ACC, the immediate precursor of ethylene in the biosynthetic pathway for ethylene in plants. ACC deaminase activity would decrease ethylene production in the roots of host plants and result in root lengthening. More importantly, *Actinobacteria* alleviate plant stresses by reducing the ethylene level in the root by secreting 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase enzyme (Hamedi et al. 2015).

To combat with biotic and abiotic stress, *Actinobacteria* produces ACC deaminase which inhibits auto-catalytic ethylene synthesis (Grichko and Glick 2001, Mayak et al. 2004a, b, Belimov et al. 2005). ACC deaminase, 1-aminocyclopropane-1-carboxylate deaminase (EC 3.5.99.7), is one of the plant growth-promoting enzymes. ACC deaminase converts ethylene precursor to ammonia and α-ketobutyrate which is further utilized by bacteria for their growth. ACC deaminase activity provides induced systemic tolerance to plants against stress caused by drought, heavy metals, flooding, and high salt (Jaemsaeng et al. 2018). Symbiotic performance of PGPR depends on ACC deaminase. Reported ACC deaminase-producing strains are *Streptomyces*, *Amycolatopsis*, and *Nocardia* (Nascimento et al. 2014). ACC deaminase enzyme is an inducible enzyme and requires its substrate ACC. This enzyme encoded by gene AcdS which is present in *Actinobacteria*, found in their primary and unique chromosome (Singh et al. 2015). In stressed conditions leguminous plants produce ethylene in high concentration which leads to nodulation and growth inhibition (Nascimento et al. 2016). To overcome this stress-induced effect on crops, it is necessary to maintain the rhizospheric *Actinobacteria* which have the potential to produce ACC deaminase. Screening of *Actinobacteria* for ACC deaminase production potential can be done by growing this *Actinobacteria* in a medium containing 1-aminocyclopropane-1-carboxylate as nitrogen source. *Actinobacteria* exhibiting good growth could be further confirmed using TLC-based method (Wang et al. 2012).

14.5.1.3 Production of Volatile Compound

Bacterial volatiles have been reported to promote growth and to induce systemic resistance in *Arabidopsis*. Strain AOK-30 of *Streptomyces padanus* volatiles are associated with this induced drought tolerance. Based on these earlier reports, tissue-cultured seedlings may recognize and respond to AOK-30 as an external stimulus. Thus, if drought tolerance of tissue-cultured seedlings is enhanced by *Actinobacteria*, perhaps the seedlings can be acclimatized under a relatively lower humidity, enabling the seedlings to grow and escape diseases (Hasegawa et al. 2004). Srivastava et al. (2014) demonstrated that *Streptomyces rochei* SM3 activates ethylene-mediated defense pathway and phenyl-propanoid pathway in chickpea and therefore discharged stresses caused by both biotic (*Sclerotinia sclerotiorum*) and abiotic (NaCl) factors. Hence, this could be a potential candidate for the development of a plant growth-promoting agent (PGPA).

Heavy metal stress results in low iron supply to the plants. So the best solution for heavy metal stress is use of plant-associated siderophore-producing *Actinobacteria*.

14.6 Management of Biotic Stress (Plant Diseases)

Chemical Pesticides are used to control plant diseases from ancient days. This resulted in severe environmental pollution and decreased diversity of non-target organisms. Microorganisms as biological control agents have high potential to

control plant pathogens and no effect on the environment or other non-target organism (Sutthinan et al. 2009). *Actinobacteria*, including *Streptomyces species*, have various qualities of effective biocontrol agents. They are effective colonizers of root system. Several phytopathogen-inhibiting compounds are produced by actinomycetes, and they are also one of the major producers of antibiotics against fungi. They secrete various extracellular enzymes which inhibit plant pathogens including fungi and insects.

14.7 Mechanisms of Biocontrol

14.7.1 Effective Colonizers of Root System

Actinobacteria have the ability to colonize not only the root surfaces, but also the various parts of plants. They have been isolated from seeds and ovules also. To be used as effective biocontrol agents, colonization is one of the essential properties. *Actinobacteria* specifically *Streptomyces* form desiccation-resistant spores. So they can be formulated in powder form to be used as biocontrol agents. *Streptomyces griseoviridis* has the ability to colonize the plant rhizosphere. *Fusarium* wilt of carnation, the damping-off of *Brassica*, and the root rot disease of cucumber are controlled by *S. griseoviridis* which is an antagonistic actinomycete.

14.7.2 Production of Inhibitory Compounds Against Phytopathogens

Actinobacteria, belonging to the genus *Streptomyces* sp., appear to be good candidates to find new approaches to control plant diseases (Běhal 2000). *Actinobacteria* found in rhizosphere soil of medicinal plant may have the ability to produce new inhibitory compounds against phytopathogens. Plant root exudates stimulate rhizosphere growth of *Streptomycetes* that are strongly antagonistic to fungal pathogens. *Streptomyces* sp. Strain 5406 has been used in China to protect cotton crops against soil-borne pathogens. *Actinobacteria* are important natural producers of antibiotics or anti-fungals that could protect the plants against various devastating phytopathogens such as *Pythium ultimum*, the cause of damping-off disease in wheat seedlings (Jain and Jain 2007). Crawford et al. (1993) found that 12 actinomycetes strains isolated from *Taraxicum officinale* rhizosphere were active against *Pythium ultimum*. *Actinobacteria* are able to produce large number of agroactive metabolites that play a role as biocontrol agents exhibiting antagonism against a variety of plant pathogens (Trejo-Estrada et al. 1998; Yuan and Crawford 1995). *Streptomyces nigrescens* produce phosphazomycins that exhibit in vitro activity against *Botrytis cinerea*, *Rhizoctonia solani*, and *Alternaria kikuchiana* (Tomiya et al. 1990). The genus *Streptomyces* has been investigated as a potential biocontrol agent against fungal phytopathogens such as *Pythium ultimum* (Jensen et al. 2002).

Medicinal plants are known to be rich in secondary metabolites and are potentially useful to produce natural drugs. Medicinal plants support a great diversity of microflora in their rhizosphere including plant growth-promoting rhizobacteria. Active *Actinobacteria* may be found in medicinal plant root rhizosphere soil and may have the ability to produce new inhibitory compounds against phytopathogens. Thangapandian et al. (2007) isolated *Streptomyces* from medicinal plant in rhizosphere soils, and from this study it was observed that eight isolates were showing good antipathogenic activity. Sutthinan Khamna et al. (2009) isolated *Actinobacteria* from rhizosphere of medicinal plants and demonstrated the anti-phytopathogenic activity of *Streptomycetes* against selective plant pathogen. The antibacterial activity of *Actinobacteria* was established in many previous studies; Zamanian et al. (2012) demonstrated a high level of activity for *Streptomyces plicatus* against *E. carotovora* subsp. carotovora, while El Karkouri et al. (2017) isolated an actinomycete strain which inhibited the growth of *E. chrysanthemi* and identified it as *Streptomyces cinereoruber*. Kanini et al. (2013) isolated and identified potential antifungal *Streptomycetes* from rhizosphere and nonrhizosphere soil and carried out in vivo experiments on beans. Srividya et al. (2012) evaluated *Streptomyces* sp. as effective biocontrol against chilli soil-borne fungal phytopathogens. A research conducted by Muiru et al. (2008) evaluated the antibiotic metabolites from two antagonistic *Actinobacteria* isolates for the control of late blight of tomatoes in the greenhouse. Mildiomycin was extracted from a culture of *Streptoverticillium imofaciens*, which has antifungal activity. Mildiomycin is an excellent solution for powdery mildews on various crops). The metabolites were found to give a significant control in the management of late blight and delayed the onset of the disease. Siderophores synthesized by *Actinobacteria* residing in the rhizospheric soil are mainly studied due to their attribute as biocontrol agent against pathogens and in disease-suppressive soils (Loper and Buyer 1991).

14.7.3 Secretion of Extracellular Lytic Enzymes

Actinobacteria are producers of various lytic enzymes. Chitinases and glucanases are important for antifungal activity. Extracellular glucanases are able to hydrolyze glucans from cell wall of fungal pathogen like *Phytophthora* species. Chitin is the component of fungal cell wall and also the cuticle of insects. Chitinase-producing *Actinobacteria* are useful to control fungal pathogens and insects. *Streptomyces* has also been widely used for biocontrolling soil-borne fungal pathogens (Trejo-Estrada et al. 1998). *Streptomyces* antibiotics and lytic enzymes have proved their potential as biocontrol agents against *F. culmorum* responsible for various symptoms like damping roots, stems and spikelet fusariosis in many broadleaf and monocotyledons plants such as cereals. Previous study showed that *Actinobacteria* isolated from Malaysian soil have the potential to inhibit the growth of several plant pathogens. Oskay et al. (2004) also reported about the ability of *Actinobacteria* isolated from Turkey's farming soil. They have the ability to inhibit *Erwinia amylovora*, a

bacteria that cause fire-blight in apple, and *Agrobacterium tumefaciens*, a causal agent of crown gall disease (Jeffrey 2008).

14.8 Conclusion

The efficacy of *Actinobacteria* is not only applied in formulation of biofertilizers or biopesticides, but they also appear ideal for innumerable applications in environmental issues. Their adaptive morphology as well as excellent metabolic versatility enables them to establish their populations to all kinds of extreme environments including highly polluted locations. Evaluation of significant role of *Actinobacteria* in terms of decontamination of polyaromatic hydrocarbons has been mentioned in previous literatures. Spores of *Streptomyces rochei* strain PTL2 has been tried in wettable talcum powder, sodium alginate pellets, and sodium alginate-clay pellets to control the disease infestation caused by *Sclerotium rolfsii*. Talcum-based formulation was found to be more effective to reduce the disease and promoted growth of tomato seedlings. Commercial formulations of *Streptomyces* are also available in international market like Actinovate, Novozymes, Mycostop, and Microsat F UNO. These can be applied to soil in the form of granules and slurry. *Streptomyces* have been extensively studied for the production of antibiotics. Literature shows the significance of *Streptomyces* as plant growth promoter and biocontrol agent in agriculture sector. More emphasis on this aspect of *Streptomyces* sp. will surely change the scenario of productivity of crop and soil health.

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15 Plant Growth-Promoting Rhizobacteria (PGPRs): Significant Revolutionary Tools for Achieving Long-Term Sustainability and Combating the Biotic Stress Caused by the Attack of Pathogens Affecting Crops in Agriculture

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Abstract

The microbes residing in the soil that are beneficial for the growth of crops in terms of vegetative and reproductive growth are known as plant growth-promoting microbes (PGPMs). These PGPMs may be agriculturally promising bacterial and fungal strains which reside in the rhizosphere region of crops. Today these PGPMs are of areas of interest for research and commercialization. These PGPMs are now broadly categorized as "plant growth-promoting rhizobacteria (PGPR)." These PGPRs play a vital role in maintaining soil fertility and plant health. They can act as biofertilizers and provide immunity to the crops against invasion of pathogens and resist against different biotic and abiotic stress conditions. PGPRs are effective growth modulators for the crop as they secrete novel metabolites and growth molecules that enable the crop to sustain in adverse and stress conditions. These molecules also induce systemic resistance and anti-pathogenic effect against the soil-borne infections. These PGPRs release different metabolites such as phyto-harmones, viz., indoleacetic acid (IAA), auxins, cytokinin and gibberellic acid (GA3) for growth of crops via solubilizing the minerals and other complex compounds. Besides these molecules, these PGPRs secrete allelochemicals and metabolites, including iron-chelating siderophores, antibiotics, biocides, volatile compounds, lytic compounds, and detoxification compounds which are able to kill the soil-borne pathogens. The PGPRs are also involved in biological control of insects and pests as these PGPRs are producing enzymes and metabolites which are able to invade the prey's immune system and digest the internal organs followed by exoskeleton of insects and pests. Thus plant growth-promoting rhizobacteria are the promising candidates for agriculture

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in enhancing soil fertility and protecting the crops against soil-borne pathogens, insects, and pests. These PGPRs are therefore regarded as significant revolutionary tools for achieving long-term sustainability.

Keywords
Plant growth-promoting rhizobacteria (PGPR) · Plant growth promotion activity · Antagonistic activity · Soil-borne pathogens · Insecticidal and pesticidal activity

15.1 Introduction

PGPR is the term coined by Kloepper around the 1970s, and in due duration it is gaining lot of attention in the modern world. Plant growth-promoting rhizobacteria (PGPRs) are the wonder microbial strains responsible for the growth promotion activity and invasion against pathogens and are a great resource of natural molecules and metabolites. These strains are adaptable to grow in adverse climatic ranges within the rhizosphere region of soil and are able to degrade toxic compounds present within the soil. The genetic potential of PGPR is considered to be the main resource for driving all beneficial activities (Cook 2002). The PGPRs are now regarded as the microbial strains which are able to perform aggressive colonization, root colonization, plant height elongation and biocontrol against pests and pathogens (Weller et al. 2002; Vessey 2003). Generally PGPRs associations are positive interactions, while some negative interactions are seen in phyto-pathogenic rhizobacteria which produce phytotoxic substances such as hydrogen cyanide or ethylene, thus, negatively influencing on the growth and physiology of the plants. The PGPRs are forming a significant association and a strong network in the rhizosphere region of the soil and perform activities in terms of phosphate solubilization, potash mobilization, nitrogen assimilation, nutrient exchange to crops, interaction with root exudates, secretion of novel metabolites for plant growth, invasion of attack of soil-borne pathogens, etc. PGPRs are now of versatile and variable type comprising different genera, viz., *Arthrobacter*, *Variovorax*, *Azospirillum*, *Alcaligenes*, *Enterobacter*, *Bradyrhizobium*, *Burkholderia*, *Serratia*, *Azotobacter*, *Klebsiella*, *Mesorhizobium*, *Rhodococcus*, *Streptomyces*, *Flavobacterium*, *Bacillus*, *Pseudomonas*, *Acinetobacter*, *Alcaligenes*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Proteus*, *Rhizobium*, *Serratia*, and *Xanthomonas* (Glick 1995; Kaymak 2011) exhibiting successful rhizosphere colonization. It is also explored that, a large number of surface molecules secreted by the bacteria are able to help in colonization. The rhizospheric colonization of these PGPRs is involved in beneficial purposes such as biofertilization, phytostimulation, biocontrol, and phytoremediation.

15.2 Mechanism of Action of PGPRs

There are different mechanisms of PGPRs which support the growth of crops by secretion and releasing of metabolites, phyto-harmones, production of siderophores, root colonization, nitrogen fixation, mineral solubilization, lowering the ethylene levels, elongation, and strengthening the crops against adverse effects of biotic and abiotic stresses. These PGPRs are believed to stimulate the growth of crops directly or indirectly (Castro et al. 2009). PGPRs also work by the mechanism of quorum sensing by which different secretions and molecules secreted by one bacterium signals and interacts with others and forms a network. Different studies have also revealed the mechanism of plant growth by PGPR in terms of ACC deaminase production which reduces and regulates the level of ethylene in roots; secretion of phyto-harmones, viz., IAA, auxins, cytokine, and gibberellic acid, etc.; antagonistic activity against pathogenic microbes by production of siderophores and enzymes, viz., chitinases, glucanase, cyanides, etc.; and solubilization of minerals such as phosphates, potash, silicates, etc. Different biochemical and molecular approaches are utilized for the information for the regulation of biosynthetic pathways in PGPRs for biological control mechanisms (Joshi and Bhatt 2011).

15.3 Synthesis and Secretion of Different Metabolites by PGPRs

The microbiota of PGPR synthesizes and secretes different allelochemicals which are involved in colonization and interaction of multiple strains and colonies of PGPRs. The colonization within the rhizosphere niches is enabled by production of bacterial allelochemicals and metabolites, including iron-chelating siderophores, antibiotics, biocides, volatile compounds, lytic compounds, and detoxification compounds. Besides the secretion of these molecules, PGPRs release the enzymes, phyto-harmones and other degrading metabolites for solubilization of minerals and other complex compounds.

15.3.1 Role of Siderophores and Competition for Iron

It is already well defined that iron is an important element for the growth of all living organisms. The scarcity of bioavailable iron in the soil and soil habitat develops a strong competition amongst soil flora which resides in the form of PGPRs. In iron-limiting conditions, PGPRs produce low-molecular-weight compounds called siderophores to competitively acquire ferric ion. Different bacterial siderophores differ in their abilities to sequester iron. Several environmental factors also modulate siderophores synthesis, including pH, the level of iron and their irons, the presence of other trace elements, and an adequate supply of carbon, nitrogen, and phosphorus.

15.3.2 Antibiosis

The antibiosis is the biocontrol mechanism of PGPRs which includes different compound secretions, viz., amphisin, 2,4-diacetylpholorglucinol, phenazine, oomycin, and tensin, and different types of cyclic lipopeptides secreted by *Pseudomonas* sp. Different compounds such as oligomycin A, kanosamine, zwittermicin A, and xanthobaccin are produced by *Bacillus* and *Streptomyces* specifically (Mpiga et al. 1997). The antibiotics synthesized by these PGPRs are helpful as pharmacological agents and also provide a path to develop new antimicrobials against drug-resistant pathogens. The secretions of such molecules are also influenced by the effect of pH, metabolic status of the microbes, nutrient availability, trace elements, and environmental stimuli.

15.3.3 Lytic Enzyme Production

Different PGPRs secreted lytic enzymes such as hydrolases, chitinases, proteases, glucanases, etc. which are able to inhibit the growth of soil-borne fungal pathogens such as *Fusarium oxysporum*, *Sclerotinia sclerotiorum*, *Botrytis cinerea*, and other soil-borne pathogens (Singh et al. 1999). Previous studies also reported that the endophytic PGPR strains, viz., *Streptomyces* NRRL 30562 isolated from *Kennedia nigriscans*, can inhibit the growth of *Streptomyces scabies* and *Xanthomonas campestris* by production of siderophores and other volatile compounds (Kamensky et al. 2003).

15.3.4 Nitrogen Fixation

The nitrogen is an essential element for all forms of life and it is the most vital nutrient for plant growth and productivity. Symbiotic nitrogen fixation is a mutualistic relationship between a microbe and the plant. The microbe first enters the root and later on forms nodules in which nitrogen fixation occurs. Rhizobia are a vast group of rhizobacteria that have the ability to lay symbiotic interactions by the colonization and formation of root nodules with leguminous plants, where nitrogen is fixed to ammonia and make it available for the plant (Gaby and Buckley 2012). The mode of nitrogen fixation is symbiotic or non-symbiotic. The plant growth-promoting rhizobacteria widely presented as symbionts are *Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, and *Mesorhizobium* with leguminous plants and *Frankia* with non-leguminous trees and shrubs. Non-symbiotic nitrogen-fixing rhizospheric bacteria belong to genera including *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus*, *Enterobacter*, *Gluconacetobacter*, and *Pseudomonas* and cyanobacteria (*Anabaena*, *Nostoc*) (Zahran 2001). Non-symbiotic nitrogen-fixing rhizospheric bacteria belonging to genera include *Azoarcus*, *Azotobacter*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Diazotrophicus*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas* and cyanobacteria (*Anabaena, Nostoc*) (Reed et al. 2011). The genes for nitrogen fixation, called *nif* genes, are found in both symbiotic and free-living systems.

15.3.5 Phosphate Solubilization

Phosphorus is the most important key element in the nutrition of plants, next to nitrogen. It is abundantly available in soils in both organic and inorganic forms. It plays an important role in virtually all major metabolic processes in plant including photosynthesis, energy transfer, signal transduction, macromolecular biosynthesis and respiration (Ahmad and Kibret 2014). Phosphate-solubilizing PGPR belong in the genera *Arthrobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium*, *Rhodococcus*, and *Serratia* (Sharma et al. 2013).

15.3.6 Potassium Solubilization

Potassium (K) is the third major essential macronutrient for plant growth. The concentrations of soluble potassium in the soil are usually very low and more than 90% of potassium in the soil exists in the form of insoluble rocks and silicate minerals (Parmar and Sindhu 2013). Moreover, due to imbalanced fertilizer application, potassium deficiency is becoming one of the major constraints in crop production. Without adequate potassium, the plants will have poorly developed roots, grow slowly, produce small seeds and have lower yields. This emphasized the search to find an alternative indigenous source of potassium for plant uptake and to maintain potassium status in soils for sustaining crop production (Kumar and Dubey 2012). Plant growth-promoting rhizobacteria are able to solubilize potassium rock through production and secretion of organic acids (Han and Lee 2006). Potassium solubilizing plant growth-promoting rhizobacteria such as *Acidithiobacillus ferrooxidans*, *Bacillus edaphicus*, *Bacillus mucilaginosus*, *Burkholderia*, *Paenibacillus* sp., and *Pseudomonas* have been reported to release potassium in accessible form from potassium-bearing minerals in soils (Liu et al. 2012). Thus, application of potassium solubilizing plant growth-promoting rhizobacteria as biofertilizer for agriculture improvement can reduce the use of agrochemicals and support ecofriendly crop production.

15.4 Common PGPRs That Have Been Recognized as Biological Control

15.4.1 Fungi

15.4.1.1 Trichoderma

It is a predominant fungal genus which belongs to many ecosystems. It possessed the ability to reduce the severity of different plant diseases by inhibiting plant pathogens, mainly in the soil or on plant roots, through their high antagonistic and mycoparasitic potential (Viterbo and Horwitz 2010). The recent comparative genome sequence analysis of two recognized biocontrol species – *Trichoderma atroviride*

and *Trichoderma virens* – has afforded us a better understanding of how mycoparasitism arose in a common Trichoderma ancestor as a lifestyle of the genus (Kubicek et al. 2011). The presence of fungal prey and the availability of root-derived nutrients may have been major attractors for the ancestors of Trichoderma to establish them in the rhizosphere and to facilitate the evolution of positive interactions with plants (Druzhinina et al. 2011). The control of a broad range of plant pathogens, including fungi, oomycetes, bacterial and viral diseases, through elicitation by Trichoderma of ISR or localized resistance has been reported (Harman et al. 2004). Some Trichoderma rhizosphere-competent strains have been shown to have direct effects on plants, increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination, and stimulation of plant defenses against biotic and abiotic damage (Shoresh et al. 2010). Trichoderma cells enhance root colonization, the coordination of defense mechanisms and increased rate of leaf photosynthesis (Vargas et al. 2009). Solute transporters such as a di/tripeptide transporter and a permease/intracellular invertase system involved in the acquisition of root exudates have been described in Trichoderma (Vizcaıno et al. 2006; Vargas et al. 2009)

15.4.2 Mycorrhizae

Mycorrhizae is a symbiotic plant-fungus association which plays a significant role in colonization and association with roots of higher plants. The fungus provides mineral nutrients, water, protection against pathogens, alleviation of abiotic stresses such as salinity, drought and pollution, to the plant which, in return, provides carbon as an energy source to fungus. The Mycorrhizal association is able to maintain the sustainable agriculture by exchanging the nutrients and strengthening the roots and crops. Mycorrhizae interact with plant growth-promoting rhizobacteria (PGPRs) in order to develop strong interactions for exchange of nutrients and strengthening roots (Diedhiou et al. 2005, 2009, 2010; Hijri 2016). Mycorrhizal- and PGPR-based commercial inoculants are utilized as biofertilizers in a variety of formulations in agriculture, horticulture and even in forestry. Ecto-mycorrhizal and endo-mycorrhizal associations can influence plant community assembly and facilitate plant coexistence in boreal and temperate regions, but little is known in tropical and neotropical forests (Ba et al. 2012).

15.4.2.1 Metarhizium anisopliae

It is identified as a biocontrol agent in the 1880s, found in soil, and used as a biocontrol agent against different insects and pests including beetles, spittle bugs, and locusts (Zimmerman 1993). Different spores or conidial formulations of M. anisopliae are prepared and applied. After achieving the initiation of the fungal epizootic control, new spores and the vegetative cells are created in the infected insect. These spores rapidly extend to the healthy insect population and encourage persistent control.

15.4.2.2 Paecilomyces lilacinus

It is a nematode egg parasite currently used as a biological control agent against various plant-parasitic nematodes particularly the *P. lilacinus* strain 251 for which a commercial formulation is available (Kiewnick 2013). *P. lilacinus* successfully control the nematode infections causing severity in soil. *P. lilacinus* is found to invade and attack the initial stages of nematode, particularly nematode eggs. It is observed that, *P. lilacinus* is able to control the mobile nematode *R. similis* on banana and on betel vine when introduced into the soil prior to nematode inoculation.

15.4.2.3 Beauveria bassiana

It is also known as thread-like fungi; different strains of Beauveria are introduced in the insect. The fungus infects the insect cuticle in a high humid environment. Insects are infected by conidia (asexual propagules) which adhere to the host cuticle. Successful infection of *Beauveria bassiana* is dependent mainly on various enzymatic activities for degradation of proteins, chitin and lipids in the insect integument.

15.4.2.4 Verticillium lecanii

It is a cosmopolitan fungus which was first described in 1861. It is also known as a "white-halo" fungus because of the white mycelial growth on the scale and cuticle of insects. The fungus infects the insects by generating the hyphase from the spores which gets penetrate into the insect's gut and destroys all the internal parts of the insects. The fungus protrudes grows out from the insect's body and burst the insect. The fungus needs high humidity of 85% to 90%. The fungus cannot work well in low humid conditions. The fungus mycelium produces a cyclodepsipeptide toxin called bassianolide, other toxins such as dipicolinic acid. The fungus infects aphids, white flies while also destroys rust fungi. The fungus activity has dose-dependent activity. Significantly higher doses of the fungus result in faster killing. The most effective results can be observed on the spores count and when produced via submerged fermentation technique. Virulence also depends on the density of spores and rate of sporulation, which is also dependent on environmental conditions. Fungal virulence varies with the method of conidial production.

15.4.3 Bacteria

15.4.3.1 *Pseudomonas* Species

Pseudomonas sp. performs biocontrol mechanism, such as colonization and proliferation within the plant, competition with other microorganisms, adaptation against environmental stresses, and the production of a wide range of active biometabolites such as antibiotics, siderophores, volatile substances, and growth-stimulant compounds (Srividya and Sasirekha 2016). *Pseudomonas* is also found efficient in phosphate-solubilizing property within the soil. The strains, viz., *Pseudomonas fluorescens*, *P. chlororaphis*, *P. aureofaciens*, *P. putida*, and *P. syringae*, are found to be significant in invasion against the pathogens.

15.4.3.2 *Bacillus* Species

Bacillus sp. are the important genera of PGPR which are having nutrient solubilization and mobilization activities. Besides these activities, *Bacillus* sp. are effective in controlling the fungal phyto-pathogens. The compound released by *Bacillus* strains is cyclic lipo-peptide that has an inhibitory effect on fungal growth while has a little toxic effect on humans. Studies determined that the antifungal metabolites of *Bacillus* species show that these metabolites are resistant to temperature and pH changes and thus fungal activity remains unchanged (Hang et al. 2005; Helbig 2001). *Bacilli* strains such as *Bacillus megaterium* were found to have anti-pathogenic effect on white blotch in wheat, while *Bacillus circulans* causes the death of date seedlings, and *Bacillus polymyxa* inhibits blight tomato (Naim and Ibrahim 2014). *Bacillus* is found to be an effective agent for biological control as they produce antimicrobial metabolites and also can be easily formulated. The *Bacillus* is found to secrete different novel antimicrobial compounds, viz., cyclic lipo-peptides such as surfactin, etorin, and fengycin for biotechnology and pharmacy applications. The strains, viz., *Bacillus subtilis*, *Bacillus cereus*, *Bacillus megaterium*, and *Bacillus mycoides*, are found to be significant in terms of disease and pathogen control (Bettiol et al. 2011).

15.5 Studies on Different PGPR Strains

Different PGPR strains were reported with the passage of time. *Azarcus* has been seen along with crop named rice and has been known for nitrogen fixation (Roger et al. 1985). *Azotobacte*r was reported to enhance the cytokine synthesis in cucumber. *Azorhizobium* and *Azospirillum* have been isolated from fields of wheat and sugarcane, respectively, and have been helpful in nitrogen fixation. *Azotobacter* isolated from a number of crops like maize, barley, wheat, oats, etc. has undergone nitrogen fixation. *Bacillus* has been obtained from various crops fields like potato, cucumber, pepper, peanuts, maize, etc. with wide array of its mechanism like auxin synthesis and cytokinin synthesis, gibberellin synthesis, potassium solubilization, induction of plant stress resistance, antibiotic production, and siderophore production. *Beijerinckia* and *Burkholderia* isolated in associated form from sugarcane and rice crops respectively have been reported to perform nitrogen synthesis. *Chryseobacterium* has been associated with tomato crop and acts through siderophore production. *Frankia*, *Gluconacetobacter*, and *Herbaspirillum* isolated from *Alnus*, sugarcane, and rice have been helpful in nitrogen fixation. *Paenibacillus* isolated from lodgepole pine and black pepper has been reported for indoleacetic acid production and potassium solubilization, respectively, as a mechanism for enhanced growth and stress management. *Phyllobacterium* has been reported for phosphate solubilization and siderophore production. *Pseudomonas* also has been associated with large varieties of crop and has been proved to beneficial in stress management through the number of mechanism and production it could associate to or could lead to. Some of the reported mechanisms are chitinase and glucanase production, ACC deaminase synthesis, induction of resistance to stress and antibiotic production.

Rhizobia isolated from legumes and peanuts crop has been reported for nitrogen fixation, induction of resistance to various stresses and hydrogen cyanide formation. *Rhizobium* isolated from pepper, tomato, lettuce, carrot, tomato mung beans, etc. has been reported too for some common mechanism like nitrogen fixation, indole-acetic acid synthesis, ACC deaminase production, and siderophore production.

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Plant-Bacterial Association and Their Role as Growth Promoters and Biocontrol Agents

16

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Abstract

Bacteria are common among the microorganisms that colonize both the aerial and underground parts of plant systems. The colonization could result in a benefit to improve fitness in the ecosystem they live by a variety of positive activities. Both the gram-positive and gram-negative bacteria were found in association with plants. It was found from the available literature number of evidence recorded that the plant-associated bacteria were able to reduce the burden of pathogens and support plant growth promotion. Researchers proved that bacterial isolates for plant growth promotion and biocontrol of pathogens from different domesticated plants were efficient antifungals, antibacterials and antinematicidal than synthetic agrochemicals. The use of beneficial bacteria is an eco-friendly approach to develop a sustainable environment.

Keywords

Biocontrol · Inoculants · KSB · PSB · PGPR · Nitrogen fixers

16.1 Introduction

Progress in time made man from food gatherer from natural wild resources to food cultivator by farming systems for a variety of crops. Improvement in tools and techniques better yields in farming that serves both food and source of economy. The farming or agriculture covers rearing of plants, animals, fungi and other living forms for food, fiber, fuel, drugs, and products that sustain and enhance man's life. The farming or cultivation of crops remain under uncertain obstacles like soil

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erosion, water deficit, scanty rain, scarce of productive seed supply, pest/microbial attack, weeds, loss of fertility, progress in the industrial sector, lack of sufficient manpower that need to be conquered at warfare. The advances in modern agronomy such as breeding, agrochemicals (fertilizers, pesticides, weedicides/herbicides, antifungals/antibacterials), and the use of equipment (harvester/thrasher) reduced physical burden on farm owners and sharecroppers with increased yields. The sophistication in cultivation practices brought an increase in total farmlands with significant demand for inputs with negative impacts around the globe (Khan et al. 2010, 2011, 2016, 2017).

To set the practices of preservation of natural resources/biodiversity minimum use of synthetic agrochemicals and maximum practices of conserve like crop rotation, natural fertilizers/manures, biological control and integrated pest management (IPM) were introduced. World Commission on environment and development (WCED) earlier known The Brundtland Commission, introduced word 'sustainable development' to preserve the environment with the necessities to be met for generations together for the environment, local people, future as ELF. The agriculture practices are simplified as sustainable, organic, climate-smart and resource-conserving to fulfill the requirement of conservation of resources. These approaches use eco-friendly systems to overcome the detrimental practices in crop productivity.

16.2 Plant Growth Promotion by Beneficial Bacteria

The mixing of different soil samples could remediate the defects in farmland was suggested by Theophrastus. Virgil recorded that legume crops increase soil fertility and better yields from successive crops are early reports. Microorganisms and plants exist in nature as an indispensable intimate association. These organisms remain free, attached or enter into symbiosis with host plants (commensalism/mutualism, parasitism). Kloepper and Schroth introduced termed beneficial bacteria that surround the root system of plants as plant growth-promoting rhizobacteria (PGPB). This group of bacteria was further made into three categories: neutral, negative, and positive. PGPB could be extracellular (ePGPB) or intracellular (iPGPB). The role of plant bacterial isolates in plant growth and biocontrol from different pathogens are highlighted as in vitro and in vivo practices (Table 16.1).

16.2.1 *Acacia dealbata*

The link between the composition of bacterial community and functional capacity in the rhizospheric microbiomes is associated with *A. dealbata* by shotgun DNA sequencing. The analysis showed that several genes associated with plant growth-promoting (PGP) traits were present in the rhizospheric metagenomes. The findings suggested that *A. dealbata* enriched rhizosphere soils with potentially beneficial microbial taxa (*Bradyrhizobium*, *Geodermatophilus*, *Koribacter*, *Kribbela*, and *Sphaerobacter*) that play an integral role in mediating PGP processes (Kamutando et al. 2019).

Table 16.1 Potential of plant-bacterial association for improved traits

Test strain	Applied value	References
Pseudomonas protegens N	Novel protein and its antifungal activity (*Alternaria*) on tomato fruits	Agrillo et al. (2019)
Herbaspirillum seropedicae	Improved plant growth and no effect on leaf anthracnose (*Colletotrichum graminicola*) in maize plants	Dall'Asta et al. (2019)
Streptomyces violaceusniger	Potato common scab (*Streptomyces scabies*) control and plant growth promotion, i.e., IAA, siderophore production, nitrogen fixation, and phosphate solubilization potential	Sarwar et al. (2019)
Actinobacterial strains	Date fruits found with high levels of sugars, organic acids, essential amino acids, unsaturated fatty acids, phenolic acids, flavonoids, vitamins, and minerals	AbdElgawad et al. (2019)
Enterobacter cloacae, *Pseudomonas* sp.	Enhanced grain yield in wheat	Khani et al. (2019)
Enterobacter, *Pseudomonas*	IAA and ammonia were plant growth-promoting features in chickpea (*C. arietinum* L.)	Brigido et al. (2019)
Bacillus amyloliquefaciens, *Pseudomonas fluorescens*	Promoted banana growth superior chemical fertilization	Gamez et al. (2019)
Bacteria (*Stevia rebaudiana*) rhizosphere	IAA production improved root and shoot biomass	Chandra et al. (2018)
Bacillus halotolerans	Date palm (*P. dactylifera* L.) Bayoud disease by *Fusarium oxysporum* f. sp. *albedinis* and phytopathogens – *Botrytis cinerea*, *A. alternata*, *Phytophthora infestans*, and *Rhizoctonia bataticola* control	Slama et al. (2019)
Pseudomonas fluorescens Sasm05	Promoted *Sedum alfredii* root development and Zn uptake	Wang et al. (2019)
Bacillus pumilus, *B. licheniformis, Enterobacter* sp., *Bacillus* sp., *Stenotrophomonas maltophilia*	Biocontrol of cucurbit downy mildew (CDM) by *Pseudoperonospora cubensis*	Zheng et al. (2018)
Bacillus altitudinis, B. velezensis	*Xanthomonas axonopodis* pv. *vesicatoria* and *Pseudomonas syringae* pv. tomato and *R. solani*, *Pythium ultimum* control	Liu et al. (2018)
Proteus mirabilis	Exhibited plant growth-promoting attributes in *Foeniculum vulgare*	Dhiman et al. (2019)
Azotobacter sp. strain Avi2	Better vegetative and reproductive growth of rice	Banik et al. (2019)
Pseudomonas protegens	Antifungal activity against B. cinerea and IAA, phosphate solubilization	Andreolli et al. (2019)
Bacillus subtilis, *Pseudomonas fluorescence*	Control infection of common bean plants by *Sclerotium rolfsii* and as biofertilizers	Mohamed et al. (2019)

(continued)

Table 16.1 (continued)

Test strain	Applied value	References
Bacillus velezensis YC7010	Improved defense against brown planthopper – *Nilaparvata lugens* Stal in rice	Harun-Or-Rashid et al. (2018)
Paenibacillus polymyxa CP-S316	Improved plant microbial community in poplar	Sui et al. (2019)
Bacillus amyloliquefaciens	Increase in shoot weight and photosynthetic efficiency – *Antennaria dioica, Campanula rotundifolia, Fragaria vesca, Geranium sanguineum, Lotus corniculatus, Thymus serpyllum, Trifolium repens,* and *Viola tricolor*	Xie et al. (2018)
Azospirillum brasilense Ab-V5. *Enterobacter* sp. ESA 57	Contributed for sorghum growth promotion through calcium phosphate solubilization and auxin and siderophore production	da Silva et al. (2018)
Pseudomonas sp. (19Fv1t, 5Vm1K, and Pf4)	Fruit of strawberry (*Fragaria* x *ananassa* var. Eliana F1) showed some elements and/or volatiles	Todeschini et al. (2018)
Bacillus cereus YL6	Growth-promoting effects in soybean, wheat, and Chinese cabbage	Ku et al. (2018)
Pseudomonas protegens pf-5	Antifungal activity	Jing et al. (2018)
Bacillus amyloliquefaciens YTB1407	Colonization, elongation of adventitious root and branch roots in sweet potato	Wang et al. (2018)
Enterobacter sp., *Serratia* sp.	Improved growth-promoting traits and inhibited phytopathogenic fungi	Sánchez-Cruz et al. (2019)
Bacillus velezensis LDO2	Peanut pathogens inhibition and growth promotion	Chen et al. (2019)
Bacillus amyloliquefaciens, *B. velezensis*, and *Acinetobacter* sp.	Anti-oomycete activity in chili	
Pantoea sp. A3, *Pantoea* sp. A34, *Kosakonia* sp. A37, *Kosakonia* sp. B7 and *Bacillus* sp. AH9	Plant growth-promoting potential	Chakdar et al. (2018)
Bacillus sonorensis RS4	Improved growth of groundnut	Ankati et al. (2018a, b)
Bacillus amyloliquefaciens BAS23	Control of dirty panicle fungal pathogens of rice (*Curvularia lunata, Fusarium semitectum* and *Helminthosporium oryzae*)	Saechow et al. (2018)
Bacillus simplex 30 N-5, *B. simplex* 11, *B. simplex* 237, and *B. subtilis* 30VD-1	Plant growth-promoting (PGP) and biocontrol attributes	Khan et al. (2018)

16.2.2 *Arabidopsis thaliana*

Yu et al. (2019) investigated multifunctional mutant of *Pseudomonas protegens* (root-colonizer with broad spectrum biocontrol activity) to improve bactericidal and nitrogen fixation by recombineering technique. The pot experiment of *A. thaliana* after inoculation of test mutant strain showed increased plant growth with production of 2,4-diacetylphloroglucinol (2,4-DAPG) and nitrogenase.

16.2.3 Aspen (*Populus tremuloides*)

Shinde et al. (2019) investigated the tripartite laboratory community comprised of *P. tremuloides* (aspen) seedlings, *Pseudomonas fluorescens* (mycorrhizal helper bacteria – MHB) and the ectomycorrhizal fungus *Laccaria bicolor*. The results demonstrated that *P. fluorescens* has MHB activity and promotes mycorrhization by *Laccaria* through the suppression of aspen root antifungal defense responses.

16.2.4 Banana (*Musa acuminata* Colla)

Gamez et al. (2019) reported that banana crop requires a high input of chemical fertilizers and rhizobacteria were assessed as an alternative for fertilizers. The seedlings of test plant from tissue culture were inoculated with *Bacillus amyloliquefaciens* and *Pseudomonas fluorescens* as plant growth-promoting rhizobacteria. The inoculated plants were evaluated for height, leaf (number, area), pseudostem (thickness), shoot (fresh and dry weight), and root (dry weight) as growth parameters and found superior responses than chemical fertilizer treatment.

16.2.5 *Bacopa monnieri*

The study was reported on the effect of microbial consortia for growth and protein content of micropropagated plants of *B. monnieri*, *Pseudomonas* sp., *Burkholderia* sp., *Bacillus* sp., and *Paenibacillus* sp. that were isolated from rhizosphere soils of chickpea, pea, red gram, wheat, rice, and mung bean. *Burkholderia* sp. was screened effective and significant for phosphate solubilization and IAA, HCN, and siderophore production (Verma et al. 2018).

16.2.6 Broccoli (*Brassica oleracea* L.)

Gadhave et al. (2018) studied the effects of seed inoculation and field application of soil bacteria, *B. cereus*, *B. subtilis*, and *B. amyloliquefaciens*. The diversity, evenness, and richness of endophytic bacterial communities in sprouting broccoli roots

were evaluated using high-throughput metagenome sequencing. The *Bacillus* inocula were found to fail as endophytes, but their effects extended on the endophytic bacterial community both generic and species-specific.

16.2.7 Bur Clover (*Medicago polymorpha*)

The role of rhizobial bacterium *Ensifer medicae* was investigated for protection of bur clover plant against antagonistic soil microbes in complex soil communities. The results of *E. medicae* treated bur clover plants showed antagonistism against soil microbes and rhizobia in root biomass, root/shoot ratio and nodule number. These findings indicated the potential of complex plant-bacterial interactions contribute for plant genetic variation and bacterial diversity in host genotype dependant manner (Jack et al. 2019).

16.2.8 Castor (*Ricinus communis* L.)

The study was made to isolate and characterize phosphate-solubilizing bacteria (PSB) from castor rhizosphere. 15 castor rhizobacteria isolates were screened in vitro for P solubilization efficiency both qualitatively and quantitatively. *Bacillus firmus* was found to be the most potential isolate and could be a source of biofertilizer for castor farming (Sandilya et al. 2018).

16.2.9 Chili (*Capsicum annuum* L.)

The phosphate-solubilizing bacteria (PSB) isolates from rhizosphere soil of chili plant were reported for their role in plant growth promotion. The *Pseudomonas aeruginosa* isolates in vitro inoculated in chili plant showed significant increase in IAA, siderophore, growth, plant nutrient uptake, phosphorus availability, and yield. This report proved use of PSB isolates as biofertilizers in single or part of integrated nutrient management practice (Linu et al. 2019).

16.2.10 Chickpea (*Cicer arietinum*)

The potential of *Mesorhizobium ciceri* isolates was evaluated for plant growth, nodulation, and yield of three different chickpea varieties. The isolates exhibited siderophore, HCN, IAA, NH_3 production, solubilize the inorganic phosphate and zinc. These strains proved to be were effective bioinoculant for the growth and yield enhancement of chickpea (Pandey et al. 2018).

16.2.11 Common Bean (*Phaseolus vulgaris* L.)

Common bean forms root nodules with a wide range of rhizobia among them *Bradyrhizobium*, that is able to induce profuse nodule formation. The study tested co-inoculating *bradyrhizobia* with standard common bean symbiont *Rhizobium tropici* that stimulate growth and nodule formation. Co-inoculated plants found to produce more nodules and accumulate shoot dry biomass and nitrogen than plants inoculated with *R. tropici* alone (Jesus et al. 2018).

16.2.12 Cowpea (*Vigna unguiculata* L.)

The cowpea seed coating of plant growth-promoting bacteria (*Pseudomonas libanensis* from the rhizosphere of *Trifolium repens*) and AMF was evaluated for effects on biomass and physiological traits. The results of cowpea treated with *P. libanensis* showed growth-promoting activities such as fixation of N_2, solubilized P, 1-aminoc yclopropane-1-carboxylate (ACC) deaminase, siderophore, IAA production, and ammonia, hence proving as efficient strain to enhance plant biomass and seed yield (Ma et al. 2019).

16.2.13 Date Palm (*Phoenix dactylifera* L.)

The rhizosphere soil samples from date palm were assessed for plant growth-promoting bacteria with IAA, mineral phosphate solubilization, siderophores, ammonia release, protease, cellulase, biosurfactant, and antimicrobial activities. *Pseudomonas vancouverensis* and *Pseudomonas brassicacearum* were among the 36 rhizopseudomonads with high rate of plant growth potential (Ferjani et al. 2019).

16.2.14 *Dianthus caryophyllus*

Gang et al. (2018) studied the effect of a rhizospheric isolate *Klebsiella* strain to promote *D. caryophyllus* growth under sterile and non-sterile conditions. The colonization of test strain in root system endophytically and its impact on the cultivatable microbial community was evaluated. The auxin indole-3-acetic acid (IAA) production of *Klebsiella* has effectively improved root traits of *D. caryophyllus* in a dose-dependent manner.

16.2.15 Dragonhead (*Dracocephalum moldavica* L.)

Asl and Hatami (2019) investigated the impacts of zeolite and bacterial biofertilizer Nitroxin (10^7 CFU/ml: *Azotobacter* sp., and *Azospirillum* sp.) and phosphate barvar-2 (phosphate-solubilizing bacteria: *Pantoea agglomerans* (p5), *Pseudomonas*

putida) on physiological characteristics, essential oil content and yield of aromatic medicinal herb *D. moldavica* under different irrigation regimes. The experimental treatments improved physiological functions of test plants.

16.2.16 Duckweed (*Lemna minor*)

This is an aquatic floating plant that proliferates with high growth rate in lakes and wetlands. This duckweed species (*L. minor*) is known to flourish in contaminated surface water bodies and it is a choice for exclusion of toxic compounds. Ishizawa et al. (2019) examined the plant growth-promoting bacteria (PGPB) and plant growth-inhibiting bacteria (PGIB) for their colonization and competition in *L. minor*. The inoculation of test plant with bacteria, i.e., *Aquitalea magnusonii*, *Acinetobacter ursingii*, and *Asticcacaulis excentricus*, and growth promotion along with inhibition were found. The results proved *A. magnusonii* was growth-promoting and other two strains were inhibitory.

16.2.17 Ginseng (*Panax ginseng* Meyer)

Ginseng-cultivated soil was used via in vitro studies of plant growth-promoting traits. The isolated strain reported to produce indole-3-acetic acid, siderophores, and solubilized phosphate. Cross-nodulation tests were performed between strain and three legume species *Glycine max*, *Vigna radiata*, and *Phaseolus vulgaris.* The results showed strain as a potential plant growth-promoting bacterium and a novel species of the genus *Rhizobium* that was named as *Rhizobium panacihumi* (Kang et al. 2019).

16.2.18 Kikuyu Grass (*Pennisetum clandestinum*)

PGPR bacterium *Paraburkholderia* genus and poultry manure-based organic fertilizer with or without PGPR were compared to conventional urea fertilizer on pasture grass growth and N leaching. The test strain improved the growth of grass and reduced the N leaching (Paungfoo-Lonhiennea et al. 2019).

16.2.19 Lentil (*Lens culinaris* L.)

Lentil crop is commonly cultivated in dryland as it requires low water supply and fungal disease incidence under these environments. The soil erosion and depletion of nutrients were negative attributes for low yield of this legume. Sepulveda-Caamano et al. (2018) isolated rhizobacteria from Chilean Mediterranean drylands. The bacterial isolates characterized by BOX-PCR and the strains screened for enzyme ACC deaminase, IAA and compatibility with rhizobia. Among the compatible ten strains of *Pseudomonas* sp. were co-inoculated with rhizobia in lentil seedlings. The results proved nodulation in test plants were improved 85% compared to control.

16.2.20 Maize (*Zea mays*)

Deodatus et al. (2019) conducted the effects of liquid inorganic fertilizer and microbiological products BioSoil Crop Booster (BSCB) (*Pseudomonas fluorescens*), and Bio Soil Nitro plus (BSN+) (*Acetobacter* sp.) on growth, nutrient uptake and yield of maize under greenhouse and field conditions. The combination of N and P fertilizers improved crop response. Mpanga et al. (2019) demonstrated that the nitrogen fertilization has a significant impact on the performance of various phosphate-solubilizing microorganism (PSM) inoculants in maize grown on neutral to alkaline soils with limited P availability. In greenhouse experiments, Proradix®, Sourcon Padena GmbH, Tübingen, Germany, with *Pseudomonas* (Pro: 1×10^9 CFU kg^{-1} substrate) and Vitalin SP11, which comprises *Bacillus subtilis*, *Pseudomonas* sp., and *Streptomyces* sp., and ABiTEP GmbH, Berlin, Germany, with *Paenibacillus mucilaginosus* (Paeni, 1×10^9 spores kg^{-1} substrate) were inoculants used on maize seeds. The findings suggested that the efficiency of PSM-plant interactions could influence the form of N fertilization and P-solubilizing potential.

A study was reported that characterized culturable 170 rhizosphere and 60 endophytic bacteria from rhizosphere soil and roots of maize. The inoculated plants with isolates were grown with industrial and municipal wastewater. The inoculated strains (*Bacillus cereus* and *Enterobacter cloacae*) enhanced plant growth-promoting (PGP) traits (IAA, siderophores, ACC deaminase, P solubilization) (Abedinzadeh et al. 2019). The study was carried on the rhizosphere of crop area such as maize (*Zea mays*), banana (*Musa paradisiaca*), tobacco (*Nicotiana tabacum*), sugarcane (*Saccharum officinarum*), pigeon pea (*Cajanus cajan*), and potato (*Solanum tuberosum*) for potassium solubilizing rhizobacteria (KSR) isolation. The use of chemical fertilizers along with KSR isolates like *Agrobacterium tumefaciens, Flavobacterium anhuiense, Rhizobium pusense,* and *R. rosettiformans* strains to evaluate the beneficial effect on growth and yield attributes in maize. The result showed significant difference in different parameters (growth, physio-biochemical, and yield attributes) in maize studied with varying doses of K and KSR strains. This study proved eco-friendly approach in reducing dependency on chemicals (Meena et al. 2018).

16.2.21 Model Grass (*Setaria viridis*)

Alves et al. (2019) evaluated the ability of *H. seropedicae* SmR1 strain and mutants defective in PHB (poly-3-hydroxybutyrate) production or mobilization to colonize the roots of the model grass. The results of study demonstrated that PHB metabolism by test strain contributed to the plant growth promotion ability as large PHB production increased root area and number of lateral roots in grass plant. The colonization (epiphytic or endophytic) of test strain was not found significant in plant growth.

16.2.22 Mustard (*Brassica juncea*)

Vishwakarma et al. (2018) isolated bacteria from rhizospheric soil samples of mustard with different PGP activities such as IAA production, phosphate solubilization,

siderophore production, symbiotic N2 fixation, and HCN production. Among the isolates three with potential PGP were characterized as *Pseudomonas* sp., *Bacillus thuringiensis*, and *B. paramycoides* based on 16s rRNA sequencing.

16.2.23 Pepper

Tao et al. (2019) used *Bacillus subtilis* and biochar obtained from agricultural waste as formulation to study pepper plant growth and improve soil fertility in pot experiment. During their study test bacteria and biochar were separately inoculated along with combination as microbial biochar formulation (MBF). The combination MBF was reported as effective to improve plant growth, physiological indices, several enzyme activities and physical-chemical properties of soil. The agri-waste biochar was beneficial to improve soil ability and inoculant bacteria as plant growth promoter.

16.2.24 Pigeon Pea (*Cajanus cajan*)

Rhizobium sp. a *Sesbania* plant growth promoter and *Cajanus cajan* nodulating strain were studied for its whole genome. 109 genes were found responsible for diverse plant growth-promoting activities like P solubilization and synthesis of acetoin, nitric oxide, indole-3-acetic acid, exopolysaccharide, siderophore, and trehalose. The genome sequence showed all the phyto-beneficial traits of test strain to use it as a biofertilizer (Iyer and Rajkumar 2019).

16.2.25 Pineapple (*Ananas comosus* L.)

A study was carried to quantify and compare the diversity of culturable microorganisms that exist in rhizospheric and rhizoplane soils of *Ananas* sp., from natural, commercial fields and ex situ conservatory (pineapple germplasm bank) for their potential biotechnological applications. The evaluation was done by using basic and selective culture media complemented with REP, BOX and ERIC-PCR molecular tools. The results showed predominant bacterial isolates in roots, stems, sheaths, and leaves samples (Souza et al. 2019).

16.2.26 Rapeseed (*Brassica napus*)

The soil in pots seeded with rape (*B. napus*) with biochar (six raw feedstocks: rice straw, rice husks, soybean straw, peanut shells, corn cobs, and wood) was inoculated with the inorganic phosphate-solubilizing bacteria (iPSB) (*Arthrobacter defluvii*, *Burkholderia cepacia*, *Bacillus megaterium*, *Pseudomonas frederiksbergensis*, *Rhodanobacter* sp., *Streptomyces prasinopilosus*, and *Variovorax paradoxus*). All seven iPSB strains were detected but the strain *A. defluvii* dominated. The abundance of the iPSB was correlated with rape biomass, P content, and P uptake

($P < 0.05$). The results demonstrated that biochar-assisted iPSB improved crop growth and P uptake (Zheng et al. 2019).

16.2.27 Raspberry (*Rubus idaeus* L., Rosaceae)

The study was carried on 2-year-old raspberry plants inoculated with bacterial (*Alcaligenes*, *Staphylococcus*, *Agrobacterium*, *Pantoea*, and *Bacillus*) suspensions by dipping method. The mineral content (leaf N content) and organic acid (malonic acid, malic acid, citric acid, and fumaric acid) in leaves and root (tartaric acid, butyric acid, and maleic acid) and amino acid content in root of raspberry were enhanced after treatment with bacterial inoculants. The results determined that PGPR treatments play a significant role in mineral nutrient uptake, organic acid and amino acid content of the test plant (Ipek 2019).

16.2.28 Rice (*Oryza sativa* L.)

Shahzad et al. (2019) reported a study on endophyte *Bacillus amyloliquefaciens* from rice seeds for methylotrophic potential and its effect on IAA, enzymatic antioxidants, and functional amino acids. The results based on metabolomics and proteomics showed methylotrophy promotes phytohormone production. A study was carried for species diversity and soil properties in 27 samples of rice rhizosphere. Among the bacteria isolated from analyzed samples 98 isolates were characterized as *Azotobacter* sp. by 16s rRNA gene sequences. The strains were analyzed for carbon source utilization and plant growth-promoting traits such as nitrogen fixation activity, IAA production, P solubilizing ability, and siderophore secretion. Among the strains isolated, *A. beijerinckii* and *A. chroococcum* showed their effects on rice growth and could be a source of biofertilizer (Chen et al. 2018).

Khanghahi et al. (2018) isolated potassium-solubilizing bacteria (KSB) from paddy rhizosphere soil and three selected isolates showed the best solubilization of potassium. These isolates were characterized using 16S rDNA sequencing as *Pantoea agglomerans*, *Rahnella aquatilis*, and *Pseudomonas orientalis* with multiple beneficial characteristics. The isolates were proved as biofertilizers that enhance the availability of potassium and improve the growth and rice yield.

16.2.29 Root Vegetables

Potato (*Solanum tuberosum*), Carrot (*Daucus sativus*), Beet (*Beta vulgaris*), Neep (*Brassica napus*), and Topinambur (*Helianthus tuberosus*).

The pulp and peel of root vegetable under study were investigated for endophytic bacteria that show impact on plant growth and source of plant probiotics. All the test samples were rich source of diverse group of bacterial species, i.e., *Alphaproteobacteria* and *Actinobacteria* from peel and pulp with *Gammaproteobacteria* and *Firmicutes* (Koiv et al. 2019).

16.2.30 Soybean (*Glycine max* L.)

The two-factor study was reported on biochar amendment and biofertilizer on soybean. The biofertilizer used was containing N-stabilizing bacteria, phosphate-solubilizing bacteria (*Bacillus coagulans*), soil pathogens controller, and plant growth-promoting rhizobacteria (PGPR) including *Azospirillum* sp., *Pseudomonas fluorescens*, and *Bacillus subtilis* applied in two levels of inoculation and non-inoculation through foliar feeding. The results demonstrated that after harvest biochar affected the amount of residual nitrogen, cation exchange capacity (CEC), acidity (pH), and electrical conductivity (EC) in the soil. The grain yield was high in the biochar treatment with inoculated biofertilizer (Arabi et al. 2018).

16.2.31 Sulla (*Sulla coronaria*)

Sulla is a legume pasture with high protein content used as forage crop for grazing. This plant possesses tolerance to alkalinity, salinity, and drought stress and is intercropped to improve fertility. The nodules were screened for culturable bacterial endophytes and isolated 63 isolates. *Rhizobium sullae*, *Pseudomonas* sp., *Microbacterium* sp., and *Pantoea agglomerans* were among the strains from nodule. These strains exist along with symbionts and support for plant growth promotion (Muresu et al. 2019).

16.2.32 Chili and Tomato

Desai et al. (2019) investigated plant growth promotion of tomato and chili by using bacterial strain *Bacillus sonorensis* and mycorrhiza sp.; the results revealed that under polyhouse the inoculated test plant seedlings were healthy and vigorously growing compared to uninoculated.

16.3 Antifungal Action

16.3.1 Avocado (*Persea americana* Mill)

Avocado plants are attacked by fungal diseases like *Fusarium* dieback (FD) and *Phytophthora* root rot (PRR). These diseases in avocado are caused by fungal pathogens: *F. euwallaceae* in association with beetle species (*Euwallacea fornicatus*) in dieback and root rot by *P. cinnamomi*. The bacterial isolates from avocado rhizosphere were tested for antifungal activity in dual culture assays against causative agents of FD-*Fusarium euwallaceae, Graphium euwallaceae* and *Graphium* sp., and PRR -*P. cinnamomi*. The bacterial isolates that inhibited the mycelial growth of both *Graphium* species were *Bacillus subtilis/Bacillus amyloliquefaciens* species complex and *Bacillus mycoides*, inhibiting the growth of *P. cinnamomi* (Guevara-Avendano et al. 2018).

16.3.2 Banana (*Musa* sp.)

The mixtures of beneficial microbes (endophytes and rhizobacteria) in the plantlets in the rooting medium under in vitro conditions to control *Fusarium* wilt in banana (*Musa* sp.) were investigated. The different banana cultivars and plantation areas were screened and characterized for endophytes and rhizobacteria. The in vitro conditions of banana tissue culture plantlets were bacterized with the prospective endophytes, *Bacillus subtilis* strains, and the rhizobacteria, *Pseudomonas fluorescens* strain, and effects were investigated against *Fusarium oxysporum* f. sp. *cubense* race 1 under glasshouse and field conditions. The results proved that tissue culture along with the establishment of beneficial microbes could supplement the micropropagated plants for easier adaptation under field conditions (Kavino and Manoranjitham 2018).

16.3.3 Cape Gooseberry (*Physalis peruviana* L.)

The study was to determine the influence of biotic factors, including soil sterility and concentration of both BCA and pathogen in the soil, on the biocontrol activity of *Bacillus velezensis* against fusarium wilt (*Fusarium oxysporum* f. sp. *physali* (Foph)) and the plant growth promotion activity of cape gooseberry. The test strain showed antagonistic potential against Foph (Moreno-Velandia et al. 2018).

16.3.4 Cassava (*Manihot esculenta*)

Phytopathological analyses revealed that shoot-propagated cassava after inoculated with *Bacillus amyloliquefaciens* or Microbacterium imperiale induced increase in shoot and root growth compared to control. The inoculation lowered disease incidence (root rot caused by *Fusarium solani*) in greenhouse-grown plants. The results demonstrated the role of beneficial bacteria to increase plant growth and protect against pathogen infection by *F. solani* (Freitas et al. 2018).

16.3.5 Chickpea (*Cicer arietinum*)

A study on association of two distinct rhizobacteria, i.e., *Bacillus altitudinis* and *Pseudomonas chlororaphis*, for growth promotion and yield improvement of chickpea was reported. Both test isolates showed inhibition of radial mycelial growth of *Fusarium oxysporum* (wilt disease). *B. altitudinis* and *P. chlororaphis* were beneficial as single and in combination for growth and yield improvement of chickpea (Baliyan et al. 2018).

16.3.6 Common Bean (*Phaseolus vulgaris* L.)

This plant is an important legume with high nutritional value but its yield is affected by anthracnose (*Colletotrichum lindemuthianum*). Martins et al. (2019) evaluated the in vitro and in vivo effects of microbial volatiles (mVOCs) from *Bacillus amyloliquefaciens* strains to control anthracnose (*C. lindemuthianum*). Among the volatiles 3-methylbutanoic acid and 2-methylbutanoic acid were potent against bean anthracnose.

16.3.7 Cucumber

The in vitro biocontrol activity was investigated for *Fusarium oxysporum* f. sp. *cucumerinum* (Foc) (*Fusarium* wilt in cucumber) by using bacteria isolated from rhizosphere of five different crop/vegetable plants, i.e., mustard (*Brassica campestris*), pea (*Pisum sativum*), bathua (*Chenopodium album*), lentil (*Lens culinaris*), and radish (*Raphanus sativus*), grown in agricultural fields. Among the 35 isolates screened for antagonistic activity, one isolate showed the highest antagonistic activity Foc. This isolate was found to produce siderophores, volatiles, hydrocyanic acid, and protease and exhibited plant growth-promoting traits like phosphate and zinc solubilization, ammonia production, organic acid production, and in vitro biofilm formation. The isolate was characterized as *Pseudomonas aeruginosa* based on the morphological, physiological, biochemical characteristics, phylogeny analysis, and 16S rDNA sequence (Islam et al. 2018).

16.3.8 Flax (*Linum usitatissimum* L., Linaceae)

Flax is a fiber- and oil-yielding crop that is affected by fungal blight disease (*Rhizoctonia solani*) at seedling stage. Tan et al. (2019) isolated bacterial strain, i.e., *B. subtilis* (characterized by morphological, physiological, biochemical, and 16S rDNA sequence analysis), from the rhizosphere soil of flax crop area. This strain was antagonistic against the pathogen by production of enzymes (protease, cellulase), volatiles, and lipopeptides. The results proved that the test strain control efficiency of 83.34% against seedling blight (*R. solani*).

16.3.9 Grapevine (*Vitis vinifera*)

In viticulture, the plant death is common due to grapevine trunk diseases (GTDs). Among the GTDs, *Botryosphaeria* dieback is major devasting problem caused by pathogenic fungi from family–botryosphaeriaceae i.e., *Diplodia seriata, D. mutila* and *Neofusicoccum parvum*. Trotel-Aziz et al. (2019) investigated the biocontrol ability of dieback disease pathogen (*Neofusicoccum parvum*) by using *Bacillus subtilis* isolate from rhizosphere soil of grapevine field. The test pathogen phytotoxins (terremutin and mullein) were detoxified in vitro plantlets and enhanced immunity by *B. subtilis*.

Andreolli et al. (2019) isolated bacterial isolate, i.e., *Pseudomonas protegens*, from soil sample from warm-temperate deciduous forest in Italy. This strain was reported for abilities like production of 2,4 diacetylphloroglucinol (2,4-DAPG), pyoluteorin, pyrrolnitrin, siderophores, ammonia, IAA and phosphate solubilization. The phytopathogens from grapevine such as *Botrytis cinerea, Alternaria alternata, Aspergillus niger, Penicillium expansum, Neofusicoccum parvum, Phaemoniella chlamydospora, Phaeoacremonium aleophilum* were effectively controlled by *P. protegens*. The results suggested test strain as an antifungal for application in viticulture to eliminate devasting tracheomycosis/esca disease.

16.3.10 Maize (*Zea mays*)

Maize crop is affected by leaf anthracnose disease caused by *Colletotrichum graminicola.* Dall'Asta et al. (2019) reported biocontrol of maize leaf pathogen by using an endophyte and diazotroph *Herbaspirillum seropedicae*. The disease severity was evaluated by necrosis, chlorosis, and quantification of test strain (PCR) on leaf. The inoculant along with humic amendment showed effective colonization and leaf growth promotion.

The potential of *Bacillus amyloliquefaciens* as biocontrol agent against fungal pathogens *Rhizopus stolonifera*, *Penicillium variable*, and *Fusarium verticillioides* infesting maize was evaluated. The strain was selected based on its in vitro antagonistic activity with potential to secrete fungitoxic lipopeptides. The biocontrol activity was further tested on infected plantlets in growth chamber and under field conditions. The results showed strong protective effect of the strain at two different locations with specific agroecological conditions (Kulimushi et al. 2018).

16.3.11 Melon (*Cucumis melo*)

The study evaluated in vitro and in vivo antifungal activity of endophytic bacteria, *Achromobacter xylosoxidans*, against isolates of *Fusarium oxysporum* and *F. solani* responsible for *Fusarium* wilt of melon. The results of in vitro assay showed reduced 80% of pathogen mycelial growth and in vivo assay 60% of disease severity on melon (Dhaouadi et al. 2018).

16.3.12 Mexican Orange (*Choisya ternata*)

The study on potential benefit of chemical treatment (Mefenoxam) and biological control agents (*Glomus intraradices*, *Gliocladium catenulatum*, *Trichoderma atroviridae*, and *Bacillus amyloliquefaciens*) in the protection of *C. ternata* against *P. parasitica* (root rot disease) was evaluated. BCAs were applied as individual and/or combined treatments. The effect of the treatment was observed by monitoring *C. ternata* symptoms. The potential of biological control by the rhizosphere microbes for their effective use was a replacement for chemical treatment (Manasfi et al. 2018).

16.3.13 *Mimosa pudica*

The root nodules of *M. pudica* were evaluated for plant growth promotion microorganisms (PGPM) and test their growth promotion potential along with inhibition of phytopathogens (*Fusarium* sp., *Alternaria solani*, and *Phytophthora capsici*). The isolated strains, i.e., *Enterobacter* sp. and *Serratia* sp., were found with growth-promoting traits like phosphate solubilization; production of auxin (IAA), cellulase, and chitinase; and ability to colonize *Phaseolus vulgaris* with increase in shoot height (Sánchez-Cruz et al. 2019).

16.3.14 Mulberry (*Morus atropurpurea*)

The mulberry fruit is affected by a devasting disease called sclerotiniose caused by *Ciboria shiraiana*, *Ciboria carunculoides*, *Sclerotinia sclerotiorum*, and *Scleromitrula shiraiana.* Xu et al. (2018) investigated the role of *Bacillus subtilis* an endophyte from healthy mulberry stem in biocontrol of fruit sclerotiniose disease in greenhouse conditions. The results showed test strain with genes for non-ribosomal peptides (surfactin, fengycin, bacillibactin, and bacilysin) and ribosome-dependent bacteriocins (subtilin, subtilosin A) of numerous antimicrobial metabolites production and release of plant growth-promoting substances (indole-3-acetic acid, volatile substances, and siderophores). The test strain proved as plant growth promoter and disease control agent.

16.3.15 Peanut (*Arachis hypogaea* L.)

Peanut is an important food and oilseed crop that is susceptible to bacteria and fungi. The plant leaves, roots, fruits, seed, and seedling are reported for fungal attacks that reduce yield, quality, and loss. The common diseases by fungi are leaf blight, damping-off, crown rot, pod rot, and root rot (*Alternaria tenuissima*, *Aspergillus* sp., *Fusarium oxysporum*, *Rhizoctonia solani*, *Rhizopus* sp., and *F. moniliforme*). The peanut endophytic bacterial strain *Bacillus velezensis* was investigated for antimicrobial and growth-promoting abilities. Based on genomic evaluation, the gene clusters for antimicrobial metabolites (fungi, fengycin, surfactin, bacilysin; bacteria, butirssin, bacillaene, difficidin, macrolactin) were detected and exhibited phosphate solubilization and siderophore production. The results prove *B. velezensis* as biocontrol agent to enhance peanut production (Chen et al. 2019).

A study was carried on groundnut (*A. hypogaea* L.) associated bacterial isolate *Pseudomonas* sp. (*P. aeruginosa*) that promoted growth and induced resistance against the stem rot (*Sclerotium rolfsii*). The bacteria colonized root exudates (RE) increased levels of chitinase, thaumatin-like protein, ascorbate peroxidase, and glutathione S-transferase benzoic and salicylic acid. The exudation of metabolites enabled root colonization, suppressed fungal growth, promoted plant growth, and increased the defense-related proteins in the roots (Ankati et al. 2018a). A study was reported on the efficacy of plant growth-promoting rhizobacteria (PGPR) to control

root rot (RR) (*Macrophomina phaseolina*) and late leaf spot (LLS) (*Phaeoisariopsis personata*) in groundnut and to test the ability of plant systemic acquired resistance (SAR) inducers and plant extracts in greenhouse experiments. The treatment of seed and soil with talc-based formulation of *B. subtilis* strain reduced the incidence of root rot (Shifa et al. 2018).

16.3.16 Pine (*Pinus thunbergii* Parl)

The biocontrol activity of *Bacillus licheniformis* (rhizosphere isolate) against *Fusarium* root rot (*F. oxysporum*) in pine seedlings was reported by Won et al. (2019b). The results showed increased nitrogen and phosphorus in soil, fungal cell wall lytic enzymes (chitinase and β-1,3-glucanase), and phytohormone (auxin) production and support high nutrient uptake and control of pathogen by *B. licheniformis* for pine seedlings.

16.3.17 Potato

A product Rhizoflo Premium® mixture of two plant growth-promoting bacteria (*Pseudomonas fluorescens* and *Azospirillum brasilense*) was investigated for its effect on the yield of three potato varieties. The influence of the PGPB mixture of test strains in potato by different treatments i.e., soaked tubers, sprayed tubers, and untreated tubers on potato for susceptibility by pathogens *Phytophthora infestans*, *Alternaria solani* (early and late leaf blight), and Colorado potato beetle (*Leptinotarsa decemlineata*). The results showed that PGPB mixture posed potential to support potato yields under dry conditions and under low levels of infection by foliar fungal diseases and attacks by foliar insects (Trdan et al. 2019).

A study was reported for the management of the late blight disease by using the antagonistic bacterium. Twenty-five isolates of antagonistic bacteria were collected from rhizospheric soils of Solanaceous crops (potato, tomato and *Solanum nigrum*). These isolates were tested in vitro for its efficacy against *Phytophthora infestans* (late blight disease in potato) along with the strain of *Bacillus subtilis* var. *amyloliquefaciens*. The potato plants after treatment showed higher activity of plant defense-related enzymes, i.e., peroxidase, polyphenol oxidase, and phenylalanine ammonia lyase, that could accumulate total phenols in comparison with untreated control plants. The treated plants were less susceptible to disease and high tuber yield (Keerthana et al. 2018).

16.3.18 Purple Yatay Palm (*Butia purpurascens* Glassman)

The microbial diversity and four functional traits were evaluated: solubilization of calcium phosphate (CaHPO4) and iron phosphate (FePO4), synthesis of IAA and suppression of seed and fruit-spoilage fungi (*Neodeightonia phoenicum* and *Penicillium purpurogenum*) from purple yatay palm. The culturable endophytic and

rhizospheric microbiota was isolated and evaluated. Among the isolates of the genus *Bacillus* were efficient to suppress the growth of spoilage fungi tested, *B. subtilis* higher inhibition rates (Silva et al. 2018).

16.3.19 *Radix pseudostellariae* L. (Caryophyllaceae)

Wu et al. (2019) investigated the effects of artificially applied root exudates on seedling growth, rhizosphere soil microbial communities, and soil physicochemical properties of *R. pseudostellariae*. The results showed root exudates increased for relative abundance of *Fusarium*, *Xanthomonadales*, *Micrococcales*, and *Gemmatimonadales* and decrease of *Trichoderma*, *Penicillium*, *Pseudomonadales*, and *Streptomycetales*. The treatment reduced the densities of beneficial *Pseudomonas* and *Burkholderia* spp. and had positive effect on pathogenic *F. oxysporum*, *Talaromyces helices*, and *Kosakonia sacchari* in soil.

16.3.20 Rice

A study for potential of antifungal activity against five rice pathogenic fungi (*Magnaporthe oryzae*, *M. salvinii*, *Fusarium verticillioides*, *F. fujikuroi*, and *F. proliferum*) was investigated under in vitro conditions by the use of 550 cultivable bacterial isolates from rhizosphere and endorhiza of rice, berseem clover, and oilseed rape. The isolates were characterized based on biochemical tests and comparison of 16S rDNA sequences. The endophytic and rhizosphere isolates (*Bacillus mojavensis*, *B. amyloliquefaciens*, *B. subtilis*, and *B. cereus*) showed strong inhibitory effects against the mycelial growth of all the five fungal rice pathogens. The test strains could be biocontrol agents for control of the rice pathogenic fungi tested (Etesami and Alikhani 2018).

16.3.21 *Smilax bona-nox* L.

The study characterized the bacterial isolates based on 16S rRNA gene sequencing (*Proteobacteria*, *Actinobacteria*, and *Firmicutes* distributed over 7 families – *Burkholderiaceae*, *Caulobacteraceae*, *Xanthomonadaceae*, *Pseudomonadaceae*, *Enterobacteriaceae*, *Microbacteriaceae*, and *Bacillaceae* – and 12 genera different genera *Burkholderia*, *Pseudomonas*, *Xenophilus*, *Stenotrophomonas*, *Pantoea*, *Enterobacteriaceae*, *Kosakonia*, *Microbacterium*, *Curtobacterium*, *Caulobacter*, *Lysinibacillus*, and *Bacillus*) associated with *Smilax bona-nox* L, a wild plant with invasive growth habits. Among them *Pseudomonas* sp. displayed the highest potential for inhibition of fungal species – *Phytophthora* sp. (*P. cinnamomi*, *P. tropicalis*, *P. capsici*, and *P. palmivora*) and *Alternaria alternata*, *Rhizoctonia solani*, and *Fusarium oxysporum*. The chromatographic profile and bioactivity assays showed the secretion of glucanolytic enzymes (β-1,3 and β-1,4 glucanases) by *Pseudomonas* sp. (El-Sayed et al. 2018).

16.3.22 Sugarcane

Smut disease is caused by pathogen *Sporisorium scitamineum* that is primarily transmitted through sett of sugarcane. The study was investigated for the potential of endophytic bacteria as a biocontrol agent against the disease. The isolates from internode, bud, and root were screened for their in vitro antagonist potential against *S. scitamineum*. Among the isolates two endophytes – *B. pumilus* and *B. axarquiensis* – showed strong antagonism for smut disease of sugarcane (Jayakumar et al. 2018).

16.3.23 Tea (*Camellia sinensis* L., *Camellia oleifera*)

Pramanik et al. (2018) evaluated K-solubilizing bacteria (KSB) from soil samples of tea plantation and supplement as K in soil as biofertilizer. *Bacillus pseudomycoides* was strain identified as potassium solubilizer, and its inoculation improved height, collar diameter (girth), leaf numbers, length, and breadth of leaves of tea plants in greenhouse. Won et al. (2019a, b) investigated the control of foliar fungal diseases by *Botrytis cinerea, Glomerella cingulata, Pestalotia diospyri*, and *Pestalotiopsis karstenii* and growth promotion of oil tea (*C. oleifera*) seedlings through the use of *Bacillus licheniformis*. The test strain was found to produce lytic enzymes (chitinase and β-1,3-glucanase) and inhibited foliar pathogens and promoted growth of plant seedlings.

16.3.24 Tomato (*Lycopersicon esculentum*)

The cultivated crop of tomato was reported most valuable agricultural crops for global production of 3.7 million hectares. The tomato crop species is susceptible to a variety of pathogens that reduce both yield and quality, and late blight (affects the leaves, stems, and fruits) by *Phytophthora infestans* is the most devastating disease.

Three bacterial isolates, i.e., *Acinetobacter* sp., *B. amyloliquefaciens*, and *B. velezensis*, from soil were evaluated by Syed-Ab-Rahman et al. (2019a, b) for growth promotion and suppression of *Phytophthora capsici* (causative agent: root, crown, and fruit rot on tomato and other species from Solanaceae). The test organisms were inoculated (pre- and postinfection with pathogen) as seed treatment. Inoculation improved root length, total fresh weight, and seedling vigor along with decrease in pathogen load.

The tomato crop was found affected with late blight, early blight, stem rot, and wilt diseases and to counter these diseases *Pseudomonas fluorescens* along with other biocontrol agents were applied through different modes of treatment (soil application, seedling treatment, foliar spray). The results proved for growth promotion and disease control by reduced plant mortality, increased yield (Kabdwal et al. 2019). Mohammed et al. (2019) investigated biocontrol and plant growth promotion (IAA and phosphate solubilization) study of tomato fusarium wilt (*Fusarium oxysporum*) with endophytic rhizobacteria *Pseudomonas fluorescens* and *Bacillus*

subtilis from healthy tomato plants. The results proved that application both test endophytes were promising in biological control of wilt disease.

The effect of oak-bark compost, *Bacillus subtilis* subsp. *subtilis*, *Trichoderma harzianum*, and two commercial products (FZB24 and FZB42) was investigated on tomato growth, production of metabolites, and resistance for infection with *Phytophthora infestans*. The results showed *B. subtilis* subsp. *subtilis* and *T. harzianum*, along with oak-bark compost, enhanced plant growth and immunity against *P. infestans* (Bahramisharif and Rose 2018).

16.3.25 Turmeric (*Curcuma longa* L.)

The antagonistic potency of rhizobacteria isolates, i.e., *Bacillus tequilensis*, *B. subtilis*, *B. cereus*, *B. amyloliquefaciens*, and *Pseudomonas aeruginosa*, from turmeric fields was evaluated against rhizome rot fungal pathogens (*Rhizoctonia solani*, *Fusarium solani*, *F. graminearum*, *Schizophyllum commune*, *Macrophomina phaseolina*). 2,4-Diacetylphloroglucinol, pyrrolnitrin, pyoluteorin, bacillomycin D, fengycin, hydrogen cyanide, and enzyme cellulase genes were assessed to detect biocontrol action. The strains were used as bioformulations (*B. subtilis, P. aeruginosa*) in field trials and results proved plant growth promotion and management of turmeric rhizome rot (Chenniappan et al. 2019).

The rhizobacteria were isolated from *Curcuma amada* and screened for their antifungal activity against phytopathogens (*Rhizoctonia solani*, *Corynespora cassiicola*, *Pythium myriotylum*, *Phytophthora infestans*, *Sclerotium rolfsii*, *Colletotrichum acutatum*, and *Fusarium oxysporum*). Among the isolates *Serratia plymuthica* was found to have antagonistic activity against phytopathogens in dual culture. The antifungal compound purified by column chromatography was identified as pyrrolnitrin by ultraviolet spectrum (UV), infrared spectrum (FT-IR), and mass spectroscopic (LC-MS) analyses. *S. plymuthica* was found to be effective against ginger rhizome soft-rot (*P. myriotylum*) (John and Radhakrishnan 2018).

16.3.26 Wheat (*Triticum aestivum*)

The rhizosphere of wheat isolate *Pseudomonas protegens* and it's potential to produce an antifungal compound in the culture medium for biocontrol was carried by Bajpai et al. (2018). The gas chromatography-mass spectrometry analysis of the methanolic extract revealed antifungal compound as pyrrole-type antifungal molecule 3-(2-methylpropyl)-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione ($C_{11}H_{18}N_2O_2$) that significantly inhibited the growth of fungal pathogen (*Sclerotinia sclerotiorum*). The study was reported to investigate and determine the effect of the inoculation of bacterial isolates from roots of wild wheat (*Aegilops triuncialis* and *A. speltoides*) on grain yield and the nutrient content (N and P) of wheat plants (*T. aestivum*) grown under pot culture and dryland farming. The bacterial isolates on the NFb (N-free malate) medium showed ability to produce phytohormone (IAA) and fix atmospheric

nitrogen. The bacterial strains from wild wheat root were characterized as *Azospirillum brasilense* and *A. zeae* by 16S rRNA sequence analysis. These isolates were reported for applied value as biofertilizers based on inoculation for wheat growth promoting ability (Karimi et al. 2018). The effect of two *Pseudomonas* bacteria strains, isolated from earthworm coprolites, was investigated for treatment of soft wheat seeds (*T. aestivum* L.) with *Bipolaris sorokiniana* (Sacc.) Shoemaker. The antifungal and growth-promoting action (guaiacol-dependant peroxidase) under phytopathogenic load was evaluated. The results showed decrease ($P < 0.05$) in root rot disease incidence and severity during bacterization that indicated both antifungal activity of test bacterial isolates and their successful colonization (Minaeva et al. 2018).

16.4 Antibacterial Action

16.4.1 Citrus

Citrus plants pose bacterial diseases, i.e., blast and black pit, by *Pseudomonas syringae*, *P. orientalis*, *P. simiae*, *P. lurida*, *P. moraviensis*, and *P. monteilii*. Mougou and Boughalleb-M'hamdi (2018) evaluated the biocontrol of blast and black pit disease (*P. syringae*) in citrus by using indigenous *Bacillus* sp. and garlic extract. The inoculation of test bacterial strain reduced the stem necrosis under greenhouse conditions.

16.4.2 Common Bean (*P. vulgaris* L)

The study determined the functional diversity of soil bacteria *Pseudomonas* genus and their effects on bean (*P. vulgaris* L.) seed germination and their biocontrol potential of common bean blight (*Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* – Xapf). The PGP traits of isolates like HCN, IAA, siderophore, and phosphate solubilization and the antibacterial activity against blight pathogen were evaluated. The results revealed that *Pseudomonas grimontii* and *P. cepacia* protected bean seedlings against blight pathogen and are found as promoter of bean germination (Mokrani et al. 2019).

16.4.3 Cucumber (*Cucumis sativus* L.)

Endophytic bacteria (EB) were isolated from healthy cucumber plant tissues (e.g., root, stem, leaves) to evaluate their biological control of angular leaf spot disease (*Pseudomonas syringae* pv. *lachrymans*) in cucumber. Twenty-four endophytic bacteria were selected for tests based on biocontrol traits such as indole-3-acetic acid and siderophore production, solubilization of phosphate, and inhibition growth of *P. syringae* pv. *Lachrymans* in vitro. The efficient biocontrol agents among selected endophyte isolates were characterized as *Ochrobactrum pseudintermedium* and *Pantoea agglomerans* based on 16S rRNA primers and sequence analysis (Akbaba and Ozaktan 2018).

16.4.4 Potato

Potato is among the important solanaceous tuber crop used as vegetable and starch source worldwide. This crop plant yield is affected by diseases like late blight, bacterial wilt, soft rot, and common scab. The gram-positive bacteria belonging to genus *Streptomyces* (*S. scabies*, *S. acidiscabies*, *S. turgidiscabies*, *S. ipomoeae*) were found as causative agent of common scab. Lin et al. (2018) investigated biological control of common scab (*S. scabies*) by using *Bacillus* sp., i.e., four *B. amyloliquefaciens* isolates, a *B. subtilis* isolate, and a *Bacillus* sp. isolate with species unidentified. The results showed that *B. amyloliquefaciens* effective as biocontrol agent as it inhibited the growth of test pathogen and detected the secretion of surfactin, iturin A, or fengycin.

The study on growth, yield and develop resistance for brown rot disease (*Ralstonia solanacearum*) of potato crop by using *Pseudomonas fluorescens, P. aeruginosa, Bacillus subtilis,* and *Trichoderma* sp., was reported. The inoculation of biocontrol agents showed protection to the infected plants with increase in growth parameters and yield of potato, and these strains prove for their use as biological control of potato wilt (Elazouni et al. 2019). Gerayeli et al. (2018) studied 235 *Bacillus* strains isolated from the potato rhizosphere screened for control of *Pectobacterium carotovorum* subsp. *Carotovorum* (Pcc), causative agent for soft rot in potato. The results showed 15 strains inhibited the test Pcc strains in vitro. The antagonistic strains (*Bacillus subtilis*, *B. pumilus*, *B.megaterium*, *B. amyloliquefaciens*, and *B. thurigiensis*) were found to produce auxin, biosurfactant, mobility, enzymatic activities, and production and inactivation of acyl-homoserine lactones.

16.4.5 Sugar Beet

The bacterial pathogen (*Pseudomonas syringae* pv. *aptata*) strain known for leaf spot disease from sugar beet was investigated for antibacterial ability by using crude lipopeptide (CLE) extracted from *Bacillus* species (*B. amyloliquefaciens* and *B. pumilus*). The CLE from strain *B. amyloliquefaciens* was effective biocontrol against test pathogen (Nikolic et al. 2019).

16.4.6 Tomato

The biocontrol of tomato bacterial wilt disease (causative agent: *Ralstonia pseudosolanacearum*) was reported by using *Mitsuaria* sp. and *Ralstonia* (non-pathogenic) from Welsh onion (*Allium fistulosum* L.) and Chinese chives (*A. Tuberosum*). The co-inoculation of test strains contributed to improved suppression of wilt pathogen in tomato (Marian et al. 2018).

16.5 Anti-nematode Action

16.5.1 Maize

The maize plant root colonization was investigated with inoculation of *Bacillus pumilus* strain and *Heterorhabditis bacteriophora* (entomopathogenic nematode: EPN) in presence or absence of *Diabrotica virgifera* (root herbivore). The results showed that bacterial coating of maize seeds with the tested strain shaped tritrophic interactions. The test strain proved effective in maize rootworm management (Disi et al. 2019).

16.5.2 Mustard (*Brassica juncea*)

A study evaluated the herbicidal potential of rhizospheric bacteria *Bacillus* species (*Bacillus flexus*, *B. simplex*, *B. megaterium*, and *B. aryabhattai*) against *Lathyrus aphaca* L. weed in mustard. The strains showed plant growth-promoting traits such as indole-3-acetic acid (IAA) production, phosphorus and potassium solubilization, 1-aminocyclopropane-1-carboxylate deaminase enzyme, and antagonistic activity against potential pathogens. The pot studies revealed significant reduction up to 92% in root and shoot dry weight of *L. aphaca*. The rhizospheric bacterial isolates could be potential candidate for suppression of weed growth and bioherbicide application (Phour and Sindhu 2019).

16.5.3 Tomato

The nematode-infested and noninfested rhizosphere soils from four different plants (cucumber, eggplant, tomato, and bitter melon) were sequenced on the Illumina Hi-Seq platform for 16S rRNA genes of the bacterial communities. The rhizosphere of tomato plants was inoculated with microbiome slurry/bacterial culture, followed by root-knot nematode (RKN), *Meloidogyne incognita.* The result showed *Pseudomonas* sp. and *Bacillus* sp. from noninfested eggplant soil exhibited biocontrol activity to RKN on tomato (Zhou et al. 2019).

The mycorrhizal colonization (*Rhizophagus irregularis*) and phosphorus uptake in presence of PGPRs (*Pseudomonas jessenii* and *P. synxantha*) were evaluated for nematode infection (root-knot nematode: *Meloidogyne incognita*) in tomato. AM and PGPRs were proved to be effective as biocontrol agents against rootknot a soil-borne disease. These inoculants enhanced mycorrhizal colonization and P uptake in tomato (Sharma and Sharma 2019). The behavior in tomato rhizosphere of *Bacillus amyloliquefaciens* and *B. velezensis* strain was analyzed for the surfactin production, the use of tomato roots exudate as substrates, and the biofilm formation. The rhizosphere colonization by *B. velezensis* was found dependent on surfactin production and on root exudates composition (Al-Ali et al. 2018).

The effect of bacterial isolates (*Bacillus subtilis*, *B. pumilus*, *Mycobacterium confluentis*, *M. immunogenum*, *Paenibacillus castaneae*, *Pseudomonas fluorescens*, *P. viridilivida*, and *Tsukamurella paurometabola*) against root-knot nematode *M. incognita* was determined on tomato in the greenhouse. *P. castaneae* isolates reduced the number of egg masses and root galling without effect on plant growth but *M. immunogenum* increased plant height and shoot fresh weight. The results indicated that among bacterial strains of *P. castaneae* and *M. immunogenum* were the promising biocontrol agents (Cetintas et al. 2018).

The role of plant growth-promoting bacteria – *Pseudomonas aeruginosa* and *Burkholderia gladioli* – on growth and antioxidative potential in nematode (*Meloidogyne incognita*)-infected *Lycopersicon esculentum* seedlings were investigated. The inoculation of test microbes (*P. aeruginosa* and *B. gladioli*) reduced nematode infection, improved the growth of seedlings, and reduced the number of galls in tomato seedlings. These strains modulated growth characteristics and antioxidative defense expression of *L. esculentum* (Khanna et al. 2019).

16.6 Antifungal and Antibacterial Action

16.6.1 Potato

The soil samples from potato fields were sampled for screening of bacteria with antagonistic ability. Among the 600 isolates, *Bacillus velezensis* was found to be effective against microbial pathogens, bacteria (*Bacillus cereus*, *Clavibacter michiganensis*, *Erwinia amylovora*, *Pantoea agglomerans*, *Pseudomonas syringae*, *Ralstonia solanacearum*, *Xanthomonas campestris*, and *Xanthomonas euvesicatoria*) and fungi (*Alternaria solani*, *Cochliobolus carbonum*, *Fusarium oxysporum*, *Fusarium solani*, *Gibberella pulicaris*, *Gibberella zeae*, *Monilinia fructicola*, *Pyrenochaeta terrestris*, *Pythium mamillatum*, and *Rhizoctonia solani*), and in vivo control of root colonizer *P. syringae* inoculated in *Arabidopsis thaliana* (Grady et al. 2019).

16.7 Conclusion

In the era of genomics, researchers documented the genes located in the beneficial bacteria for a variety of phenotypes like IAA, HCN, NH3, lytic enzymes, siderophores, and ACC deaminase, P and K solubilization, and organic acid and amino acid production that support plant growth promotion and biocontrol in agroecosystems. *Bacillus* sp., *Pseudomonas* sp., dominates the studies by investigators to find plant growth promotion and biocontrol from available citations. Tomato, potato, and chili plants were the choice of the study to control pathogens and improve growth. The citations found strong commendation to improve the path for eco-safe practices with plant-associated beneficial bacteria, and such formulations are readily available in the market. These formulations need to be popularized among common people and set their mind-set for sustainable agroecosystems.

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