Rizwan Ali Ansari · Irshad Mahmood
Editors

Plant Health Under Biotic Stress

Volume 2: Microbial Interactions

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Preface

In order to understand the plant fitness entirely, it is high time for researchers to relinquish the obsolete theories and must unravel unexplored aspects pertaining to plant health. The book "Plant Health Under Biotic Stress" is being published in two volumes to provide the articulated, justified and updated information which are either directly or indirectly related to soil and plant health. Plant Health Under Biotic Stress – Volume 2 (Microbial Interactions) accounts for the application of plant strengtheners, biofertilizers, bio-inoculants, phytostimulators, biopesticides, biocontrol agents, etc in the amelioration of plant fitness. There is a wide spectrum of bio-inoculants which are used in various plant protection strategies. Literature on microbial root colonization, plant growth enhancement, and also on rescue of plants from attack of various soil-borne pathogens have been presented in a well manner. Potentiality of biocontrol endophytic fungi, bacteria, and actinomycetes enhancing the crop resistance ability against pathogens attack leading to improved plant health has also been underpinned. It is anticipated that the book will be useful to advisers, extension officers, educators, and advanced researchers who are concerned about the protection of plant health as well as environment.

A sincere acknowledgment is extended to Prof. Tariq Mansoor, Hon'ble Vice Chancellor, Aligarh Muslim University, Aligarh, India, for being a constant source of inspiration for the researchers.

Professor Akhtar Haseeb, Ex-Vice Chancellor, Narendra Deva University of Agriculture & Technology, Kumarganj, Faizabad, India; Prof. Saghir A. Ansari, Dean, Faculty of Agricultural Sciences; Prof. M. Yunus Khalil Ansari, former Chairperson, Department of Botany; Prof. Nafees A. Khan, Chairperson, Department of Botany; Prof. Mujeebur Rahman Khan, Chairperson, Department of Plant Protection; Prof. Zaki A. Siddiqui; Prof. Iqbal Ahmad; Prof. A. Malik; Prof. P. Q. Rizvi, Prof. M. S. Ansari; Prof. M. Haseeb; Prof. S. Ashraf; and Dr. R.U. Khan of Aligarh Muslim University, Aligarh, India, deserve special thanks for providing us critical suggestion during the write-up of this book.

This book would have remained just a dream if Dr. Rose Rizvi has not come and taken up each hurdle translating it into an enjoyable moment. She assisted us from onset of this journey and therefore indeed deserves to be acknowledged with great

appreciation. In addition, Dr. Sartaj A. Tiyagi, Dr. Safiuddin, Dr. Aisha Sumbul, Mr. Hari Raghu Kumar, and Ms. Aiman Zafar were constantly surrounded with us whenever we felt like giving up – sincere thanks to all of them.

Editors would have not completed this task without endless support, prayers, and encouragements of their elders during light and dark situations.

We can never forget our "little doctor," Mr. Ayan Mahmood, who used to practically look up and smile at us with two lovely and twinkling eyeballs, each time muttering words of comfort and encouragement.

We hope that our efforts to forward the readers toward the better state of plant science shall be fruitful.

Aligarh, India

Rizwan Ali Ansari
Irshad Mahmood

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About the Editors

Dr. Rizwan Ali Ansari is a young and active faculty member of Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, India. He obtained his Ph.D. from the same university and has been involved in the research and development strategies of plant pathology/nematology. He has been engaged in the formulation of management modules involving various microorganisms, antagonists and organic additives active against a wide range of soil-borne plant pathogens infesting several agricultural crops. He has also attended several national and international conferences so far and received prestigious awards by various scientific societies like Society of Plant Protection Sciences (SPSS) and Nematological Society of India (NSI) for his outstanding contribution in the field of plant pathology/nematology. He has published several book chapters, research and review articles pertaining to the utility of organic additives, mycorrhizal fungi as well as plant growth-promoting bacteria in the sustainable management of plant pathogens in various journals/books of great repute. Judicious application of organic additives and biological agents in the management of plant diseases, amelioration of soil and crop health and survey on disease prevalence caused by soil-borne pathogens on various economically important crops are the current research domain of Dr. Ansari.

Dr. Irshad Mahmood is working as a professor of plant pathology and nematology in the Department of Botany, Aligarh Muslim University, Aligarh. He obtained his PhD from Aligarh Muslim University in. the field of plant pathology and nematology. Promotion of organic farming across the world by utilizing organic additives and potent microorganisms for the sustainable management of phytoparasitic nematodes and plant pathogenic fungi resulting to augment soil and plant health is the domain of his research. He has been engaged with teaching programme of undergraduate and postgraduate-level students for the last 30 years and has many overseas visits including the United States, France and the United Kingdom. He has attended a significant number of national and international conferences pertaining to wide area of agricultural sciences and published around 150 original research papers, review articles and book chapters in various refereed national and international publication media, most of them in very high impact factors. He has successfully completed many training courses in various ICAR-sponsored research institutes in India and also in North Carolina State University, Raleigh, USA. He is also an active member of national and international scientific organizations; an expert for selection committee; a reviewer of journals, doctoral theses and funding agencies; and a recipient of Scientist of the Year award in the field of plant pathology and nematology. He has guided ten PhDs, several MPhil and a large number of MSc dissertations. He has also been engaged in the establishment of a joint government project with Aligarh Muslim University for improvement of infrastructural facilities in botanical garden to facilitate ex situ conservation and propagation of rare, endangered and threatened plants and the plants endemic to the region.

Chapter 1
Endophytic Bacteria: Prospects and Applications for the Plant Disease Management

P. Latha, M. Karthikeyan, and E. Rajeswari

Abstract Biological control of plant diseases has metamorphosed into a unique field of science and development, and this field is fast happening in recent years. Bacterial endophytes are a group of microorganism which can colonise in any part of a plant devoid of symptoms or harmful effects in the plant in which they inhabit for their survival. The endophytic bacterial species have been identified by numerous researchers, and they have increasingly been reported to reduce the growth and activity of a plethora of plant pathogens. The interest of the researchers in this field is ever expanding given the potential it possesses to serve as an alternative to synthetic fungicides. The primary aim of this review is to trace the development in endophytic bacterial research and to communicate the researchers with updated information which will serve as a catalyst for their research endeavours. The review started with a prologue about endophytes, their diversity and existence. A systematic review on the colonisation of endophytic bacteria has been given which unravels the processes involved in their entry into the rhizosphere, then cortex and xylem and further their movement to the vegetative and reproductive organs of plants. This has followed the review on the control of various plant diseases through endophytic bacteria, viz. wilt, damping off and rot, foliar fungal diseases and bacterial diseases. The control of postharvest diseases and nematodes by endophytic bacteria has also been discussed. The major processes involved in the mode of action or mechanism of control of diseases have been discussed in different heads, namely, competitive root colonisation, competition for ferric iron ions, antibiosis and antibiotics suppressing pathogens, induced systemic resistance (ISR), signal interference, food and space competition, and minimization of the factors responsible for virulence of pathogens. Quite a few literatures have been discussed on the application of bacterial endophytes through different modes of applications. The review ends with future thrust which will go long way in indicating the future niche research areas on endophytic bacteria.

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1.1 Introduction

Plant diseases pose humongous biotic stress to plants which results in huge economic loss for farmers besides spoiling the food through toxin production during storage also. The deliberate urge of farmers to combat the diseases resulted in the invention of several fungicides and a bactericidal molecule, the application of which culminates in environmental degradation ultimately endangering the health of human kind. Several plant pathogens developed resistance to these chemicals and render plant health management difficult. In order to get rid of these problems, biocontrol of plant diseases assumed greater significance.

The biocontrol interventions have been concentrated in the rhizosphere for a very long time, and plant growth promoting rhizobacteria (PGPR) have intensively been researched by various researchers. The microbes colonising internal tissues have recently been given laser beam focus by the researchers due to the ever-increasing scope of them being exploited for enhancing the growth of the plants and reduction of disease causing pathogens. Among these microbes the role of bacterial endophytes in suppression and control of plant diseases has been intensively reported by researchers in the recent past. Though enough review has been attempted, still there existed scope for updating the reviews in order to enlighten the researchers working in this area. Hence, this review is an attempt to comprehensively cover the research work which has been carried out in bacterial endophytes and to link what has been done and what is to be done in the future.

It would be appropriate to define 'endophytic bacteria' from previous literature before discussing the mode of action. Holliday (1989), Schultz and Boyle (2006) were of the view that endophytic bacteria are colonisers of internal tissue of crop plants which do not exhibit any sort of external symptoms or inimical effect on the plants in which they live and colonise. Almost all plant species that exist on earth harbour one or more than one endophyte in their system (Strobel et al. 2004). Wilson (1995) defined endophytic bacteria as prokaryotes that tried to colonise the xylem and phloem vessels of disease free plants which do not cause any harm to the plant in which they reside. In recent past, researchers defined endophytes as 'endosymbionts' which inhabit the inner parts of plant tissues and do not damage or inflict diseases which could be isolated through adherence of aseptic methods (Arnold and Lutzoni 2007; Khan et al. 2015).

The earlier works of researchers indicated the mutual benefits among plants and microorganisms, and they were of the view that the fungi which were not known for inflicting diseases in crop plants possessed the forte of the traits of microbial endophytes (Carroll 1988; Clay 1988). Despite the fact that Hollis (1951) identified bacteria in disease-free potato tissues seven decades back, the bacterial endophytes

were less researched than fungal endophytes. Bacterial species could be isolated from seeds and fruits of agricultural and horticultural crops (Mundt and Hinkle 1976; Kirchhof et al. 1997). Sturz et al. (1997) examined crop plants with big bacterial population of 10^7 colony forming units (cfu) g^{-1} of plant matter in wet weight, whereas Hallmann et al. (1997) reported that population sizes of 10^2 and 10^6 cfu g^{-1} were predominantly observed in most parts of the plants.

The dwelling of endophytic bacteria inside the plant parts has been well documented by researchers. Andrews (1992) while commenting on the dwelling place of endophytes did report that endophytes survive in a totally secluded milieu, when compared to microorganisms living in the root zone and above root zone, whereas the researchers like Schulz et al. (2002) and Arnold and Lutzoni (2007) reported that endophytic bacteria could survive in roots, stem, leaves, flowers, seeds and fruits of the crop plants.

A growing body of literature indicated an array of advantages of endophytes. Kang et al. (2007) detailed the growth-promoting characteristics of endophytes, while Kloepper et al. (2004) and Senthilkumar et al. (2007) demonstrated the disease-inhibiting traits of endophytes. The nature of endophytes in strengthening the defence mechanism of crops to various plant diseases was researched upon by Bargabus et al. (2002), Mishra et al. (2006) and Bakker et al. (2007). Anti-herbivory products were found to be instigated by endophytes (Sullivan et al. 2007) besides catalysing biological nitrogen fixation in plants (Martinez et al. 2003; Jha and Kumar 2007) and enhancing the upward movement of plant mineral (Malinowski et al. 2000). Backman et al. (1997) discussed various factors influencing endophytes as biocontrol agents against various plant diseases like specific bacterial species colonising in a particular crop species, the changing population in different seasons, the pattern with which they have been colonising and their capacity to mobilise inside the tissues and to stimulate systemic resistance.

1.2 Diversity of Endophytic Bacteria and Their Existence in Plant Parts

The dwelling of endophytic bacteria and the diversity of their genera have been a research issue taken up by many researchers, and maiden credible findings came out about the separation of endophytic bacteria from parts of plants which were sterilised using sodium hypochlorite or similar agents as reported by Samish and Dimant (1959) which was endorsed by Mundt and Hinkle (1976) and Miche and Balandreau (2001). Since then almost 200 bacterial genera from 16 phyla were reported as endophytic bacteria (Malfanova 2013). Sun et al. (2017) and Sessitsch et al. (2012) meticulously grouped them into cultural and uncultural bacteria, and majority of them were found to be associated with the species, namely, *Acidobacteria*, *Actinobacteria*, *Aquificae*, *Bacteroidetes*, *Cholorobi*, *Chloroflexi*, *Cyanobacteria*,

Deinococcus-Thermus, *Firmicutes*, *Fusobacteria*, *Gemmatimonadetes*, *Nitrospira*, *Planctomycetes*, *Proteobacteria*, *Spirochaetes* and *Verrucomicrobiae*.

Malfanova (2013) reviewed in depth the diversity of entophytic bacteria and reported that three major phyla were studied predominantly by the researchers, namely, *Actinobacteria*, *Proteobacteria* and *Firmicutes*. Taghavi et al. (2010), Deng et al. (2011), Weilharter et al. (2011) and Pedrosa et al. (2011) analysed the bacterial species in different parts of plants and observed that *Azoarcus*, *Acetobacter* (renamed as *Gluconobacter*), *Bacillus*, *Enterobacter*, *Burkholderia*, *Herbaspirillum*, *Pseudomonas*, *Serratia*, *Stenotrophomonas* and *Streptomyces* were the predominant bacterial endophytes colonised in plant tissues.

Hallmann and Berg (2006) were of the opinion that the species of the above genera are found to colonise in most of the soil and rhizosphere of the plants, whereas Compant et al. (2010) in their study confirmed the presence of endophytes above the root zone, flowers and also seeds. Hallmann et al. (1997) reviewed the diversified host plants of endophytic bacteria which was updated by Rosenblueth and Martinez-Romero (2006) and Berg and Hallmann (2006) who presented a rather comprehensive list of bacterial endophytes which were reported to be isolated from a wide range of plants.

Jesus and Lugtenberg (2014) reported that bacterial endophytes are omnipresent and can be identified from many sites in the plant, such as the root, stem, leaf, berry, seed and xylem sap, which was endorsed by a score of researchers like Rosenblueth and Romero (2006), Mercado-Blanco and Bakker (2007), Malfanova et al. (2013), Berg and Hallmann (2006) and Weyens et al. (2009). Endophytes population are always greater in the roots than any other organs of plants. In the root the average density is 10^5 cfu per g fresh weight, whereas average values of 10^4 and 10^3 are reported for stem and for leaf, respectively (Jesus and Lugtenberg 2014). Vendan et al. (2010) analysed the presence of endophytic bacteria in ginseng and reported that *Staphylococcus* spp. and *Bacillus* spp. were predominant in the stems of 1- and 4-year-old plants, respectively. The dominant endophytic groups of *Sphagnum* mosses were associated with the bacterial endophytes, namely, *Burkholderia*, *Pseudomonas*, *Flavobacterium*, *Serratia* and *Collimonas* (Shcherbakov et al. 2013). The upper part of poplar tree (*Populus* spp.) harbours abundant *Pseudomonas* and *Curtobacterium* spp. of bacterial endophytes (Ulrich et al. 2008).

Ryan et al. (2008) indicated that endophytic bacteria can be isolated from all kinds of plants in the plant kingdom irrespective of the nature of plants like trees, herbs, shrubs, etc. Lodewyckx et al. (2002) elaborated the main methods used for the isolation and characterisation of bacteria and reported at least 81 bacterial species which were found to be associated with crop plants. The presence of a variety of endophytic bacteria in a toluene-contaminated field was reported by Porteous-Moore et al. (2006) isolated endophytic bacteria from poplar tree and tried to find out the effectiveness of endophytic bacteria in phytoremediation which was endorsed by the findings of Loy et al. (2007).

1.3 Colonisation of Endophytic Bacteria in Rhizosphere and Rhizoplane

Colonisation of endophytic bacteria in plants started with the rhizosphere and moves on to the other parts of plants. The rhizosphere and rhizoplane colonisation of endophytic bacteria has been extensively reviewed. A variety of plant growth-promoting bacteria were said to be colonised in the rhizosphere, and they gained entry into other plant parts which was first reported by Galippe (1887) and proved again by di Vesta (1888). Smith (1991) reported that before this, it was thought that the healthy plants did not harbour microorganisms. In the previous decade many researchers demonstrating a wide range of endophytic bacteria possessed growth promotion and characters of suppression of pathogens. Many researchers including James et al. (2002), Compant et al. (2005b) and Hardoim et al. (2008) were concomitant with the opinion that endophytic bacteria tended to colonise the roots first followed by other parts of the plants. Notwithstanding, the researchers like Sessitsch et al. (2002) and Berg et al. (2005) argued that prominent and unique endophytic bacterial strains were found in all parts of plants starting from roots to flowers, fruits and seeds indicating differential capacities of bacterial strains to grow in various parts of plants. Population densities of bacterial species in the rhizoplane were in the range of 10^5 to 10^7 cfu g^1 of fresh weight (Bais et al. 2006). Gamalero et al. (2004) indicated that root zones of different crop species were reported to colonise endophytic bacteria in varied density of population.

Gamalero et al. (2003) reported that the cells of the bacterium first find a niche in the root zone which could be seen as a unitary cell clinging onto the root surfaces consequently observed as doublets in the rhizodermis. Benizri et al. (2001) pointed out that endophytic bacteria could stabilise themselves as microcolonies or microfilms once they colonise the entire rhizoderm. Root exudation in the form of amino acids, organic acids and other components which nourish bacterial species in the rhizosphere and rhizoplane helped colonisation. Lugtenberg and Kamilova (2009) argued that the endophytic bacterial strains were observed to be chemoattracted and migrated towards the exudates which catalyse the colonisation and multiplication. Further research on the root exudates revealed that variation in crop variety, differential stage of crop and varied amount of biotic and abiotic stresses amounted to varied nature of release of root exudates which were found to facilitate the growth of differential endophytic bacteria in the root zone. Besides, the research on root exudates indicated that some of the exudates were inimical for bacterial strains which may spoil colonisation (Bais et al. 2006; Haichar et al. 2008). The infection of phytopathogen also influenced the secretion of exudates from roots, which was proved by a study of Rudrappa et al. (2008) who found that the secretion of malic acid attracted *Bacillus subtilis* and catalysed the colonisation of the endophytic bacteria in the root zone of the plant resulting in the formation of a biofilm which guarded the roots from the virulent pathogens causing diseases. Bacterial colonisa-

tion was also affected by root mucilages, and it was found in a study conducted by Mandimba et al. (1986) that *Azospirillum* spp. strains were reported to be attracted by the root mucilage produced in the root zone of maize, whereas another study conducted later on by Humphris et al. (2005), in maize crop, reported the negative effect of root mucilage which averted colonisation of the strain SBW25 of *P. fluorescens* strain and their interaction in the root zone of maize.

Various mutational studies proved that the prerequisite for endophytic establishment depends on the attachment of bacterial cells to the root. A huge number of components which are found in the exterior of bacterial strains are involved in the process of attachment of bacterial cells to the roots. These views were supported by the findings of Dorr et al. (1998) who reported that BH72, an endophytic diazotroph of rice, and type IV pili which could be encoded by *pil*AB are needed for the connection of *Azoarcus* sp. in the root zone of rice. The dependence on liposaccharide for the attachment of *Herbaspirillum seropedicae*, to root surfaces of maize, was reported by Balsanelli et al. (2010). In their study they found that juxtaposing a wild type of maize, a mutated strain of maize with varied starch composition, exhibited lesser root sticking and endophytic spreading. An analogous study carried out by Meneses et al. (2011) reported the importance of exopolysaccharide for the adhesion of endophytic bacteria *Gluconacetobacter diazotrophicus* to the root zone of rice plants.

1.4 Entry Mechanism of Endophytic Bacteria

The review on penetration process suggested active and passive mechanisms. Hardoim et al. (2008) were of the view that the endophytic bacteria can also follow passive mechanism and it need not be always active mechanism for the penetration into plant tissues and hence at one or other stages of their life all bacteria that colonise the rhizosphere can be expected to be an endophytic bacteria. According to Reinhold-Hurek and Hurek (1998), cracks which are formed at the tips of the roots or the infection inflicted by harmful microbes could serve as a passive entry for endophytic bacteria. Combined with active penetration, this mode of entry has been reported by Reinhold-Hurek and Hurek (1998) for *Azoarcus* sp. BH72, and the entry of *Burkholderia vietnamiensis* in rice was reported by Govindarajan et al. (2008). In grapes the entry of *B. phytofirmans* PsJN was reported by Compant et al. (2005). In mulberry the access of *B. subtilis* Lu144 and *B. cepacia* Lu10–1 to the root zone was reported by Ji et al. (2010). James et al. (1994) found *Gluconacetobacter diazotrophicus* Pal5 gained entry through cracks in sugarcane. Hardoim et al. (2008) reviewed specific adaptations nodulating bacteria possessed for active penetration of the root system, an example of which was elucidated by Goormachtig et al. (2004) wherein *Azorhizobium caulinodans* entered the root of semiaquatic *Sesbania*

rostrata via splits likely to happen in the lateral root and gained entry through cortical and intercellular cracks.

Garg and Geetanjali (2007) while discussing the colonisation process in legumes known for nodulation, indicated that the preferred entry is through hairy roots. They also reported that prior to the formation of infection thread, they used to penetrate the tissues in the rhizosphere and consequently penetrate the nodules which are specialised organs developed by legumes.

Numerous works done by researchers like Compant et al. (2005a), Haas and Défago (2005), Raaijmakers et al. (2008) and Lugtenberg and Kamilova (2009) revealed a common finding that secondary metabolites produced by bacterial strains did provide a competitive advantage for those bacterial strains against other microorganisms and could catalyse the colonisation in roots. Van Loon and Bakker (2005) indicated that the antibiotics produced by certain bacterial strains were very much helpful for rhizosphere colonisation. The research papers of Nakayama et al. (1999), Nielsen et al. (2002), Raaijmakers et al. (2002) and de Souza et al. (2003) supported this view and quoted several antibiotics like 2,4-diacetylphloroglucinol (DAPG), hydrogen cyanide, phenazine, etc., which were found to be helpful in colonisation of bacterial strains in the rhizosphere. Duijff et al. (1997) and Bohm et al. (2007) reported in their work that lipopolysaccharides, flagella, pili and twitching motility were found to affect endophytic colonisation and bacterial mobility within host plants. A review of Lodewyckx et al. (2002) elaborated the enzymes responsible for degradation of cell wall which aid in the penetration of bacterial strains and spreading within the plant which has been confirmed by the work of Krause et al. (2006) wherein genome analysis of the non-nodulating endophyte *Azoarcus* sp. BH72 was carried out which revealed that the these endophytes carried genes possessing cell wall-degrading enzymes such as cellulases and polygalacturonases.

1.4.1 Colonisation of Endophytic Bacteria in the Cortex and Xylem Vessels of Plants

In order to move from the rhizoplane to the cortex or the root system, the endophytic bacteria have been reported to involve in translocation processes through active or passive mechanisms. Gregory (2006) reported in his study that the endodermis in the root zone hinders the further colonisation of endophytic bacteria and very few bacterial species could find an entry through and proved the report of the previous workers in this area. James et al. (2002) reported that either some endophytic bacteria entered through the endodermis through secretion of cell wall dissolving enzymes or some of them took a passive way during the disruption created in the root phase for the formation of secondary roots (Gregory 2006).

James et al. (2002) explained that the species of endophytic bacteria, namely, *Herbaspirillum seropedicae* Z67, need to pierce the pericycle after the endodermis in the root zone to reach the xylem vessel in rice. Compant et al. (2005b,

2008) confirmed this process of penetration of *B. phytofirmans* strain PsJN in grapes. This phenomenon holds good for most of the endophytic bacteria colonising internal tissues of the root. Further James et al. (2002), Compant et al. (2005) and Gasser et al. (2011) opined that the piercing of endodermis in the root zone of crop plants to gain an entry into xylem vessels could be possible for only a small number of species of endophytic bacteria. Reviews revealed that, despite the endophytic bacteria reaching the root xylem vessels passing all hurdles, the inducement of defence mechanism in the host plants by the bacteria is significant for colonisation in internal tissues (Rosenblueth and Martínez-Romero 2006). James et al. (2002), Compant et al. (2005b) and Miché et al. (2006) reported that the defence mechanism could result in cell walls of plants getting strengthened and the materials encircling the xylem vessel got established besides the development of gum inside the tissues of xylem.

Sattelmacher (2001) and Bacon and Hinton (2006) argued that the nutrient availability is enough to facilitate the growth of endophytic bacteria though its availability is minimal in xylem which has been evidenced from several radioactive labelling experiments in potato plants with $13CO_2$ which detected the isotope in photosynthetic metabolites and in varied bacterial endophytes (Rasche et al. 2009). Malfanova et al. (2013) found that the endophytic bacteria available in the root zone of cucumber was able to make use of Larabinose, a predominantly available sugar found in xylem fluid of an array of plants which is very much differing with *Pseudomonas* spp. found in other crops. Bartz (2005) contemplated the movement of beneficial endophytic bacteria and reported that these bacteria could move from one to another xylem element through perforated plates. This mechanism does not involve the enzymes catalysing the dissolvement of cell walls as the sizes of the holes in the plates were large enough to push the bacteria inside xylem vessels. Further work of James et al. (2002) and Compant et al. (2005b) who tracked the movement of endophytic bacteria reported the involvement of bacterial flagella to further aid their migration into the tissues of plants.

1.4.2 Colonisation of Endophytic Bacteria in Vegetative and Reproductive Parts of Plants

The inflorescence and fruits of some plants were reported to harbour endophytic bacterial species according to the studies of Mundt and Hinkle (1976) as well as Misaghi and Donndelinger (1990). Endophytic bacterial species could be found in seeds of rice according to Okunishi et al. (2005). Cankar et al. (2005) and Barac et al. (2004) were able to isolate the species of endophytic bacteria, namely, *Pseudomonas* and *Rahnella*, from seeds of Norway spruce and yellow lupine.

Compant et al. (2008) in their experiment in cv. Chardonnay grapevine variety, after application of *B. phytofirmans* strain PsJN in soil, observed that the endophytic

bacterial species was found to move from roots to flowers and tried to colonise in aerial parts of the grapevine. Graner et al. (2003), Okunishi et al. (2005), Furnkranz et al. (2012) and Compant et al. (2011) offered credible evidence of presence of endophytic bacterial species in reproductive organs of plants including inflorescence, seeds and fruits which were confirmed through isolation and microscopic observation.

1.5 Biocontrol Mechanisms Exhibited by Endophytic Bacterial Strains

The mode of action of endophytic bacterial strains has been enunciated by various researchers, and voluminous literature is available on this aspect. An attempt has been made to classify those mechanisms and detailed in the following section.

1.5.1 Competitive Root Colonisation

The applications of biocontrol agents resulted in the competition of the microbes present in biocontrol agents and the microflora already existing in the soil. The potential of the endophytic bacteria depends on, over a period of time, how efficient the colonisation happens in the root zone, the ability of them to survive the competition and their multiplication all through the tissues of roots (Whipps 1997). There are certain traits which facilitate competitive root colonisation, namely, differential phase of growth, ability to stick onto the roots, ability to move, effective use of the organic acids present in root exudates and the synthesis of various components including amino acids, type III secretion system (TTSS), lipopolysaccharides, nucleotides, etc. (Lugtenberg and Kamilova 2009).

The efforts of scientists to untangle the mechanism with which the endophytic bacteria safeguard plants from various diseases resulted in significant findings. Especially plant growth-promoting bacteria (PGPB) dwelling in the rhizosphere have been identified by many researchers as protectors of plants from various diseases. It has been observed by researchers that the epidermis of the root harbours lot of nutrients which pull a large variety of microorganism including the ones which cause diseases also. The hectic competition which persists among beneficial and harmful microorganisms for food resulted in the inhibition of disease-producing microorganism to inflict diseases in plants. There were reports which indicated the role of flagella in the migration of PGPB towards the nutrient-rich root surfaces, and these PGPB were adept in making use of the nutrients

which are primarily the root exudates oozing from root surfaces (Duffy 2001; Turnbull et al. 2001).

1.5.2 Competition for Ferric Iron Ions

Iron is an important element of survival of microorganisms which is in high demand as mostly the iron exists in unavailable form in root zone. Studies of Loper and Henkels (1997), Whipps (2001) reported the emitting of siderophores by plant growth-promoting bacteria, a compound with lesser molecular weight, which facilitated the PGPB to effectively attain the iron in the ferric ion which will be easily available to them. He further elaborated that notwithstanding the effectiveness of siderophores produced by bacterial species varied in gaining iron, their presence will check the fungal pathogens to make use of siderophores which endanger the disease-producing pathogen by making them starve for iron which is an important element for survival. This mechanism has been very much observed in the suppression of *Erwinia carotovora* through application of *P. fluorescens*, an endophytic bacterium which actively competes with the pathogen for bioavailable iron.

1.5.3 Competition for Nutrients and Niches (CNN)

There were several benefits for those endophytic bacteria controlling disease causing pathogens through the mechanism of competition for nutrients and niches. The foremost benefit is that this mechanism is being liked by researchers as the bacterial strains which possess these mechanisms can easily be selected for experiments. Secondly, the endophytic bacteria classified under CNN are not known for production of antibiotics, which facilitates their registration by regulatory authorities, as usually the antibiotic-producing microbes are not preferred to be allowed into soil environment. Thirdly, supposing a situation has arisen wherein the merger of the two mechanisms, namely, CNN and production of antibiotics, is preferred, the bacterial strains which are known for exhibiting both the mechanisms can be isolated and utilised for experiments (Malfanova 2013). This combination of mechanism was demonstrated by Pliego et al. (2008) who recorded the suppression of root rot disease in avocado through the combination of these mechanisms.

1.5.4 Antibiosis and Antibiotics Suppressing Pathogens

Antibiosis is an important mechanism which was reported to curtail the growth of pathogens in crop plants, and several researchers worked on this mechanism and tried to demystify the processes involved in it. Antibiosis is the process of the release of secondary metabolites like antibiotics and other volatile compounds by the beneficial microorganism to check the pathogenesis of disease producing microorganisms (Fravel 1988).

Haas and Défago (2005) highlighted the antibiotics like volatile HCN, phenazines and pyoluteorin which are responsible for antibiosis. Later, Dandurishvili et al. (2011) have identified newer antibiotics, namely, D-gluconic acid, 2-hexyl-5-propyl resorcinol and the volatiles 2,3-butanediol, 6-pentyl-α-pyrone and DMDS which are produced by endophytic microbes facilitating faster antibiosis.

Tabbene et al. (2009) reported that *Bacillus* species could produce peptide antibiotics in abundance, whereas Zhang et al. (2013) found out that *Bacillus* species could synthesise volatile compounds with lesser molecular weight and several lipopeptides with specific activities against phytopathogenic fungi. Among these lipopeptides, surfactin, fengycin, polymyxin, bacitracin and the group of iturin can elicit relevant properties (Ongena and Jacques 2008). The lipopeptides' structural differences are strongly related to their antifungal and antibacterial activities (Ramkumar et al. 2013). Thus, fengycin and iturin are known for having antifungal activities (Savadogo et al. 2011).

The effectiveness of iturins to suppress the bacterial pathogens causing diseases was studied by Zeriouh et al. (2011) who recorded the reduced incidence of *Pectobacterium carotovorum* and *Xanthomonas campestris* by the antibiosis of iturins. Fengycin, yet another antibiotic produced by bacterial endophytes, could be observed in apple plant and found to be useful in checking the population of *Botrycis cinerea* (Toure et al. 2004). The role of fengycin in reducing the incidence of brown rot in peach was reported by Yanez-Mendizábal et al. (2011).

Bais et al. (2004) found that surfactin, an antibiotic known for the control of plant pathogens, was found to be effective against *Pseudomonas syringae* on *Arabidopsis.* Ongena et al. (2007) and Henry et al. (2011) were the researchers who tried to find the combination of fengycin and surfactin in suppressing plant pathogens and reported that in bean and tomato plants, these two antibiotics could be able to prompt the various pathways responsible for resistance to diseases. Consortia of antibiotics including surfactin, iturin and fengycinin were observed to be produced by endophytic bacterial species *Bacillus* species PGPBacCA1 in soybean to suppress the growth of pathogen producing charcoal rot (Torres et al. 2016).

Dwivedi and Johri (2003) identified another group of antibiotics, phloroglucinols, which could strengthen the defence mechanism of plants by way of serving as elicitor of phytoalexins. Plenty of literature supported the ability of phenazines, a heterocylic secondary metabolite, as antibiotic which can lessen the virulence of pathogens in plants (Pierson and Pierson 2010). Phenazine-1-carboxamide,

phenanazine-1-carboxylic acid and phenanzine-1-carboxamide are some of the phenazine compounds released as antibiotics in plant system and reported by researchers to control *R. solani*, *X. oryzae* in rice and *P. myriotylum* in cocoyam and *P. splendens* in beans (Pierson and Thomashow 1992; Perneel et al. 2008; Shanmugaiah et al. 2010). The scientists have observed endophytic bacterial species *P. fluorescens*, *P. chlororaphis* and *P. aeruginosa* PNA1 in the plants which were reported to produce the various phenazine compounds.

Pyrrolnitrin, cyclic lipopeptides and massetolides are the antibiotic substances produced by a wide range of endophytic bacterial species. Pyrrolnitrin could suppress a wide range of fungal pathogens belonging to three fungal families, namely, deuteromycete, ascomycete and basidiomycete. Massetolide could facilitate biofilm formation which is an important defence mechanism towards plant pathogens. *P. fluorescens* BL915, *P. fluorescens* SS101 and various *Pseudomonas* strains were found to be responsible for the production of these antibiotics (Ligon et al. 2000; Katz and Demain 1977; de Bruijn et al. 2008).

Phenols are another group of antibiotics involved in antibiosis in crops and reduced the incidence of plant diseases. Saidul et al. (2001) reported about the formation of 2-acetamidophenol catalysed by *Pseudomonas fluorescens* strain 2–79 (NRRL B-15132) which could lessen the virulence of most of the disease-causing pathogens in wheat. Salicylic acid, yet another phenolic derivative, was reported to inhibit plant pathogens by serving as a messenger (Wildermuth et al. 2001). The research work of Liechti and Farmer (2002) and Diaz et al. (2003) brought to light another phenolic compound, jasmonic acid, which can suppress pathogens by way of regulating and mediating the response of plants to pathogens.

Gao Zhenbeng et al. (2017) reported that volatile organic compounds pyrazine (2,5-dimethyl), benzothiazole, phenol (4-chloro-3-methyl) and phenol-2,4-bis (1,1-dimethylethyl) from *Bacillus velezensis* ZSY-1 exhibited significant antifungal activity against *Alternaria solani*, *Botrytis cinerea*, *Valsa mali*, *Monilinia fructicola*, *Fusarium oxysporum* f. sp. *capsicum* and *Colletotrichum lindemuthianum* and the inhibition rates were found to be 81.1%, 93.8%, 83.2%, 80.9%, 76.7% and 70.6%, respectively.

1.6 Plant Growth Promotion

Endophytes were found to accelerate plant growth through a plethora of mechanisms. It includes primarily phytostimulation (e.g. by hormone production) followed by biofertilisation (e.g. by fixation of atmospheric nitrogen, solubilisation of minerals such as phosphorus and formation of siderophores to scavenge Fe3+ ions under Fe3 + −limiting conditions). The third mechanism is the induction of stress tolerance (e.g. by regulation of the release of quantity of stress hormone by the enzyme 1-aminocyclopropane-1-carboxylate deaminase), and the fourth mechanism is the rhizoremediation (i.e. protection of plants by rhizobacteria against environmental pollutants).

Lugtenberg et al. (2013) reported the production of hormones by bacteria like ethylene, cytokinins, gibberellins, auxins, etc. Majority of rhizosphere bacteria are found to produce auxins which are very much important for lateral root formation (Pliego et al. 2011). Spaepen et al. (2009) in their paper published in *Annals of Botanical Research* explained about different pathways of synthesis of plant growth-promoting hormones. They reported the secretion of tryptophan, a constituent of exudates of roots, as the antecedent for the initiation of synthesis of indole acetic acid pathway which is being utilised by the bacteria present in the root zone. This view of Spaepen et al. (2009) was confirmed by the study of Kamilova et al. (2006) who found that the growth of radish got enhanced through tryptophan-induced IAA secretion from a bacterial strain WCS365 of *P. fluorescens* which has increasingly been recommended for biological control of diseases. Further, it was recorded by Spaepen et al. (2009) that IAA production was enhanced due to the presence of *Azospirillum brasilense* which spiked the formation of lateral roots and root hair formation ultimately resulting in increased production of exudates from roots.

Numerous rhizosphere bacteria are reported to produce gibberellins (Pliego et al. 2011) which are responsible for cell division, cell elongation and seed germination. The studies carried out by researchers to analyse the growth promoting ability of bacteria living in the root indicated the secretion of growth promoting substances, namely, cytokinin, GA, acetoin and 2,3-butanediol, by *Acinetobacter calcoaceticus*, *Bacillus* spp. and other rhizosphere-dwelling bacterial species in various crops including cucumber, Chinese cabbage, etc. (García de Salome et al. 2001; Kang et al. 2009; Ryu et al. 2003).

Hardoim et al. (2008) documented an array of bacteria in the root zone which were found to produce an enzyme called 1-aminocyclopropane-1-carboxylate deaminase which was responsible for removing stress induced in crop plants due to the production of ethylene as a result of various biotic and abiotic stresses in crop plants. According to Ryu et al. (2003) endophytic bacteria secrete some volatile compounds, namely, acetoin and 2,3-butanediol, to enhance the growth of plants in general. Genomic sequencing of *Enterobacter* sp. 638 indicated the production of such components in poplar, a biofuel feedstock plant, which was helpful in the availability of sucrose facilitating the production of phytohormones which could enhance growth of plants (Taghavi et al. 2010).

Many of the endophytic bacterial strains were found to facilitate the availability of nutrients like nitrogen and phosphorus to the plants via soil. Vendan et al. (2010) and Shcherbakov et al. (2013) reported the ability of endophytic bacteria to fix atmospheric nitrogen in plants. Phosphorus is an important growth-promoting nutrient for various crops whose availability is a biggest problem, and whatever phosphorus applied to soil in organic or inorganic form could not be readily taken by the plants. Researchers have been able to isolate the endophytic bacterial species which are useful in converting the unavailable nutrients into available form. Studies indicated that phosphate-solubilising *Pseudomonas* spp., *Bacillus megaterium* and *Bacillus* spp. were found to provide phosphorus in available form and increased the growth and yield of maize, sugarcane and canola, respectively (De

Freitas et al. 1997; Sundara et al. 2002; Rodriguez et al. 2006; Vyas and Gulatti 2009; Smyth 2011).

Reinhold-Hurek and Hurek (1998) in their research paper in *Trends in Microbiology* detailed the role of siderophores as a response to overcome iron-limiting conditions in plants which was reported in many studies. It was found that endophytic bacteria could synthesise siderophores to cope with microenvironments such as the root interior which is highly depleted of bioavailable iron. Several reports indicated production of siderophores by bacterial species may affect iron plant nutrition. For example, Becker et al. (1985) reported that iron uptake in pea (*Pisum sativum* L.) and maize (*Zea mays* L.) is inhibited when purified pseudobactin is applied to plants. In peanuts (*Arachis hypogaea* L.) amendment with Fe^{3+} pseudobactin resulted in lime-induced chlorosis amelioration (Jurkevitch et al. 1998).

Iron availability to plants grown in hydroponics and pot culture was also facilitated by endophytic bacterial strains. Duijff et al. (1994) observed that the plants could make use of Fe^{3+} −pseudobactin-358 which also enhanced the synthesis of chlorophyll in plants. Sharma et al. (2003) conducted a pot experiment in mung bean (*Vigna radiata* L. Wilczek) inoculated with *Pseudomonas* sp. strain. The bacterial strain was able to synthesise siderophore which was reported to enhance the iron available to the plant system which could increase the level of chlorophyll and reduction of chlorosis in bean plants.

Pirttila et al. (2004) reported the ability of endophytic bacterial species to provide necessary vitamins to crops which can enhance the growth of crops. Compant et al. (2005) identified several physiological processes which were catalysed by endophytic bacteria, thus improving the growth and yield potential of crops. In the leaves of plants, the endophytic bacterial species could facilitate adjustment of osmotic pressure and regulation of stomatal openings. In roots the bacteria could alter the biochemical processes of availability of nutrients to the plants. Besides, the role of endophytic bacteria for the remediation of polluted soils with heavy metals and regeneration of forest has been increased in the recent past, and there were several instances that endophytes are being used for such purposes.

1.7 Induced Systemic Resistance (ISR)

Resistance in crop plants for phytopathogens has been debated widely, and numerous research findings were evolved to decipher the mechanism. There was a consensus among researchers that induced systemic resistance (ISR) could be offered by microorganisms to combat pathogens. ISR is the immunity response mechanism inherent in crop plants which is triggered by the beneficial bacteria present in the rhizosphere such as *P. fluorescens* strains WCS417R and WCS365 (van Loon and Bakker 2003; Kamilova et al. 2005; Van Wees et al. 2008).

Stadnik (2000) defined ISR as the external agents mediating enhanced resistance and altering the genome of the plant. ISR is different from systemic acquired resistance (SAR) in several physiological and biochemical phenotypes (Van and Elsas 2008) and can be induced by many different bacterial surface molecules, secreted metabolites and volatiles (Lugtenberg et al. 2013). Examples of bacterial endophytes which have been suggested or claimed to induce ISR are *Bacillus amyloliquefaciens*, *Bacillus pumilus*, *Bacillus subtilis*, *Pseudomonas fluorescens*, *Pseudomonas syringae* and *Serratia marcescens* (Kloepper and Ryu 2006).

The plants which got immunised through ISR can guard the plants against a score of disease causing pathogens of different origins. In plants which possess stronger ISR, the response for defending the pathogens entering the plants used to be swifter which offers high level of resistance to the plant for diseases. Numerous studies portrayed the event of ISR in different crops inoculated with varied bacterial species dwelling in root zone (Van Peer et al. 1991; Liu et al. 1995; Raj et al. 2003; Halfeld-Vieira et al. 2006; Van Loon 2007).

Bonaldo et al. (2005) listed the advantages of ISR wherein they pointed out the efficiency against an array of pathogens, exhibition of varied resistance methods, efficient utilisation of energy and exploitation of genetic ability to induce resistance in the plants which are vulnerable for diseases. Several studies demonstrated that the different crop plants exhibit differential ISR and the efficiency also varied from plant to plant which was reported to be regulated by jasmonic acid and ethylene in most of the plants (van Wees et al. 2000; Van Loon and Bakker 2003). De Weert et al. (2007) reported that toll-like receptors were utilised by the ISR mechanism which was analogous to inherent immunity. Studies indicated that complete colonisation of bacteria in root zone is not necessary for initiation of ISR which indicated even partial colonisation can bring out ISR. Further, apart from living endophytic bacterial species, even dead microorganism can activate ISR (Dekkers et al. 2000). A long list of literature indicated that ISR can be activated through several compounds produced by endophytic bacteria like salicylic acid, c-LPs, pyocyanins, siderophores, etc. (Audenaert et al. 2002; Ryu et al. 2003; Schuhegger et al. 2006; Pérez-García et al. 2011).

Hallmann et al. (1995) reported that ISR mechanism was enhanced in plants treated with endophytic bacteria which resulted in enhanced protection against parasitic nematodes responsible for extensive damage to crops. They further stressed that a huge potential is there for researchers to venture into research linking ISR and plant parasitic nematode control in several crops.

Endophytic bacteria treated with chitosan, which is available in the cell wall of fungi, could accelerate the ISR which effectively check the growth of pathogens, and research studies involving such chemical elicitors for enhanced ISR in crops would pave way for designing disease management protocol with a combination of methods (Benhamou et al. 1998).

Induction of resistance promoted by plant growth-promoting rhizobacteria (PGPR) is active according to the researchers, Hoffland et al. (1995) and Pieterse et al. (1998) and Romeiro (2000); the ISR is facilitated via production of salicylic acid with induction of PR proteins via the production of the jasmonic acid and eth-

ylene. They further explained the process that during the colonisation of endophytic bacteria in the rhizosphere region, the elicitors produce certain bacterial molecules which served as biochemical signal which culminates in the encoding of genes responsible for these processes and the ISR is initiated in the plant. Wei et al. (1991) who worked on the plants exhibiting ISR reported that cucumber is the best example of exhibitor of ISR mechanism and demonstrated the suppression of anthracnose caused by *Colletotrichum orbiculare* through the activation of ISR.

Chen et al. (2000a, b) and Saikia et al. (2004) contemplated that the formation of enzymes like peroxidases, lipoxygenases, chitinases and glucanases which are responsible for the inhibition of the growth of pathogens is the forte of the qualities of endophytic bacterial species. The scientists recorded the production of the enzymes like peroxidases in cucumber plant effectively reduced the incidence of *Pythium aphanidermatum*, and similar mechanism was observed by Young et al. (1995) in rice and wheat. Yet another mechanism indicated by Li et al. (1991) was the induction of phytoalexins enhanced by the formation of the enzyme called lipoxynase which was inhibitory to the incidence of diseases. Daniel and Purkayastha (1995), Nakkeeran et al. (2006) and Saikia et al. (2006) in their research papers emphasised that the more production and involvement of enzymes, the more would be the ISR, ultimately resulting in pathogenesis which differed based on the nature of host and disease-inflicting pathogens.

1.7.1 Signal Interference

Dong et al. (2004) identified a mechanism wherein the production of exoenzymes could be controlled by inactivating the N-acyl homoserine lactone molecule which is essential for exo-enzyme production. Dandurishvili et al. (2011) reported the control of crown gall disease in tomato inflicted by the pathogen *Agrobacterium* through reduction of transcription of N-acyl homoserine lactone synthase genes *phzI* and *csaI* activated by root zone bacterial strains *P. fluorescens* B-4117 and *S. plymuthica* IC1270.

1.7.2 Detoxification and Degradation of Virulence Factors

Detoxification of toxins secreted by pathogens would serve as a way to suppress the activity of pathogens which has been displayed by several endophytic bacteria (Compant et al. 2005). Toyoda and Utsumi (1991) reported that fusaric acid, a toxin secreted by *Fusarium* species, a major wilt-causing pathogen, could be suppressed by the endophytic bacterial strains of *B. cepacia* and *Ralstonia solanacearum.*

Compant et al. (2005) reported that the virulence factor of pathogens could be deprived by some of the endophytic bacteria. Uroz et al. (2003) discussed about the quorum-sensing capacity of bacterial endophytes through inhibiting the expression

of genes responsible for virulence of pathogens. Von et al. (2003) remarked that autoinducer-mediated quorum- sensing is an important mechanism that has been relied upon by the pathogens as this mechanism could bring down the virulence of pathogen to inflict diseases. This mechanism has been considered to be of paramount importance since the pathogen could be taken cared of by the mechanism of quorum-sensing after the pathogen gets established in the plant system.

A summary of the literature pertaining to the mode of action of endophytic bacteria is provided in Table 1.1 for better understanding of readers.

Table 1.1 Summary of mode of action of endophytic bacteria

Broad mode of action	Mechanism involved	References
Competitive root colonisation	Differential phase of growth, ability to stick onto the roots, ability to move, effective use of the organic acids present in root exudates and the synthesis of various components including amino acids, type III secretion system	Whipps (1997), Lugtenberg and Kamilova (2009), Duffy (2001) and Turnbull et al. (2001)
Antibiosis and antibiotics suppressing pathogens	Production of antibiotics like phenazines, pyoluteorin, pyrrolnitrin and the volatile HCN	Gupta et al. (2001), Fravel (1988), Haas and Défago (2005), Dandurishvili et al. (2011), Tabbene et al. (2009), Zhang et al. (2013), Ongena and Jacques (2008), Ramkumar et al. (2013), Caldeira et al. (2011), Savadogo et al. (2011), Zeriouh et al. (2011), Touré et al. (2004), Yánez- Mendizábal et al. (2011), Ongena et al. (2007); Henry et al. (2011), Torres et al. (2016), Dwivedi and Johri (2003), Pierson and Pierson (2010), Shanmugaiah et al. (2010), Pierson and Thomashow (1992), Perneel et al. (2008), Ligon et al. (2000), Katz and Demain (1977), Wildermuth et al. (2001), Liechti and Farmer (2002), Diaz et al. (2003) and Gao Zhenbeng et al. (2017)
	Production of antibiotics, namely, D-gluconic acid, 2-hexyl-5-propyl resorcinol and the volatiles 2,3-butanediol, 6-pentyl-α-pyrone and DMDS	
	Among lipopeptides, surfactin, fengycin, polymyxin, bacitracin and the group of iturin can elicit relevant properties of disease control	
	Production of phloroglucinol, pyrrolnitrin, phenols and volatile organic compounds like pyrazine (2,5-dimethyl), benzothiazole, phenolic derivatives	
Signal interference	Inactivation of AHL molecule required for exo-enzyme production	Dong et al. (2004) and Dandurishvili et al. (2011)

(continued)

Table 1.1 (continued)

Broad mode of action	Mechanism involved	References
Competition for ferric iron ions	Production of siderophores to catch hold of ferric ion and to deprive the pathogens for iron	Loper and Henkels (1997) and Whipps (2001)
Competition for nutrients and niches (CNN)	The mechanism involved in competitive root colonisation applies for CNN also	Pliego et al. (2008) and Malfanova (2013)
Detoxification and degradation of virulence factors	Fusaric acid detoxifies the toxins produced by pathogens.	Toyoda and Utsumi (1991), Uroz et al. (2003), Von et al. (2003) and Compant et al. (2005)
	Quorum-sensing ability by degrading autoinducer signals, thereby inhibiting expression of numerous virulence genes	
Induced systemic resistance (ISR)	Resistance induced by the production of salicylic acid, c-LPs, pyocyanins, siderophores, etc.	Li et al. (1991), Wei et al. (1991), Van Peer et al. (1991), Daniel and Purkayastha (1995), Young et al. (1995), Hoffland et al. (1995), Hallmann et al. (1995), Liu et al. (1995), Van Wees et al. (1997), Benhamou et al. (1998), Pieterse et al. (1998), Romeiro (2000), Chen et al. (2000a, b), van Wees et al. (2000), Dekkers et al. (2000), Audenaert et al. (2002), Stadnik (2000), Iavicoli et al. (2003), Ryu et al. (2003), Van Loon and Bakker (2003), Raj et al. (2003), Silva et al. (2004), Kloepper et al. (2004), Saikia et al. (2004), Campos et al. (2004), Kamilova et al. (2005), Halfeld-Vieira et al. (2006), Saikia et al. (2006), Kloepper and Ryu (2006), Schuhegger et al. (2006), Nakkeeran et al. (2006), de Weert et al. (2007), Ongena et al. (2007), van Loon (2007), Van Wees et al. (2008), Van and Elsas (2008), Pliego et al. (2011) and Pérez-García et al. (2011)
	Combined application of endophytic bacteria and chemical elicitors such as chitosan, a chitin derivative, will enhance ISR	
	Increased production of peroxidases, PPO and PAL enhances ISR	
	The action of lipoxygenase products which contributes to induction of phytoalexins	

1.8 Endophytic Bacteria Suppressing Wilt-Causing Pathogen in Plants

Among the diseases, wilt is a prominent disease caused by pathogens of fungal and bacterial origin which could bring huge economic loss to the farmers. The prominent fungal pathogens causing wilt are *Fusarium* and *Verticillium* species, the control of which is onerous since these pathogens are soilborne. Often, the chemical

measures to control wilt do not bear fruit as the pathogen has a wide range of host and sustained in soil for a very long time. Hence, the biological control of wilt assumed greater importance which resulted in many scientists venturing into the research on finding suitable endophytic bacteria to control wilt diseases.

A number of studies showed endophytic bacteria were reported to suppress the growth of wilt-producing pathogen in cotton. Lin et al. (2013) conducted a pot experiment with 60 strains of endophytic bacteria isolated from *Sophora alopecuroide* to control *Verticillium* wilt (*Verticillium dahliae*), and the mean control effect of two strains, namely, *Bacillus subtilis* KDRE01 and *Bacillus megaterium* KDRE25, was worked out. The results indicated that the mean control effect of the two endophytic bacteria was 84.91% and 78.82%, respectively, and the strains differed significantly at 5% level of significance.

Chen et al. (1995) reviewed earlier studies on cotton involving endophytic bacterial strains, *Aureobacterium saperdae*, *B. pumilus*, *Burkholderia solanacearum*, *Phyllobacterium rubiacearum* and *Pseudomonas putida*, which were isolated from internal tissues of cotton and were found to suppress vascular wilt in cotton caused by *F. oxysporum* f. sp. *vasinfectum*.

Xia et al. (1996) observed that the endophytic bacteria had more potent antagonistic activity against *V. dahliae* than the rhizosphere bacteria and elicited induced response in cotton against wilt pathogen, and the findings were endorsed by Fu et al. (1999a, b), and reported the toxin produced by *V. dahliae* was effectively suppressed by antagonistic activity of endophytic bacteria.

Sturz et al. (1999) reported that the disease causing wilt pathogens, namely, *F. avenaciarum, F. sambucinum* and *F. oxysporum*, were found to be controlled by endophytic bacteria isolated from potato tubers. Further in vitro antagonism was exhibited by endophytic bacteria isolated from live oak stems which could lessen the virulence of *C. fagacearum* (Brooks et al. 1994).

Amaresan et al. (2014) in their study in chillies pertaining to the isolation and characterisation of endophytic bacteria on chilli diseases found that the antagonistic activity against *Fusarium oxysporum* was to the tune of 37.8%. In the study the authors could identify the ability of bacterial isolates BECS7, BECS4 and BECL5 in terms of catalysing the growth, suppressing the pathogenesis and promoting enhanced yield. Further the authors argued that the bacterial strains that produced different hydrolytic enzymes, such as protease, had inhibited the growth of pathogenic fungi *F. oxysporum*. Besides reducing the pathogenesis of *Fusarium oxysporum*, the proportion of endophytes was found to enhance the germination potential of seed and crop growth. These findings were in line with the results reported by Nielson and Sorensen (1999) in their study on barley and sugar beet and Nejad and Johnson (2000) in oilseed rape and tomato.

Literature on application of endophytic bacteria in isolation was found to arrest the growth of *Fusarium* minimally, and it was suggested by several researchers to use a combination of endophytic bacteria which yielded desired results. Smith et al. (2003) in their study on management of *Fusarium* wilt in banana using bacterial endophytes reported that de-flasking stage was optimal for allowing the bacterial strains. In their study, they found that under greenhouse conditions, the incidence of

Fusarium oxysporum f. sp. *cubense* was found to be reduced through the application of two strains of *Pseudomonas* 84 and 4B into the rhizosphere of banana.

Similarly, studies conducted by Ayyadurai et al. (2006) and Getha et al. (2005) using singular soil antagonistic bacteria *Pseudomonas fluorescens*, *P. aeruginosa*, *Burkholderia cepacia* and *Streptomyces* sp. to investigate the efficiency of bacterial species for the suppression of the *Fusarium oxysporum* f. sp. *cubense* did not result in complete control of the disease. Earlier Guetsky et al. (2001) also advocated through their studies that combination of biocontrol agents with multiple traits could be very useful to combat the biotic and abiotic stress in the field.

Taking a cue from these studies recommending the use of combination of bacterial isolates, Thangavelu and Gopi (2015) conducted a study to evaluate the effectiveness of bacterial isolates in suppressing of *Fusarium* wilt in cv. Grand Naine banana. The authors of the study took 24 different combinations of both rhizospheric and endophytic bacterial isolates, and they conducted experimental trials in pot culture. Results of the study indicated that five combinations involving four endophytic bacterial isolates, namely, *Pseudomonas putida*, *Acromobacter* spp., *Rhizobium* spp. and *Bacillus flexus*, and two bacterial isolates live in the root zone of plants, namely, *Bacillus cereus* and *Pseudomonas putida*, were reported to control the *Fusarium* wilt fully. The study was conducted in the field with the same set of treatment wherein the bacterial isolates were applied in the soil and the data were recorded. The field study results indicated that the same five combinations which were found to be effective in controlling *Fusarium* wilt in pot culture experiments were also effective in field conditions. Bunch weight and number of banana hands were the yield parameters estimated in the study. The data pertaining to these two parameters indicated that the average number of banana hands increased up to 155% and average bunch weight increased up to 214% when compared to control.

Sundaramoorthy et al. (2012) conducted a similar study in chilli pepper using the combinations of rhizospheric and endophytic bacterial strains for the control of *Fusarium* wilt incidence. They found that endophytic bacterial strain *P. fluorescens* (Pf1) and rhizospheric bacterial strains *B. subtilis* (EPCO16 and EPC5) were found to reduce the incidence of wilt in the range of 17% to 30% when compared to control. The reports of Ganeshmoorthi et al. (2008) and Latha et al. (2009) were in conformity with their previous researchers who reported that combination of biocontrol agents would be more effective in controlling plant diseases rather than a single biocontrol agent.

Nagarajkumar et al. (2004) indicated that the wilt pathogens, *F. oxysporum* and *R. solani*, could be effectively controlled by the application of *Pseudomonas* strains through the formation of secondary metabolites, enzymes and siderophores which were produced in abundance.

Wang et al. (2013) investigated the antagonistic ability of *Bacillus amyloliquefaciens* W19 on *Fusarium* wilt of banana and reported that W19 strain was found to observably suppress *Fusarium* wilt and enhance the development of banana plants when combined with the organic fertiliser (OF). Two kinds of antifungal lipopeptides (iturin and bacillomycin D) produced by W19 strain were detected and identified using HPLC-ESI-MS. Another lipopeptide, called surfactin, was also produced

by the thick biological film forming W19 strain. In addition to lipopeptide, 18 volatile antifungal compounds with significant antagonistic effect against *F. oxysporum* were detected and identified.

Many research studies conducted in tomato revealed that different endophytic bacterial isolates were found to control the *Fusarium* and *Verticillium* wilt of tomato. Endophytic bacteria *Bacillus* sp. could be able to control *V. dahliae* and *F. oxysporum* f. sp. *lycopersici* in rape and tomato plants, respectively. They found that *Bacillus* sp. could inhibit mycelial growth and reported 75% reduction in infection. Further, they reported production of volatile metabolites other than hydrogen cyanide. *Pseudomonas* sp. strain PsJN was reported to enhance resistance against *Verticillium* wilt in tomato up to 5 weeks (Hall et al. 1986; Sharma and Nowak 1998; Nejad and Johnson 2000).

M'Piga et al. (1997) studied the suppression of *F. oxysporum* f. sp. *radicis-lycopersici* by *P. fluorescens* in tomato. While explaining the mode of action, they reported the combined effect of structural and biochemical barriers to the growth of plant pathogens suppressing the incidence.

Duijff et al. (1997) confirmed the control of *Fusarium* wilt in tomato by *P. fluorescens* WCS417r and found that colonisation of epidermal or hypodermal cells or cortical intercellular spaces by WCS417r led to the thickening of cortical cell walls in tomato plants which checks the entry of pathogen producing *Fusarium* wilt. Another study which used *B. pumilus* SE-34 for the reduction of pathogenesis of *F. oxysporum* f. sp. *radicis-lycopersici* in tomato provided evidence of suppression of the wilt-producing pathogen through induction of resistance either alone or in combination with chitosan (Benhamou et al. 1998).

Endophytic bacteria from *Datura stramonium* could be used as an effective suppressor of *Fusarium* wilt in tomato as reported by Abdallah et al. (2016). The authors of the study screened ten bacterial isolates from *D. stramonium* for the containment of *Fusarium* wilt in tomato which is inflicted by the pathogen *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and to accelerate the growth. The study revealed that the bacterial isolates S37 and S40 were found to reduce the leaf yellowing symptom within the range of 88% to 94%. There was 95–96% reduction in vascular browning due to the effect of these bacterial isolates juxtaposing the data from untreated control.

Vitullo et al. (2012) studied the mechanisms of BO7, a strain of *Bacillus amyloliquefaciens* taken from orchard soil, to reduce the incidence of vascular wilt fungus *Fusarium oxysporum* f. sp. *lycopersici* (FOL) and reported that three of the surfactin lipopeptides which are similar in structure were involved in the antibiosis. Further the study results revealed that one of the three compounds was found to possess huge antifungal properties to control FOL.

Realising the scope of *B. amyloliquefaciens* claimed to possess extensive antagonistic potential against pathogens which has been documented by several researchers (Zouari et al. 2016; Chen et al. 2016), Shahzad et al. (2017) did experiment the antagonistic potential of *B. amyloliquefaciens* RWL-1 on the *Fusarium* wilt of tomato (*Fusarium oxysporum* f. sp. *lycopersici*). The in vitro experimental study involving the dipping of tomato roots in bacterial culture revealed that *B. amyloliq-*

uefaciens RWL-1 could not only suppress the pathogenic fungal growth significantly but also reduced the incidence of disease symptoms in the field.

The authors of this study found that the introduced RWL-1 could produce bioactive constituents, siderophores and organic acids, which could assist plants to counteract disease-induced stress. The study results also found that RWL-1 inoculation increased the production of plant defence hormones like salicylic acid, which was not observed in control. Further amino acids like glutamic acid and aspartic acid were produced in abundance in RWL-1-inoculated plants in comparison to the control. The study results were in line with the findings of Pratelli and Pilot (2014), Khan et al. (2015), Waqas et al. (2015) and Shahzad et al. (2016).

A summary of the literature pertaining to the endophytic bacteria controlling wilt-producing pathogens is provided in Table 1.2 for better understanding of readers.

1.8.1 The Endophytic Bacterial Cultures Suppressing the Pathogens of Damping Off and Rot

Damping off and rot are the important soilborne diseases caused by fungal pathogens, and their inhibition by endophytic bacteria through various mechanisms has been studied by researchers. A nutshell of literature is given in the following section.

1.8.2 Endophytic Bacteria Controlling Damping Off

Melnick et al. (2008) tested four *Bacillus* spp. to assess their efficacy in restricting the pathogen *Phytophthora capsici* which caused damping off in cacao seedlings. The study results revealed that two species, namely, *B. cereus* BT8 and BP24, applied with a surfactant were found to significantly reduce the incidence of the disease.

Muthukumar et al. (2010) to assessed the efficacy of ten endophytic isolates of *Pseudomonas fluorescens* to control damping-off disease in chillies caused by *Pythium aphanidermatum.* Among the ten isolates tested, *P. fluorescens* EBS 20 was found to produce bigger inhibition zone, and the mycelial growth in petridish was minimal. Further, the authors found that the inhibition of damping-off disease could be mainly attributed to the secretion of salicylic acid, siderophore and hydrogen cyanide in abundance by *P. fluorescens* EBS 20 which are known for blocking the incidence of diseases and progression of symptoms. These findings were cor-

Table 1.2 Endophytic bacteria and wilt disease control

S. No	Crop	Pathogens causing wilt	Endophytic bacteria reported to control/ reduce wilt incidence	Mode of action	References
1.	Tomato	*Verticillium dahliae* *F. oxysporum* f. Sp. *lycopersici* *F. oxysporum* f. Sp. *radicis-lycopersici*	*Pseudomonas* sp. strain PsJN *P. fluorescens* WCS417r *B. pumilus* SE-34 *Bacillus amyloliquefaciens* BO7 *B. amyloliquefaciens* RWL-1	Production of volatile metabolites. Combined effect of structural and biochemical barriers to reduce the pathogenesis. Effective colonisation and thickening of cortical cell walls in tomato plants. Induction of resistance either alone or in combination with chitosan. Antifungal action of surfactin lipopeptides. Combating effects of siderophores and organic acids. Production of plant defence hormones, jasmonic acid and salicylic acid which enhance ISR.	Hall et al. (1986), Nejad and Johnson (2000), Sharma and Nowak (1998), M'Piga et al. (1997), Duijff et al. (1997), Benhamou et al. (1998), Vitullo et al. (2012) and Shahzad et al. (2017)
2.	Cotton	*F. oxysporum* f. Sp. *vasinfectum* *Verticillium dahliae*	*Aureobacterium saperdae* *Bacillus pumilus* *Burkholderia solanacearum* *Phyllobacterium rubiacearum* *Pseudomonas putida* *Bacillus subtilis* KDRE01 *Bacillus megaterium* KDRE25	Antibiosis through production of antibiotic components. Inhibition of mycelial growth and toxin production causing wilt in cotton.	Chen et al. (1995), Lin et al. (2013), Xia et al. (1996) and Fu et al. (1999a, b)

(continued)

Table 1.2 (continued)

S. No	Crop	Pathogens causing wilt	Endophytic bacteria reported to control/ reduce wilt incidence	Mode of action	References
3.	Banana	*F. oxysporum* f. Sp. *cubense* race 4 *Fusarium oxysporum* f. Sp. *cubense*	*Burkholderia cepacia* *Pseudomonas* strains 84 and 4B *Pseudomonas putida strains* *Bacillus cereus* strains *Acromobacter* spp. *Bacillus cereus* *Bacillus flexus* strains *Rhizobium* spp. *Bacillus amyloliquefaciens* W19	Colonise hyphae of the fungus and its macrospores. Mycelial deformation with terminal and intercalary swellings resulted in reduced disease incidence. Secretion of secondary metabolites and siderophores which was reported to suppress pathogen growth. Thick biological film forming iturin and bacillomycin D and surfactin control growth of pathogen.	Pan et al. (1997), Smith et al. (2003), Thangavelu and Gopi (2015), Sundaramoorthy et al. (2012) and Wang et al. (2013)
4.	Chillies	*Fusarium oxysporum*	BECS7, BECS4 and BECL5 *P. fluorescens* (Pf1) *B. subtilis* (EPCO16 and EPC5) *Pseudomonas* spp.	Inhibition of pathogens through production of hydrolytic enzymes	Amaresan et al. (2014) and Sundaramoorthy et al. (2012)
5.	Potato	*F. Avenaciarum* *F. sambucinum* *F. Oxysporum*	*Bacillus* spp.	In vitro antibiosis	Sturz et al. (1999)
6.	Oak	*C. Fagacearum*	*P. denitrificans* and *P. putida*	In vitro antagonism and competitive colonisation of microbes	Brooks et al. (1994)
7.	Peas (*Pisum sativum* L.)	*F. oxysporum* f. Sp. pisi	*B. pumilus* strain SE34	Strengthening of the epidermal and cortical cell walls	Benhamou et al. (1996)

roborated with the similar findings of the authors. Muthukumar and Bhaskaran (2007) in yet another study screened 12 isolates of *P. fluorescens* and observed that isolates 3 and 4 were found to be very effective against *Pythium* spp. In a similar study carried out a little earlier by Nakkeeran et al. (2006), it was found that two endophytic bacterial strains, namely, *P. chlororaphis* strain PA23 and *B. subtilis* strain BSCBE4, were found to arrest the growth of *P. aphanidermatum*, the causal organism of damping-off disease in chillies.

The studies of Buysens et al. (1996) and Kraus and Loper (1992) reported that restricted growth of damping-off-producing pathogens in tomato and cucumber was attributed to the production of siderophore. The production of antibiotic components like polyphenol oxidase, peroxidases and phenylalanine ammonia-lyase as part of the mechanism of induced systemic resistance exhibited by bacterial species present in the rhizosphere is reported to be behind the suppression of damping off in cucumber caused by *Pythium aphanidermatum* (Chen et al. 2000a).

A study on biological control of damping off caused by *Rhizoctonia solani* in cucumber was taken up by Huang et al. (2012). The authors of the study used an endophytic bacterial strain, *Bacillus pumilus* SQR-N43, for the study. Two experiments were conducted in the study wherein the first experiment has seen the utilisation of only which was applied on the cucumber field. In the second experiment, the researchers added fermented organic fertiliser along with the *Bacillus pumilus* SQR-N43 and applied in the field. The observations were recorded after 20 days, and the results revealed that the second experiment involving the organic manure along with *Bacillus pumilus* SQR-N43 performed better than the first experiment which involves only *Bacillus pumilus* SQR-N43 in terms of number of CFUs, percentage of spores and control efficiency.

Fiddaman and Rossall (1993) and Yangui et al. (2008) observed hyphal vacuolisation and deformation in *R. solani* and in *Pythium ultimum* after treatment with a *B. subtilis* strain and *Bacillus* spp. which resulted in reduced growth of pathogen. Selim et al. (2017) evaluated the antifungal potentialities of three endophytic bacterial strains in greenhouse and found that a significant increase of seed emergence and seedling survival with a clear reduction of disease severity was achieved with the endophytic bacterial treatments.

1.8.3 Endophytic Bacteria Controlling Rot

Disease incidence (DI) (Campbell and Madden 1990) and disease severity (DS) (Liu et al. 1995) were studied in oil palm plants inoculated with two strains of endophytic bacteria, namely, *Burkholderia cepacia* (B3) and *Pseudomonas aeruginosa* (P3), for their ability to reduce the symptoms of basal stem rot caused by *Ganoderma boninense* (Sapak et al. 2008). The study revealed that these endophytic bacteria could keep the *G. boninense* incidence below threshold level through the inhibition of entry and movement of the pathogen into the plant. The epidemic rate of pathogens in treated and control field of 4-month-old oil palm fields was tested, and the

results indicated that the selected bacterial species performed better in treated field either solitary or in combination. In 8-month-old oil palm fields inoculated with the two species of endophytic bacteria, the incidence of basal stem rot reduced to 76%. Similar results were already recorded by Dikin et al. (2003) in oil palm fields which were inoculated with the bacterial endophytes *P. aeruginosa* and *B. cepacia.*

Dikin et al. (2003) who recorded that combination of *P. aeruginosa* with *B. cepacia* was less effective than *P. aeruginosa* alone were found to be contradictory to the findings of Lemanceau et al. (1993), Pierson and Weller (1994) and Crump (1998). These researchers established that rather than solitary biocontrol agent, consortia of them would be more beneficial and effective in controlling plant diseases. The biocontrol agents are being tested for their antagonistic behaviour individually instead of testing in combination which was endorsed by earlier studies (Leeman et al. 1996; Meyer and Roberts 2002).

Barka et al. (2002) found *Pseudomonas* sp. strain PsJN, an endophytic bacteria found in onion, suppressed the incidence of *Botrytis cinerea* Pers. (*Botrytis* bunch rot) and enhanced vine growth in colonised grapevines. Jetiyanon (1994) reported that cabbage colonised by endophyte *Xanthomonas campestris* pv. *campestris* in the greenhouse could suppress the symptoms of black rot in the field which is facilitated by inducement of resistance mechanism. The progression of disease in treated field was slow when compared to non-treated fields. The antifungal activity of *B. lentimorbus* was studied, and the bacterium has been reported to produce the antifungal substances alpha- and beta-glucosidase and volatile substances which suppress the pathogenicity of *Botrytis cinerea* Pers. in grapevine and suppressed the development of *Fusarium sambucinum* Fuckel in potato tubers, respectively (Kim et al. 2002; Sadfi et al. 2001).

Torres et al. (2016) in their study, analysed the ability of endophytic bacteria *Bacillus subtilis* subsp. *subtilis* PGPMori7 and *Bacillus amyloliquefaciens* PGPBacCA1 strains against three fungal species of *M. phaseolina* causing the charcoal rot disease in soybean and identified three different mechanisms through which the pathogen get suppressed. They were cell suspension, production of cell-free supernatant and the secretion of lipopeptide fraction. Irrespective of the fungal strains, the mechanism of suspension of the cell wall was found to possess more than 50% of the suppressive ability for the charcoal rot disease in soybean than other mechanisms.

The virulence of *Colletotrichum gloeosporioides* the causal organism of anthracnose in strawberry was found to be drastically reduced by *Bacillus amyloliquefaciens* strain S13–3 (Yamamoto et al. 2015). Colonisation of black pepper vine with endophytic *Pseudomonas* species resulted in 90% reduction in lesion lengths and 60% of plantlets free from infection caused by *P. capsici* (Aravind et al. 2012).

Sian (2013) isolated endophytic bacteria from Australian native plant species and studied their ability to check the infestation of pathogen *Phytophthora cinnamomi.* The in vitro studies revealed that six of the selected bacterial endophytes were found to suppress *P. cinnamomi* infesting *L. augustifolia* effectively by significantly reducing the length of lesions produced by the pathogen. The mechanism of suppression of diseases was found to be the production of antibiotics.

Paenibacillus polymyxa PB71 isolated from the spermosphere of the Styrian oil pumpkin (*Cucurbita pepo* L. subsp. pepo var. styriaca Greb.) was able to reduce disease severity of the Styrian oil pumpkin caused by the phytopathogenic fungus *Didymella bryoniae* (black rot) under greenhouse conditions (Furnkranz et al. 2012).

Sun et al. (2017) reported that out of 19 strains of PGPR strain tested for antifungal ability, LHS11 efficiently antagonised *S. sclerotiorum* in rapeseed and its inhibition rate reached 85.71%. In greenhouse experiments, the control efficiency (LHS11 + FX2) reached 80.51%. Previous studies revealed that the inhibitory rate of *B. subtilis* CKT1 reached 74.71% against *S. sclerotiorum* in vitro.

Yamamoto et al. (2015) studied the effectiveness of the antagonistic endophytic bacterial strain in lessening the virulence of anthracnose in strawberry brought about by the pathogen *Colletotrichum gloeosporioides*. The study results revealed that the spray of *Bacillus amyloliquefaciens* S13–3 on the leaves of strawberry was able to induce the production of chitinase and β-1,3-glucanase which were responsible for suppressing the anthracnose-producing pathogen.

Bacillus amyloliquefaciens PGPBacCA1 was studied to prove its ability to protect common bean seeds from their intrinsic pathogens, and the findings of the study indicated that it had the potential to inhibit the development of the following phytopathogenic fungi *Sclerotium rolfsii* (35%), *Sclerotinia sclerotiorum* (76.5%), *Rhizoctonia solani* (73%), *Fusarium solani* (56.5%) and *Penicillium* spp. (71.5%) (Torres et al. 2017).

1.8.4 Control of Bacterial Diseases Using Endophytic Bacterial Strains

Chen et al. (2016) studied the biocontrol effects of *Brevibacillus laterosporus* AMCC100017 on potato common scab and found that the bacterial strain significantly ($P < 0.05$) reduced the pathogen population of *Streptomyces bottropensis* from 4.54 to 4.28 Log_{10} CFU g^{-1} soil in the harvesting stage of potato and the biocontrol efficacy against common scab reached as great as 70.51%.

Sturz et al. (1999) identified certain strains of endophytic bacteria, namely, *Pantoea agglomerans* and *Pseudomonas* sp. and *Curtobacterium luteum*, which were found to reduce the virulence of *Erwinia carotovora*, a bacterial pathogen causing disease in crops. A recent study conducted by Sharifazizi et al. (2017) with the selected antagonists to reduce the *Erwinia carotovora* found that all antagonists were able to reduce the disease severity on fruit and flowers. On immature fruit assay, isolates Pa21 and En23 with 83% and 25%, respectively, had the highest and lowest effects on disease incidence compared to the control. On flowers, isolates Ps170 with 92% and En23, Ps89 and Se111 with 25% reduction of infection, respectively, had the highest and lowest effects under condition tested. Based on results obtained in this study, Ps170, Ps117, En113 and Pa21 strains have potential to be used for fire blight control.

Crown gall in grapevines, caused by the phytopathogenic bacterium *Agrobacterium vitis*, has been reported to be prevented by endophytes of the xylem sap of vine plants, including *Enterobacter agglomerans*, *R. aquatilis* and *Pseudomonas* spp. strains (Bell et al. 1995). Symptom development of Pierce's disease caused by *Xylella fastidiosa* can be reduced by virulent, endophytic *X. fastidiosa* strains (Hopkins 2005).

Assis et al. (1996) in their paper on the management of *Xanthomonas campestris* pv. *campestris* (Xcc) which is the causal organism of black rot reported to inflict heavy damage in most of crucifer plant species indicated that endophytic bacteria *Bacillus* spp. isolated from disease free cabbage and radish could able to suppress the black rot in the same host plants.

Araujo et al. (2002) identified endophytic bacterial species which brought about resistance to citrus variegated chlorosis (CVC) in citrus and explained that *Curtobacterium flaccumfaciens* could reduce the incidence of CVC. Sturz et al. (1999) argued that endophytic bacterial strains could safeguard potato against soft rot, a disease caused by pathogenic bacterium. Further, Reiter et al. (2002) have identified noteworthy correlation between the incidence of *Erwinia carotovora* and the endophytic bacterial strains present in the soil of potato and colonised in the root zone. It was found that more the presence of such endophytic bacterial strains in the soil of potato, more will be its resistance to the pathogenic bacterial species *Erwinia caratovora.*

Feng et al. (2013) discussed the strength of association between the quantity of endophytic bacteria present in the soil and the resistance of tomato plants against the bacterial wilt caused by *Ralstonia solanacearum* in tomato. The researchers argued that the suppressive ability of endophytic bacterial species towards bacterial wilt varied among resistant and susceptible tomato cultivar at varied stages of tomato plant which was confirmed through traditional MPN counting method. Findings suggested that the population was found to be more in bacterial wilt-resistant tomato cultivar than susceptible cultivar. Further, they found that the antibiotic producing ability of endophytic bacterial species also was higher in resistant cultivar of tomato than susceptible cultivar. This finding of relationship between variety and resistance to disease will go a long way in designing management protocol for bacterial wilt in tomato caused by *Ralstonia solanacearum.*

1.8.5 Endophytic Bacteria Suppressing the Foliar Fungal Diseases

The studies on the antifungal activity of endophytic bacteria on the fungal pathogens of the foliar diseases were found to be scarce and an attempt is made to review those available findings and they are presented below.

The results of the studies conducted by Bargabus et al. (2002) and Bargabus et al. (2004) in successive years revealed that *Cercospora* leaf spot in sugar beets was effectively controlled by the application of two endophytic bacterial strains, namely, *Bacillus mycoides* isolate BacJ and *Bacillus pumilus* isolate 203–7. Despite the plant surfaces of rice were devoid of entophytic bacteria, their presence in internal stem led to the effective curtailing of the pathogen causing sheath blight disease (*Rhizoctonia solani*) in rice through induced systemic resistance as reported by Krishnamurthy and Gnanamanickam (1997). Garita et al. (1988) in their survey identified 8 bacteria and 24 fungi which were found to be antagonistic to *Phytophthora infestans* in the phyllosphere, rhizosphere and endosphere of tomato.

Fifty-five bacterial strains antagonistic to *Phoma tracheiphila* the causal agent of citrus mal secco disease were screened, out of which nine of the most effective antagonistic strains were tested by inoculating them into the stem of sour orange seedlings 15 days before pathogen inoculation. Three isolates of *B. subtilis* and one isolate of *P. fluorescens* significantly lowered the disease symptoms and maintained higher populations in the internal tissues of the plants in which they colonise (Lima et al. 1994).

Shiomi et al. (2006) rust isolated certain endophytic bacterial strains from the phyllosphere of two coffee species, namely, *Coffea arabica* L. and *Coffea robusta* L. They found that two bacterial endophytes, *Bacillus lentimorbus* and *Bacillus cereus*, were reported to inhibit rust development and to control germination of urediniospore responsible for pronouncement of rust in coffee leaves. The study results indicated that the leaf samples collected from coffee were found to exhibit 50% reduced infection of coffee rust due to the suppressive ability of *Bacillus lentimorbus* and *Bacillus cereus.*

Wilhelm et al. (1997) and Yue et al. (2000) in their studies on the suppression of pathogen *Cryphonectria parasitica* causing chestnut blight by bacterial endophytes reported that *Bacillus subtilis* strains isolated from the xylem sap of healthy chestnut trees and cultures of *Epichloe* and *Neotyphodium* species were found to possess inhibitory ability towards blight-causing pathogen *Cryphonectria parasitica* in chestnut. Colonisation of black pepper vine with endophytic *Pseudomonas* species resulted in 90% reduction in lesion lengths and 60% of plantlets free from infection caused by *P. capsici* (Aravind et al. 2012).

Muthukumar and Venkatesh (2013), Karthikeyan et al. (2005) and Rao (2006) who studied the control of *Alternaria* leaf blight in ribbon plant, onion and sunflower, respectively, identified various endophytic bacterial strains which were found to be effective in inhibiting the blight producing pathogens in these crops. In ribbon plant, Muthukumar and Venkatesh (2013) screened ten endophytic bacterial isolates and reported that EBL 5 was found to be efficient in lowering the infection of *Alternaria alternata* which was substantiated by the largest inhibition zone and the least mycelial growth. Similarly, *P. fluorescens* Pf1 and another strain of *P. fluorescens* were found to be effective against *A. palandui* causing leaf blight of onion and *A. helianthi* causing leaf blight of sunflower.

Gao Zhenbeng et al. (2017) studied the volatile organic compounds produced by *Bacillus velezensis* ZSY-1 and tested their suppressive ability towards disease-

causing fungus. Volatile organic compounds from ZSY-1 exhibited significant antifungal activity against *Alternaria solani*, *Botrytis cinerea*, *Valsa mali*, *Monilinia fructicola*, *Fusarium oxysporum* f. sp. *capsicum* and *Colletotrichum lindemuthianum*; the inhibition rates were 81.1%, 93.8%, 83.2%, 80.9%, 76.7% and 70.6%, respectively. Based on the study, the antifungal activity of pyrazine (2,5-dimethyl), benzothiazole and phenolic compounds was proved to be significant, and they are promising bioagents for controlling tomato fungal diseases such as early blight and grey mould.

Researchers across the world have been studying the control of leaf blast in rice using beneficial bacterial endophytes. The studies of Krishnamurthy and Gnanamanickam (1998) and later on by Radjacommare et al. (2004) on rice blast in irrigated rice indicated that the virulence of the blast-producing pathogen was drastically reduced and the symptoms of blast disease in rice were minimally observed in the fields inoculated with plant growth-promoting rhizosphere bacterial strains. Lucas et al. (2009) tried seed treatment of rice with two strains of plant growth promoting rhizobacteria found in the root zone of rice in Spain and observed a strong correlation between seed treatment and disease control and enhancement of yield.

Marta Cristina et al. (2011) conducted a study in aerobic rice fields in Brazil to find out the effectiveness of different rhizobacterial culture in controlling the blast causing pathogen (*Magnaporthe oryzae*) in rice. The screening of 18 strains of rhizobacteria which were tested for the suppression of blast pathogen revealed that almost all strains were found to lessen the spore formation and suppress the disease in the range of 16% to 95%. Further evaluation in greenhouse trials with three replications and three application methods revealed that two isolates, namely, Rizo-46 and Rizo-55, were found to be significantly effective and drastically reduced the incidence of blast in rice. Further, the study results showed that there exist marked differences in inhibitory ability of these strains in three application methods. The study indicated that the secretion of enzymes like peroxidase, b-1,3-glucanase and chitinase were accelerated by the inoculation of Rizo-46 and Rizo-55.

1.8.6 Nematode Control Through Endophytic Bacteria

Few studies could be traced about the endophytic bacteria colonising roots of plants and suppressing the growth of nematode. Siddique and Shaukat (2003) in their review indicated that the colonising ability of endophytic bacteria and their traits of easy culturability in vitro, reducing initial root damage and influencing host's response to pathogen attack, accelerated the development of plants and production of abundant root exudates for faster growth of microbes in the soil and offered lot of scope for biological management of nematodes causing damage to plants.

Combined application of endophytic *Fusarium oxysporum* and *Bacillus firmus* resulted in 76.2% reduction in the density of the pathogenic nematode *Radopholus similis* in banana plants (Mendoza and Sikora 2009). Kluepfel et al. (1993) reported reduction of *Criconemella xenoplax* nematode population in peach trees by the

antagonistic activity of bacterial strains found in rhizosphere soil. Hallmann et al. (1997) in their study on management of nematodes through biological means indicated that in cotton, gall production in roots due to root-knot nematode, *Meloidogyne incognita*, was considerably reduced by the activity of endophytic bacteria in roots which lowers the infection of nematodes. Further, they reported that the root-knot nematodes in cotton aided the entry of endophytic bacteria in roots and thus helped the establishment of endophytic bacteria in the root system.

Siddiqui and Ehteshamul-Haque (2001) in their research paper published in *Phytopathologia Mediterranea* discussed the control of nematode, *Meloidogyne javanica*, in tomato using bacterial endophytes. The results of the study indicated that inoculation of endophytic bacterial strain *Pseudomonas aeruginosa* strain IE-6 and another strain IE-6SC were found to dent the growth of *Meloidogyne javanica* in tomato which were grown in greenhouse and also in the main field.

Hallmann (2001) identified two potential endophytic bacterial strains *Rhizobium etli* G12 and its genetically modified strain G12 (pGT-trp) in potato which were found to inhibit the gall formation inflicted by the nematode *M. incognita* which was assisted through production of green fluorescent protein by the identified bacterial strains. Reitz et al. (2000) and Hallmann et al. (2001) in their study in potato reported the role of liposaccharides produced by endophytic bacteria *Rhizobium etli* G12 which facilitated the potato plants to defend against cyst nematode *Globodera pallida*.

Siddiqui et al. (2002) found in their study that the population of *M. javanica* nematodes and consequent development of root knot in tomato were considerably reduced by the synergistic effect of combined application of endophytic bacterial strains *E. solani* and P. *aeruginosa* IE-6S+.

1.8.7 Biocontrol of Postharvest Diseases by Endophytic Bacteria

The influence of endophytic bacteria in postharvest disease control has been mostly conducted on fruits and vegetables, and they are found to be limited. A few literatures have been perused and given in this section.

Elshafei et al. (2012) indicated several disease-suppressing endophytic bacteria including *Paenibacillus brasilensis*, *Bacillus subtilis*, *Burkholderia gladioli* pv. *agaricicola* and *Streptomyces* sp. and their antagonistic ability in their study which were reported to control a plethora of pathogens causing postharvest losses in crop plants.

In vitro studies of management of *Penicillium digitatum*, a causal organism of a postharvest disease citrus mould, conducted by Mohammadi et al. (2017) revealed that among ten endophytic bacterial isolates, *Bacillus subtilis* and *Agrobacterium radiobacter* were reported to be superlative in controlling citrus mould. The authors further observed that the two effective bacterial endophytes were found to check the

development of mycelium and germination of spores of fungus through the production of important enzymes, namely, chitinase and glucanase.

Parveen et al. (2016) in their recent review on postharvest fungal rots of rosaceous fruits gave an insight into the endophytic bacterial species and their mode of action against pathogens causing postharvest losses especially moulds and rots. Mikani et al. (2008) in their study reported the reduction in the incidence of grey mould caused by *Botrytis* sp. by the antifungal ability of *Pseudomonas fluorescens* applied on the harvested produce. Mari et al. (2014) in their study on postharvest diseases of apple and pear reported that BiosaveTM, trade name of a formulation of an endophytic bacteria *Pseudomonas syringae*, could reduce the incidence of two types of moulds, viz. grey mould and blue mould. It was reported that *Pseudomonas syringae* could take care of Mucor rot, a postharvest disease common in apple and pear.

Smilanick et al. (1993) reported from their study that brown rot in stone fruits, a postharvest disease, could be reduced by the application of *Bacillus subtilis and Pseudomonas* sp. which is effective and safe. Trias et al. (2010) and Wang et al. (2010) identified two endophytic bacterial strains, namely, *Pantoea agglomerans* and *Bacillus subtilis*, which were reported to suppress the activity of a number of fungal pathogens *Botrytis cinerea*, *Alternaria alternata*, *Penicillium expansum* and *P. malicorticis* inflicting fruit rot in damaged apples during harvesting and transit.

Twenty one strains of endophytic bacteria were identified by Pratella et al. (1993) which were drawn from a wide range of fruits, viz. tomato, brinjal, etc., to control a fungal pathogen *M. laxa* which was reported to infect the harvested plum, peach and apricot fruits. The study indicated that *M. laxa* could be controlled effectively than *R. stolonifer.* Calvo et al. (2007) in another study on apple reported that disease producing fungal species of *Penicillium expansum*, *Botrytis cinerea and Alternaria alternata* could be effectively controlled by the application of *Rahnella aquatilis* as biocontrol agent.

1.9 Methods of Application of Endophytic Bacterial Strains

Relevant and appropriate methods of application of endophytic bacteria are to be selected for increasing the efficiency of biocontrol ability. Several methods were used in different crops with different bacterial cultures. The reviews revealed that the methods which are being adopted for the application of microbial inoculants on the various parts of plants including root zone and phyllosphere hold good for endophytic bacteria also (Andrews 1992). An array of methods including seed treatment, soil trenching, stem injecting and spraying on foliage were tried by researchers and reported the differential efficiency of these methods (Fahey et al. 1991).

Musson et al. (1995) conducted experiments to evaluate the effectiveness of several application methods of 15 endophytic bacteria into the stem and root tissues of cotton. Seven application methods were experimented, *viz*. inoculation of bacteria

into stem of cotton plants, seed coating with methyl cellulose, cotton seed soaking in bacterial suspensions, application of bacterial suspension on the leaves of cotton, furrow application of granules containing bacterial consortia, vacuum infiltration and application on the pruned-root dip. Among the seven method of application, inoculation into stems or radicles was proved to be effective as ten isolates could be recovered from the cotton plants inoculated with the isolates though the method was labour intensive and the process involves wounding the plant which may reduce the growth of the plants. The pruned-root dip was the most efficient method to deliver bacterial endophytes into maize (Bresson and Borges 2004).

Efforts were made by scientists to find a seed inoculation technique to increase the shelf life of seeds and to improve the compatibility with commonly used fungicides, ensuring the survival and efficacy of bacterial inoculations. In this line, a seed inoculation technique was developed by Crop Genetics International Ltd. in which the seeds were treated with bacterial suspension and redrying of seeds through application of differentiated pressure (Turner et al. 1993). Zakaria et al. (2008) experimented two methods of application of endophytic bacterial culture, *viz.* inoculation in root zone and root tip method, in cultivated and wild rice. Among the two methods experimented, the population of bacterial species increased drastically in root dip method, and the colonisation was found to be more pronounced in cultivated rice than wild rice.

Significant increases in rice yield were achieved by seed inoculation with the endophytic bacterium *Achromobacter xylosoxidans*, which suppressed symptoms of rice blast disease by stimulating production of plant defence-related enzymes (Joe et al. 2012). Similarly, inoculation of seeds with a wide range of putative endophytic bacterial isolates improved shoot dry weights in maize seedlings (Montanez et al. 2012).

Bashan and Holguin (1997) reported that addition of certain nutrients in specific forms may improve the endurance of bacteria present in a formulation. For example, skim milk can improve the survival rate of beneficial microorganisms which can reduce disease risk in crops.

The effective plant colonisation by the endophytic bacteria through inoculation into plant cell suspension and regenerated embryo is a useful option as reported by Bashan and Holguin (1997). Knudsen and Spurr (1987) experimented with lyophilised bacteria which were sprayed in dust formulations or suspensions on fruits and flowers, and the study did not specify the technicalities involved in spraying.

Marta Cristina et al. (2011) in their study tested three methods of application of bacterial cultures to reduce leaf blight in rice. Among the methods studied, soil drenching with isolate Rizo-55, 15 days prior to application of virulent isolate of blast-producing pathogen *M. oryzae*, was found to control 90% of leaf blast, and soil trenching with isolate Rizo-46 applied 2 days before the application of virulent plant pathogen could reduce blast by 95%. The findings of the study indicated that differential method of application resulted in differential control of blast in rice irrespective of the bacterial cultures applied.

Selim et al. (2017) analysed the suppression of damping-off (*Rhizoctonia solani*) disease in cotton through the application of the bacterial strains as a soil drench or

talc-based bioformulation, and the results indicated that the soil drench treatment was more efficient than talc-based bioformulation. The foliar application of *Bacillus amyloliquefaciens* S13–3, a bacterial endophyte known for producing antibiotics, was found to suppress anthracnose (*Colletotrichum gloeosporioides)* in strawberry (Yamamoto et al. 2015).

Sundaramoorthy et al. (2012) recorded 17–30% of suppression of *Fusarium* wilt in chilli pepper when compared to control through application of talc-based bioformulation of *P. fluorescens* (Pf1) and *B. subtilis* (EPCO16 and EPC5 strains). Soil application of these bacterial combinations of *Bacillus* and *Pseudomonas* resulted in significant lowering of incidence of *Fusarium* wilt in cv. Grand Naine banana (Thangavelu and Gopi 2015).

Analysing the pros and cons of different methods, Hallman et al. (1997) reported that among different modes of application of bacterial cultures, the most economical, dependable and swift method is seed treatment which will directly let the beneficial bacterial species into the soil and subsequently into the plant system. They also argued that combining of application methods, namely, seed treatment with soil drenching and foliar application, will enhance the colonisation potential of different endophytic bacterial species and could multiply the benefits of these species inside the plant system.

1.10 Conclusion and Future Prospects

The primary objective of this chapter is to sensitise and update the researchers in the field of biological management of plant diseases about the bacterial endophytes; their diversity; mechanism of colonisation in different plant parts; ability to suppress an array of fungal, bacterial and postharvest diseases; mode of action; and method of application. Despite efforts were made to update the literature, it seems unwieldy to include all possible dimensions of plant disease management using endophytic bacteria.

The chapter ellucidated some of the important research areas which need to be explored by scientists in the future. The chapter also suggested that colonisation of endophytic bacteria is the key for the suppression of disease causing pathogen. Much of the research needs to be focussed on how individual species of endophytic bacteria colonise different parts of plants and the traits involved in colonisation. Since majority of the bacterial species are identified in the rhizosphere, enhancing the colonisation of PGPR in other parts of the plants would be a good strategy, though onerous could be a strategic option for controlling foliar diseases. Plant host-specific endophytic bacteria are to be identified; population dynamics are to be studied which should logically culminate in the production of inoculum for specific disease of a specific plant. This means the optimisation of dosage for effective control which would avoid bulk production of inoculum. One step further to this type of research is the genome sequencing and identifying genes responsible for the suppression of plant diseases. Studies suggested that these information will provide a

strong base for furthering the studies in plant-microbe interactions. The efforts have already been taken to untangle the biotechnological potential which needs to be further exploited by more meaningful research. Ultimately, more exclusive studies on the endophytic microbiome would reveal useful information on biocontrol of plant diseases.

The information presented in this chapter further informed about the current scope and importance of organic amendment of soil with substances like chitin that would improve the inducement of resistance, and further studies on this line would open up several possibilities. The studies on favourable edaphic factors to suppress plant pathogens would go a long way in identifying appropriate species for appropriate soil conditions. Though the postharvest diseases could inflict huge economic loss, the available literature on controlling them with endophytic bacteria were found to be limited, and research in this area needs to be strengthened. Finally, the application of these endophytic bacteria in appropriate formulations and identifying appropriate method of application are pivotal for effective disease management. Experimental studies on finding effective formulation and method of application of bacterial endophytes are the need of the hour.

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Chapter 2
Helpful Linkages of *Trichoderma*s in the process of Mycoremediation and Mycorestoration

Manoj Kumar Solanki, Brijendra Kumar Kashyap, Anjali Chandrol Solanki, Mukesh Kumar Malviya, and Kanakala Surapathrudu

Abstract Toxic soil and polluted water enhanced the infertility of soil and directly affected the balanced ecosystem, which causes a destructive effect on the human society. Utilization of microorganism for the agricultural and soil management is a beneficial object, and many microorganisms have shown significant impact in the laboratory, but they failed in large-scale application. However, past reports discussed that *Trichoderma* is a potential organism for the plant disease management and plant growth promotion. To extend the consequence regarding *Trichoderma*, this chapter focused on the role of *Trichoderma* in the mycoremediation and mycorestoration. In this chapter, we discussed the *Trichoderma* linkages in bioremediation of pollutants like fungicides, pesticides, and heavy metals. Moreover, utilization of *Trichoderma* for the restoration of saline, acidic and metal contaminated soil. To balance the energy resources and ecosystem, we need to look forward with a microbial substitute like *Trichoderma* at large scale.

Keywords Abiotic factors · Biotic factors · Bioremediation · *Trichoderma* · Soil management

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2.1 Introduction

Environment pollution due to toxic substance becomes a major global problem. Polluted soil and water have a direct influence on the plant and microbes as well as human growth. The major contaminants are heavy metals, fungicides, and organic solvents that are mostly released as industrial waste. Bioremediation is a microbial process to clean the environment. Human from the past has utilized these processes. Currently, worldwide researchers are paying attention to keep pollution-free ecosystem. In ancient time, farmers used the plants to recover saline soil arable again. Das and Chandran (2011) discussed the use of beneficial bacteria to clean the Exxon Valdez oil spill in Alaska. Normally researchers used the term "phytoremediation" to state that plants have a significant role in remediation of the toxic substance of soil and water, and, like that, "mycoremediation" is used to discuss the role of fungi in bioremediation. Nowadays, the term "bioremediation" discusses the use of different microbes such as bacteria, protozoans, etc. The basic purpose to use plant and microbes is to harness natural habitats to solve the all ecological problems or to identify an organism or to combine a genetic material to get potential microbes that can cope with all environmental situations.

Mycoremediation is a complex process of bioremediation that is driven by living fungi. Fungal biomass has been used in situ and ex situ cleanup process to destabilize the contaminated sites (Thakur 2014; Ali et al. 2018). Mycoremediation has been applied to contaminated and polluted soil by chemical, heavy metal, or industrial waste and contaminated water by the toxic substance and is used to manage the industrial and other forms of waste to protect the environment (Bennett et al. 2001). The process includes the application of fungal biomass to the contaminated soil surface that biologically degrades the organic contaminants. First, we need to collect the potential fungal culture then screen against the contaminants like heavy metals, petroleum hydrocarbons, organophosphates, abiotic factors, and biotic pathogens (Thomas et al. 1999). After screening, potential fungi have been grown in large quantity by suitable growth media such as wood chips, and biomass has been obtained. Afterward, fungal biomass is mixed or applied to the contaminated lands directly or incorporated with the plants and applied in biofiltration and bioretention chambers, riparian buffer or stream buffer zones, and bank's soils. Mycoremediation has been used in a bioretention cell (e.g., rain garden), mixed with local flora, mixed with soil, and with the native microbial community to eliminate contaminants or kill the other pathogens. Chishimba (2013) reported the use of wood chips with mycorrhizal fungi enhanced the bioremediation process of plants.

Trichoderma, an anamorphic *Hypocreaceae* (*Ascomycota*), is a cosmopolitan group of fungi, universally present in the diverse types of environment, and has a number of agricultural and industrial importance (Harman et al. 2004; Tripathi et al. 2013). It has achieved an exceptional place in agronomy as a strong disease protector and soil health stabilizer (Lorito et al. 2010; Solanki et al. 2011). Several previous reports have discussed the bioremediation abilities of *Trichoderma* in soil and water (Kredics et al. 2001; Harman et al. 2004; Ezzi and Lynch 2005). To kill the

pathogen, parasitism and antibiosis are the major mechanisms of *Trichoderma* (Harman 2006), and hydrolytic enzymes such as amylases, cellulases, 1, 3 β-glucanases, and chitinases helped in antagonism (Howell 2006; Tripathi et al. 2013). Moreover, *Trichoderma* has remarkable industrial applications such as hydrolytic enzymes used in paper and pulp industries and food industry (Tripathi et al. 2013).

2.2 Advantageous Role in Mycoremediation

Environmental pollution has a direct impact on human health. Chemically polluted soil and water gained a global attention, and cleaning contaminated sites has now become a universal objective. Fungal candidates have the ability to degrade toxic and xenobiotic substance (Thakur 2014). Mycoremediation process is comprehensively associated with phytoremediation and rhizoremediation, and these cleaning processes played a significant role in the organic contaminant remediation from soil and water (Tripathi et al. 2013; Ali et al. 2018). Therefore, there are so many advantages of using *Trichoderma* for mycoremediation. The obvious advantages lie with the minimal cost and effort of setting up a treatment. Cost of inoculation of a fungi spore to a polluted site is less than other treatments (López Errasquín and Vázquez 2003; Argumedo-Delira et al. 2012). Another advantage of *Trichoderma* for mycoremediation is that it often works where other bioremediation techniques do not, because it has a diverse substrate range and also sustains in extreme conditions, for example, in situations where recalcitrant compounds exist or when bioavailability is a problem. Other bioremediation techniques such as microbial remediation require specific environmental conditions for them to work. But *Trichoderma*-based mycoremediation techniques offer a much more flexible approach as fungi have the ability to survive in extreme situations (Tripathi et al. 2013).

Nevertheless, soil microbes followed a number of methods for metal resistance like metal efflux by cell membrane (Kamizono et al. 1989), glutathione-derived peptides named phytochelatins (Tripathi et al. 2007), metal-binding protein metallothionein (Presta and Stillman 1997), and metal compartmentalization in vacuoles (Volesky et al. 1993). In the literature, it is already proven that *Trichoderma* spp. efficiently colonized in soil and was able to degrade organic substance (Harman et al. 2004; Lorito et al. 2010). Fungi have metabolized the complex organic compounds through the hydrolytic enzymes like amylases, cellulases, 1,3 β-glucanases, and chitinases (Solanki et al. 2011; Kumar et al. 2012). Tengerdy and Szakacs (2003) reported that *Trichoderma* spp. have the ability to decompose lignocellulose and pre-inoculation of *Trichoderma* spores enhanced the composting process (Mohammad et al. 2012; Mukhlish et al. 2013). There are numerous characteristic variations between *Trichoderma* strains, with different strains that have the capability to break down and metabolize the different contaminants and toxic compounds (Table 2.1), and combined application of different *Trichoderma* species allows us to develop new technologies to clean the toxic substance.

Table 2.1 List of *Trichoderma* species that are mostly utilized for bioremediation

Strains	Country	Pollutants	References
Trichoderma spp.	Hungary	Pesticide and heavy metal remediation from polluted soil	Kredics et al. (2001)
T. atroviride	Spain	Removal of heavy metals (Copper-Cu, Zink-Zn and Cadmium-Cd) from contaminated sludge	López Errasquín and Vázquez (2003)
Trichoderma spp.	Belgium	Restoration of diesel-contaminated soil	Van Gestel et al. (2003)
Trichoderma spp.	United Kingdom	Degradation of cyanide	Ezzi and Lynch (2005)
T. harzianum and *T. atroviride*	Hungary	Degradation of pesticide polyresistance	Hatvani et al. (2006)
Trichoderma spp.	Japan	Degradation of phenanthrene and pyrene	Matsubara et al. (2006)
T. harzianum	United Kingdom	Heavy metal remediation from polluted soil	Adams et al. (2007)
T. koningii	China	Cyanide and ferrocyanide degradation	Zhou et al. (2007)
T. atroviride	China	Augmentation of phytoextraction of cd and nickel (Ni) from contaminated soils	Cao et al. (2008)
T. harzianum	Chile	Increase the arsenic (Ar) tolerance in plants	Arriagada et al. (2009)
T. viride	Egypt	Removal of metal (chromium, Cr)	El-Kassas and El-Taher (2009)
Trichoderma isolate SP2F1	Malaysia	Removal of cu II from Penchala River, heavily contaminated with effluents from nearby industrial areas	Ting and Choong (2009)
T. harzianum, *T. hamatum* and *T. virens*	Iran	Removal of heavy metal-containing compounds and fertilizers from soil	Hajieghrari (2010)
T. atroviride	China	Degradation of organophosphorus pesticides	Tang et al. (2010)
T. asperelloides and *T. harzianum*	United States of America	Degradation of fungicides (captan-thiabendazol and the mixture captan-carboxin)	Chaparro et al. (2011)
Trichoderma spp.	Nigeria	Elimination of lead (Pb), Ni, and Cd from contaminated refinery effluent	Machido et al. (2011)
Trichoderma spp.	Malaysia	Degradation of phenanthrene	Safiya et al. (2012)
T. viride	India	Elimination of cd and Pb contamination	Sahu et al. (2012)
T. pseudokoningii	Pakistan	Extraction of Cd, Cr, Cu, Fe, Na, and Zn	Firdaus-e-Bareen et al. (2012)
T. harzianum and *T. viride*	Egypt	Degradation of pesticide (oxamyl)	Afify et al. (2013)

(continued)

Table 2.1 (continued)

Strains	Country	Pollutants	References
T. harzianum, *T. aureoviride*, and *T. virens*	Malaysia	Removal of heavy metal Zn, Pb, Ni and Cu	Siddiquee et al. (2013)
Trichoderma spp.	Mexico	Degradation of pesticide (atrazine)	Pelcastre et al. (2013)
T. asperellum	Iran	Removal of Cd-polluted media	Mohsenzadeh and Shahrokhi (2014)
Trichoderma sp.	Malaysia	Degradation of pentachlorophenol	Sing et al. (2014)
T. reesei	China	Removal of Cd from contaminated soil	Teng et al. (2015)
T. asperellum	Mexico	Degradation of phenylalanine hydroxylase (PAH) from heavy crude oil-contaminated soils	Zafra et al. (2015)
Trichoderma sp.	India	Removal of Ni and Cd	Nongmaithem et al. (2016)
T. logibrachiatum WT2	India	Removal of Pb	Devi et al. (2017)

Some content of this table adopted by Tripathi et al. (2013) and Patil and Solanki (2016)

2.3 Advantageous Role in Mycorestoration

The term "Mycorestoration" defines the utilization of fungal mycelium, spore or fungi based byproducts to restore the polluted habitats and multiple steps needed for restoration. Mycorestoration is applied to prevent oil spill sites, soil banks and saline/chemical/metal affected lands. Microbes played important role in complex soil processes such as soil texture formation, organic matter decomposition, and mineral cycles like carbon, nitrogen, phosphorus, and sulfur (Okoth et al. 2007).

Saprophytic soil microbes used plant exudates or plant residues as food and symbiotically recycled the soil nutrients for plant and other microbes. Soil nutrient availability and plant diversity influenced the microbial abundance, activity, and diversity that rotate the mineral cycle. Moreover, agricultural practices like organic and inorganic fertilizer application, pesticides, insecticides, and microbial inoculates have been shown to have an impact on microbial structures of soil (Okoth et al. 2007; Hur et al. 2011; Trabelsi and Mhamdi 2013; Guan et al. 2016). Mycoparasitic *Trichoderma* abundant in soil fungi played important role in organic matter degradation with multiple activities (Elad and Kapat 1999; Harman et al. 2004; Harman 2006; Mastouri and Harman 2009; Mastouri et al. 2010) able to tolerate extreme conditions (Howell 2003; Liu et al. 2008; Korolev et al. 2008; Rawat and Tewari 2010). *Trichoderma* species have several important features such as higher reproduction rate, growth stability in extrema environment, broad range of substrate utilization efficiency, and capability to transform the rhizosphere and other existing banks (Woo et al. 2006) and control the pathogens by antagonism and work like a

plant growth promoter (Ousley et al. 1993; Inbar et al. 1994; Shanmugam and Kanoujia 2011).

2.3.1 Soil Conservation and Plant Growth Promotion

To prevent the soil from erosion, chemical alteration, acidification, salinization, or other several pollutants, we used several management strategies; all these strategies come under the soil conservation. It is a component of environmental soil science and nutrient management. To retain the soil selection of agriculture practices is very important. Common practices are the rotation of crops, protection by cover crops, and windbreak planting. The major effects of soil erosion are soil nutrient depletion and soil structure deformation. Crop rotation inhibits nutrient depletion by repetitive chemical uptake/deposition through monotonous cropping system. In the modern agriculture practices, utilization of microbes becomes a valuable substitute. Chishimba (2013) reported that *Trichoderma*-treated wheat plant showed tolerance of acidic soils and improved P uptake and grain yield. The acidic condition may increase mycelial growth, spore production, and secretion of antimicrobial compounds such as antibiotics and lytic enzymes (Chang et al. 1986; Singh and Nautiyal 2012). Moreover, alkaline soil decreases conidial germination of *Trichoderma* spp. and lead to a decreased biocontrol activity of *T. harzianum* (Sabaratnam and Traquair 2002; Alfano et al. 2007; Saud et al. 2013). Plant growth promotion and antimicrobial activity of *Trichoderma* are well accepted, and *Trichoderma* condition activates the unique antimicrobial compounds under stressed conditions that help plants to survive stress and protect the plants from pathogens (Chang et al. 1986; Baker 1988; Ousley et al. 1993, 1994; Inbar et al. 1994). Universally, *Trichoderma* inhibited the root-associated pathogens that enhance the nutrient uptake and plant growth by phytohormones (Ousley et al. 1993; Wilberforce et al. 2003), and immobilization and solubilization of soil nutrients and minerals by *Trichoderma* improved the plant nutrient uptake (Liu et al. 2008). Furthermore, *Trichoderma* induced the seed growth and germination (Celar and Valic 2005; Harman 2006). Recently, it is predicted that *Trichoderma* spp. have the autonomous skills to promote the plant growth in the absence of pathogen (Celar and Valic 2005). Therefore, modern agriculture practices considered that these fungi have the direct effects on the plant growth and development.

2.3.2 Role in Abiotic Stresses

Endophytic *Trichoderma* is a well-known plant growth promoter hence applied extensively as a bioinoculant (Harman 2006), and direct antagonism to pathogen helps *Trichoderma* to proliferate in the soil and root zone of the plant as well as inside the root/plant (Wilberforce et al. 2003). Symbiotic association of *Trichoderma*

with plant improved the plant growth, nutrient uptake, and tolerance of biotic and abiotic pathogens. Abiotic factors influenced the plant growth and yield that cause a significant economic loss. The use of microbial candidates against abiotic stresses is a major area of research, and several fungi are documented for their stress tolerance activities (Singh et al. 2011; Li et al. 2017). Several reports discussed the abiotic stress tolerance activity of *Trichoderma* (Rawat and Tewari 2010; Rawat et al. 2011; Hashem et al. 2014; Ahmad et al. 2015). *Trichoderma* treatments help the seed to germinate uniformly and faster under drought stress (Mastouri and Harman 2009; Mastouri et al. 2010). Several reports discussed that *Trichoderma* have plant growth promotion ability and survival ability in extreme condition that help the associated plants to fight against the abiotic factors (Bae et al. 2009; Gamalero et al. 2009; Mastouri and Harman 2009). *Trichoderma* treated plant regulates the growth under abiotic stresses (Yildirim et al. 2006), through root growth and surface enrichment, reduced water evaporation rate and improved water holding capacity, and nutrient uptake enrichment (i.e., potassium). Recently, Mastouri et al. (2010) also reported that *T. harzianum* induced the tomato seed germination under various stresses, such as water, osmotic, salinity, chilling, and heat, and *Trichoderma*-treated plants showed the higher amount of antioxidants; it provides the linkage of plant protection by inducing systemic resistance. Likewise, *Trichoderma* showed systemic resistance against *Paravalsa indica* under different abiotic factors (Vadassery et al. 2009). Bailey et al. (2006) and Alfano et al. (2007) reported that *Trichoderma*-treated plants showed expression of biocontrol-related genes under the abiotic stresses or oxidative damage, and more study is needed to prove the biotic stress tolerance mechanism of *Trichoderma*.

2.3.3 Role in Biotic Stress

Biotic stress is the damage caused by biotic pathogens like bacteria, fungi, virus, insect, pest, unwanted weeds, etc. to the plant by destructing the plant's metabolic process. In the present time, the impact of biotic pathogen is raised due to resistance development against the fungicide and pesticides that affected the production and quality of cash crops. Biotic pathogens have a direct effect on the economy of the country by enhancing the production losses. Biotic pathogen management through eco-friendly tools and technology is a global need to optimize for sustainable agriculture. In the narrowest sense, potential plant health protectors are needed, which regulate the plant growth against the biotic pathogens. *Trichoderma*-based biocontrol agents established most prominent against fungal and bacterial diseases of the plant. *Trichoderma* certified for the multiple mechanisms like nutrient and space competition, antimicrobial compounds production, hydrolytic enzyme secretion, direct or indirect parasitism, and most importantly plant defense induction. Some *Trichoderma* spp. known as biocontrol agents are *T. harzianum*, *T. viride*, and *T. hamatum*. Most of the biocontrol agents applied in soil proliferate extensively in the root zone/surface; this way they can protect the plant roots and enhance the plant

growth. Moreover, the foliar application also helps to protect foliar pathogens. The commercial formulation of *Trichoderma* species comprehensively utilized against the numerous plant disease causing fungi such as *Botrytis cinerea*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, *Pythium* spp., and *Fusarium* spp. (Howell 2003; Almeida et al. 2007; Shoresh et al. 2010; Shanmugam and Kanoujia 2011; Boukaew et al. 2011; Solanki et al. 2011).

Mayo et al. (2015) reported that *Trichoderma harzianum*-treated bean seedlings revealed the significant reduction of preemergence damping off disease. *Trichoderma*-treated seedlings showed higher plant growth in *R. solani*-infested soil. Contreras-Cornejo and Macías-Rodríguez (2009) documented the advantages of *Trichoderma* fungi in agriculture: (1) extensive colonization in rhizosphere and root of the plants to help the plant in nutrient cycling, (2) multifarious mechanisms of antagonism and plant defense activation, and (3) plant growth stimulator. Therefore, several *Trichoderma* strains are commercially available in the market as best bioformulation to protect the different economic crops. Among all mechanism, mycoparasitic activity of *Trichoderma* is main. *Trichoderma* is grown toward the host and usually forms the coil around the host then penetrate in the host by hook-like structures and appressoria and cell wall-degrading enzymes that help to degrade the cell wall of the host (Elad and Kapat 1999). Finally, *Trichoderma* utilized the host intracellular contents (Gajera and Vakharia 2012). Moreover, the spectrum of hydrolytic enzyme secretion ability and antimicrobial compound production ability helps the *Trichoderma* to degrade the cell wall of the host. Application of *Trichoderma* is also effective against the soil-borne as well as foliar pathogens like *Botrytis cinerea*, *Fusarium*, *Rhizoctonia*, and *Pythium* that causes diseases in soil or foliar plant parts. Currently, researchers accepted two main mechanisms of *Trichoderma* in biocontrol; first is mycoparasitism and second is induced systemic resistance (ISR). *Trichoderma* strains are able to protect the plant from nematodes and insects also. Golzary et al. (2011) reported the significant reduction of *Meloidogyne* spp. population through the *T. harzianum* Rifai. Rodríguez-González et al. (2017) stated that *T. harzianum* significantly inhibited the *Xylotrechus arvicola* and *Acanthoscelides obtectus* pests of common bean (*Phaseolus vulgaris*). Recently, Zahran et al. (2017) reported that *T. harzianum* inhibited the 90% growth of tropical bed bugs *Cimex hemipterus*. Therefore, *Trichoderma* was also known as plant guard fungi in the commercial market. Moreover, extensive study is needed in regard to genetic manipulation and application to explore all the mechanisms and construct a strong technology that can work as a guard to the multiple stresses.

2.4 Conclusion and Future Prospects

Global climate change and population pressure are the major threats for the sustainable agriculture. Remediation and restoration methods by using living organism single or in combination with plant might help to produce enough quality food for the society. Increased percentage of contaminated land due to overindustrialization

in the Asian territory raises several environmental problems that cause the significant impact on the human health. Soil microbes have fast-growing cycle, and they are prominent players of biodegradation. Modern molecular tools and genetic manipulation might lead to the environment cleaning activities. Application of *Trichoderma* species as bioremediator against pollutants like xenobiotic organic compounds such as pesticides and polyaromatic hydrocarbons has been noticed and applied as formulation since 1985, and over time *Trichoderma* accepted suitable candidates for bioremediation due to its several mechanisms like salinity, drought tolerance, and able to survive and proliferate at different pH, temperature, nutritional stress, and antimicrobial compound production. However, *Trichoderma* linkages with plant and soil are needed to be explored more to develop eco-friendly *Trichoderma*-based approach to recover damaged ecosystems. In the future, some more research work is needed on the multiple organisms and how to utilize bioremediation, mycoremediation, and phytoremediation together for the cleanup of contaminants and pollutants from the soil and water, myco-consortia-based approach and how to utilize the microbial enzymes for degradation of the complex xenobiotic compound in short time, how to leach out the metal and oil contaminants in marine and submarine systems, and, moreover, how to utilize the microbial gene pools for the biodegradation. Modern tools and techniques might help to optimize the eco-friendly techniques by using the *Trichoderma* strains.

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Chapter 3
Biofilmed Biofertilizer for Sustainable Agriculture

M. C. M. Zakeel and M. I. S. Safeena

Abstract The pressure due to global population increase and rising environmental damage has the unfortunate consequence that world food production may shortly become inadequate to feed all the mouths of the world. It is therefore indispensable that agricultural productivity be significantly improved within next couple of decades. To achieve this, agricultural practices are approached in a more sustainable and eco-friendly manner. Further, the substantial use of chemical fertilizers and pesticides in conventional farming has led to the accumulation of harmful chemical remnants and heavy metals in the environment leading to degradation of agroecosystem and incidence of unpredictable chronic diseases in human. Therefore, biofilmed biofertilizers (BFBFs) have become a viable alternative for chemical fertilizers in agriculture. BFBFs, in addition to their fertilizing task, accomplish a variety of processes such as reinstating agroecosystem, maintaining regulated metabolic and biochemical processes, improving soil quality, suppression of pests and diseases, amelioration of plants from stress and synthesis of plant hormones. The consortia of microbes in BFBFs add an array of benefits together for the soil-plant system to support plant growth and development thereby to enhance the yield. Moreover, BFBF itself is a sustainable system which can ensure the sustainability of agroecosystem. Therefore, the use of BFBFs in agriculture would lead to a more eco-friendly approach in crop production with many health, environmental and economic benefits.

Keywords Biofilmed biofertilizer · Plant-microbe interaction · Sustainable agriculture · Biological nitrogen fixation · Agroecosystem

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3.1 Introduction

The current world population of 7.6 billion is projected to reach 8.6 billion in 2030 (Anonymous 2017). It is clear that, due to the expected worldwide population growth and increased environmental damage as a consequence of higher levels of industrialization, there will be a huge challenge to feed all the people in the world (Glick 2012). To feed this significantly increasing population, the world needs to upsurge the agricultural productivity. However, the present conventional agricultural practices that include the indiscriminate use of chemical fertilizers, pesticides and various other agrochemicals significantly contribute tremendously to the pollution of soil, air and water environments and also threaten the lives in these habitats. Sustainability of agriculture can be ensured by augmenting the soil health for enhanced productive capacity and also by increasing the soil quality. Increased microbial diversity of soil plays a pivotal role in boosting the health and the quality of soil (Sharma et al. 2011; Bastidia et al. 2008; Fernandes et al. 1997). Therefore, enhancing the agricultural productivity should be approached in a sustainable and environmentally friendly manner (Glick 2012). Sustainable agriculture will likely depend on the use of transgenic plants (Anonymous 2016) and plant growth-promoting bacteria in the form of biofertilizers (Buddhika et al. 2016; Lucy et al. 2004).

Application of chemical fertilizers, particularly phosphate fertilizers, and other agrochemicals including herbicides and insecticides, which contain large amounts of heavy metals such as cadmium (Cd), arsenic (As) and uranium (U) (Chandrajith et al. 2010; McLaughlin et al. 2000; Jones and Jarvis 1981), has resulted in a big havoc in human health causing chronic kidney diseases in addition to the increased incidences of toxicity in many countries including Sri Lanka (Jayasumana et al. 2013; Kim et al. 2015; Chandrajith et al. 2010; Johri et al. 2010; Bandara et al. 2008; Gillera et al. 1998). As a result of both growing population and industrialization, edaphic, atmospheric and aquatic environments of the earth are increasingly contaminated with a wide range of toxic metals and organic pollutants (Glick 2010; de Rosa et al. 1996; Ziegler 1993) as these environments are insufficient to absorb or breakdown the increasing amount of waste that is accumulated (Glick 2012). Identifying the nature and level of the problem is an imperative step to set the way forward to mitigate the problem, mainly by remediating the contaminated environments either by phytoremediation, the use of plants that can absorb and bioaccumulate or degrade a variety of environmental pollutants (Pilon-Smits and Freeman 2006; Pilon-Smits 2005; Alkorta and Garbisu 2001; Salt et al. 1995) or through the use of certain bacteria in the form of biofertilizers (Gamalero and Glick 2012; Glick and Stearns 2011; Glick 2010), particularly in biofilmed mode (Zakeel 2015).

Conventionally, biofertilizers have been produced as monocultures or mixed cultures of beneficial microbes (Saharan and Nehra 2011; Mahdi et al. 2010). However, the significance of using these biofertilizer microbes as surface-attached biofilms has been recently emphasized, and many researches into the importance of biofilmed

mode of biofertilizers over the biofertilizers alone have been undertaken (Manawasinghe et al. 2014; Seneviratne and Jayasinghearachchi 2003). Fungal-bacterial biofilms (FBBs) developed with mycelia of *Penicillium* colonized by *Bradyrhizobium elkanii* under in vitro condition have shown a significant increase in biological nitrogen fixation (BNF) over *B. elkanii* alone in a study conducted in Sri Lanka (Jayasinghearachchi and Seneviratne 2004). Biofertilizers in biofilmed mode have also shown augmented P-solubilization through increased amounts of organic acid production (Jayasinghearachchi and Seneviratne 2006; Seneviratne and Indrasena 2006) and plant growth benefits via increase in growth hormone production (Bandara et al. 2006). Since then, the concept of FBBs has become an important area in biofertilizer research. Nadell et al. (2009) reported that this capability of biofertilizers in biofilmed mode was attributed to the ability of biofilms to secrete extracellular substances compared to mixed cultures. These biofertilizers were then named as biofilmed biofertilizers (BFBFs) (Seneviratne et al. 2008).

The heterogeneity of biotic and abiotic factors of conventional biofertilizers and the competition with indigenous organisms make the survival and function of the biofertilizer microbes unpredictable, thus necessitating new inoculants for commercialization (Nelson 2004). However, BFBFs have demonstrated successful fertilizing capacity in many crop species such as rice, maize, tea, rubber and a variety of vegetables under both field and greenhouse conditions. In addition to offering various benefits for the sustainability of agroecosystem, BFBFs have been able to reduce the chemical fertilizer use by 50% in many crops under field condition, something conventional biofertilizers have not achieved (Seneviratne and Kulasooriya 2013; Hettiarachchi et al. 2012; Seneviratne et al. 2011). Though the role of naturally available biofilms in plant growth enhancement (Rudrappa et al. 2008) and their ecological importance (Rudrappa et al. 2008; Ramey et al. 2004; Davey and O'Toole 2000) have been widely reviewed, their use as biofertilizers has not been adequately evaluated. Therefore, in this chapter, we discuss the importance of BFBFs for sustainable agriculture.

3.2 Plant-Microbe Interaction

Soil is a rich source of microflora, the biologically active powerhouse of soil, which include an incredible diversity of microorganisms such as bacteria, cyanobacteria, fungi, actinomycetes and algae. Plant-microbe interaction can be either beneficial (BNF, P-solubilization, etc.) or detrimental (plant pathogens). Under natural environment, all plants are supported by a massive invisible world of microorganisms that live in and around roots, leaves and stem of plants (Zakeel 2015). Soil and rhizosphere microbes carry out many beneficial activities for plants, i.e. (1) decomposition of plant residues, manures and organic waste, humus synthesis, mineralization of organic nitrogen (N), phosphorus (P) and sulphur (S) and improving the soil aggregation; (2) biological nitrogen fixation; (3) production of plant growth hormones; (4) increasing the availability of plant nutrients such as P, manganese (Mn),

iron (Fe), zinc (Zn) and copper (Cu), symbiotic mycorrhizal association, production of organic chelating agents and oxidation-reduction reactions; (5) protection against pests and pathogens; and (6) degradation of synthetic pesticides and industrial wastes (Zakeel 2015).

A large number of bacterial and fungal species among these soil microflora are well known to be responsible to promote plant growth and thus referred to as plant growth-promoting microorganisms (PGPM). These species do not generally exhibit even distribution in soil. The concentration of PGPM distribution around the root zone, generally known as rhizosphere, is much greater than the rest of the soil (Glick 2012). PGPM, such as *Azotobacter* spp., *Rhizobium* spp., *Pantoea agglomerans*, *Rhodospirillum rubrum*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Bacillus subtilis*, *Lactobacillus*, *Trichoderma*, *Acinetobacter calcoaceticus* and *Aspergillus*, are likely to contribute to the plant growth and development through the production of various phytohormones, such as cytokinins, gibberellins, indoleacetic acid and ethylene (Glick 2012; Spaepen and Vanderleyden 2011; Kang et al. 2009; Tsavkelova et al. 2006; Joo et al. 2005; Lorteau et al. 2001; Patten and Glick 1996; Atzorn et al. 1988). In addition, these hormones play a vital role in both biotic and abiotic stress tolerance in plants (Kaur et al. 2016; Glick 2012; Cheng et al. 2009; Zahir et al. 2008; Reed and Glick 2005; Glick 2004; Mayak et al. 2004; Grichko and Glick 2001; Glick et al. 1997).

Another promising way of soil microbial contribution for plant growth is by making nutrients such as N, Fe and P available for plants to absorb. Besides free-living nitrogen-fixing bacteria, symbiotic nitrogen fixers (N-fixers) also play a major role in plant growth. They convert unavailable form of atmospheric dinitrogen (N_2) into available forms in large quantities compared to the amount fixed by free-living N-fixers (James and Olivares 1997). N-fixing (diazotrophic) bacteria contain nitrogenase (*nif*) genes in a cluster of about 20–24 kb with 7 operons encoding 20 different proteins, including structural genes required for the activation of Fe proteins, iron molybdenum cofactor biosynthesis, electron donation and regulatory genes required for the synthesis and function of the enzyme (Glick 2012).

Different *Rhizobium* species show varying level of nodulation in host legume. Infection of legume by *Rhizobium* spp. causes the plant to slightly increase the localized production of ethylene which in return inhibits rhizobial infection and subsequent nodulation (Ma et al. 2002). However, certain rhizobial strains reduce the ethylene production in their hosts by synthesizing rhizobitoxine which inhibits ethylene biosynthetic enzyme called ACC (1-aminocyclopropane-1-carboxylate) synthase (Yuhashi et al. 2000), thereby boosting root nodulation. On the other hand, some rhizobial strains confiscate some of the ethylene precursor called ACC by an enzyme ACC deaminase and thus lessen the ethylene biosynthesis (Ma et al. 2002). Some research has proven that reducing the ethylene production in legumes has enhanced nodulation and plant biomass production by 25–40% (Ma et al. 2004; Ma et al. 2003). As certain rhizobial strains naturally contain ACC deaminase, it is possible to engineer ACC deaminase gene into *Rhizobia* strains that lack ACC deaminase. In an attempt, a strain of *Sinorhizobium meliloti*, which lacked ACC deaminase, engineered with ACC deaminase gene from *R. leguminosarum* bv. *viciae* showed

augmented nodulation and biomass production in the host plant alfalfa (Ma et al. 2003). However, due to regulatory concerns, these engineered strains are no longer in field application.

Phosphate solubilization is another key role played by microbes for the promotion of plant growth. Though large amount of phosphorus (400–1200 mg kg^{-1}) is available in soil, a significant proportion is in unavailable forms (either in organic form or as inorganic mineral form) for plants to absorb. Moreover, soluble inorganic phosphorus applied as chemical fertilizers is also immobilized soon after its application. However, some soil bacteria and fungi solubilize and mineralize these unavailable forms of phosphorus and thus contribute for plant growth. Certain organic acids (gluconic acid and citric acid) produced by soil bacteria such as *Azospirillum* spp. help solubilize inorganic phosphorus (Rodriguez et al. 2004; Rodríguez and Fraga 1999; Bnayahu 1991). Organic phosphorus mineralization happens via the hydrolysis of phosphoric esters by a variety of phosphatase enzymes synthesized by soil microbes (Rodríguez and Fraga 1999). Some bacteria can perform both mineralization and solubilization of phosphate (Tao et al. 2008).

Though iron is abundantly available in soil, it is not readily assimilated by living organisms in soil as iron in aerobic soil is available in ferric iron (Fe^{3+}) form which is rarely soluble in water. Therefore, iron available in soil in useable form by plants and soil microbes is extremely limited (Ma 2005). As microbes and plants require large amount of iron for their growth and development, we can see a competition among plants, bacteria and fungi for iron particularly in the rhizosphere (Guerinot and Ying 1994; Loper and Buyer 1991). To survive in iron-limited environment, bacteria produce a molecule called siderophore which has high affinity to Fe^{3+} and membrane receptors which can bind to Fe-siderophore complex to facilitate iron absorption by microbes (Hider and Kong 2010; Neilands 1981).

Some bacteria become highly useful during biotic and abiotic stress in plants. Indoleacetic acid (IAA) produced in plants makes them resistant to stressful conditions (Spaepen and Vanderleyden 2011; Tsavkelova et al. 2006). Plants generally produce stress ethylene in response to the presence of phytopathogens, and these stress hormones aggravate the stress effects on plants (Abeles et al. 1992). Lowering the plant's ethylene response can reduce the damage to plants (Glick and Bashan 1997). Treating plants with microbes having ACC deaminase activity can mitigate the damage to plants (Glick et al. 1998). This approach has produced successful results in greenhouse and growth chamber experiments with potato, tomato, cucumber, carrot, soybean and castor bean against *Pythium ultimum*, *Fusarium oxysporum*, *Erwinia carotovora*, *Agrobacterium tumefaciens*, *Agrobacterium vitis*, *Sclerotium rolfsii* and *Rhizoctonia solani* (Hao et al. 2011; Husen et al. 2011; Toklikishvili et al. 2010; Hao et al. 2007; Wang et al. 2000).

3.2.1 Biofertilizer

Biofertilizers are formulations of living beneficial microorganisms which may include one or more combinations of nitrogen-fixing bacteria, P-solubilizers, iron-siderophore synthesizers, cyanobacteria and mycorrhizal fungi (Khan et al. 2007; Wu et al. 2005; Richardson 2001; Ansari and Mahmood 2017; Loper and Buyer 1991). They can be classified based on the organisms, biological activity linked to biofertilization and the symbiotic nature (Fig. 3.1). They can be found in rhizosphere or interior of the plant and contribute to its growth in many ways, either directly or indirectly, such as fixation of atmospheric nitrogen and making nitrogen available for plants (Jayasinghearachchi and Seneviratne 2006; Marroquí et al. 2001; James and Olivares 1997; Bashan and Levanony 1990), modulating the effects of environmental stresses (both biotic and abiotic), and regulation of plant growth and development through the production of phytohormones (Biofertilizer manual 2006; Tien et al. 1979). Biofertilizers are well known to be a viable alternative for chemical fertilizers to enhance soil fertility and crop yield in sustainable agriculture.

The heavy use of chemical fertilizers and pesticides has led to the accumulation of detrimental chemical remnants and heavy metals (available in those agrochemicals) in the environment, which has as a result been destroyed significantly, and also caused unpredictable human health issues. Therefore, biofertilizers at present have become much popular among farmers. Biofertilizers can be a complete or partial substitute for chemical fertilizers and other agrochemicals for eco-friendly practices to increase crop yield in a sustainable way. Biofertilizers bring about many advantages over the use of conventional fertilizers in agriculture.

Biofertilizers are generally incorporated with seeds, soil or both to enhance microbial activities such as decomposition, nitrogen fixation, mobilizing nutrients, hormone synthesis and stress alleviation. Research on biofertilizers and their use

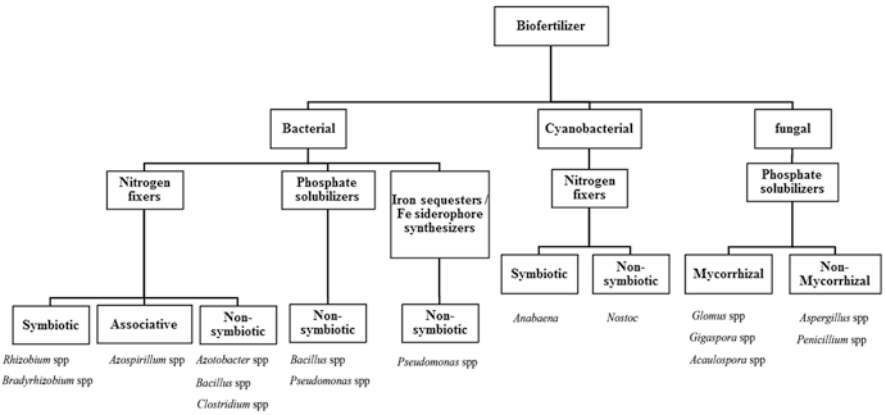

Fig. 3.1 Classification of biofertilizers

has been extensively carried out, for instance, Varma (1993) reported that inoculation of *Azospirillum* to paddy, sorghum and sunflower fields reduced nitrogen requirement by 25%, and Bashan et al. (2004) observed high N level in plants treated with biofertilizers. Biofertilizers having P-solubilizing and P-mineralizing microbes help convert unavailable forms of phosphate to available form, thereby reducing the requirements for phosphate fertilizers (Mikanová and Nováková 2002). Microbes of different genera in biofertilizer formulations are capable of producing plant growth regulators such as IAA, gibberellin and cytokinins (Glick 2012). These hormones support plant growth and development in a variety of ways (Cassan et al. 2009; Barea et al. 1976). Moreover, some biofertilizers, having antagonistic microbes, are popular among farmers to control plant pathogens as they show antibiosis and mycoparasitism against a large array of phytopathogens (Yu et al. 2009; Badri et al. 2008; Bailey et al. 2008). In addition, some microbes in biofertilizers help improve soil properties, such as organic matter content (Wu et al. 2005), soil porosity (Czarnes et al. 2000) and soil aggregation (Six et al. 2004).

Biofertilizers as mixed cultures of different microbes are much beneficial for plants over monocultures as the mixed cultures provide balanced nutrients, particularly for crops such as rice (Tiwary et al. 1998), maize (Pal 1998) and other cereals (Afzal et al. 2005). Mixed cultures of microbes have been found to have enhanced levels of different processes which are important for crop growth and higher production. For instance, *Azospirillum brasilense* in a mixed culture with *Staphylococcus* sp. was found to fix more N than its monoculture (Holguin and Bashan 1996). Some soil microbes which are capable of detoxifying heavy metals (He et al. 2010) and defensive against plant pathogens (Mazzola 2007) are also incorporated with biofertilizer formulations having other N-fixers and P-solubilizers as mixed cultures. In a metagenomic analysis of antagonistic soil bacteria, it was revealed that the disease resistance was exhibited by a variety of microbes (Mendes et al. 2011). However, this study did not attempt to evaluate interaction between these microbes nor the community arrangements among them.

3.2.2 Biofilm

Biofilm is microbial communities in which microbial cells irreversibly attach to each other and often to a surface by a matrix of extracellular polymeric substance (EPS) (Donlan 2002). This matrix is produced by the cells themselves, and it provides the structure and protection to the microbial community (Kokare et al. 2008). Biofilms can be found in a wide variety of surfaces including living tissues, medical instruments and industrial or natural water systems, and sometimes highly complex biofilms can be found in water systems (Donlan 2002). Microbial communities which produce biofilm consist of alga, fungi, bacteria and/or other microbes. When these microbes get transformed from free-living stage to biofilm mode, they undergo profound changes, and these changes can be related to biotechnological applications to obtain favourable effects (Seneviratne et al. 2008).

3.2.3 Soil Microbial Biofilms

Microbial communication through quorum sensing (exchange of signaling molecules) and metabolic trading makes soil microbes to be glued to soil particles and surfaces of plant roots (Nadell et al. 2009; Danhorn and Fuqua 2007; West et al. 2007). Further, plant polysaccharides serve as substrates for biofilm exopolysaccharide formation and also induce matrix gene expression to enhance biofilm formation (Beauregard et al. 2013). It delineates that plants can select biofilm-forming microbes to colonize on their surfaces. However, the naturally formed beneficial biofilms are found to be at very low levels to have a significant effect on plant growth compared to the application of developed biofilms (Seneviratne et al. 2013; Seneviratne et al. 2009). Soil microbial biofilms can be categorized according to the contribution of microbes to form microbial community. Accordingly, there are three types of biofilms, i.e. (1) bacterial biofilms (BBs), (2) fugal biofilms (FBs) and (3) fungal-bacterial biofilms (FBBs) (Seneviratne et al. 2008). BBs and FBs get attached to abiotic surfaces in soil, whereas bacterial cells attach to biotic surfaces of fungi in FBBs (Seneviratne et al. 2008). When the FBBs are formed by nonfilamentous fungi, both bacteria and fungi act as biotic surfaces (Seneviratne et al. 2008).

Developed microbial biofilms (also known as biofilmed biofertilizers) are found to produce different exudates such as hormones, siderophore and hydrogen cyanide (HCN) and also to show activities like nitrogenase activity, antagonistic activity against pathogens and solubilization and mineralization of soil organic and inorganic forms nutrients (Herath et al. 2013; Triveni et al. 2013; Bandara et al. 2006). These biochemical functionalities of biofilmed biofertilizers (BFBFs) contribute to the enhanced growth of plants (Seneviratne and Jayasinghearachchi 2003). BFBFs can also optimize the production of hormones such as IAA by having regulated metabolism via signal transfer mechanisms (Bandara et al. 2008; Seneviratne et al. 2008; West et al. 2007). This optimal production of IAA promotes root growth, thereby enhancing nutrient uptake (Appanna 2007). This shows the direct and indirect contribution of BFBFs for plant growth.

3.3 Biofilmed Biofertilizer

Biofilmed biofertilizers (BFBFs) are developed microbial communities in biofilm mode. It regenerates depleted soil and enhances the soil fertility (Manawasinghe et al. 2014). BFBFs are also capable of increasing soil biodiversity by improving ecosystem functioning and sustainability. This is attributed to the breaking of microbial and plant seed dormancy in soil by BFBFs (Manawasinghe et al. 2014; Seneviratne and Kulasooriya 2013). BFBFs are also used as safer biocontrol agents (Seneviratne 2012), in addition to their biofertilizing and growth-promoting abilities. BFBFs are already in use for many crops in different countries including Sri

Lanka, and a BFBF formulation for tea (Biofilm-T) has been recently commercialized by a private company in Sri Lanka.

3.3.1 Role of BFBF in Sustainable Agriculture

Application of BFBFs increases soil organic carbon, microbial biomass carbon, moisture retention capacity and nitrogenase activity in roots or rhizosphere, thereby reinstating the agroecosystems which get exhausted and degraded due to agronomic practices associated with conventional farming (Seneviratne et al. 2011). Pseudonodules (nodule-like structures) which are capable of fixing atmospheric N_2 through BNF (Fig. 3.2) are formed by biofilms (Seneviratne et al. 2008). BNF is triggered by oxygen-limited environment created by exopolysaccharides produced in biofilms (Seneviratne et al. 2008). The observations by Seneviratne et al. (2011) in tea and by Buddhika et al. (2012b) in maize confirmed the ability of BFBFs to trigger BNF. Nitrogenase activity positively correlates with leaf N content, perhaps due to adequate supply of N through BNF for the synthesis of chlorophyll. Bacterial and cyanobacterial BFBFs have shown increased colonization of N-fixing microbes in roots and extended nitrogenase activity until the crop maturation (Swarnalakshmi et al. 2013; Buddhika et al. 2012b). Further, the application of BFBFs increases ammonium (NH_4^+) availability (Buddhika et al. 2012b) and reduces nitrate (NO_3^-) availability in soil (Seneviratne et al. 2011). This shows the prospects of biofilms to

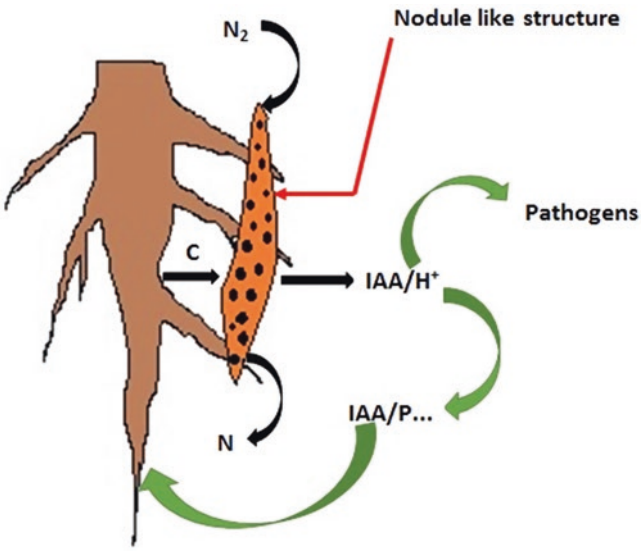

Fig. 3.2 Conceptual model showing the association established between the root and the biofilm when BFBFs are applied to roots of a non-legume

increase N-use efficiency and reduce the health and environmental knock-backs due to excessive availability of NO_3^- in soils.

Soil microbial diversity contributes immensely to agriculture to make it sustainable. Different soil microbes play a variety of roles in developing the physical structure of soil and also to enhance the soil fertility in a sustainable way to support plant growth. Conversely, the application of chemical inputs alone as conventional agronomic practices in agriculture destroys the diversity of soil microbiome. However, the application of BFBFs has shown to restore the microbial diversity of soil (Buddhika et al. 2013) and render many beneficial effects which are essential for agricultural sustainability. Various biochemicals secreted by the BFBFs cause breaking of the dormancy of microbial seed bank in soil and augment soil microbial diversity (Seneviratne and Kulasooriya 2013).

Soil microbial diversity is an important facet in sustainable agriculture as it contributes to an array of activities such as BNF, biosolubilization and mineralization of nutrients (Yao et al. 2000; Brookes 1995; Pankhurst et al. 1995), bioremediation and natural disease suppression (Sharma et al. 2011) and hormone production (Glick 2012; Spaepen and Vanderleyden 2011; Kang et al. 2009; Tsavkelova et al. 2006; Joo et al. 2005; Lorteau et al. 2001; Patten and Glick 1996; Atzorn et al. 1988). All these activities are highly important to support plant growth and development and to increase crop productivity with no mutilation to the environment. Higher microbial diversity in a pot experiment with potato has shown greater suppression of disease caused by *Rhizoctonia solani* (Garbeva et al. 2004). Therefore, microbial diversity is considered as an important indicator of soil quality (Sharma et al. 2011; Bastidia et al. 2008) and a determinant of soil health for higher crop productivity (Fernandes et al. 1997). Application of certain plant pathogenic fungi has produced a significant control of water hyacinth (*Eichhornia crassipes*), a prominent aquatic weed in water bodies and an invasive alien species in Sri Lanka (Cheanieha Queene et al. 2016). Moreover, the application of cyanobacteria in biofilm mode has regressed the loss of cyanobacteria in agricultural soils (Swarnalakshmi et al. 2013; Prasanna et al. 2009). Broad-spectrum biological activities of developed cyanobacterial biofilms tremendously contribute for effective development of inocula which is highly favoured in sustainable agriculture. However, the application of BFBFs in the absence of cyanobacterial inocula has also revealed to boost cyanobacterial diversity in crop lands (Buddhika et al. 2013). This is due to the dormancy breaking of microbial seed bank of soil by the secretions of BFBFs (Seneviratne and Kulasooriya 2013).

In addition to the important roles played by BFBFs in crop growth, they also contribute to increased seed germination and improved seedling vigour in many crops compared to the application of monoculture of the microbe (Herath et al. 2013; Triveni et al. 2013; Buddhika et al. 2012a). Maize germination and seedling growth was improved by the application of BFBFs which show regulated IAA production that supports improved crop performance (Buddhika et al. 2014). The regulated production of hormones, particularly IAA, in biofilms is important for microbial interactions that uphold various functions of biofilms (West et al. 2007). Higher-order biofilms, which possess large number of bacterial species, get prop-

erly established in the plant-soil system (Swarnalakshmi et al. 2013) and enhance plant growth (Seneviratne et al. 2009). Therefore, BFBFs are regarded as important biofertilizers to rehabilitate the degraded agroecosystem by conventional agricultural practices to ensure sustainability of agriculture in long run.

3.3.2 BFBFs as Potential Fertilizer for the Future

Bacterial and fungal components of BFBFs play a vital role in increasing the availability of soil nutrients through various processes like P-mineralization, solubilization and BNF to enhance soil fertility status. Different combinations of N-fixing bacteria and rhizosphere fungi have been used to produce a wide range of biofilms to be used as biofertilizers in agriculture (Triveni et al. 2013; Seneviratne et al. 2011; Jayasinghearachchi and Seneviratne 2004). BFBFs have been tested and in use for many crops in Sri Lanka. Fungal-rhizobial BFBF application has increased N-fixation by 30% in soybean along with other beneficial effects such as increased shoot and root growth, enhanced nodulation and soil N-accumulation compared to the sole application of *Rhizobium* (Jayasinghearachchi and Seneviratne 2004).

Generally the application of BFBFs alone is not recommended at the very onset of cropping as the fungal-bacterial biofertilizers may at the beginning incorporate a considerable amount of plant-available soil nutrients to the fungal biomass, thus sacrificing the growth of plants. Therefore, BFBFs as soil or seed inoculation with 50% chemical fertilizer (CF) have been found to be an optimum combination to maximize yield in many crop species under different soil types in Sri Lanka (Seneviratne et al. 2009). Application of BFBFs to rubber nursery has also shown a 50% reduction in CF use (Hettiarachchi et al. 2012). In an experiment in India, application of cyanobacteria and pant growth-promoting rhizobacteria (PGPR)-based BFBFs was found to augment plant growth and yield of green gram and soybean (Prasanna et al. 2014) and to improve biofortification of micronutrients in wheat (Rana et al. 2012a, b). With these significant results in growth enhancement and yield improvement together with significant reduction in CF that ensures numerous health, economic and environmental benefits, the use of BFBFs has become much popular among farmers. Hence, there will be a huge demand for BFBFs in the near future.

3.4 Conclusion

Biofertilizers support plant growth and development via a range of processes such as BNF, mineralization and solubilization of nutrients, plant hormone synthesis, pest and diseases suppression and stress tolerance. However, biofertilizers in biofilm mode show extended array of biochemical and regulated metabolic processes which enable the microbes in the BFBFs to sustain the biological system. Through

breaking dormancy of microbial seed bank in soil, BFBFs can reestablish degraded agroecosystems due to heavy use of CF and synthetic agrochemicals in conventional farming practices. As people are now more concerned about health and environment and also there is a need to increase global food production to meet the ever-growing world population, it is vital to find a sustainable alternative for chemical inputs, particularly CF. In this context, BFBFs are of immense use for sustainable agriculture in addition to other benefits to the environmental conservation.

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Chapter 4
Role of Rhizospheric Microbes in the Management of Phytopathogens

Mohammad Zuhaib, Shabbir Ashraf, Nasreen Musheer, and Mohd Ali

Abstract Medicinal plants play very crucial role in the life of people, and they are used in official and various traditional systems of medicines throughout the world, benefitting people to prevent disease, maintain health, and cure ailments. Nearly all modern pharmaceuticals are considered to be natural products or derived from plants. Fungal diseases are the major constraints in the profitable cultivation of medicinal plants. Phytopathogenic problem of medicinal plants not only reduces the yield, but it is also responsible for the deterioration of biochemical and secondary metabolites which are of immense therapeutic value. Imprudent use of insecticides, fungicides, agrochemicals, and fertilizers poses serious threat to environment. Scientists have reported various mechanisms regarding plant rhizospheric microbes, i.e., fungi and bacteria, which colonize the roots of plant and thus help the plants in maintaining its health. In the present scenario, rhizospheric microbes (biocontrol agents) have gained popularity due to their effectiveness, safety, and eco-friendliness, and hence their demand has gradually increased. Rhizospheric microbes not only manage plant diseases but at the same time also boost plant growth by different mechanisms. Many scientists have already reported the beneficial role of rhizospheric microbes on the health of various medicinal plants. Research on medicinal plants and rhizospheric microbes is inadequate as far as biotic stresses are concerned. The mechanisms of plant disease management such as mycoparasitism, antibiosis, induced systemic resistance, plant growth promotion, root colonization, siderophore production, phosphate solubilization, etc., have been studied well in reference to medicinal plants. Still due to the distinct features of medicinal plants, future research could be a major breakthrough in the significant increase in the production of medicinal plants.

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4.1 Introduction

About 80% people of the globe rely on herbal medicine for their health problems according to the World Health Organization (Goto et al. 1998). Due to severe side effects of modern medicines, drugs extracted from medicinal plants are gaining popularity in many developed countries. The basics of herbal medicines mainly depend on plant diversity and past studies of their use in maintaining human health (Table 4.1). Nearly all herbal medicines are natural products or derivatives of plants; interestingly it has also been acknowledged that the discovery of artemisinin, which is an antimalarial drug extracted from medicinal plant "sweet wormwood," has earned a 2015 Nobel Prize in medicine (George et al. 2016). Fungal diseases pose a serious threat to the profitable cultivation of crop as well as the medicinal plant. In 1845 potato late blight fungus was responsible for Irish famine which led to millions of people to migrate from Ireland. The *Plasmopara viticola* causal organism of downy mildew of grapes devastated the wine industry in France. In 1943 brown spot disease of rice was solely responsible for Bengal famine, and other catastrophic examples of phytopathogens also include apple scab; Panama wilt; wheat rust; wilt of *Cajanus cajanus*, chickpea, castor, and guava; rust; and smut of cereals. Phytopathogenic problem is not only a threat to commercial crop plants, but they are also a threat to our important medicinal plants. *Alternaria* leaf spot and *Fusarium* cause severe wilting in Ashwagandha which leads to enormous yield losses (Sharma et al. 2013; Zuhaib et al. 2016; Ansari and Mahmood 2017; Sharma and Trivedi 2010). Apart from Ashwagandha other medicinal plants were also reported to be infected by *Fusarium* wilt.

Various options of disease management such as chemical, botanical, and biological are available, and among them, chemicals are considered one of the best and reliable options, but they pose serious health and environmental risks, which have limited their use. About 0.1% of the agrochemicals used in crop protection reach to the target pest, rest 99.9% enter into the environment causes greater damage to the ecosystem (Ashraf and Zuhaib 2013). The indiscriminate use of chemical fungicides has consequently caused several health issues, such as toxicity in food, water, and soil, which ultimately leads to pollution of the ecosystem; hence it was recommended by the scientists to use nonchemical methods for the management of phytopathogenic problem of medicinal plants.

Plant rhizospheric microbes including soil fungi and bacteria colonize plant roots and, in turn, help in maintaining plant health. In the present scenario, rhizospheric microbes (biocontrol agents) have gained popularity due to their effectiveness, safety, and eco-friendliness, and hence, their demand has gradually increased. Rhizospheric microbes not only manage the plant diseases but also enhance plant growth by adhering and multiplying at the root hair surface; increase in seedling emergence, functioning of premature nodules, and nodulation; and increase in area

Table 4.1 Medicinal plants and their uses in human health

Traditional name	Scientific name	Family	Part in use	Medicinal use
Amla	*Emblica officinalis*	Euphorbiaceae	Fruit	Rich source of ascorbic acid, cough, cold, hyperacidity, laxative, prevention of cancer
Ashok	*Saraca asoca*	Caesalpiniaceae	Bark, flower	Diabetes disorder, menstrual pain, uterine problems
Ashwagandha	*Withania somnifera*	Solanaceae	Root	Curative tonic and helps in nerves disorder
Guggul	*Commiphora wightii*	Burseraceae	Gum resin	Rheumatism, laxative, hyperlipidemia
Aloe	*Aloe vera*	Liliaceae	Leaves	Used in cosmetic industry
Bael/bilva	*Aegle*	Rutaceae	Fruit, bark	Diarrhea, dysentery, constipation
Tulsi (perennial)	*Ocimum sanctum*	Lamiaceae	Leaves/ seed	Helps in bronchitis, acts as expectorant, anticancerous
Sarpagandha (H)	*Rauvolfia serpentina*	Apocynaceae	Root	Hypertension, insomnia
Bhumiamla (H)	*Phyllanthus amarus*	Euphorbiaceae	Whole plant	Provide strength, lower the bilirubin
Shatavari	*Asparagus racemosus*	Liliaceae	Tuber, root	Pregnant women, anti-fatigue, lowers blood sugar
Brahmi	*Bacopa monnieri*	Scrophulariaceae	Whole plant	Anxiety, improve the memory enhancer,
Makoi/ Kakamachi	*Solanum*	Solanaceae	Fruit/ whole	Dropsy, general weakness, anticancerous
Isabgol	*Plantago ovata*	Plantaginaceae	Seed coat	Constipation and gastrointestinal irritations. Also used in food industry
Coleus	*Coleus forskohlii*	Lamiaceae	Tuberous root	Used in glaucoma, heart functioning, and various types of carcinoma
Henna/mehndi	*Lawsonia inermis*	Lythraceae	Leaf, seed	Burning, steam, anti-inflammatory
Pashanbheda	*Coleus barbatus*	Lamiaceae	Root	Stone problems, diabetes
Peppermint	*Mentha*	Lamiaceae	Leaves,	Digestive, painkiller
Sadabahar	*Vinca rosea*	Apocynaceae	Whole plant	Blood cancer, blood pressure muscle spasm
Vringraj	*Eclipta alba*	Compositae	Seed/ whole	Anti-allergic, digestive, hair
Neem	*Azadirachta*	Meliaceae	Whole plant	Sedative, analgesic, epilepsy

Modified from of Shahzad et al. (2015)

of leaf surface, vigor, biomass, phytohormone, and nutrient, water, and air uptake, hence stimulating the accumulation of important nutrients in plants (Shrivastava et al. 2015). This review will focus on major fungal diseases of medicinal plants and also the recent developments in the field of biological control of medicinal plant diseases by rhizospheric microbes, which will emphasize on the mechanism. In this chapter we will limit our discussion on important rhizospheric microbes, viz., species of *Trichoderma*, *Pseudomonas*, and *Bacillus*.

4.2 Biotic Stresses on Medicinal Plants

Since there is a great demand for herbal medicinal in the international market, many biotic factors are responsible for the low productivity of medicinal plants. Biotic factors liable for the low productivity of medicinal plants include an attack of insects, arthropods, fungi, bacteria, and nematodes. Among them the fungi cause major yield losses after insects; in this chapter, we will focus on economic yield losses caused by fungi. Black leaf spot diseases of *Aloe vera* were caused by *Alternaria alternata*; two different isolates A and B of *Alternaria alternata* were isolated from the diseased leaf of *Aloe vera* (Alam et al. 2007). Similarly Shukla et al. (2008) also reported *Pythium* leaf spot of *Aloe vera* which was caused by *Pythium aphanidermatum*. An occurrence of leaf spot of Kalmegh (*Andrographis paniculata*) was also observed which causes severe yield loss of 30–45% (Alam et al. 2007). Leaf blight of *Mentha piperita* and *Ocimum sanctum* was also reported by (Alam et al. 2007) and (Ashraf and Zuhaib 2009), respectively. Same workers (Zuhaib et al. 2016) also reported leaf spot of *Withania somnifera* by *Alternaria alternata* and also screened the resistant cultivars of *Withania somnifera* for the pathogen. Twig blight of periwinkle (*Catharanthus roseus*) caused by *Sclerotinia sclerotiorum* was also studied; similarly foliar infection in the form of leaf spot of *Rauvolfia serpentina* was also studied by Alam et al. (2007). Medicinal plants are not only attacked by phyllospheric pathogens, but they are also attacked by rhizospheric pathogens. *Fusarium* causes severe wilting in Ashwagandha which leads to enormous yield losses (Bharti et al. 2014; Zuhaib et al. 2016; Sharma and Trivedi 2010). Apart from Ashwagandha other medicinal plants were also reported to be infected by *Fusarium* wilt. Important medicinal plants reported to be infected with *Fusarium* wilt include *Atractylodes lancea*, *Dioscorea zingiberensis*, *Euphorbia pekinensis*, *Ophiopogon platyphyllum*, *Pinellia ternata* (Dai et al. 2009), *Curcuma manga* (Khamna et al. 2009), *Launaea nudicaulis* (Mansoor et al. 2007), Jerusalem artichoke (sunchoke) (Jina et al. 2013), *Panax quinquefolius* (Song et al. 2014), *Coleus forskohlii* (Zheng et al. 2012), *Papaver somniferum* (Kishore et al. 1985; Sattar et al. 1995), *Calotropis gigantea* (Selvanathan et al. 2011), *Basilicum* (Elmer et al. 1994; Katan et al. 1996), and *Asparagus* (Lamondia and Elmer 1989). *Coleus forskohlii* is an important medicinal plant; wilt of *Coleus forskohlii* is a disease complex caused by *Rhizoctonia bataticola*, *Fusarium chlamydosporum*, *Sclerotium rolfsii*, and *Ralstonia solanacearum* (Bhattacharya and Jha 2012).

4.3 Rhizospheric Microbes as Biocontrol Agents

Plant health may be ameliorated by rhizospheric microbes (naturally present soil fungi and bacteria) by colonizing the plant roots. In the present scenario, rhizospheric microbes (biocontrol agents) have gained popularity due to their effectiveness, safety, and eco-friendliness, and hence, their demand has gradually increased. Vigor, biomass, nutrients and water absorption, yield, root hair proliferation, root hair branching, increase in seedling emergence, increase in area of leaf surface, nodulation, and promoted accumulation of carbohydrates are some of the ways in which rhizospheric microbes supplement plant growth besides providing protection to plants from diseases (Shrivastava et al. 2015). Usage of fungicides is not recommended as it is neither economical nor environmentally friendly. Moreover, its long-term use can cause the development of resistant strains of a pathogen (Ashraf and Zuhaib 2014a, b; Vinale et al. 2008). However, research on biological control gained momentum in the last quarter of the tenth century, and several books (Baker and Cook 1974; Cook and Baker 1983) and review articles (Papavizas 1985) have come up stressing the potential of microorganisms in disease management. Numerous microorganisms have been reported to cause antagonism against plant pathogenic fungi in laboratory and in vivo condition. A perfect biocontrol agent/rhizospheric microbe must have the subsequent qualities (Lucy et al. 2004; Mukerji 2000).

1. Prolonged survival, either in active or passive form.
2. Greater probability of contact with the pathogen.
3. Functional under variable environments.
4. Mass multiplication should be easy, feasible, and economical.
5. Proficient and cheap.
6. Eco-friendly.

A number of rhizospheric microbes such as *Trichoderma*, *Bacillus*, and *Pseudomonas* have been found successful against a number of important fungal diseases of medicinal plants (Scher and Baker 1982; Strashnov et al. 1985; Kaur et al. 2006; Abo-Elyousr et al. 2014; Dubey et al. 2007). The most common species of *Trichoderma* which have been successfully exploited in biological control of pathogenic fungi are *T. virens*, *T. viride*, and *T. harzianum* (Benitez et al. 2004). *T. viride* has been found to significantly reduce mycelial growth, a formation of spores, and germ tube formation of *A. solani* and *A. alternate* (Latha et al. 2009). *T. harzianum* has been found active against *F. oxysporum* inciting wilt in Ashwagandha (Sharma and Trivedi 2010). Moreover, *Trichoderma* can even stimulate plant growth; reports of which have been found in the case of *T. virens* (Kumar et al. 2011) and the stimulation of plant defense mechanisms (Chet et al. 1997).

Mechanism of disease suppression by rhizospheric microbes *Trichoderma* spp. is reported to suppress plant pathogenic fungi through a combination of different mechanisms (Table 4.2) such as mycoparasitism, synthesis of antibiotics (Harman 2006; Harman et al. 2004), enzymes degrading cell wall (Jayalakshmi et al. 2009),

Table 4.2 *Trichoderma* species, their target organism, and mechanisms involved in suppression of plant pathogens

Trichoderma species	Target organism	Factor responsible for biocontrol	Disease control
T. harzianum 1051, *T. harzianum* 39.1	*Crinipellis perniciosa*	Chitinase, *N*-acetylglucosaminidase, β-1,3-glucanase,total cellulase, endoglucanase, aryl-β-glucosidase, β-glucosidase, protease, and amylase	Witches' broom disease (*Crinipellis perniciosa*) of cocoa
T. lignorum, T. virens, T. hamatum, T. harzianum, T. pseudokoningii (Rifai)	*Rhizoctonia solani*	Extracellular, metabolites or antibiotics, or lytic enzyme action	Damping-off disease of bean plants
T. viride, T. harzianum	*Aspergillus flavus* and *Fusarium moniliforme*	Lipolytic, proteolytic, pectinolytic, and cellulolytic enzymes. Unknown (mycotoxins) antibiotic compounds (e.g., peptides, cyclic polypeptides)	Fungal seed-associated
T. harzianum, BAFC 742	*Sclerotinia sclerotiorum*, BAFC 2232	β-1,3-Glucanase and chitinase	Fungal-soybean plant
T. harzianum 25*, T. viride*	*Serpula lacrymans*	Antibiotic; anthraquinones	Fungal wood decay
T. virens "*Q*" *strain*	*Rhizopus oryzae/ Pythium* sp.	Plant phytoalexin induction by antibiotic compound, gliovirin	Seedling disease of cotton
T. virens isolates GL3 and GL21; *T. harzianum* T-203	*Rhizoctonia solani, Pythium ultimum, Meloidogyne incognita*	Antibiotics gliovirin and gliotoxin and other inhibitory metabolites	Damping-off disease of cucumber

Source: Leng et al. (2011)

contesting for the availability of important nutrients and increase in plant health (Zimand et al. 1996), parasitism of host fungus (Komatsu 1968; Gao et al. 2001), inducing plant defense (Jayalakshmi et al. 2009), and/or induced systemic resistance (Harman et al. 2004; Sriram et al. 2009).

4.3.1 Mycoparasitism

The most common mechanism used by *Trichoderma* for the suppression of phytopathogens is mycoparasitism (Howell 2003; Vinale et al. 2008). Mycoparasitism is a diverse process involving recognition of the host by the mycoparasite; hyphal attachment and coiling of pathogen hyphae (Whipps 2001; Woo and Lorito 2007).

The biocontrol of *R. solani* by *T. lignorum* through mycoparasitism was very well described by Weindling (1932). Enzymes such as chitinases, proteases, and β-1, 3-glucanases lyse hyphal cell walls of pathogens during mycoparasitic activity (De La Cruz et al. 1993; Schirmbock et al. 1994). β-1, 3-Glucanases have properties for degrading the cell wall and inhibit the mycelial growth and spore germination of phytopathogenic fungi (Benítez et al. 2004; Lin et al. 2007). Degradation of pathogen hyphal membranes and cell walls was achieved by proteases produced by *T. harzianum*. Application of T. harzianum may inhibit the synthesis of hydrolytic enzymes such as endo-polygalacturonase and exo-polygalacturonase, produced by *Botrytis cinerea*, a causal agent of gray mold, resulting in reduced disease severity (Elad and Kapat 1999). Mustafa et al. (2009) and Kotze et al. (2011) also observed the mycoparasitic activity of *Trichoderma* species against wide range of plant pathogenic fungi.

4.3.2 Competition and Rhizosphere Competence

Biocontrol agents multiplication and their multiplication depends upon various factors like rhizosphere competence, successful root colonization, proliferation along the growing plant roots (Chet 1990; Irtwange 2006). Rhizospheric competence is very crucial which provides appreciable understanding pertaining to mode of action of rhizospheric microbes against wide range of plant pathogens (Whipps 2001; Bais et al. 2004; Howell 2003). *Trichoderma*, *Pseudomonas*, and *Bacillus* are considered as potent biocontrol agents and offer excellent competition in terms of food and space to the pathogens (Wells 1988). Among these three rhizospheric microbes, *Pseudomonas* was reported to be more effective comparatively *Trichoderma* followed by *Bacillus* (Weller 1988).

The mass culture of *Trichoderma* can be prepared by using different media which can be thereafter used directly either by mixing with the soil or indirectly by biopriming methods (Zhang et al. 1996; Howell et al. 2000). *T. viride* have been reported to reduce the disease severity of *Chondrostereum purpureum*, the silver leaf pathogen of plum trees (Corke and Hunter 1979).

A race for obtaining carbon in the rhizosphere was also observed in the evaluation of antagonistic activity of *Trichoderma* spp. against different plant pathogens, especially *F. oxysporum* (Sivan and Chet 1989). Competition for carbon is involved in the suppression of *F. oxysporum* f. sp. *vasinfectum* and *F. oxysporum* f. sp. *melonis* by *T. harzianum* T-35 in the rhizosphere of cotton and melon, respectively (Sivan and Chet 1989). The case of root colonization by bacteria consists of two phases, attachment to roots followed by colonization of roots (Howie et al. 1987). It was also reported that motile isolates were far more better colonizers than non-motile isolates (Toyota and Ikeda 1997). The capability of bioagents to synthesize certain antibiotics has a direct relation to being a good colonizer. Mazzola et al. (1992) sug-

gested that phenazine antibiotic production contributes to the ecological competence of *P. fluorescens* in the rhizosphere of wheat. A decrease in disease severity for take - all disease of wheat and radish wilt caused by *Fusarium* has a direct relation with the establishment *of Pseudomonas* strains (Bull et al. 1991; Raaijmakers et al. 1995). Berger et al. (1996) after thorough studies have drawn a conclusion that decrease in disease severity has a direct relation with the rhizospheric establishment by *B. subtilis*.

4.3.3 Antibiosis

Suppression or destruction of diseases producing propagules (spores, conidia, conidiophore) by the synthesis of antibiotics or other chemicals synthesizing the bioagents (fungi or bacteria) is known as antibiosis (Irtwange 2006; Viterbo et al. 2007; Haggag and Mohamed 2007). Most of the biocontrol agents including *Trichoderma*, *Pseudomonas* spp., and *Bacillus* species produce several types of antibiotics (Kumar et al. 2011; Handelsman and Stabb 1996). The antibiotics produced by *Trichoderma* species include gliotoxin (Anitha and Murugesan 2005), harzianic acid (Vinale et al. 2014), trichoviridin (McAlees and Taylor 1995), viridian (Zafari et al. 2008), viridiol (Phuwapraisirisan et al. 2006), alamethicins (Aidemark et al. 2010), and others (Goulard et al. 1995). Gliovirin an antibiotic isolated from *Trichoderma* (*Gliocladium*) *virens* shows a strong inhibitory effect against *Pythium ultimum* and *Phytophthora* species (Howell and Stipanovie 1983). *Thielaviopsis basicola*, *Phymatotrichum omnivorum*, *Rhizopus arrhizus*, or *Verticillium dahliae*. *B. thuringiensis* was not inhibited by gliovirin. Secretion of *T. harzianum* strain against *Gaeumannomyces graminis* var. *tritici* exhibited inhibtory effects supporting the fact that bioagent synthesizes antibiotics plays a vital role in the inhibition of the pathogen.

Bacillus and *Pseudomonas* species are also effective microbes in managing plant diseases by the production of antibiotics (Weller 1988; Kumar et al. 2011). Plant disease suppression due to *P. fluorescens* may be due to synthesis of pyoluteorin, phenazine, oomycin A, IAA, siderophores, phenazine, siderophore (Whistler et al. 2000; Schoonbeek et al. 2002; Suzuki et al. 2003; Johri et al. 2003; Rachid and Ahmed 2005), extracellular hydrolytic enzymes (Siddiqui 2006), alginate, HCN (Bagnasco et al. 1998), and pseudomonic acid. The antimicrobial compounds discussed above are responsible to cause fungistasis, inhibition of spore germination, and degradation of a mycelial wall and also induce other fungicidal effect (Thomashow and Weller 1990). Production of iturin and surfactin by *B. subtilis* RB 14 played important role in the protection of tomato plant against *R. solani* (Asaka and Shoda 1996). *B. subtilis* synthesize about five antibiotics, namely, subtillin, bacitracin, bacillin, subtenolin, and bacillomycin (Young et al. 1974). Pukall et al. (2005) isolated four toxin-producing strains of *Bacillus* spp., such as *B. pumilus*, *B. fusiformis*, *B. subtilis*, and *B. mojavensis* apart from *B. cereus*.

4.3.4 Plant Growth Promotion

PGPR helps in improving the plant health by the producing of different metabolites such as siderophore and hydrocyanic acid (HCN) (Bhatia et al. 2008); other metabolites also include phytohormones like indole acetic acid, gibberellins, cytokinins, and ethylene (Patten and Glick 2002). Another mechanism is the breaking of ethylene molecules which inhibits the growth of roots by certain rhizobacteria and also improves the plant health (Glick et al. 1999). Great number of rhizospheric microbes produces the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which breaks down the ACC molecule, the direct originator of the plant hormone ethylene (Belimov et al. 2001; Glick 1995). They kindle the root propagation of different crop plants (Belimov et al. 2001). The abovementioned mode of action (breakdown of ACC) is most efficient in plants which undergo stresses like flooding, drought, and phytopathogens (Grichko and Glick 2001; Wang et al. 2000). The plant health improvement by rhizospheric microbes lies in the fact of initiation of phosphorous plant nutrition (Bertrand et al. 2001). (The increase in yield of groundnut by *Pseudomonas* strains is the best example of initiation of phosphorous plant nutrition which leads to easy uptake of soil phosphorus by plants) (Dey et al. 2004). Effect of rhizospheric microbes on plant growth is because of synthesis of siderophores; synthesis of phytohormones which leads to increase in plant growth (Garcia de Salamone et al. 2001); and initiation of phosphorous plant nutrition leading to readily available phosphorous (Richardson 2001). Sen et al. (2012) reported that *Stevia rebaudiana* Bertoni, a natural sweetener, is composed of two main sweetest compounds which make it 300 and 450 times sweeter than sucrose. *Pseudomonas* BRL-1 isolated from the rhizosphere showed both in vitro and in vivo antagonistic activity against the pathogen *Alternaria alternata* inciting leaf blight disease in *Stevia rebaudiana.* Siderophore produced by fluorescent *Pseudomonas* has very high affinity for ferric ion and is secreted during growth under low iron conditions (Johri et al. 2003) which is then converted to ferrous ions and thus reduces iron availability to pathogens. However, the producing strain can utilize this via a very specific receptor in its outer cell membrane (Buyer and Leong 1986). In this way the bacteria may restrict the growth of deleterious bacteria and fungi at the plant root surface (Loper and Buyer 1991). Consequent iron starvation condition prevents the germination of fungal spores. Elad and Baker (1985) have demonstrated the direct relationship between synthesis of siderophores and their tendency to control the germination of chlamydospores of *Fusarium oxysporum*. Johri et al. (2003) have also reported that fluorescent pseudomonas during low iron concentration secrete siderophores which reduces ferric into ferrous ions, and thus no more iron is available to pathogens. However, the synthesizing strain has a tendency to use this with the help of specific receptors in its outer cell membrane (Buyer and Leong 1986). In this is how the bacteria can check the growth of harmful bacteria and fungi at the surface of roots (Loper and Buyer 1991) and hence promote the plant growth.

4.4 Conclusions

Now it is very much clear that rhizospheric microbes have a positive trend in increasing the growth and yield of medicinal plants under biotic stresses. Although understanding the mechanism of rhizospheric microbes as a plant growth promoter is still an interesting field of qualitative research, therefore, it is the right time to think about the potential candidate of microbes which can improve the plant health even under biotic stresses. Application of suitable strain of microorganisms in the field infested with the soil borne pathogens may exert some reliable results. Consortium of microorganism of different origin can enhance the potentiality of the bioagents which may be very useful in the disease management. However, mechanisms behind the control of diseases are still the matter of research as this will unravel various important facts related to disease management. Due to the distinct features of medicinal plants, future research could also pave a new platform in understanding the subject. Adequate research in this thrust area could be a major breakthrough in the improvement of health of various economically important medicinal plants.

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Chapter 5
Microbe-Assisted Plant Growth Ameliorations

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Abstract Diverse microbes present in soil play a remarkable role in symbiotic action under different plant ecosystems. The use of prominent microbes against the pathogenic microorganisms affecting plant health helps in preventing the potential harmful effect of chemical pesticides on environment and human kind. Plant growth promoting rhizobacteria (PGPR) are one of the beneficial microbial groups under biocontrol agents for the best alternative to avoid the hazardous effect of chemicals and help in maintaining the plant health. PGPR colonize plant roots and help in plant health ameliorations using various bacteria. They play a significant role in enhancing the production of plant growth hormone substances, fixation and availability of plant nutrients and modulate the defence activity for inhibiting the effect of various pathogens through production of antimicrobial metabolites. Integration of PGPR and plant induces the defence mechanisms against the variety of pathogenic group. The exploitation of productive and efficient PGPR community helps in achieving plant growth and protection from plant pathogens significantly to avoid the crop loss.

Keywords PGPR · Plant health · Biological control · Rhizosphere · Rhizoplane

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5.1 Introduction

Soil is a matrix which shelters the life of almost all types of living organisms and also provides the food security to the human kind. Soilborne pathogens are the living organisms which badly affect plant health and cause drastic reductions in the quantity and quality of various agricultural commodities (Ansari and Mahmood 2017). Application of chemical pesticides and inorganic fertilizers kills the pathogens, weed, rodents, etc. However, on the other hand, introduction of huge amount of pesticides in the soil causes great impact in the natural agroecosystem and eventually on human health (Ansari and Mahmood 2017). Due to human health issues, researchers are walking for suitable alternatives. Biological agents are the suitable alternatives in the management of soilborne pathogens. PGPR play a remarkable role in the ameliorations of soil and plant health (Ansari et al. 2017a). Application of PGPR has excitingly been accepted by wide-scale levels of farmers and so far been commercialized at global market (Patni et al. 2018). PGPR thrive in the vicinity of root surface and are directly or indirectly implicated in the plant growth promotions. Generally, PGPR improve the plant yield by accumulating the various nutrients such as nitrogen, phosphorus, potassium, etc. The other mode of plant growth and yield improvement is by reducing the plant pathogen populations in the soil (Ramamoorthy et al. 2001). A large number of studies have been conducted, and the results obtained from such experiments have revealed that application of PGPR may improve plant health being grown under stressed environment (Yang et al. 2009; Ramamoorthy et al. 2001). The PGPR-ridden commodities may cut down the use of hazardous agricultural chemicals, and therefore present chapter accentuates the current progress in the application of the PGPR in relation to plant health amelioration. Various common mechanisms being used by the PGPR have been discussed; also recent development and future outlooks have also been illustrated.

5.2 Rhizosphere: The Battlefield/Playground of the Microorganisms

The root systems are surrounded by a narrow zone of soil and are termed as rhizosphere. The result provides the mechanical support to the plants and facilitates the water and nutrient mobilizations. Besides, plant root also secretes some root exudates containing diverse array of organic molecules and attracts the microorganisms towards the root system (Bacilio-Jiménez et al. 2003; Marschner 2012). These exudates regulate the biological, chemical and physical properties of soil and modify the structure of the soil microbial community in the surrounding region of roots. These exudates sometimes act as repellent against plant pathogenic microorganism.

The composition of root exudates totally depends upon the physiology, biology and chemistry of plant roots and environment. In addition, plants release some free organic molecules which are later on metabolized by the microorganisms as a source of carbon and nitrogen. On the other hand, microbial mediated organic molecules are eventually taken up by the plant roots and used in their machinery which helps in the growth and development of plants (Bais et al. 2004).

5.3 Plant Growth-Promoting Rhizobacteria (PGPR)

PGPR holds certain distinct features which make them separate from other groups of organisms. Some distinct features such as PGPR are potential in (1) root colonization and (2) survival and multiplication rate which are much higher especially when these groups are nurtured in heterogeneous environments. The PGPR should pass plant growth-promoting activities. The PGPR when reintroduced in a competitive atmosphere exert a beneficial impact in plant growth promotions. The PGPR are currently used in various commercial levels against diverse array of plant pathogens. Commercialized derived products from PGPR have also been used in the alleviations of abiotic and biotic stress. The most prominent and researched PGPR are in *Pseudomonas fluorescens* in diverse range of environments. Some other pseudomonads are also used in the growth promotion and protection from soilborne pathogens (Haas and Défago 2005). The habitation of PGPR comprises extracellular organisms (bacteria on rhizoplane, rhizosphere or space of cortical cells), for example, *Agrobacterium*, *Erwinia*, *Arthrobacter*, *Caulobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Chromobacterium*, *Flavobacterium*, *Micrococcus*, *Pseudomonas* and *Serratia*. On the other hand, intracellular organisms are located in the nodular regions of the roots which belong to the family of *Rhizobiaceae* such as *Allorhizobium*, *Bradyrhizobium*, *Mesorhizobium* and *Rhizobium*.

5.4 Mechanisms Implicated in Plant Growth Promotions

PGPR-induced plant health improvement may occur by altering the microbial communities of rhizosphere. The microbial community produces a wide range of chemical substances which promotes the plant growth and development. Generally, PGPR improve the plant growth and yield components either directly or indirectly.

5.4.1 Direct Mechanisms

PGPR improve plant growth and development by enhancing the nutrient uptake, mineralization and mobilization of organic molecules. Production of plant growth-promoting molecules is the determining factor which ameliorates the plant health (Ahemad and Kibret 2014).

5.4.1.1 Nitrogen Fixation

Nitrogen is an essential element for all forms of life, and it is the most vital nutrient for plant growth and productivity. Although nitrogen presents ~78% of the atmosphere, it remains unavailable to the plants. Regrettably, no plant species is capable for fixing atmospheric dinitrogen into ammonia and extend it directly for plant growth (Cornwell et al. 2008). Nevertheless, the atmospheric nitrogen is fixed through biological nitrogen fixation which changes nitrogen to ammonia by nitrogen fixer using nitrogenase enzymes (Masson-Boivin et al. 2009). In addition, nitrogen are fixed through symbiotic and non-symbiotic associations. Nitrogen-fixing organisms are normally categorized into two forms: (1) symbiotic nitrogen-fixing bacteria and (2) non-symbiotic nitrogen-fixing organisms. Nitrogen-fixing bacteria including members of family *Rhizobiaceae* generally establish symbiotic associations (Zahran 2001). Moreover, non-symbiotic nitrogen-fixing bacteria are common such as *Azospirillum*, *Gluconacetobacter diazotrophicus* and *Agaricus* and cyanobacteria (Bhattacharyya and Jha 2012). Non-symbiotic bacteria provide very little amount of fixed nitrogen to the plant. The bacteria fixing the nitrogen through symbiotic associations infect and establish symbiotic relationship with the roots of leguminous plants. The establishment of symbiotic relationship involves a compatible cross talk between host plant and symbiotic bacteria (Giordano and Hirsch 2004). This relationship forms nodules where rhizobial cells colonize the intracellular parts of the plant cell. The nitrogenase enzyme is the driving enzyme which plays important role in nitrogen fixations (Kim and Rees 1994).

The complex enzyme (nitrogenase) is composed of two components of metalloenzymes which consist of (1) dinitrogenase reductase (an iron protein) and (2) dinitrogenase (metal cofactor). The nitrogen reductase gives off electron having high reducing ability; however, on the other hand, dinitrogenase utilizes these electrons for reducing N_2 to NH_3. These are the main metal cofactors which have been identified as (1) Mo-nitrogenase, (2) V-nitrogenase and (3) Fe-nitrogenase. The nitrogen fixation ability varies from bacterial genera to genera and even species to species. Most of the biological nitrogen fixations are carried out by the molybdenum nitrogenase activity, found in almost all diazotrophs (Böhme and Masepohl 2018). Application of biological nitrogen-fixing organisms registers greater improvement in plant growth promotion and disease management and thus maintains the nitrogen status of agricultural soil.

5.4.1.2 Phosphate Solubilization

Phosphorus is also an important element playing crucial role in several metabolic processes in plants. Photosynthesis, energy transfer, signal transduction, macromolecule synthesis and respirations are the biochemical reactions where phosphorus presence is necessary. Phosphorus is present into two forms, i.e. organic and inorganic. The plants are not able to utilize phosphate because around 95–99% phosphate present in the insoluble, immobilized and precipitate form (Pandey and Maheshwari 2007). In addition, plants can use phosphate in monobasic (H_2PO_4) and dibasic (HPO_4^{2-}) ions (Bhattacharyya and Jha 2012). PGPR harbours in the soil adopt different strategies to facilitate the phosphorous availability to the plants. The chief mechanisms implicated in the phosphate solubilization are (1) release of organic mineral components such as organic anions, protons, hydroxyl ions and CO_2, (2) extracellular enzyme liberations and (3) phosphate liberations during phosphate degradations (Sharma et al. 2013). Some important PGPR such as *Beijerinckia*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium*, *Rhodococcus* and *Serratia* have been studied in details in terms of plant health ameliorations (Bhattacharyya and Jha, 2012).

5.4.1.3 Potassium Solubilization

As far as potassium ion as macroelement is concerned, it is the third major essential element for plant health improvement. The amount of usable potassium is very low, and around 90% of rest potassium is present in unavailable form to the plants (Parmar and Sindhu 2013). Moreover, due to non-judicious application, potassium deficiency is the major trouble in different types of soil. Potassium-deficient soil develops poor root, produces small seeds and has lower yield. Therefore, this is high time to search for an alternative to maintain the potassium status in soil for crop productivity (Kumar and Dubey 2012). PGPR have been reported to solubilize the rock through the production of some organic molecules, making it available to the plants (Han and Lee 2006). Some important PGPR like *Acidithiobacillus ferrooxidans*, *Bacillus edaphicus*, *Bacillus mucilaginosus*, *Burkholderia*, *Paenibacillus* sp. and *Pseudomonas* are the key example which play an important role in phosphate solubilization (Ansari et al. 2017a).

5.4.1.4 Production of Siderophore (Iron-Chelating Compound)

Siderophore, an iron-chelating compound, plays an important role in the sustenance of soil microorganisms except some bacilli (Neilands 1995; Ansari et al. 2017b). Fe^{3+} in the aerobic atmosphere forms insoluble hydroxide and oxyhydroxide which

ultimately utilize by the host plants and microorganisms (Rajkumar et al. 2010). Generally, bacteria take up the iron by the low molecular compounds referred as siderophores having great associations' ability for complexing the iron. These iron chelators, i.e. siderophores, are either extracellular or intracellular in nature (Ansari et al. 2017b). Moreover, some rhizobacteria are proficient in the utilization of homologous siderophores (produced by the same genus), while other bacteria utilize those produced by other rhizobacteria, i.e. heterologous siderophores (Khan et al. 2009). Moreover, Fe^{3+} is reduced into Fe^{2+} in Fe^{3+}-siderophore complex on bacterial membrane which is released from the siderophore molecules into cells through gating mechanisms (Rajkumar et al. 2010). Also, siderophores act as sometimes solubilizing agent from minerals or some organic molecules especially in iron-deprived atmosphere (Indiragandhi et al. 2008). Binding of siderophores with a metal enhances the solubility of metal concentrations and assists in the alleviations of the metal stress (Rajkumar et al. 2010). Moreover, plants assimilate the iron-chelating agents by different mechanisms and improve the plant productivity. Generally, plants accumulate the iron either by direct mode or by ligand exchange, metabolic reactions (Rajkumar et al. 2010). Siderophore-mediated iron transport system improves the iron delivery in many crop plants especially in iron-limited environment (Crowley and Kraemer 2007). *Pseudomonas fluorescens* C7 synthesize the Fe-pyoverdine complex which ameliorate the *Arabidopsis thaliana* plant health (Vansuyt et al. 2007). Application of siderophore-producing *Pseudomonas* strain GRP3 enhances the plant health and physiology like chlorophyll a and chlorophyll b of *Vigna radiata* (Sharma et al. 2013).

5.4.1.5 Production of Plant Growth-Promoting Molecules

A wide range of microbial agents including PGPR produces plant growth-promoting molecules which helps in plant health ameliorations by regulating the plant growth and development. PGPR produces the hormones such as auxin, cytokinin, gibberellins and ethylene which may affect the root structure and cell growth/proliferations (Arora et al. 2013).

There are a wide number of plant growth regulators, for example, IAA (indole acetic acid) is the most commonly occurring phytohormone which exerts positive effects on growth and development of plant. Around 80% of rhizobacteria are able to synthesize phytohormones like IAA and stimulate cell proliferations and also improve the accumulations of minerals and nutrients capacity of host plant from soil (Duca et al. 2018). IAA also influences the cell division, extensions and differentiations of root structure. This hormone enhances the rate of xylem and root development, regulates the vegetative growth and begins the formations of lateral and adventitious root. A significant role in photosynthesis pigment formations and biosynthesis of various metabolites to stressful conditions has also been observed (Miransari and Smith 2014). The biosynthesis of IAA by PGPR involves the formations of indole pyruvic acid and also indole-3-acetic aldehyde, and this is the most

common mechanism in some bacteria such as *Pseudomonas*, *Rhizobium*, *Bradyrhizobium*, *Agrobacterium*, *Enterobacter* and *Klebsiella* (Shilev 2013).

Likewise, a large number of PGPR such as *Azotobacter* sp., *Rhizobium* sp., *Pantoea agglomerans*, *Rhodospirillum rubrum*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Paenibacillus polymyxa* may produce cytokinins and gibberellins or both which may improve the plant growth and yield attributes of various agriculturally important plants (Kang et al. 2010). The interesting fact is that some strains of phytopathogens can also synthesize the cytokinins; however, PGPR produce lower amount of cytokinins which is plant growth stimulatory in nature. On the other hand, cytokinin produced by plant pathogens is inhibitory due to its higher amount (Ahemad and Kibret 2014).

In addition, a judicious amount of ethylene ameliorates the plant growth and yield components in a remarkable amount (Khalid et al. 2006). This hormone is synthesized and produced endogenously. The level of ethylene is significantly increased in stresses such as salinity, drought, waterlogging, heavy metals and pathogenicity grown plants which becomes later on phytotoxic. The increased levels of ethylene cause defoliations and reductions in other cellular performances which may reduce ultimately the crop-level performance (Saleem et al. 2007). PGPR strain having 1-aminocyclopropane-1-carboxylate (ACC) facilitates plant growth and development by reducing ethylene level and enhancing salt tolerance and minimizing drought stress (Nadeem et al. 2007; Zahir et al. 2008). Currently, some bacterial strain showing ACC deaminase has been identified in a wide range of bacterial species, for example, *Acinetobacter*, *Achromobacter*, *Agrobacterium*, *Alcaligenes*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Pseudomonas*, *Ralstonia*, *Serratia*, *Rhizobium*, etc. (Shaharoona et al. 2007a, b; Nadeem et al. 2007; Zahir et al. 2008, 2009; Kang et al. 2010).

5.4.2 Indirect Mechanisms

Application of PGPR in soilborne disease management is a good approach (Lugtenberg and Kamilova 2009). The biocontrol activity is the major indirect mechanisms of plant growth promotions (Glick 2012). PGPR show the biocontrol activity by creating the atmosphere of food and space competitions, induction of systemic resistance and secretion of antipathogenic metabolites (Lugtenberg and Kamilova 2009). A large number of rhizobacteria are reported to produce antifungal metabolites such as HCN, phenazines, pyrrolnitrin, 2, 4-diacetylphloroglucinol, pyoluteorin, viscosinamide and tensin (Bhattacharyya and Jha 2012). Interaction of some rhizobacteria with the plant root may result in the development of plant resistance against plant pathogenic bacteria, fungi, nematodes and viruses. Interaction of some rhizobacteria with the plant roots can result in plant resistance against some pathogenic bacteria, fungi and viruses (Lugtenberg and Kamilova 2009). The phenomenon is called as induced systemic resistance (ISR) which involves jasmonate and ethylene signalling which after all stimulates plant growth and improves plant health (Glick 2012).

5.5 Applications of PGPR in Plant Health Improvement

Application of PGPR improves plant growth and yield components. However, performance of PGPR varies under laboratory, green house and field conditions. The variations in the PGPR's performance are due to the unpredictable nature of soil under different agroclimatic conditions (Zaidi et al. 2009). PGPR do not work individually but additively consisting of multiple mechanisms like phosphate solubilization, dinitrogen fixation, ACC deaminase and antifungal activity. IAA and iron-chelating molecule (siderophores) syntheses are most important leading to improved plant growth and yield (Bashan and Holguin 1997). The wide-scale application of PGPR may decrease the use of various hazardous chemicals. This may also be a tool which may be easily available to the farmers of developing as well as developed countries (Gamalero et al., 2009). There are some reports which revealed that *P. fluorescens* may be used as potential biological control agents in the management of various economically important soilborne plant pathogens. The *P. fluorescens* may also act as growth and yield enhancer leading to improved plant health (Ansari and Mahmood 2017).

5.6 Conclusion

Application of PGPR exerts a beneficial impact on plant health performance, however, at varying degrees. This variation is seen due to prevailed diverse agroclimatic conditions in the locality. Putative PGPR introduction in the crop yield performance may yield good remunerations. The effectiveness of the strains may highly increase, if application of suitable/compatible PGPR is introduced in the stressed environments. Judicious application of PGPR may cut down the introduction of chemical fertilizers, pesticides and artificial plant growth regulators. Moreover, emphasis on mechanisms of action behind each activity is the need of the hour which may pave the way in plant health management being grown under different stressful environments.

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Chapter 6
Plant Growth Promoting Rhizobacteria (PGPR): Modern Prospects for Sustainable Agriculture

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Abstract Plant and soil microbiome interactions are in the great demand around the globe. Bacteria that colonize in the plant roots or in the rhizosphere and promote plant growth directly by nutrient immobilization or worked as defense regulator are referred to as plant growth-promoting rhizobacteria (PGPR). During the past couple of decades, PGPR have emerged as a potent alternative to chemical fertilizer in an eco-friendly manner. Therefore, they are abundantly accepted in agriculture, horticulture, silviculture, and environmental cleanup strategies. The rhizosphere ecology is influenced by a myriad of abiotic and biotic factors in natural and agricultural soils, and these factors can, in turn, modulate PGPR effects on plant health. Manipulating this rhizospheric microbiome through rhizo-engineering has materialized as a contemporary methodology to decipher the structural, functional, and ecological behavior of rhizospheric PGPR populations. In this chapter, we have

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tried to explore the latest developments in the technologies related to PGPR, for its well acceptance for sustainable agriculture and plant health.

Keywords PGPR · Rhizospheric microbiome · Ecology · Sustainable agriculture · Plant health

6.1 Introduction

6.1.1 Concept and Definition

The soil is a dynamic living matrix, and it is not only an essential resource in agriculture and food security, but it is also toward the maintenance of all the life process. The soil is home to thousands of bacterial species. Root colonizing bacteria (rhizobacteria) that exert beneficial effects on plant development via direct or indirect mechanisms have been defined as plant growth promoting rhizobacteria (PGPR). These root colonizing bacteria (endophytic and epiphytic) have been proven to exert influence on soil security (Ahkami et al. 2017; Wallenstein 2017), seed germination under drought stress (Delshadi et al. 2017), and cleanup strategies (Thijs et al. 2016); antagonize pathogens; decrease plant diseases; enhance plant resistance to diseases, salt stress, coldness, and heavy metal toxicity; and improve crop growth, development, yield, and quality through directly synthesizing hormones, antibiotics, and other secondary metabolites and by regulating plant related gene expressions and others (Gupta and Dikshit 2010; du Jardin 2015; Haymer 2015; Kumary and Raj 2016; Vejan et al. 2016).

6.1.2 Agriculture and PGPR

The role of these PGPR formulations has been well documented this decade to improve crop productivity, plant health, and soil quality as well as in many agricultural crops, vegetable, and fruits (von der Weid et al. 2000; Orhan et al. 2006; Rana et al. 2011; Zhang et al. 2012; Sharma et al. 2014) (Table 6.1). The microbes (PGPR) and rhizosphere have interaction, i.e., rhizo-engineering and other techniques are the recent advances in this sector to meet global food and eco-friendly strategies for green earth/global warming (Haymer 2015; Thijs et al. 2016; Ahkami et al. 2017; Ahmadi et al. 2017; Reeves 2017; Timmusk et al. 2017; Wallenstein 2017). According to the Food and Agriculture Organization (FAO), the estimated world population for 2025 will be nearly 8.5 × 109 inhabitants. Such an increase will inevitably require the substantial additional agricultural production of 2.4 × 109 t/year (Timmusk et al. 2017). The changing climate and overpopulation have led to the crisis of nutrient availability and food security for humans especially in developing countries (Çakmakçi et al. 2007; De et al. 2015; Reeves 2017).

Table 6.1 List of PGPR used in different crops (grain, pulses, fruits, vegetable, and herbs)

PGPR	Crop	Country	Mechanism of action	Significance	References
Azospirrilum spp., *Azoarcus*, *Brevibacterium*, *Bradyrhizobium*, *Burkholderia* sp., *Diazotroph*, *Herbaspirillum*, *Lysinibacillus*, *Pseudomonas* sp., *Rhizobium* spp., *Xylanilyticus*	Rice (*Oryza sativa*)	India, Egypt, Malaysia, Pakistan, Indonesia	Indole acetic acid (IAA) production, overexpression of RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase), resulted in growth promotion activities, siderophore production, nitrogenase activity, phosphate solubilization	Increased yield of rice, few proteins induced growth promotion, seedling vigor enhanced, antibiotic and fungicide resistance induced, drought resistance, increased nutrient uptake, antibiotic, salt and fungicide resistance	Kandasamy et al. (2009), Gopalakrishnan et al. (2012), Hasan et al. (2014), Sharma et al. (2014), Elekhtyar (2015), Tan et al. (2015) and Yuwariah (2017)
Arthrobacter, *Azorhizobium* ORS 571, *Azospirillum brasilense* SP245, *Bacillus* spp., *A. brasilense* sp. 245, *Bacillus* spp., *Bacillus* spp.,[a] *Providencia* spp., *Brevundim pnatas*., *Bacillus subtilis* SU47, *Klebsiella*, *Enterobacter*, *Flavobacterium* sp., *Pseudomonas* spp., *Dietzia natronolimnaea*	Wheat (*Triticum aestivum*)	Germany, Belgium, India, Pakistan, Turkey, Korea, China, Uzbekistan	IAA production, phosphate solubilization, increased nutrient uptake, ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, siderophore activity, nitrogen fixation	Increased yield of wheat, induced growth promotion, quality enhancement, seedling vigor, antibiotic and fungicide resistance, drought resistance, salt tolerance, antioxidant activity	Elliott and Lynch (1985), Egamberdieva (2010), Rana et al. (2011), Zhang et al. (2012), Yandigeri et al. (2012), Abbasi (2015), Hassan et al. (2015), Bharti et al. (2016), Gontia-Mishra et al. (2016), Ahmad et al. (2017) and Qiu et al. (2017)
Bacillus strains	Raspberry (*Rubus idaeus*)	Turkey	IAA production, nitrogenase activity, phosphate solubilization	Increased plant growth and nutrient in raspberry, increased nutrient uptake	Orhan et al. (2006)
Lipoferum, *Azospirillum*, *A. brasilense*, *Pseudomonas putida*, *P. fluorescens*, *Paenibacillus polymyxa*, *P. thivervalensis*, *Serratia marcescens*	Maize *(Zea mays)*	Iran, Pakistan, Brazil, India	IAA production, siderophore, phosphate solubilization, hydrolytic enzymes	Increased plant height and leaf area, increased salt tolerance	von der Weid et al. (2000), Gholami et al. (2009), Nadeem et al. (2009), and Shahzad et al. (2013)

(continued)

Table 6.1 (continued)

PGPR	Crop	Country	Mechanism of action	Significance	References
Agrobacterium rubi, *Burkholderia gladioli*, *Pseudomonas putida*, *Bacillus subtilis*, *Bacillus megaterium*	Mint (*Mentha piperita L.*)	Turkey	IAA production, nitrogenase activity, phosphate solubilization	Increased overall growth and dry mass yield, increased nutrient uptake	Zhang et al. (2004)
Bacillus megaterium TV-3D, *B. megaterium* TV-91C, *Pantoea agglomerans* RK-92, *B. subtilis* TV-17C, *B. megaterium* TV-87A, *B. megaterium* KBA-10	Cauliflower (*Brassica oleracea L.*)	Turkey	IAA production, nitrogenase activity, phosphate solubilization	IAA production, nitrogenase activity, phosphate solubilization, increased nutrient uptake	Ekinci et al. (2014)
Bacillus spp., *Paenibacillus* spp., *Bacillus subtilis*	Pepper (*Piper nigrum*)	Korea, USA, India	IAA production, siderophore activity nitrogenase activity, phosphate solubilization	Antibacterial and antifungal activities were induced, yield in plant growth parameters like fruit quality, etc., increased nutrient uptake, antibiotic, salt and fungicide resistance	Kokalis–Burelle et al. (2002), Paul and Sarma (2006) and Lim and Kim (2013)
Bacillus, *Pseudomonas*, *Rhizobium*, *Azotobacter*	Green gram (*Vigna radiata*)	India	IAA production, siderophore activity nitrogenase activity, phosphate solubilization	Increased plant growth and yield of gram, increased nutrient uptake, antibiotic, biocontrol, salt and fungicide resistance	
Bacillus spp., *Paenibacillus*, PGPR *strains* 90-166, SE34, and C-9	Tobacco (*Nicotiana tabacum*)	China, India, USA	IAA production, siderophore production, nitrogenase activity, phosphate solubilization	Antiviral and overall growth promotion, increased nutrient uptake, antibiotic, salt and viral resistance	Zhang et al. (2004)

Azotobacter chroococcum, *Bacillus subtilis*, *Aeromonas salmonicida*, *Burkholderia cepacia*, *Ochrobactrum anthropi*, *Pseudomonas* sp., *Shewanella putrefaciens*, *Sphingomonas paucimobilis*, *Stenotrophomonas maltophilia*, *Brevibacterium*, *Burkholderia*, *Delftia*, *Leucobacter*, *Pseudomonas*, *Sinorhizobium and Variovorax*	Sugarcane (*Saccharum officinarum*)	India, China, Mexico	Indole acetic acid production, siderophore production, nitrogenase activity, phosphate solubilization	Saline resistance, increased growth, enhance soil properties, nitrogen fixation, increased nutrient uptake, antibiotic, salt resistance	Dhanraj (2013), De et al. (2015), Wang et al. (2016) and Solanki et al. (2017)
Pseudomonas jessenii PS06, *Mesorhizobium ciceri* C-2/2, *Pseudomonas putida*, *Burkholderia*, *Bacillus amyloliquefaciens*	Chickpea (*Cicer arietinum*)	India, China, Spain	IAA production, siderophore production, phosphate solubilization, hydrolytic enzymes	The co-inoculation treatment increased the seed yield and nodule fresh weight, antagonistic against pathogens	Kokalis–Burelle et al. (2002), Paul and Sarma (2006), and Joseph et al. (2012)
Pseudomonas fluorescens japonicum CB 1809, USDA110, *Azospirillum* sp., *Bacillus pumilus*, *Rhizobium japonicum, Azotobacter chroococcum*, *Azospirillum brasilense*	Soyabean (*Glycine max*)	India, Thailand, Myanmar, Argentina, Romania, Iran, Ethiopia, USA	Production of phytohormones (auxins, cytokinins, and gibberellins), N-fixing and phosphate solubilizing, antibiotics production, and antagonism against microbes	Increased plant growth, more nodulation, increased seed protein, water stress tolerance, drought resistance	Aung et al. (2013), Naseri et al. (2013), Masciarelli Llanes and Luna (2014) and Zahedi and Abbasi (2015)
Enterobacter cloacae, Pseudomonas sp., *Bacillus* sp.	Indian mustard and pumpkin	India	Production of phytohormones (auxins, cytokinins, and gibberellins), N-fixing, and phosphate solubilizing	Stimulated plant growth and decreased Cr (VI) content	Ahemad and Kibret (2014)

(continued)

Table 6.1 (continued)

PGPR	Crop	Country	Mechanism of action	Significance	References
Bacillus, *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas fluorescens*, *Serratia marcescens*	Tomato (*Solanum lycopersicum*)	India, AL, Egypt, Brazil	IAA production, siderophore production, nitrogenase activity, phosphate solubilization, antiviral activity	Increased yield and nutrient content, biocontrol of viral pathogens, increased nutrient uptake	Gagné et al. (1993), Murphy et al. (2000), Mena-Violante and Olalde-Portugal (2007), Vinothkumar et al. (2012), Solanki et al. (2012a, b), Agrawal and Agrawal (2013), Almaghrabi et al. (2013), Hyder et al. (2015), Solanki et al. (2015) and Moustaine et al. (2017)
Bacillus amyloliquefaciens, *Bacillus* spp.	Cotton (*Gossypium*)	USA, China, Germany, Iran	Production of phytohormones (auxins, cytokinins, and gibberellins), N-fixing and phosphate solubilizing, antibiotic activity	Overall growth and biocontrol of pathogens	Fahimi et al. (2014)
Pseudomonas fluorescens, *Azotobacter chroococcum* HKN-5, *Bacillus megaterium* HKP-1, *Bacillus mucillaginosus* HKK-1 sp. 4MKS8, *Klebsiella*, *Enterobacter sakazakii* 8MR5, *Pseudomonas oxytoca* 10MKR7	Indian mustard (*Brassica juncea*), Rape seed (*Brassica napus*)	India	Production of phytohormones (auxins, cytokinins, and gibberellins), N-fixing and phosphate solubilizing, antibiotic activity	Promoted the plant growth under chromium stress, protected plant from metal toxicity	Ahemad and Kibret (2014)

Pseudomonas stutzeri, *Bacillus subtilis*, *Stenotrophomonas maltophilia*, *B. amyloliquefaciens*, *P. fluorescens*, *B. megaterium*, *Variovorax paradoxus*, *Stenotrophomonas maltophilia* HW2	Cucumber (*Cucumis sativus*)	India, Pakistan, USA, China	ACC-deaminase activity, siderophore, and IAA production	Enhanced growth, significantly suppressed phytophthora crown rot, increased salt tolerance, antiviral against mottle mosaic virus	Zhang et al. (2004)
Bacillus amyloliquefaciens, *Bacillus megaterium* M3 *and* MIX *(Bacillus subtilis* OSU142, *B. megaterium* M3, *Azospirillum brasilense* Sp245	Barley (*Hordeum vulgare*)	Egypt, Turkey	Biofilm formation	Increased yield, growth of seedlings, salinity tolerance	Kasim et al. (2016)
E. cloacae, *B acillus drentensis*, *Rhizobium*, *B. pumilus* Sol-1, *Alcaligenes sp.* Mal-4, *Providencia vermicola* Ama-2, *Brevundimonas* Kro13, *Kluyvera ascorbata* SUD165, *Pseudomonas putida*, *Ochrobactrum*, *B. cereus*	Mung bean (*Vigna radiata)*	Saudi Arabia, Egypt, Bangladesh, Pakistan, India	N-fixing and phosphate solubilizing, IAA production, acetylene reducing activity, siderophore production, ACC-deaminase activity, nitrogenase activity, phosphate solubilization, increased nutrient uptake	improving gaseous exchange, water relations, photosynthetic pigments, growth, and seed yield for mung bean under saline irrigation conditions, stimulated the plant growth reduced Pb and Cd uptake, lowers the toxicity of chromium to seedlings by reducing Cr (VI) to Cr (III)	Akhtar and Ali (2011) and Mahmood et al. (2016)

(continued)

Table 6.1 (continued)

PGPR	Crop	Country	Mechanism of action	Significance	References
Pseudomonas putida BA-8, *Bacillus simplex* T7	Grapes (*Vitaceae family*)	Turkey	IAA production, N- fixing and phosphate solubilizing, antibiotic activity	Improvement in the grafting capacity at nursery conditions	Sabir (2013)
Pseudomonas BA-8, *Bacillus* OSU-142, *Agrobacterium rubi* A-18, *Burkholderia gladioli* OSU-7, *Pseudomonas putida* BA-8	Apple (*Malus domestica*)	Turkey, Brazil	Production of phytohormones (auxins, cytokinins, and gibberellins), N-fixing and phosphate solubilizing, antibiotic activity	Increased fruit quality, nutrient enhancement, biocontrol agent, antagonistic activity	Haden et al. (2007) and Karakurt and Aslantas (2010)

[a]Reported first time as PGPR in particular crop

6.2 Mode of Action

Plant roots exude a huge diversity of organic nutrients (organic acids, phytosiderophores, sugars, vitamins, amino acids, nucleosides, mucilage) and signals that attract microbial populations, especially those able to metabolize plant-exuded compounds and proliferate in this microbial habitat (Ahemad and Kibret 2014; Hasan et al. 2014). The rhizospheric soil bacteria which surrounds the plant root competes for this nutritional boon and in turn the effect plant's growth, yield, and defense mechanisms either as free living microbes or in the mutualistic relationship with plant root (Endophytic/epiphytic) (Vejan et al. 2016). These rhizobacteria affect plant development. About 2–5% of rhizobacteria, when reintroduced by plant inoculation in a soil containing competitive microflora, exert a beneficial effect on plant growth and are termed plant growth promoting rhizobacteria (PGPR).

The mode of action of PGPR is mainly of two types: the direct mechanism which directly supports the plant growth in a direct mode. This mechanism includes nitrogen fixation, phytohormone production, phosphate solubilization, and increasing iron availability used for plant growth promotion. PGPR can indirectly enhance plant growth by eliminating pathogens or by inducing plant defense responses (Narasimhan et al. 2003; Gupta and Dikshit 2010; Haymer 2015; Thijs et al. 2016; Delshadi et al. 2017; Reeves 2017; Tariq et al. 2017; Timmusk et al. 2017).

6.3 Recent Developments in the Application of PGPR

6.3.1 Role of PGPR as Biostimulant

According to European Commission, agroecology, i.e., studying and designing agricultural systems based on the interaction of their biophysical, technical, and socioeconomic components, is recommended to meet the food security of increasing population and to maintain soil security. The word biostimulant was apparently coined by horticulture specialists for describing substances promoting plant growth without being nutrients, soil improvers, or pesticides (du Jardin 2015). PGPR-based biostimulants are widely accepted in agricultural practice this decade (Brown and Saa 2015). According to *Global Biostimulant Strategic Business report 2016–2022*, there are more than 80 global companies involved in biostimulant production and manufacturing covering Canada, Japan, Europe, Asia Pacific, Latin America, and the rest of the world (Novozymes, Monsanto, Lallemand, IIsa sPA etc). According to a report, the biostimulant market is projected to reach USD 2.91 billion by 2021, at a CAGR of 10.4% from 2016 to 2021.

PGPR based biostimulants enhance nutrient uptake and stress tolerance like drought, salinity, etc. and improve crop quality by direct or indirect mechanisms (Brown and Saa 2015; du Jardin 2015). There are many registered formulations of PGPR in the market including the species *Pseudomonas*, *Bacillus*, *Enterobacter*,

Klebsiella, *Azobacter*, *Variovorax*, *Azosprillum*, and *Serratia* (Nakkeeran et al. 2006; Barea 2015; Bishnoi 2015; FAO 2016; Fixers and Solubilizers 2016; Le Mire et al. 2016), but the utilization of PGPR in the agriculture industry represents only a small fraction of agricultural practice worldwide (Meena et al. 2016).

6.3.2 Cleanup Strategies (Role in Phytoremediation)

The green technology to improve the contaminated soil involves mutual interactions of plant and microorganisms. Phytoremediation is an environmentally sustainable, solar-powered, and cost-effective soil remediation technology which relies on the ability of plants to intercept, take-up, accumulate, sequestrate, stabilize, or translocate contaminants. Phytoremediation is influenced by various abiotic and biotic conditions like pH of soil, soil components, nutrient availability, type of plant selection, and type of contaminants (Thijs et al. 2016). Recently, it has been documented that phytoremediation success rate is highly dependable on plant microbiome (Hou et al. 2015). When PGPR are introduced to a contaminated site, they increase the potential for plants that grow there to sequester heavy metals and to recycle nutrients, maintain soil structure, detoxify chemicals, and control diseases and pests; PGPR also decreases the toxicity of metals by changing their bioavailability in plants. The plants, in turn, provide the microorganisms with root exudates such as free amino acids, proteins, carbohydrates, alcohols, vitamins, and hormones, which are important sources of their nutrition (Tak et al. 2013). Biological application of PGPR for phytoremediation of heavy metals and salt-impacted soil has been reported by researchers (Nakkeeran et al. 2006; Barea 2015; Le Mire et al. 2016). Plant and microbiome interactions are nowadays being studied as the metaorganism approach, to find most promising ways to improve the success rate of phytoremediations. PGPR-based metaorganism approach assembles the role of (a) plant host selection, (b) root exudates, (c) study of single or microbial consortium in situ, and (d) molecular study of PGPR strains (Narasimhan et al. 2003; Arora 2015; Thijs et al. 2016).

6.3.3 As Biocontrol

According to Beattie, 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 (Beneduzi et al. 2012). The major disadvantage of chemical pesticides is its residual persistence in the soil which raises food safety concerns among the consumers. In recent years, PGPR-based biocontrol agent has proven its ecologically sound and effective solution to Integrated Pest management Programs (IPM) with so many beneficial advantages like cost-effectiveness, biodegradability and self-perpetuating, host specific, easy in

handling, and safe to use (Beneduzi et al. 2012). The PGPR synthesis hydrolytic enzymes, increases competition for nutrients, regulates the plant hormone ethylene level through ACC-deaminase enzyme, and produces siderophores to counteract the plant pathogens present surrounding the rhizosphere (Kumari et al. 2016; Yang et al. 2009; Haghighi et al. 2011; Anand et al. 2016; Le Mire et al. 2016). There are many examples of effective control of soil-borne diseases by means of PGPR (Haas and Defago 2005). Several species have been reported to show antagonistic activity in major crops like wheat, tomato, soya bean, tobacco, pepper, etc. (Zhang et al. 2004; Haas and Defago 2005; Domenech et al. 2006; Gupta and Dikshit 2010). There are a large number of biocontrol agents available in the international market (Bio-Save®, RhizoVital ® 42 liquid, Galltrol-A, BlightBan C9-1 etc.), but currently, the scenario is not good as only 7% of total biocontrol formulation made per year is reaching in the hand of farmers. According to the international bio-intelligence reports (2017), global biocontrol market is $2.8 Bn today growing to over $11 Bn in 2025. It is estimated that microbial will continue to make up nearly 60% of the market through 2025. North America and Europe itself will cover 2/3 part of the whole biocontrol international market. The drastic climatic changes have affected the plant microbe interactions in the recent decade; this is one of the most challenging aspects in studying PGPR strains for the formulation as biocontrol agents (Reeves 2017). Recently, researchers around the globe are focusing toward implementation of new technologies for the development of effective biocontrol agents. The latest applications of molecular genetic technologies in the area of genetically based control methods now also include cutting-edge systems for genome editing and the use of RNA inhibition for selectively knocking out the expression of individual genes (Haymer 2015). Nanomaterial-based biocontrol has also proven its impact as upcoming biocontrol agents in years.

6.4 Current Scenario of PGPR Research

6.4.1 Challenges

The role of PGPR based bio-formulations has shown great potential toward sustainable agriculture and the most accepted alternative to chemical fertilizers, biopesticide/biocontrol agents, and other chemical-based simulators. During the past couple of decades, PGPR have begun to replace the use of chemicals in agriculture, horticulture, silviculture, and environmental cleanup strategies. They have the positive impact of plant's physiological conditions through the mechanism of action of these microbes. During the years 1990–2000, most of the researches on PGPR was based on the isolation and inoculation of PGPR into rhizosphere to get better yield in crops (wheat, rice, maize some vegetables, fruits, and herbs), some reports are available about the molecular mechanism of action of these microbes; in the later decade, biotechnological approach to modify isolated PGPR was also reported (Gagné et al.

1993; Murphy et al. 2000; von der Weid et al. 2000). During the decade 2000–2010, researchers were more focused on the application part, i.e., cleanup strategies, as defense inducer and as biofertilizer mainly, during this phase few of commercial product of PGPR came into international market (Zhang et al. 2004; Haden et al. 2007; Gupta and Dikshit 2010). Recently, 2010 onward, a new term "rhizo-engineering" has been introduced to uncover the microbiome interaction that is still not clearly elucidated (Ahmadi et al. 2017). The use of nanoparticle in PGPR research has also shown a promising technology, but cost-effective and quality nano-product is still expected (Delshadi et al. 2017; Reeves 2017). 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 (Vejan et al. 2016). Majority of researches are confined either to laboratory or green house scale; hence these should be taken up to the field level. However, there are few reports on transition of PGPR-based bioformulations, but has limited success rate (Gagné et al. 1993; Murphy et al. 2000; von der Weid et al. 2000). Another major challenge in the application of this microbial product's application is the screening of microbes, their formulation, and its marketing. Researches have to trigger the following aspects to accelerate the PGPR commercialization (Fig. 6.1).

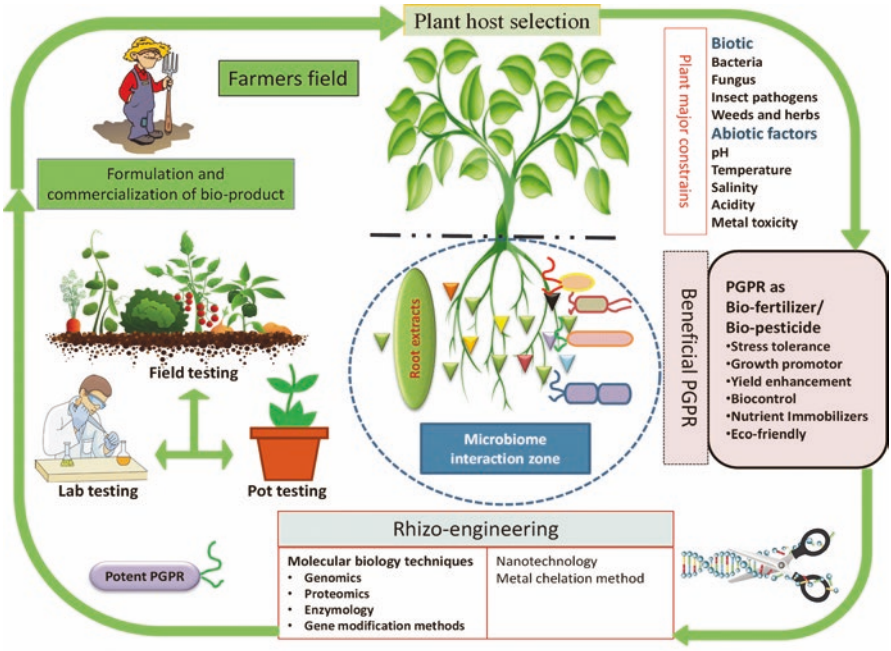

Fig. 6.1 Different challenges and applications of PGPR research

6.4.2 Future Work Should Be Focused On

6.4.2.1 At Laboratory and Field Level

- Understanding of microbiome interactions especially their diversity.
- Molecular data availability.
- Study on the effect of environmental stresses on microbiome and the mechanism of action.
- Application of recent technologies like rhizo-engineering, nanotechnology, and metaproteomics to get the most efficient and eco-friendly formulations.
- However, the approaches focused for a long time on each organism individually rather than an integrated metaorganism approach in an ecological perspective.
- The formulation is also an important parameter to be focused in the coming years, like the type of formulation and their acceptance at physiological and ecological level.
- Field level experiments to be taken up at large scale.
- The addition of ice-nucleating plant growth-promoting rhizobacteria could be an effective technology for enhancing plant growth at low temperature.

6.4.2.2 For Commercialization

- Cost-effective products with good shelf life.
- Eco-friendly.
- Safety database availability for easy registration process.
- Farmers need more knowledge about this product: like why it is better than chemical fertilizers because beneficial effects attract farmers' interest.
- Changing farmer perception may bring about the change.
- The farmers and feild person must have been trainted about PGPR bioformulations, its advantages and of-course economical acceptibility.
- Growth in commercialization is hindered by lack of thorough research so the transition of laboratory work to the farmers of field is a must.

In the near future, it is expected that metatranscriptomics and metaproteomics will develop significantly and will allow further progress in the understanding of the activity and ecological behavior of natural PGPR populations within the rhizosphere.

6.5 Conclusions

The campaign for the application of PGPR has been started from the last few decades, to achieve sustainable agriculture and plant's health under biotic and abiotic stress. However, before PGPR can contribute the desired benefits, scientists

need to learn more and explore ways and means for their better utilization in the farmers' fields. Future research should focus on managing plant-microbe interactions, for example, innovative improvements in root environments, particularly with respect to their mode of action and adaptability to conditions under extreme environments. Rhizo-engineering and metatranscriptomics use of safest nanoparticle to introduce new formulation and screening of bacterial strains through molecular techniques like proteomics and docking methods will be the focused area of researchers in the coming years. Another major aspect is the transition of this product in the hand of local farmers, which will depend on easy registration regulatory processes.

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Chapter 7
Biocontrol Potential of *Trichoderma* spp.: Current Understandings and Future Outlooks on Molecular Techniques

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Abstract *Trichoderma* species are ubiquitous ascomycetous fungi that have a wide distribution in diverse ecological zones and display remarkable interactions with other microbes and plants in the rhizosphere. Biotic stress is raised as a major problem in front of the agricultural economist. In this context, *Trichoderma* strain-based biocontrol practices could help to achieve the goal of sustainable agriculture. Modern biotechnological tool-based analysis locks out the inherent information of *Trichoderma* persistence in extreme conditions. Advance biotechnological tools have been developed to map the genome and transcriptome of *Trichoderma* spp. that will unlock the information of novel genes and their significant role in disease protection, abiotic stress tolerance, and plant growth promotion. In the present chapter, we are discussing the molecular mechanisms of *Trichoderma* that helps the plant in growth promotion as well as pathogen defense.

Keywords Biocontrol application · Molecular prospectus · Plant diseases · *Trichoderma*

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7.1 Introduction

Despite the successes of modern agriculture, there are cultural practices that face challenges in crop production: shortage of essential resources, the destructive potential of biotic stimuli, and use of excess amount of pesticides. These agricultural practices are also affected by climatic changes, pathogen reproduction, dispersion, persistence, and pathogenicity (Pautasso et al. 2012; Bebber 2015). To improve the crop productivity, quality chemical fertilizers are used extensively that damaged the ecological systems and human health (Harman et al. 2004). Nowadays, there is a true interest in the development of alternative ways to control the diseases. In this context, modern biological methods are applied with the conventional agricultural practices by utilizing the bio-pesticides and bio-fertilizers. Several researchers conveyed that biotic stimuli are the principal cause for deterioration of different crops' yield, which causes 10–50% annual yield losses (Sharma et al. 2017; Zelicourt et al. 2016; Fita et al. 2015). Nowadays, wide ranges of biocontrol, mainly bacteria and fungi that counteract important agronomical pests and diseases, have been used for sustainable agriculture.

Plant disease management is a process of one or more organisms (natural enemies, competitors, and antagonist) or part of them to inhibit the development of another organism, or their disease causing effects on the crops. Biocontrol agents mostly mixed or primed to the seeds or soil before the plantation may colonize in the root sounding rhizosphere of plant, and therefore they are able to protect the plant from the borne phytopathogens. These biocontrol agents may participate in a variety of trophic (food webs) and nontrophic (such as antagonism, amensalism, competition, commensalism, mutualism, and neutralism) interactions including host parasitism, antibiosis, cross-protection, microbial compound production, predation, competition for site and nutrient, and stimulation of diseases tolerance and plant growth regulation (Heydari and Pessarakli 2010; Shoresh et al. 2010). Currently, mycoparasitic *Trichoderma* and *Verticillium* species (Harman 2006; Fenice et al. 1998) and *Pseudomonas*, *Bacillus*, *Lecanicillium*, and *Streptomyces* are frequently described as potential disease protector agents against the biotic pathogens (Fenice and Gooday 2006; Solanki et al. 2012a, b, 2014, 2015, 2016, 2017; Yandigeri et al. 2012; Patil and Solanki 2016; Wang et al. 2016), *Lecanicillium* species. Fungal species against pathogens have received considerable research as potential biocontrol agents in many crops; one of the most well-studied fungal genera *Trichoderma*/*Hypocrea* has been applied as a biocontrol agent against several pathogens (Herrera-Estrella and Chet 2003).

Trichoderma spp. have gained significant attention as plant disease protector against different plant pathogens (Harman et al. 2004; Verma et al. 2007). The filamentous fungi *Trichoderma* (*Ascomycetes*, *Hypocreales*) are saprophytic microorganisms, ubiquitously diverse soilborne species that commonly exist on litter and manure or in soil (Harman et al. 2004; Samuels 2006). In this context, *Trichoderma* species is a well known fungi for the mycoparasitism, antagonism against pathogens for food and space, plant growth stimulator, or a plant disease protector thru

induce systemic resistance (Harman et al. 2004; Bailey et al. 2006; Błaszczyk et al. 2011; Kubicek et al. 2001; López-Bucio et al. 2015). Mycoparasitic *Trichoderma* directly grows toward the host and forms coiling to the host hyphae. Then, it secretes mycolytic enzymes, penetrates the host, and utilizes host cellular components as a nutrient (Viterbo et al. 2002). *Trichoderma* secreted mycolytic enzymes such as Chitinases, β -1,3-glucanases, cellulose, and protease as a weapon against the host to break down the cell wall polysaccharides (Kubicek et al. 2001; Benitez et al. 2004; Harman et al. 2004; Solanki et al. 2011; Rai et al. 2016a). *Trichoderma* species have secreted antibacterial or antifungal metabolites (Kubicek and Penttila 1998; Sivasithamparam and Ghisalberti 1998), peptaibiotics, and toxins such as trichodermamides (Garo et al. 2003; Liu et al. 2005; Nielsen et al. 2005; Degenkolb et al. 2008). In addition, *Trichoderma* species are playing an important part in reducing the toxicity of heavy metals, pesticides, oil spills, etc. (Anand et al. 2006; Yazdani et al. 2010).

To unlock the molecular networks of mycoparasitism, plant defenses activation against pathogens or plant growth regulation in stress condition (Benitez et al. 2004; Harman 2011). Currently, genome and transcriptome sequencing of seven *Trichoderma* species have delivered essential insights into the molecular machinery of this genus that included several biocontrol genes and biochemical and physiological pathways that facilitate biotechnologist for the improvement of plant transgenics (Baroncelli et al. 2016; Rai et al. 2016b; Lorito et al. 2010). Many mycoparasitic, hydrolytic, and elicitor genes are expressed before and during colonization/contact with the plant/pathogen in different *Trichoderma* spp. that encode the protein, enzymes, elicitors, and oligopeptide transporters (Seidl et al. 2009; Suárez et al. 2007; Siddiquee et al. 2012). Hence, most of the *Trichoderma* genes (Table 7.1) are significantly overexpressed in plants to mitigate the disease susceptibility and better plant health management, so that transgenic plants are more valuable and economic (Nicolás et al. 2014). Briefly, the present chapter describes the silent feature of *Trichoderma* species, regarding their skill to antagonize pathogens, stimulate plant growth, and defense responses and molecular mechanism involved in it.

7.2 Multifarious Biocontrol Mechanisms of *Trichoderma* spp.

Biological control is a process to manage plant diseases, which has benefits to plants; nonbeneficial microbes interact with one other for food and space, and it could have occurred via several mechanisms like antibiosis, mycoparasitism, nutrient and space competition, and stimulation of plant defense (Pal and Gardener 2006). As a whole, the current literature adopted *Trichoderma* spp. as biocontrol agents, and Coley-Smith et al. (1974) decipher the first report on the process by which microtome sections have shown that chlamydospores of *T. hamatum* colonized inside the sclerotia of *Sclerotium delphinii*. Likewise, Henis et al. (1984) also reported chlamydospores of *T. hamatum* colonized inside the mycelium and sclerotia of *Sclerotium rolfsii*.

Table 7.1 Mycoparasitic genes of *Trichoderma* species involved in biocontrol mechanism and their functions

Mycoparasitic gene	Genes encoded	*Trichoderma* spp.	Function of hydrolytic gene	Effect of gene in biocontrol	References
prb1 and ech42 genes	Proteinase and endochitinase	*T. harzianum* strain IMI206040	Parasitic activity against *Sclerotium rolfsii* and *Rhizoctonia solani*	Expression of this gene helps in regulation of hydrolytic enzymes	Cortes et al. (1998)
gluc78 gene	1,3-β-glucosidase	*T. atroviride* strain P1 (ATCC 74058)	Cell wall degradation of pathogens *Pythium* and *Phytophthora*	Exhibits moderate biocontrol activity.	Donzelli et al. (2001)
Xyl gene	Xylanase gene	*Trichoderma* strain SY	Helps in breakdown of hemicellulose	Cell wall degradation of the pathogens	Min et al. (2002)
β-1-6-gla gene	Endo-β-1-6-glactanase	*T. viride* IFO31137	A type of arabinogalactan proteins that involves in cell-cell adhesion, expansion and cell death	Expression of gene enhances the production of proteins.	Kotake et al. (2004)
Tvsp1 gene	Serine protease	*T. virens*	Pathogen cell wall degrading enzyme	Pathogen cell wall destruction	Pozo et al. (2004)
creI gene	Glucose repressor	*T. harzianum*	Repression of cellulase and xylanase	Cell wall degradation of the pathogens	Saadia et al. (2008)
TvBgn2 and TvBgn3 genes	β-1,3 and β-1,6 glucanase	*T. virens* Gv29-8	Secrete cell wall degrading enzyme	Cell wall degradation of the pathogens shows enhanced biocontrol activity	Djonovic et al. (2007)
SL41 gene	Serine protease	*T. harzianum*	Secrete cytoplasmic material degrading enzyme	Shows enhanced biocontrol activity	Liu et al. (2009)
ThPG1 gene	Endopolygalacturonase	*T. harzianum*	Secrete cell wall degrading enzyme	Shows enhanced biocontrol activity	MoranDiez et al. (2009)

Th-Chit gene	Chitinase	*T. harzianum*	Antifungal activity in transgenic tobacco	Cell wall degradation of the pathogens shows enhanced biocontrol activity	Saiprasad et al. (2009)
Tag 3 gene	–	*T. asperellum*	Production of cell wall degrading enzyme glucanase	Shows significant biocontrol Activity	Marcello et al. (2010)
exc1 and exc2 gene, chit42 and chit33 gene, prb1 gene and bgn13.1 gene	NAGases, chitinases, proteases and b-glucanases	*T. harzianum* CECT 2413	Mycoparasitic activity against *F. oxysporum*	Expression of this gene helps in regulation of hydrolytic enzymes	LopezMondejar et al. (2011)

7.2.1 Mycoparasitism

The antagonistic interaction is established between two fungal species, where one fungus can attack step by step: (1) recognition of the host, (2) attachment and penetration, and (3) killing the host by antimicrobial compounds or enzymes (Druzhinina et al. 2011; Howell 2003). *Trichoderma* spp. have been effectively studied to control phytopathogens that attack agronomic and economically important crops. On the basis of parasitic aggressiveness of *Trichoderma* toward its host, these antagonistic interactions start from the host attachment, and finally the host is utilized as a feed of the antagonist. Consequently, these mycoparasites cannot be successfully used as biological control agents. Necrotrophic interactions are the second class of mycoparasitic relationships where hosts are more aggressive toward their preys. *Trichoderma* species exercise necrotrophic mycoparasitism, so they are considered as effective biocontrol agents due to their antagonistic capacities against a broad range of phytopathogens. The mechanisms employed by *Trichoderma* are very complex, which involve chemotrophic recognition and growth toward pathogen, the direct contact, and coiling around the pathogen. The remote sensing is revealed that during the degradation of host cell wall, different CWDEs played sequential expression as per the cell wall structure. Table 7.1 represents the example of different mycoparasitic *Trichoderma* strains that enhanced the plant resistance by the expression of expressed sequence tags (ESTs) in the transcriptomic analysis and genomic sequences of *Trichoderma* grown under biocontrol conditions, and by using these genes, genetic engineers accelerated stress tolerance of crops for sustainable agriculture (Suárez et al. 2007; Bhatnagar-Mathur et al. 2008; Gill et al. 2013.

7.2.2 Sensing of Pathogen and Morphological Changes

Mycoparasitism is known as the foremost antagonistic tool of *Trichoderma* spp. (Kubicek et al. 2001). The pathogen releases small molecules and peptides which are recognized by *Trichoderma*, and in response proteases secreted before contact to the pathogen and sequential mechanism started, viz., coiling, penetrate into the cell wall by different lytic enzymes and secretion of microbial compounds (Viterbo et al. 2002). The signaling cascade of *Trichoderma* that includes G proteins and mitogen-activated protein kinases (MAPKs) is elicited when pathogen secreted small molecules which bind to nitrogen-sensing receptors or G protein-coupled receptors (such as Gpr1) that are located on the *Trichoderma* hyphae and ultimately sets of transcription factors (TFs) are activated. Then, these factors stimulated the CWDEs and secondary metabolites synthesis genes (McIntyre et al. 2004; Druzhinina et al. 2011). The most apparent morphological changes are already discussed before in the mycoparasitism section. In brief, pathogen cell wall carbohydrates (lectins) help the *Trichoderma* hyphae in attachment and coiling, and then *Trichoderma* forms the appressoria that penetrate inside the host pathogen by

mycolytic enzymes and peptaibols (Howell 2003) that disrupt the host lumen to enter *Trichoderma* hypha. Recent studies have demonstrated that *T. harzianum* coiling is enhanced by the low concentration of pachybasin and emodin to the *Rhizoctonia solani* hyphae and nylon fibers. Moreover, a close contact of *Trichoderma* host encouraged the accumulation of cAMP in *Trichoderma.* However, atropine application inhibited the cAMP accumulation that negatively affects *T. harzianum* coiling around nylon fibers even in the presence of pachybasin and emodin; hence, these experiments revealed that these molecules have involvement in *Trichoderma* mycoparasitism via cAMP signaling (Zeilinger et al. 2005; Lin et al. 2012).

7.2.3 Role of Hydrolytic Enzymes

Hydrolytic enzyme-based mycotrophism is a well-known mechanism of *Trichoderma*, and these enzymes such as chitinase, β-(1, 4)-, β-(1, 3), and β-(1, 6)-glucanases, N-acetylglucosaminidase, and protease played important role in antagonism and are frequently detected in the *Trichoderma* genome (Table 7.1). Gruber and Seidl-Seiboth (2012) reported some interesting facts of CWDEs that they are not only involved in the mycoparasitism or host cell wall degradation to extract the nutrients but have also recycled during aging and remodeling the hyphal branches by autolysis. Whole genome mapping analysis described the gene pools that encoded CWDEs in the *Trichoderma* spp., and it provided depth of complex enzymatic degradation mechanism (Kubicek et al. 2011).

7.2.3.1 Chitinases

Fungal cell wall contains the chitin a β-(1, 4)-linked N-acetylglucosamine polymer and β-(1, 3)-glucan (Latge 2007). Several researchers revealed the critical role of chitinase in mycoparasitism mediated by *Trichoderma* (Yang et al. 2009). The hydrolytic activity of *Trichoderma* is driven by the several enzymes, and among them chitinase is the key enzyme, and several genes reported the involvement of different kinds of chitinases during the antagonism of *Trichoderma*. The endochitinases, exochitinases, and 1, 4-β-acetylglucosaminidases (GlcNAcases) are the three types of chitinases that are commonly reported from *Trichoderma*. Harman et al. (2004) and Kim et al. (2002) have reported different kinds of GlcNAcases from *T. harzianum* T25-1, *T. atroviride* P1, and *T. virens* Tv29-8 species that also contain their genes-*exc1* (=*nag1*), *exc2*, *tvnag1*, and *tvnag2*. In addition, *chit33*, *chit37*, and *chit42* genes were cloned by the *Trichoderma* strain 2413 that expressed the extracellular endochitinases. Carsolio et al. (1999) also reported chitinase coding genes such as *Chit42*, chitinase-*ech42*, *cht42*, and *ThEn4* from the *T. atroviride* IMI206040, and Howell (2003) has cloned gene *Gv2908* from the *T. atroviride* P1. Moreover, Harman et al. (2004) discussed that chitinase genes *Chit37* and *Chit36* of *T.*

harzianum TM have 89% similarity at the amino acid level and Chit36 played important role in inhibition of the growth of *B. cinerea*, *Fusarium oxysporum*, and *Sclerotium rolfsii* (Viterbo et al. 2002).

7.2.3.2 Glucanases

After chitin, β-1, 3-glucan is the second major polymer of fungal cell walls (Latge 2007), and *Trichoderma* spp. genome contain β-1, 3-glucanase genes that hydrolyzed the β-1, 3-glucan, and compared to other fungi, *Trichoderma* genome had a higher number of glucanases (Kubicek et al. 2011). The available transcriptomic data revealed the significance of β-1, 6-glucanases during antagonism between *Trichoderma* and host pathogen, and a biocontrol strain *T.* cf. *harzianum* CECT 2413 has showed overexpression of the β-1, 6-glucanase Bgn16.3 to inhibit the growth of different plant pathogens such as *Phytophthora citrophthora*, *B. cinerea*, and *R. solani* (Djonovic et al. 2006; Montero et al. 2007; Ihrmark et al. 2010; Dubey et al. 2011). The *Trichoderma* mostly inhibits the pathogen spore germination by antibiosis through a cocktail of β-1, 3-glucanases, chitinases (Benítez et al. 1998; El-Katatny et al. 2001), and antibiotics (Harman et al. 2004; Howell 2003). Among all β-1, 3-glucanase genes, Benítez et al. (1998) cloned *bgn13.1* gene, and Cohen-Kupiec et al. (1999) cloned *lam1.3* gene from *T. harzianum*, Donzelli et al. (2001) cloned *glu78* gene from *T. atroviride*, and Kim et al. (2002) cloned two genes such as *Tv-bgn1* and *Tv-bgn2* from *T. virens*. However, cellulases (β-1,4-glucanases) are also important component of fungal and plant cell wall, and as compared to cellobiohydrolases, endoglucanase (*egl1*, *egl2*) and β-glucosidase cellulases have not been explored for plant disease management (Bartnicki-García; 1968; Benítez et al. 1998; Cruz et al. 1995; El-Katatny et al. 2001). Romao-Dumaresq et al. (2012) demonstrated the significance of N-acetylglucosaminidase encoding gene that not only enhances the mycoparasitism and defense mechanism but also *Trichoderma*-plant symbiosis.

7.2.3.3 Proteases

Trichoderma proteases also played a significant role in mycotrophism and destruction of host cell wall; distribution of proteases varied as per the species such as *T. reesei* which have more proteases as compared to the *T. atroviride* and *T. virens*. Among the proteases, subtilisin-like proteases' dominance enhanced the mycotrophism. Moreover, during the *Trichoderma* interaction with pathogen aspartyl protease, proteases such as *PRB1* played important role to enhance the mycoparasitism (Geremia et al. 1993; Viterbo et al. 2005; Szekeres et al. 2004; Suárez et al. 2007; Seidl et al. 2009). Additionally, due to nitrogen limitation among *Trichoderma*, host pathogen-derived peptides work as sensors for the *Trichoderma*, and these kinds of peptides have been described (Delgado-Jarana et al. 2002; Howell 2003), and Elad et al. (2000) reported protease *Pra1* that helps the *T. harzianum* in the

attachment to the pathogen and degrade the cell wall. For example, Benítez et al. (1998) reported alkaline protease *Prb1* from *T. harzianum* IMI 206040 played an important role in biological control, and when this gene (*prb1*) transformed in *Trichoderma* strains, the biocontrol efficacy of transformed strains enhanced five-fold against *R. solani* as compared to the wild type. Pozo et al. (2004) also discussed that an extracellular serine protease (*tvsp1*) gene of *T. virens* has been cloned, and this gene also overexpressed during the biocontrol of *R. solani* root rot of cotton seedlings. Antal et al. (2000) reported that chitinases, β-glucosidases, and trypsin-like proteases producing strains can also antagonize against the pathogen in low-temperature zones.

7.2.4 Signal Transduction in Mycoparasitism

The mitogen activated protein kinase (MAPK) pathways are a major and conserved signaling pathway of the fungal system. Basically, complete genome analysis of *Trichoderma* spp. revealed the information about the cascade that encodes MAPKs. In *Trichoderma* spp., three MAPKs are involved in the regulation of several genetic and biochemical approaches: the pathogenicity-regulated MAPK (TmkAor Tvk1 and Tmk1), the cell integrity kinase (TmkB), and the osmoregulatory MAPK (Hog1). To better understand the application, MAPKs can demonstrate either by deleting or blocking expression of candidate gene; tmkA deletion in a "P" strain of *T. virens* ("P" strains secretes gliovirin) showed negative impact on the antagonistic activity of *Trichoderma* against *S. rolfsii* (Mukherjee et al. 2003; Viterbo et al. 2005); on the contrary, Mendoza-Mendoza et al. (2007) reported that deletion of tmkA in *T. virens* "Q" strain ("Q" strains produces gliotoxin at high quantity) showed positive influence of antagonism against the two pathogens *R. solani* and *Pythium ultimum*. Similarly, Reithner et al. (2007) reported that deletion of tmk1 homologue in *T. atroviridis* enhances the production of mycolytic enzymes, viz., chitinase and antifungal compounds, as a result of reduction in mycoparasitism against the *R. solani*. The rest of other two MAPKs, TmkB and Hog1, are less studied because, these mutants are growing very slow; hence, *T. virens* mutants of TmkB showed significant reduction of mycoparasitism against *S. rolfsii* and *T. atroviridis* mutants of Hog1 which showed losses in mycoparasitic ability (Reithner et al. 2007; Kumar et al. 2010). Another essential protein that involves in signal transduction is G protein-coupled receptors (GPCRs) involved in pathogen sensing. The G protein signaling cascade that contains three Gα subunits, one Gβ subunit, and one Gγ subunit, as well as the second messenger cAMP (cyclic adenosine monophosphate), has been associated with pathogenicity (Li and Yang 2007). The hypothesis was demonstrated at the molecular level by Rocha-Ramírez et al. (2002) that the G-α gene (*tga1*) has been expressed and controlled by own promoter or promoter of the basic proteinase *prb1* in *T. atroviride*. Transformed *T. viride* strain with *tga1* showed enhancement in coiling on nitrogen, and it also enhanced the mycoparasitism against the *R. solani* (Benitez et al. 2004). The previous investigation showed that

cAMP and MAPK such as G-α played a significant role during mycoparasitism by the production of extracellular enzymes and antibiotics and coiled around the host hypha (McIntyre et al. 2004). Molecular analysis of a class III G protein indicated its participation in the morphological change process during the *Trichoderma*-host interaction. Mutants affected in the corresponding gene (*tga3*) contained low cAMP intracellular levels and were unable to develop contact areas and coil around the host hyphae (Zeilinger et al. 2005). Recent research on silencing of gpr1 gene (encoding cAMP-receptor protein) reduces levels of intracellular cAMP and protein kinase activity in *T. atroviride*; as a result *Trichoderma* loses the pathogen detection, antagonism, and mycoparasitism abilities (Omann et al. 2012).

7.2.5 Detoxification Through ROS-Signal Transduction

Detoxification of plant cells and oxidative stress management during plant-pathogen interaction also played a significant role in plant disease management by *Trichoderma* that induce the genes of secondary metabolites and reactive oxygen species (ROS) during the interaction. The ROS are generated during oxidation of L-amino acid by the action of L-amino acid oxidase that forms α-oxoacid, H_2O_2, and ammonia. Several previous reports indicated that radical oxygen species used as signaling molecules by *R. solani* during sclerotia formation process released antifungal metabolites, and the synergistic actions of radical oxygen species and antifungal metabolites may elicit the stress response that is observed by *Trichoderma* spp. (Papapostolou and Georgiou 2010; Aliferis and Jabaji 2010). On the other hand, NOX (NADPH oxidases) also produce reactive oxygen species as defense response as well as a vital role in the development of sexual structures and appressoria in fungi. The overexpression of the *nox1* gene in *Trichoderma* spp. showed an increased hydrolytic pattern and ROS production during interaction with *Pythium ultimum* (Montero-Barrientos et al. 2010).

7.2.6 Antibiosis (Secondary Metabolites Involved in the Biocontrol)

Antibiosis is an interaction between pathogen antagonist and pathogen via low-molecular weight diffusible organic compounds or antibiotics. Most of the *Trichoderma* species produce volatile and non-volatile toxic metabolites that hinder colonization by antagonized microorganisms, in which several different metabolites, such as 6-pentyl-α-pyrone, alamethicins, antibiotics, gliovirin, glisoprenins, harzianic acid, heptelidic acid, massoilactone, peptaibols, tricholin, viridian, etc. are involved for antimicrobial activity (Vey et al. 2001; Reino et al. 2008). Over the years, numerous *Trichoderma* spp. have been studied for the production of

intracellular siderophores, and a large number of peptaibols contain non-standard amino acids which are responsible for storage of iron and protection of cells from oxidative stress (Degenkolb et al. 2006; Wallner et al. 2009). In general, some strains (*T. virens*) have the ability to inhibit pathogens through their mytrophism and antimicrobial compounds such as gliovirin (Howell 1998). Moreover, pyrone antibiotic produced by *T. harzianum* played a significant role in inhibition of *Gaeumannomyces graminis* var. *tritici*. The cocktail of antimicrobial compounds and lytic enzymes trigger up the antagonism higher than individual mechanism. In addition, a study carried out in mutant of strain 2413 with higher levels of extracellular enzymes acts in combination with α-pyrone, which performed better inhibitory effect against *R. solani* as compared to the wild-type strain, and it also protects grape from *B. cinerea*, both under repression (only pyrones were produced) and depression conditions (enzymes and pyrones were produced) (Omero et al. 1999). Correspondingly, genetically modified strain 2413 that overexpressed *Chit42* gene (chitinase) provided evidence of a significant role of Chitinase enzyme in biocontrol activity, while a transformant which was not able to produce α-pyrone was not able to overgrow *R. solani* (Limón et al. 2004). Consecutive roles of secondary metabolites and hydrolytic enzymes during fungal interactions have also been described. When combinations of antibiotics and hydrolytic enzymes were used against *B. cinerea* and *F. oxysporum* spore formation, synergism occurred, but it was lower when the enzymes were added after the antibiotics, indicating that cell-wall degradation was needed to establish the interaction (Howell 2003).

7.2.6.1 Nonribosomal Peptides

Nonribosomal peptides are synthesized by largely integrated enzymes that are recognized as nonribosomal peptide synthetases (NRPSs). NRPSs produce a large variety of compounds composed of monomers that are from a much wider range than the 20 proteinogenic amino acids. The monomers may be nonproteinogenic amino acids (peptaibols: 11–25 amino acid linear nonribosomal peptides that are rich in α-aminoisobutyric acid), or even compounds that are not amino acids at all. The peptides can be linear or cyclic and often undergo various chemical modifications (Bushley and Turgeon 2010; Strieker et al. 2010). Several classes of peptides synthesized by *Trichoderma* spp. are amphipathic in nature that forms voltage-dependent ion channels in membranes because of their ability to self-assemble. *Trichoderma* synthesizes wide varieties of secondary metabolites; they are synergistically acted with cell wall hydrolases to antagonize the pathogens and prevent the resynthesis of the cell wall of pathogen; therefore these antibiotics played important role in mycoparasitism (Howell 2006). The mycoparasitic strains of *T. virens* and *T. atroviride* have overrepresented NRPS-encoding genes (28 and 16, respectively) compared with the non-mycoparasitic *T. reesei*, whose genome contains only 10 genes (Kubicek et al. 2011). This complementary distribution of NRPS genes among saprophytic and parasitic species accounts far the role of NRPS in biological controll (Kubicek et al. 2011).

7.2.6.2 Peptaibols

Peptaibols, a class of linear peptides that generally has strong antimicrobial activity against wide range of bacteria, yeast, and fungi, act synergistically with cell-wall-degrading enzymes (CWDEs) to inhibit the growth of plant pathogens and elicit plant resistance to pathogens. In recent research, many workers have focused on the isolation, structural conformation, biosynthetic pathway, and amino acid sequences of peptaibol that is freely accessible in the peptaibols database (www.cryst.bbk.ac.uk/peptaibol), which comprises 317 peptaibol structures that grouped into 9 distinct subfamilies, and more than 190 are produced by *Trichoderma* species (Neuhof et al. 2007; Degenkolb et al. 2008), although new compounds are being reported daily (Mukherjee et al. 2011). In tobacco plants, application of peptaibols ameliorate defense response and reduces susceptibility to tobacco mosaic virus. A recently purified gene from *T. virens* that coded peptaibol synthetase has been cloned, which will facilitate studies of this compound and its contribution in biomanagement. An extensive review pertaining to antibiosis and production of *Trichoderma* secondary metabolites has been given by Howell (2003). In *T. virens,* Viterbo et al. (2007) studied the role of the *Tex1* gene (encoded trichovirin II peptaibol) by constructing mutants, which demonstrated the application of peptides in symbiotic interaction with cucumber plant, inducing systemic protection and the upregulation of defense genes. The significant reduction observed in systemic resistance response against foliar pathogen and lower production of phenolic compounds when mutants disrupted *tex1* gene grown with cucumber plants.

7.2.6.3 Gliotoxin

Gliotoxin belongs to the nonribosomal peptides molecules (Patron et al. 2007). Gliotoxin was the first metabolite that is described from *T. virens* (Brian and Hemming 1945) and due to its antibiotic/antifungal properties implicated against antagonism of *Rhizoctonia* in the soil (Weindling and Emerson 1936). The gliotoxin gene cluster is determined in the *T. virens* "Q" strain genome, for instance, *gliZ*, *gliJ*, *gliA*, and *gliT* (putatively encoding a transcription factor, dipeptidase, transporter, and thioredoxin reductase, respectively), and showed their potential role in the disease control of cotton seedling disease (Wilhite and Straney 1996; Howell 2006).

7.2.6.4 Polyketides

The polyketides are the most diverse group of secondary metabolites that demonstrate antibiotic activity (macrolides, tetracyclines, and polyenes) or toxins (aflatoxins) produced by many microorganisms, including bacteria and filamentous fungi. Besides pharmacological importance of many PKs due to their antimicrobial,

anticancer, and immunosuppressive properties these compounds are also responsible in the facilitation of nutrient competition (Khosla 2009; Mukherjee et al. 2012). Polyketides are generally synthesized by polyketide synthases (PKSs). There are various PKS genes involved in biosynthesis pathway, and available *Trichoderma* genomes have revealed that *T. virens* and *T. atroviride* encode 18 predicted PKSs (Kubicek et al. 2011), and *T. reesei* encode 11 (Martinez et al. 2008). The physiology and biocontrol properties of *Trichoderma* spp. producing PKSs are still not very known, but some reports showed that two PKS genes in *T. atroviride* are expressed during the confrontation of *R. solani*, indicating a possible role in mycoparasitism (C. P. Kubicek, personal communication).

7.2.6.5 Isoprenoid-Derived Metabolites

Another important diverse range of compounds is synthesized from five-carbon isopentenyl units with important pharmacological activities like antiviral, antibacterial, antimalarial, anti-inflammatory actions, and anticancer activity. These important molecules have been found to be involved in the biocontrol (ergokonins and viridins) of plant pathogens and their structural function in the cell membranes (ergosterol). The fungistatic and anticancer steroid producing cluster of gene that is putatively implicated in viridin biosynthesis has been identified in *H. virens* (Druzhinina et al. 2011). As discussed by Degenkolb et al. (2008), *T. arundinaceum* and *T. brevicompactum* produce the trichothecenes harzianum A and trichodermin, respectively, which are formed by a cascade of reactions in which trichodiene synthase (*Tri5*) catalyzes the first step.

7.2.6.6 Pyrones

One of the volatile antifungal compounds with the "coconut aroma," 6-pentyl pyrone (6-PP) produced by *Trichoderma* spp., which is one of the best-studied secondary metabolites from a biocontrol perspective (Reithner et al. 2005, 2007; Vinale et al. 2008). This important molecules have the ability to restrict the pathogens multiplication and enhance the plant health status. The specific pathway responsible for the production of 6-PP is still a matter of debate, however, it is anticipated that a lipoxygenase gene (Triat1: 33350) unique to *T. atroviride* may be involved (Kubicek et al. 2011). In *T. virens* the compound 6-substituted 2H-pyran-2-one showed antifungal activity against *S. rolfsii* (Evidente et al. 2003). In *T. harzianum* disruption of the *Thctf1* gene that encodes a cutinase transcription factor 1 alpha disables *Trichoderma* to produce yellow pigmentation and two 6-PP-derived secondary metabolites. Interestingly, the Δ*thctf1* strain did show reduced antimicrobial capacity against *Fusarium* spp. (Rubio et al. 2009). Investigation of the new secondary metabolites and their fungicide bioassays revealed an interesting detoxification mechanism of secondary metabolites produced by *Trichoderma* spp. Researchers suggested that the existence of 6-PP in the

environment might not persist for a long time, so it would enhance an interest of ecological fungicidal/fungistatic compound against phytopathogenic fungi or demonstrate signaling function in the soil and rhizosphere.

7.3 Competition for Nutrients

Trichoderma spp. play a dynamic role in soil nutrient mobilization and uptake through showing excellent competition for space and nutrients. In this sense, *Trichoderma* spp. that efficiently uptake nutrients and displace other competitors by growing faster will have a noticeable advantage to colonize and survive different plant rhizospheres. Several studies indicated that a number of plant macro- and micronutrients are solubilized by *Trichoderma* spp., and as a result, microbe-microbe competition enhanced in the rhizosphere of plant (Saravanakumar et al. 2013; Khan et al. 2016). Microbial competitions are intense for acquisition of an insoluble form of iron in the rhizosphere; however, microbes have a special system to uptake and chelate iron from the soil. Whole genome analyses of *Trichoderma* spp. have shown that genes participate in the ferrocrocin synthesis and protect against oxidative stress, while *T. virens* and *T. reesei* each have two gene clusters that encoded siderophore and nonribosomal peptides which participate in the microbial competition and biocontrol (Kubicek et al. 2011). The siderophore-mediated iron acquisition is associated with biocontrol properties of *Trichoderma* spp. which is accomplished by competition for iron and, ultimately, suppression of *Fusarium lycopersici* causing wilt in tomato (Segarra et al. 2009). Another most influencing factor that augmented the microbial competition in rhizospheric soil through pH mediation, as a result, nutrient uptake and biomass of *Trichoderma* spp. support faster growth than other rhizospheric competitors. A genome-wide analysis demonstrated the role of *PacC* gene in pH regulation; deleted and a constructive mutant of *PacC* gene from *T. virens* was studied in response to pH change and biocontrol mechanism against *Rhizoctonia solani* and *Scelorosia rolfsii*. Trushina et al. (2013) revealed the increased expression of *PacC* gene through transcriptome analysis of constructive mutant against several biotic and abiotic responses.

7.4 Plant Growth Promotion and Biotic Stress Management by *Trichoderma*

Trichoderma spp. have received considerable attention throughout the world as potential biological control agents against a wide spectrum of soilborne plant pathogens, also play significant role in plant growth and yield promotion (Harman et al. 2004; Verma et al. 2007). Several *Trichoderma* species are well known to act as biocontrol agents against various fungal phytopathogens either through direct parasitism, competition with pathogens for space and nutrients, ameliorators of plant

health, or inducers of plant systemic resistance to plant pathogens (Harman et al. 2004; Bailey et al. 2006; Błaszczyk et al. 2011). To improve the soil health, suppression of plant pathogens and mineralization of nutrients to provide nutritive material for plant growth provide new insight on *Trichoderma* spp.-assisted stress management (Contreras-Cornejo et al. 2009; Lorito et al. 2010). In this context, *Trichoderma* spp. have gained a special position in biotic stress management and plant growth promotion and improving plant resistance. *Trichoderma* species is highly effective in the management of root rot, foot rot, collar rot, stem rot, damping off, wilt, blight leaf spot of crops like pulses, oilseeds, *cucurbitaceous* crops (cucumber, bottle gourds, ridge gourd), and *solanaceous* crops like tomato, brinjal, chili, capsicum, etc. *Trichoderma* spp. are also effective in controlling the disease of sheath rot, sheath blight, and bacterial leaf blight of rice. *Trichoderma* spp. have beneficial effects on plant growth and enhanced resistance to abiotic and biotic stresses. Several reports revealed that *Trichoderma* improves growth responses in cereals and legumes, vegetable, spices, oilseed, and cash crops (Contreras-Cornejo et al. 2016; Zeilinger et al. 2016; Brotman et al. 2013; Donoso et al. 2008; Bae et al. 2009). Hence, positive effects of *Trichoderma* species on agriculture crop management against plant pathogens/diseases which have been recognized in the whole world are summarized in Table 7.2.

7.5 Induction of Plant Defense by *Trichoderma* spp.

Plant health are adversely affected due to various biotic stresses. The rhizosphere provides niches for proliferation of *Trichoderma* spp. and opportunities for both biotrophy and saprotrophy on plant root exudates. Plant indicate the presence of other microorganisms by activating potential defense mechanisms which are eventually accomplished in two stages of immune response. Firstly, recognition of pathogen associated molecular patterns (PAMPs) or microorganism associated molecular patterns (MAMPs) is known as PAMP-triggered immunity (PTI), whereas the second stage responds to virulence factors from the pathogen and is called effector triggered immunity (ETI). *Trichoderma* spp. (a non-pathogenic microorganism) accelerate induced systemic resistance (ISR) in plants which culminates in the accumulation of components of the associated jasmonate and ethylene signaling pathways, such as hydroperoxide lyase, peroxidase, and phenylalanine ammonia lyase (which induces lignification) (Fujita et al. 2006; Yasuda et al. 2008; Shoresh et al. 2010; Pieterse et al. 2014; Verma et al. 2016), enhancing plant metabolism, such as photosynthetic rate or respiratory activities (Domínguez et al. 2016). In addition, second stage response of plant innate immune (systemic acquired resistance, SAR) is not elicited because *Trichoderma* spp. are not considered as plant pathogens. Various biomolecules such as xylanases, peptaibols, swollenin, and cerato-platanins are secreted by *Trichoderma* spp. which act as MAMPs. The endoxylanase Eix (also known as *Xyn2/Eix*) from "*T. viride*" ATCC 52438 was the first protein known to elicit ethylene formation in tobacco and tomato (Rotblat et al.

Table 7.2 Application of *Trichoderma* species in major crops against different plant pathogens and plant growth promotion

Crop	Botanical name	Pathogen/disease	*Trichoderma* spp.	Type of treatment	PGP attribute/ defense mechanism	References
Cereals and legumes						
Black gram	*Vigna mungo*	*Macrophomina phaseolina*, *Alternaria alternata*, *Fusarium oxysporum*	*T. viride* and *T. harzianum*	Seed pelleting	IAA, phosphate solubilization/ hydrolytic enzymes, mycelial inhibition	Raghuchander et al. (1997), Dubey and Patel (2001), Mishra et al. (2011)
Chickpea	*Cicer arietinum*	*Sclerotium rolfsii*, *Sclerotinia sclerotiorum*, and *Colletotrichum capsici*	*T. harzianum*, *T. asperellum*, *T. koningiopsis*, *T. longibrachiatum*, and *T. aureoviride*	Seed treatment	IAA, phosphate solubilization/ hydrolytic enzymes, mycelial inhibition	Saxena et al. (2015)
Rice	*Oryza sativa*	*Rhizoctonia solani*, *Fusarium* spp., *A. alternate*, *Trichoconiella padwickii*	*T. harzianum*, *T. virens*, *T. atroviride*, *T. viride*, and *T. koningii*	Spray method, seed treatment	IAA, phosphate solubilization/ hydrolytic enzymes, mycelial inhibition	Naeimi et al. (2010), Bhat et al. (2009), Gomathinayagam et al. (2010), Chakravarthy et al. (2011), da Silva et al. (2012)
Bean	*Phaseolus vulgaris L.*	*R.solani*, *S.sclerotiorum*	*T. Koningii*, *T. harzianum*, and *T. virens*	Soil treatment	Plant growth hormones, siderophore production/ hydrolytic enzymes, mycelial inhibition	El-Fiky et al. (2006), Figueirêdo et al. (2010)
Pigeon pea	*Cajanus cajan*	*F. udum*	*T. viride* and *T. harzianum*	Foliar	Hydrolytic enzymes, mycelial inhibition	Hukma and Pandey (2011)
Soybean	*Glycine max* L.	*R. solani*, *A. alternata*, *S. rolfsii*, *M. phaseolina*, *Aspergillus flavus*, *Curvularia lunata*, *Fusarium* spp., *P. arrhenomanes*	*T. harzianum* and *T. viride*	Seed and soil	Plant growth hormones, siderophore production/ hydrolytic enzymes, mycelial inhibition	Singh et al. (1973), Ray et al. (2007), John et al. (2010), Khodke and Raut (2010), Anitha (2011), Mishra et al. (2011), Jat and Agalave (2013)

Cowpea	*Vigna sinensis*	*R. solani*	*T. harzianum*	Seed	Plant growth hormones, siderophore production/ hydrolytic enzymes, mycelial inhibition	Pan and Das (2011)
Spices						
Garlic	*Allium sativum* L.	*Sclerotium cepivorum*	*T. harzianum*, *T. asperellum*, *T. hamatum*, *T. oblongisporum*, and *T. viride*	Bulbs treatments	Plant growth hormones, / hydrolytic enzymes, mycelial inhibition	Dilbo et al. (2015), Mahdizadehnaraghi et al. (2015)
Ginger	*Zingiber officinale*	*P. aphanidermatum*	*T. harzianum*	Rhizome treatment	Hydrolytic enzymes, mycelial inhibition	Gupta et al. (2010)
Cumin	*Cuminum cyminum* L	*F. oxysporum* f sp. *cumini*, *S. rolfsii*	*T. viride* and *T. harzianum*	Seed, soil, and foliar	Hydrolytic enzymes, mycelial inhibition	Chawla and Gangopadhyay (2009)
Oilseeds crop						
Groundnut	*Arachis hypogaea* L.	*Thievaliopsisbasicola*, *S. rolfsii Sacc*, *A. niger*, *R. solani*, *P aphanidermatum*, and *M. phaseolina*	*T. harzianum*, *T. viride*, and *T longibrachiatum*	Seed, soil and foliar		Biswas and Sen (2000), Kishore et al. (2001), Rakholiya and Jadeja (2010), Bagwan (2011), Sreedevi et al. (2011, 2012)
Sesame	*Sesamum indicum* L.	*A. flavus*, *Curvularia lunata*, *P. notatum*, *P. chrysogenum*, *F. moniliforme*, *F. oxysporum*, *R. nigricans*, and *M. phaseolina*	*T. viride* and *T. harzianum*	Soil and foliar	Hydrolytic enzymes, mycelial inhibition	Tamimi and Hadvan (1985), Sankar and Jeyarajan (1996a, b), Jayelakshmi et al. (2013)
Vegetables						
Lettuce	*Lactuca sativa*	*R. solani*	*Trichoderma* spp.	Seed	Hydrolytic enzymes, mycelial inhibition	Pinto et al. (2014)

(continued)

Table 7.2 (continued)

Crop	Botanical name	Pathogen/disease	*Trichoderma* spp.	Type of treatment	PGP attribute/ defense mechanism	References
Spinach	*Spinacea oleracea*	*R. solani*, *Pythium* sp, *F. graminearum*, *F. oxysporum* f. sp. *phaseoli*, and *F. oxysporum* f. sp. *lycopersici*	*T. harzianum*, *T. viride*, and *T. virens*	Soil and foliar sprays	Hydrolytic enzymes, mycelial inhibition	Siameto et al. (2011)
Mushroom	*Agaricus bisporus*	*Rhizopus stolonifer*, *Coprinopsiskimurae*, *P. glabrum*, *F. oxysporum*	*T. viride*		Hydrolytic enzymes, mycelial inhibition	Rawal et al. (2013)
Capsicum	*Capsicum annum* L	*A. alternate* and *Drechslera tetramera*	*T. viride* and *T. harzianum*	Seed	IAA, phosphate solubilization, siderophore production/ hydrolytic enzymes, mycelial inhibition	Kapoor (2008)
Cauliflower	*Brassica oleracea* L. var. *botrytis*	*R. solani* and *Pythium aphanidermatum*	*T. viride* and *T. harzianum*	Seed treatment, fungicide, and soil	Hydrolytic enzymes, mycelial inhibition	Sharma et al. (2001, 2003), Ahuja et al. (2012), Rahman et al. (2012)
Garden Sorrel	*Rumex acetosa* L.	*Alternaria tenuissima*	*T. viride*, *T. harzianum*, *T. virens*, *T. koningii*, and *T. pseudokoningii*		Hydrolytic enzymes, mycelial inhibition	Ambuse et al. (2012)
Cabbage	*Brassica oleracea* L. var. *capitata*	*S. sclerotiorum* and *R. solani*	*T. viride* and *T. harzianum*	Soil and seed	Hydrolytic enzymes	Rabeendran et al. (2000), Sharma et al. (2001), Sharma et al. (2003)

Crop	Botanical name	Pathogen/disease	*Trichoderma* spp.	Type of treatment	PGP attribute/ defense mechanism	References
Brinjal	*Solanum melongena* L.	*Fusarium solani*, *F. oxysporum* f. sp. *lycopersici*, *Sclerotinia sclerotiorum*	*T. viride* and *T. harzianum*	Seed and soil	IAA, phosphate solubilization, siderophore production/ hydrolytic enzymes, mycelial inhibition	Jadon (2009), Balaji and Ahir (2011)
Onion	*Allium cepa*	*Stemphylium vesicarium*, *Alternaria alternate*, *A. porri*, *A. tenuissima*, *Cladosporium allii-cepae*, and *C. circinans*	*T. viride*, *T. reesei*, and *T. harzianum*	Soil, seed, seedling dip, and foliar sprays	IAA, phosphate solubilization, siderophore production/ hydrolytic enzymes, mycelial inhibition	Mishra and Gupta (2012), Prakasam and Sharma (2012) Shahnaz et al. (2013), Yadav et al. (2011), Abo-Elyousr et al. (2014)
Potato	*Solanum tuberosum*	*R. solani* and *Phytopthora infestans*	*T. viride* and *T. harzianum*	Foliar and seed	IAA, phosphate solubilization, siderophore production/ hydrolytic enzymes, mycelial inhibition	Basu (2009), Selvakumar (2008), Pandey and Pundhir (2013)
Chili	*Capsicum annuum* L.	*Colletotrichum capsici* and *Alternaria tenuis*	*T. virens*, *T. Pseudokoningii*, and *T. harzianum*	Fruit treatment, seed	Hydrolytic enzymes, mycelial inhibition	Begum et al. (2010), Joshi et al. (2010), Rahman et al. (2012)
Taro	*Colocasia esculenta* (L.) Schott	*Phytophthora colocasiae*	*T. asperellum*, *T. longibrachiatum*, and *T. harzianum*	Leaf disk Method	Hydrolytic enzymes, mycelial inhibition	Nath et al. (2014)
Tomato	*Lycopersicon esculentum* Mill.	*R. solani*, *Fusarium oxysporum* f. sp. *Lycopersici*, *Alternaria solani*	*T. hamatum*, *H. virens*, *T. asperellum*, and *H. lixii*	Root dip method	Hydrolytic enzymes, mycelial inhibition	Solanki et al. (2011), Sundaramoorthy and Balabaskar (2013), El-Komy et al. (2014), Marzano et al. (2013), Selim (2015)

(continued)

Table 7.2 (continued)

Crop	Botanical name	Pathogen/disease	*Trichoderma* spp.	Type of treatment	PGP attribute/ defense mechanism	References
Cucumber, bottle gourd and bitter gourd	*Cucumis sativus*, *Lagenaria siceraria*, and *Momordica charantia*	*S. rolfsii* and *R. solani*	*T. aureoviride*, *T. viride*, *T. harzianum*, and *T. virens*	Seed treatment	IAA, phosphate solubilization, siderophore production/ hydrolytic enzymes, mycelial inhibition	Kotasthane et al. (2015), Yobo et al. (2004)
Cash crops						
Bamboo	*Dendrocalamus calostachyus*	*Polyporus sanguineus*	*T. lignorum*, *T. harzianum*, and *T. viride*	Soil	Hydrolytic enzymes, mycelial inhibition	Kundu and Chatterjee (2003)
Cotton	*Gossypium hirsutum*	*R. solani*, *S. rolfsii*, and *P. aphanidermatum*	*T. viride* and *T. harzianum*	Seed and soil	Hydrolytic enzymes, mycelial inhibition	Gaur et al. (2005)
Coconut	*Cocos nucifera* L	*Ganoderma lucidum*	*T. viride* and *T. harzianum*	Foliar	Hydrolytic enzymes, mycelial inhibition	Karthikeyan et al. (2006)

2002). Attachment of *Trichoderma* spp. to the plant roots is mediated by the protein molecule, like swollenin (a cellulose-binding domain able to recognize cellulose), that stimulates root colonization and defense responses in cucumber plant, and in return, *Trichoderma* spp. received sucrose as a carbon source that enable faster hyphal growth (Brotman et al. 2008). Another root-colonizing protein, hydrophobins (Ruocco et al. 2015; Viterbo and Chet 2006) and expansin-like proteins (Brotman et al. 2008), is required for the adherence of *Trichoderma* to the root surface, cell wall development, and induction of defense response. The 1-aminocyc lopropane-1-carboxylic acid (AAC) deaminase inhibits the ethylene formation by the plant and enhanced root growth (Viterbo et al. 2010); *Trichoderma* spp. produce enzymes and metabolites, viz., nitrilase which is further responsible for the formation of the auxin 3-indole acetic acid (IAA) (Contreras-Cornejo et al. 2009). Viterbo et al. (2010) revealed the role of *Trichoderma* spp. that possess an α-1-aminocyclopropane-1-carboxylate (ACC) deaminase gene (acc1) that encodes an enzyme which cleaves ACC (intermediate in ethylene biosynthesis) and is expressed during *Brassica napus* (oilseed rape) root colonization, while gene knockout process shows inability to promote root elongation. Similarly, the *Trichoderma* spp. genome contains many genes that encode nitrilases which hydrolyzes β-cyano-l-alanine while biosynthesis of ethylene is ongoing or during the conversion of plant metabolite, indole-3-acetonitrile to indole-3-acetic acid (IAA), a phytohormone which are responsible for the promotion of plant root growth.

7.6 Conclusion

It is important to keep in mind that all conclusions derived from genomic and transcriptomic data of *Trichoderma* spp. provide new insights into many useful genes that have a different expression pattern which support to unlock the complex molecular mechanism involved in mycoparasitism, plant growth promotion, and establishment of a new platform for the molecular ecology. However, *Trichoderma* mediated several mechanisms, such as mycoparasitism, hydrolytic enzymes and metabolites production, competition for nutrition and space, plant root colonization, and plant defense, which are better understood at the molecular level and provide new research interest at proteomic and metabolomic levels which decipher novel pathways and search of novel biotic compound of *Trichoderma* spp. and their applications in plant disease management. These molecular approaches ultimately define an ideal model for *Trichoderma*-plant-plant pathogen interaction, and released signaling molecules could be utilized as plant defense activator or plant growth promotor against several biotic stresses. Hence, new traits of transgenic crops could be developed with the help of genetic engineering by inserting novel biocontrol genes of *Trichoderma* spp. that accelerate plant transgenics to resolve the hungerness, and it may help to fight against the climate change problems.

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Chapter 8
Plant Responses to Phytonematodes Infestations

Atef M. El-Sagheer

Abstract Phytoparasitic nematodes, of which more than 4100 species have been identified worldwide, are obligate parasites that attack a wide range of plants; some of these species reduce global agricultural production. Phytoparasitic nematodes causes negative impact on plant health: Plant growth, germination, morphogenesis, generative growth, and reproductive growth, as well as functional and morphological changes are greatly affected. The feeding behavior and small size of phytoparasitic nematodes sometimes does not lead to the development of characteristic plant signs and symptoms where nematode problems often go completely undiagnosed. This chapter will focus on responses of plant health growing under nematodes infestation.

Keywords Phytonematodes · Biology · Plant health · Symptoms · Plant biomarkers

8.1 Introduction

Throughout the animal kingdom, nematodes are second after arthropods in both the numbers of species and in the numbers of individuals present (Cobb 1915). In other words, nematodes represent four of every five multicellular animals on the planet (Bongers and Ferris 1999). Nematodes are small multicellular animals with a rather simple organization and a permeable body; typically, the body model consists of a flexible cylinder which tapers at the front and rear. The body is confined by a flexible layer, but has strength because of its collagen cuticle. Phytonematodes are obligate parasites; below the cuticle is a hypodermis, which can be cellular or syncytial, and the body has a pointed tail and a blunt head, with many variations depending on different species and lifestyles. Nematodes are triplobastic and have a pseudocoel; the internal organ systems are tubular, relatively simple histologically, and mainly lie free within the body cavity (Merrifield and Ingham 1998).

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Economically, phytoparasitic nematodes, which are distributed across the world, are one of the major biological constraints in the production of various economically important crops, and cause huge losses. The worldwide annual global loss in horticultural crops due to phytoparasitic nematodes has been estimated as 8.8–14.6% of total crop production and in monetary terms, 100–157 billion US$ (Koenning et al. 1999; Nicol et al. 2011).

8.2 Biology and Life Cycle

Most species of plant parasitic nematodes have a relatively simple life cycle consisting of the egg, four larval stages, and the adult male and female. Development of the first-stage larvae occurs within the egg, where the first molt occurs. Second-stage larvae (juveniles) hatch from the eggs and the stage at which hatching occurs (second- or fourth-stage juveniles) varies depending on the nematode species. The juveniles find and infect plant roots, or in some species they infect above-ground tissues. Host finding or movement in soil occurs within surface films of water surrounding soil particles and root surfaces. Depending on the species, feeding will occur along the root surface, or in some species, such as root-knot nematodes, young larval stages will invade root tissue, forming feeding sites within the root. Second-stage larvae will then molt three times to become adult males or females (Williamson and Hussey 1996). For most species of nematodes, as many as 50–150 eggs are produced per female, while in others, such as root-knot nematodes, upwards of 2000 eggs may be produced. The life cycle length and population rate increase depending upon several factors, most important of which are soil temperature, host suitability, and soil type (Abawi and Widmer 2000). Under suitable environmental conditions, the eggs hatch and new larvae emerge to complete the life cycle within 4–8 weeks, depending on temperature. Nematode development is generally most rapid within an optimal soil temperature range of 21–26 °C (Anderson 2000; Duan et al. 2009 and Maleita et al. 2012).

8.3 How Phytoparasitic Nematodes Deteriorate Plant Health?

Since the actual definition of nematodes as pathogens, some hypotheses and theories have been developed to explain how nematodes select their destination and how they are attracted to a host plant. (i) The first of the early hypotheses was recorded by Steiner (1925), where he supposed that (a) nematodes had the ability to locate their hosts' roots over considerable distances, (b) that plant roots produced secretions that were carried by the soi1 and water and that acted as selective stimuli upon

the nematodes, and (c) that amphids were the sense organs through which nematodes responded to the stimuli. (ii) In 1961 Viglierchio concluded that there was no relationship between host efficiency and ability to attract the nematode parasite, when he noted that the larvae of *Heterodera schachtii* were weakly attracted to tomato, but were repelled by the roots of oat of the variety Kanota, while, on the contrary, *Meloidogyne hapla* larvae were strongly attracted to the roots of oat, which is not a host, and were repelled by rye roots; and attributed this to the ability of the tomato roots on the secretion of stimulating materials to attract nematodes, which have the ability to spread over a distance of 10 mm at least. (iii) Klingler (1965) noted that the nematode-host attraction was probably due, in part, to chemical agents, especially carbon dioxide, as nematodes move under orientation to its gradients, and they respond by means of chemoreception, using amphids as chemoreceptors (Steiner 1925). Also, certain amino acids liberated by roots, such as aspartic acid, threonine, serine glutamic acid, glycine, and alanine (Ivarson and Sowden 1969), play an important role in nematode attraction to the host (Bird 1959).

In open fields phytonematodes are diffused over wide areas by wind, irrigation, flooding, and the activities of farmers and agricultural animals, or due to the irregular dispersion of some larvae as a result of high population density competition around food sources (Baujard and Martiny 1994; Lehman 1994). Generally, many hypotheses describe phytonematodes' responses to root secretions, which depend mostly on the type of phytonematode and how they respond to the specific quality of the substances produced by the host's roots.

8.4 How Phytoparasitic Nematodes Affect Plant Health

Some may wonder how microorganisms such as nematodes can damage a wide range of plants. The answer is to imagine huge numbers of nematodes surrounding the plant from each side, and the nematode's stylet (stomatostyle) being used to obtain fluid from the root. Undoubtedly, the plant weakens and withers as the worms grow larger (Gheysen and Mitchum 2011). The damage is caused by the saliva produced by the worms, which enters the plant's cells as long as the worms continue to feed; the saliva dilutes the contents of the plant cells so that the contents are easy for the nematode to absorb. The salivary enzymes of root-knot nematodes sometimes cause the growth of abnormal cells, such as giant cells, or these salivary enzymes inhibit cell division. In contrast, however, some salivary enzymes may promote cell division, leading to deformation of the roots or large numbers of side roots near the sites of injury (Gheysen and Mitchum 2011). Nematodes, by weakening plants and opening gaps in the roots, create an environment that is suitable for the introduction of fungal, bacterial, and viral diseases. Certain types of nematodes carry pathogens in their digestive system and transmit them to healthy plants through their saliva.

8.5 Disease Symptoms of Phytonematode Infestation

The best time to diagnose nematode infestations by their apparent symptoms on plants is in spring and summer, when grow crops actively, and nematode feeding and reproduction is greatest, with the population density being highest at the end of the growing season. Phytonematodes vary in their feeding habits, with each species causing slightly different types of damage to the hosts' roots. But generally, depending of the location of the damage in plants, phytonematode infestation symptoms are divided into two main categories (Khan 2008).

8.5.1 Aboveground Symptoms

Aboveground symptoms include those that appear on the plant's vegetative systems; these symptoms are similar to the symptoms of lack of nutrients (Khan 2008).

8.5.2 Symptoms Resulting from Root Damage

The most severe damage caused by nematodes in plants is the result of their feeding on the roots, as this reduces the plant's ability to take up water and nutrients from the soil, and causes poor responses to fertilizers and irrigation; thus, the vegetative parts of the plant (shoot system) show one or more symptoms of nutrient deficiency. Usually these symptoms are not noticeable until considerable damage has been done to the root system, or there are symptoms of wilting or symptoms of poorly functioning roots (Khan 2008).

8.5.2.1 Stunting

Plants infested with nematodes show changes in growth parameters and are significantly smaller than other, healthy, members of their species.

The best example of this symptom of stunting, which appears in strawberry plants infested with the ectoparasitic nematode, *Aphelenchoides besseyi* (Aphelenchida: Aphelenchoididae), is commonly known as summer dwarf disease (Raski and Allen 1948). Second-stage juveniles feeding on young tissues enter the inner leaf tissues through stomata located between lower epidermis cells, and destroy cells of the palisade tissue that contain green plastids, causing them to loose their photosynthetic capacity, and destroying the spongy tissues that store carbohydrates. This process leads to the loss of these tissues' ability to carry out the storage process, and causes distortion and crinkling of the leaves, as well as the reduction of leaf size. The resulting signs are crinkled, elongated, asymmetrical curling leaf edges that wrap into the upper sides of young leaves, with the old leaves' edges wrapped into the lower side; there are also reductions in petiole length (Khan 2008).

8.5.2.2 Wilting

In the leaves and branches of plants infested with nematodes, wilt may be temporary during the day at high temperature and disappear in the evening when the temperature is low; however, withering may be persistent through periods of water stress, and then the vegetative parts die gradually, with the whole plant dying when the nematode population reaches a high density above the critical threshold (Montasser 1990). But the death of the aboveground parts of plants rarely occurs as a result of nematode infestation alone, as some other pathogens such as fungi or bacteria are involved in the development of complex disease (Ciancio and Mukerji 2007). However, the wilting symptoms differ between each plant; therefore, the symptoms of infestation may vary in one field (Johnson and Powell 1969).

Physiologically, the wilting process in plants infested by nematodes is due to the lack of efficient uptake and translocation of water that is necessary for internal biological and physiological processes to a degree that is not compatible with the amount of water lost during transpiration, so that cells in various tissues and leaf branches lose water and therefore wither and lose freshness. Such prominent wilting can be diagnosed, when *Meloidogyne* spp. infest okra, eggplant, and other vegetables (Anwar and McKenry 2010).

8.5.2.3 Yellowing (Chlorosis)

One of the important signs of nematode infestation, in simple the yellowing or whitening of normal green tissue due to partial or complete failure of chlorophyll, can develop due to various diseases caused by nematodes or other pathogens. Some genera of phytonematodes such as root-knot nematodes can reduce photosynthetic rates in infested plants (Schans 1991 and Ahmed et al. 2009).

In nematode infestations, the pigment of leaf components is sensitive to the loss of photosynthetic pigments (e.g., chlorophyll a and b) or the loss of photoprotective pigments, such as zeaxanthin or β-carotene (Demmig-Adams and Adams 1992). Plant leaf tissue and chloroplasts are damaged by various stresses (Karpinski et al. 2003), and chlorophyll degradation occurs with the cell death that is caused due to pathogens (Hendry et al. 1987). However, it is not the products of chlorophyll degradation that turn green leaves yellow or red, but mainly carotenoids and anthocyans (Takamiya et al. 2000).

8.5.2.4 Reduction of Yield

As a result of previous damage, plants health affected negatively which decreases the quality and quantity of yield, e.g. root-knot nematodes. *Meloidogyne* spp. are one of the most economically damaging genera of plant parasitic nematodes on horticultural and field crops where considerable economic losses with average 10% of loss in yield is frequently cited for vegetables (Barker and Koenning 1998 and Koenning et al. 1999). On the other hand, the situation is not much better when

nematodes invade the tree roots. Because of the small size of nematodes and difficulty of diagnosis in the first stages of the growth, the trees begin with weak yields and decline year after year until it becomes uneconomic. The farmers are then forced to uproot and replant new trees. Some common nematodes infesting trees are *Radopholus* spp., burrowing nematode infesting banana trees; *Xiphinema* spp., dagger nematodes infesting grape trees.

8.5.3 Direct Contact Signs

Direct contact signs can be described as malformations caused by nematodes feeding directly on the aboveground parts of plants. A few species of phytonematodes prefer to feed on vegetative parts, causing disease symptoms, as outlined below.

8.5.3.1 Gall Formation on Stems, Leaves, Flowers, and Buds

Some genera of plant parasitic nematodes, e.g., *Anguina*, *Aphelenchoides*, *Bursaphelenchus*, *Ditylenchus*, and *Rhadinaphelenchus*, primarily, attack plant parts on the surface of the soil. However, a few species form galls on leaves, stems, flowers and buds (Table 8.1).

The galls form as a result of the salivary secretions produced by the nematodes; these secretions contain some enzymes that affect the health of plant cells causing an increase in the size and number of epidermal cell layers or cells in vascular tissues. For example, in the cross-sections of galls induced by *Ditylenchus* sp. on *Miconia albicans*, Viana et al. (2013) noted a structure comprising tissues spanning from the epidermis through to the spongy mesophyll, including vascular tissue bundles; differences in gall composition were shown in the numbers of hollow chambers or in the presence of a parenchymatous septum (Fig. 8.1). There is no doubt that malformations reduce the viability of the leaves and therefore affect the host physiology (Hussey et al. 2002).

8.5.3.1.1 Flowers, Growing Points (Meristematic Cells), and Buds

Some genera of nematodes attack flowers, growing points and buds in some hosts causing symptoms of disease e.g., *Aphelenchoides besseyi* attacks the leaves of the *Ficus elastica* tree through interveinal leaf tissues causing discoloration lesions; within a few days the leaves become dark brown before falling from the plant.

Also, an endoparasite of foliar chrysanthemum, the nematode *A. ritzemabosi*, attacks the leaves of *Chrysanthemum morifolium* causing brown spots close to the veins (Fig. 8.1), and a gradual yellowing of the whole leaf. These symptoms appear to be similar to those in other organisms (Cayrol and Combettes 1972); the severity of damage is different between the upper and lower leaves in the same plant (Cid and Sosa 1978), this damage is caused by a mechanical stylet action and through hormones of growth and division (Cayrol and Combettes 1972).

Table 8.1 Nematode species, types of plant hosts, and sites of galls on infested plants

Nematode species	Host	Galls on	References
Ditylenchus gallaeformans	*Miconia albicans*	Leaves, stems, and inflorescences	da Silva et al. (2016)
Pterotylenchus cecidogenus	*Desmodium ovalifolium.*	Stem	Lehman (1991)
Ditylenchus oncogenus	*Sonchus bulbosus*	Leaves and stems	Vovlas et al. (2016)
Pterotylenchus cecidogenus	*Desmodium ovalifolium*	Stem	Siddiqi and Lenne (1984)
Ditylenchus drepanocercus	*Evodia roxburghiana*	Leaves	Goodey (1953)
Subanguina picridis	*Agrostis capillaris*	Stem	Subbotin et al. (2004)
	Acroptilon repens	Leaves, stems	Watson (1986)
Ditylenchus dipsaci	*Avena sativa*	Stems and leaves	McDonald and Nicol (2005)
	Zea mays	Stems	Rivoal and Cook (1993)
	Fragaria ananassa	Leaves and petioles	Barker and Sasser (1959)
Anguina graminis	*Festuca dumetorum*	Stems and leaves	Dorofeeva et al. (2002)
Anguillulina graminophila	*Agrostis tenuis* Sibth. *(Agrostis vulgaris* With.*)*	Leaves	Goodey (1933)

8.5.3.2 Disease Symptoms on Nematode-Infected Roots

8.5.3.2.1 Root Galls

Infestations by some nematode species such as *Meloidogyne*, *Nacobbus*, *Hemicycliophora*, *Longidorus*, and *Ditylenchus* causes pathological malformations such as galls, and bloating and swelling on plant roots. The malformations are formed on nematode-infested roots as a result of the formation of hypertrophic cells, hyperplastic cells, and giant cells (Favery et al. 2016).

8.5.3.2.2 Root Rot

Root rot is the decomposition of the cells in the epidermal tissue and cortex layer in infested host roots (Insunza and Valenzuela 1995). (i) Cell moldiness (deformation of wall and cell contents) may occur with the direct infestation of nematodes as a result of the secretion of the pectinase, protopectinase, which decomposes intercellular substances, and breaks down and separates the cortex layer, as occurs with, for example, *Ditylenchus dipsaci*. (ii) Alternatively, it may occur with direct nematode infestation as a result of infestation with secondary parasites, such as pathogenic fungi and bacteria, as seen with *Ditylenchus destructor* infestation.

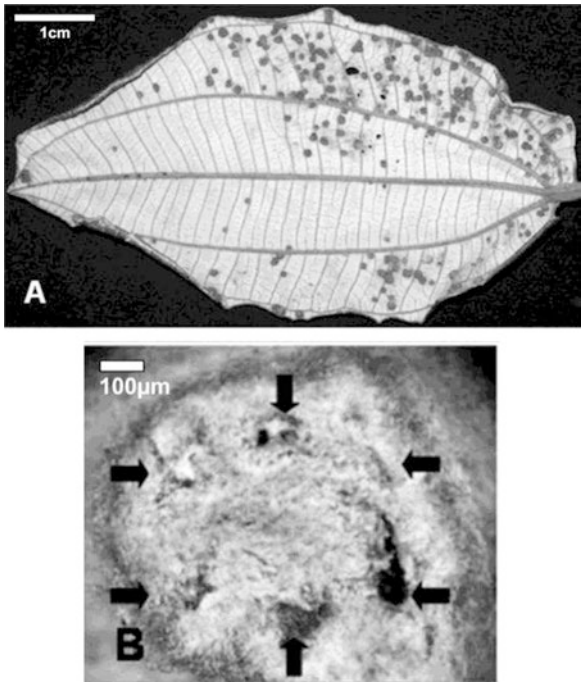

Fig. 8.1 Galls induced by *Ditylenchus* sp. on *Miconia albicans*. (**a**) Abaxial view of a *Meloidogyne albicans* leaf with galls, (**b**) leaf gall showing hollow chambers or loci (*arrows*) in cross-section. (Adapted from Viana et al. (2013))

8.5.3.2.3 Root Lesions

Lesion symptoms can be seen as a characteristic of infestations with some nematode species such as banana lesion nematode, *Pratylenchus goodeyi* (Pinochet et al. 1998) and the burrowing nematode, *Radopholus similis* (Valette et al. 1998) on banana. However, in general, almost all nematodes cause necrosis and the breakdown of the cortex layer as a result of the laying of their eggs and the development of the juveniles; nematodes can be seen scattered within the root tissue, especially outside the boundaries of ulcerated areas, where damage is often associated with the feeding site. Depending on the nematode species, the lesions in plant roots may be superficial when only the cortex layer breaks down, or 'gummy' until cylindrical tube where both the cortex and epidermal layers break down. The lesions originate from the secretion of enzymes, such as phenol oxidase, during the nematode feeding process. This enzyme which oxidizes non-oxidizing phenols in the cell contents to oxidizing phenols that cause cell death as a result of increased concentration of oxidizing phenols in the cell wall (Gheysen and Mitchum 2011).

8.5.3.2.4 Root Necrosis

As result of the epidermal cell death caused by ectoparasitic nematodes, e.g., *Helicotylenchus* and *Ditylenchus*, parts of the root surface appear as necrotic areas (Barekye et al. 2000).

8.5.3.2.5 Excessive Root Branching and Damage to Root Growing Points

Some phytonematodes in heavy infestations cause the formation of new lateral roots above the infested area rather than causing dysfunction in infected roots. In such cases, the infested plant directs all its energy to increasing the rooting volume without offsetting this increase with an increase in the rate of vegetative growth.

8.5.3.2.6 Stubby Roots

Stubby-looking roots are usually associated with some genera of nematodes like *Trichodorus* where their feeding on cells in root tips causes the suspension of growth and elongation of the roots resulting in stubby root formations (Winfield and Cooke 1975).

8.5.3.2.7 Bending of Root Tips

Where ectoparasitic root nematodes such as *Xiphinema* spp. (dagger nematodes) attack the root tips (growing points) of their hosts, this causes the death of cells in this area on one side of the root, while the cells on the other side continue to grow, resulting in the bending and deformation of the root tips (Cohn 1970).

8.5.4 Water Imbalance Due to Nematode Infestation

In light infestations of sugar beet by the cyst nematode (CN), *Heterodera schachtii* which invades the cortex layer with a stylet and migrates intracellularly toward the vascular cylinder, there was water imbalance in the plants during the day, while the imbalance disappeared during the night (Haverkort et al. 1991). However, heavy infestation caused the leaves to become dry, and then to fall; the plant then cannot recover its normal health even with increased irrigation.

8.5.5 Histopathological Nematode Damage

In root tissues infested with nematodes some cellular changes including changes in the size and shape of the cells as well as physiological, histological and chemical changes occur and some of such cells not only at the infestation site but throughout the entire plant system becomes the source of food for the nematodes (Sosa-Moss et al. 1983).

8.5.5.1 Hypertrophic Cells

Stretching in the cells size (hypertrophy) near the head of nematodes in feeding site (outside and near feeding tube) as a result of their impact on some activated auxins resulting from salivary secretions of nematodes with cytoplasm (Gheysen and Mitchum 2011).

8.5.5.2 Hyperplastic Cells

As a result of some of the contents of nematode salivary secretions, hyperplastic cells are formed; these show increased cell numbers, with abnormal cells that are smaller than healthy cells (McClure 1977).

8.5.5.3 Giant Cells

One of the most common histological symptoms of plant association with the most common nematode genera is the formation of giant cells. These abnormal cells show extremely thick cell walls. They are of different sizes and contain numerous pit fields with many plasmodesmata ranging between two and seven per cell depending on the host sensitivity to the infestation and the nematode species and stage. The cells also differ in their numbers of nuclei (Cabrera et al. 2017).

Single uninucleate giant cells are commonly associated with hosts responding to nematodes belonging to genera of the subfamily *Heteroderinae* (family Heteroderidae), e.g., *Heterodera*, *Globodera*, and *Punctodera*. The single nucleus is expanded to accommodate the increase in the nuclear and cytoplasmic exchange rate (Mundo-Ocampo and Baldwin 1984). Multinucleate giant cells, often associated with the root-knot nematodes, *Meloidogyne* spp. are formed by the expansion of approximately half a dozen cambial cells within the differentiating; in the absence of cytokinesis and repeated synchronous mitoses these cells becomes multinucleate (Wiggers et al. 1990; Endo 1991).

8.5.5.4 Syncytia

The appearance of syncytia is a histological change that is often associated with the potato cyct nematode (CN), *Globodera*, and with the sugar beet CN, *Heterodera*. Syncytia appear as single cells formed by the merging of the cytoplasm and nuclei of many hypertrophic cells where the cell walls are degraded. These cells merge and become syncytia with the condensation of cytoplasm the number of nuclei is similar to the number of hypertrophic cells involved (Endo 2012).

8.5.5.5 Syncytium

A group of more than 100 natural cells (normal healthy cells) in infested tissue is converted into a syncytium cell without the complete degradation of these cells' walls or the degradation of the cytoplasm (Rodiuc et al. 2014). Each cell appears as a self-contained syncytium cell containing dense cytoplasm; protoplasts are fused together through the partial dissolution of local cell walls and there is one enlarged nucleus (Bohlmann and Sobczak 2014). Also, the osmotic pressure inside the syncytium is greater than that in the outer syncytial cell walls (Siddique et al. 2014). There are some differences in syncytium sites as listed below:

(a) Most are commonly formed within the vascular cylinder, as associated with the CNs, *Heterodera*, *Globodera*, *Punctodera*, and *Cactodera*.
(b) Syncytium formation occurs within the cortical cells of tissue infected by the false root-knot nematode, *Nacobbus* spp.
(c) Some syncytium formation may occur in the pericycle. *Gossypium herbaceum* roots infested with reniform nematode, *Rotylenchulus reniformis is an example*.
(d) In the xylem parenchyma cells of rape, *Brassica napus* var. *oleifera* infested with beet CN, *Heterodera schachtii* Schmidt, the syncytium volume was largest 5 days after infestation (Magnusson and Golinowski 1991).
(e) As reported by Cohn et al. (1984), syncytium formation may occur in the cortex layer of buttonweed, *Diodia virginiana* infested with *Verutus volvingentis* (Heteroderidae: Tylenchida).

8.5.5.6 Nurse Cells

One of the characteristics of the nutrition site of the citrus nematode, *Tylenchulus semipenetrans*, where immature female deeply penetrates the cortical layers normally without reaching the central cylinder (or perhaps the endodermis) and becomes sedentary (Duncan 2005). Nurse cells are characterized by stable size and normal shape of the healthy cells with some of the following changes: (a) an enlarged nucleus and nucleolus larger than healthy cells, (b) thickness of cells wall to about 8–10 cells to formed nurse cells as feeding site, (c) dense cytoplasm and (d) a large vacuole (Vovlas 1987).

8.6 Conclusions and Future Prospects

In summary, development of healthy plant consider complex process in shapes and functions, and phytoparasitic nematodes in any stage represents a biotic stress factor. Nematode infestations to the crop may cause greater damage in plant health by reducing the germination, growth, morphogenesis, reproductive growth,

etc. Different nematodes causes various symptoms which can not be easily diagnosed. Therefore, a suitable techniques are needed so that identification of nematodes as well as their symptoms can be easily identified. It is high time for the researcher to think about the management practices as this pathogen is causing economic level damage to wide range of cultivated crops.

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Chapter 9
Potential Role of Plant Growth Promoting Rhizobacteria in Alleviation of Biotic Stress

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Abstract Plant growth promoting rhizobacteria (PGPR) are well known to ameliorate the plant health. A large number of rhizobacteria possess the growth promoting activities. Some of them are very common and has been also commercialised to large/industrial scale. Plant growth regulators have been found to induce the growth and development of various crop plants. Some hormones like auxin, cytokinin, IAA, etc. are the key hormones in the plant growth promotion. However, their ratio of auxin to cytokinin may be determinant in the lateral root or root hair formation. The root surface area and root lengths are also conceived to play very important role in the accumulation of nutrient and are significantly influenced by the application of PGPR. Moreover, PGPR also have the biocontrol activities against a wide range of soil-borne plant pathogens. Some organic molecules such as siderophores, antibiotics, and bacteriocins producing PGPR arrest the pathogen populations and improve the plant health indirectly. Presence of more PGPR in rhizosphere exhibits more vigour plant health. Therefore, PGPR is considered as an alternative and effective way in the management of plant pathogens and plant growth promotion.

Keywords PGPR · Biotic stress · Plant growth · Rhizobium · Siderophore

9.1 Introduction

PGPR encompass a wide range of soil bacteria with some beneficial impacts on plant and soil health (Miransari 2014; Ansari et al. 2017). Various kinds of soil microbes perform different activities in the soil, some of which are very beneficial in the sustenance of ecological services. The most beneficial microbes are PGPR including *Rhizobium* sp., nitrogen fixer. The PGPR have been researched extensively by looking at the importance of these important bacteria. Plant growth promoting activities take place directly (in the absence of the pathogens) or indirectly

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(by protecting the host plants from harmful pathogens) (Ansari et al. 2017). The other important point which pertains to PGPR is how the root colonisation takes place. It is an important part of plant growth promotion because this may affect the plant growth activities. Effective strains of PGPR efficiently control the disease caused by plant pathogens in the roots and also help in the movement of beneficial bacterial populations towards the roots (Lugtenberg and Kamilova 2009). Bacterial populations of soil have the ability to grow rapidly and use a wide array of substances as a substrate. Bacterial populations are dispersed in the soil, often attracted to the plans (Goswami et al. 2016). Moreover, rhizosphere contains maximum bacterial populations than in bulk soil. This seems to be due to bacteria possessing metabolic activity for the adsorption and utilisation of root exudates. One of the chief mechanisms, through which PGPR remain adsorbed with the soil, is ion exchange. The significant role of PGPR in the functioning of the ecological services/niches has been gaining significant importance throughout the world. A putative PGPR qualify when it exerts positive effects on plant growth promotion (Goswami et al. 2016). PGPR play significant role in achieving the sustainable crop production trend for the future. In addition, rhizosphere consists of most essential part of the ecological habitat in soil and becomes the house of a wide variety of microorganisms including PGPR dwelling in close contact with plant roots (Brink 2016). PGPR remain in close contact with the roots and influence plant growth promotion through several mechanisms including direct as well as indirect effects. Release of some plant growth promoting substances by PGPR such as phytohormones, metabolites, etc. is engaged in the modification of the rhizospheric microbial community structures leading to the deviation of rhizospheric environment towards the formation of more diverse and more beneficial microbial diversity (Ahemad and Kibret 2014; Ansari et al. 2017). Moreover, several other processes like exchange of signal molecules, mobilisation of nutrients to make them easily available to plants' absorptive surface, and creation of a protective layer on the root surface also work simultaneously for the improved plant growth and development (Ahemad and Kibret 2014).

9.2 Factors Influencing the PGPR Diversity in the Rhizosphere

Among various factors responsible for the development of a particular microbial community in the rhizosphere, root exudates are the most important one. Components of the root decide which type of PGPR community will develop in the vicinity of roots. Moreover, chemical components of the root exudates enrich the rhizosphere with essential nutrients thus providing better harmony for beneficial population leading to development of improved plant health. These exudates act as a carbon source for the proliferation of microbial population. Certain important organic molecules from the root exudates also chemotactically attract the microbes towards the root. Besides, such root exudates are also helpful in maintaining steady levels of

some flavonoids and mineral nutrients released after the degradation of organic additives (Dakora and Phillips 2002). Therefore, based on the nature of the released organics and flavonoids in the rhizosphere, development of specific diversity of PGPR takes place.

9.3 Plant Growth Promoting Activities of PGPR

The plant hormones especially auxin and cytokinin are the chief regulator of organogenesis and destine the root structure (Aloni et al. 2006). The ratio of these two phytohormones may be influenced due to PGPR activities as these microbes produce a wide array of secondary metabolites which interfere the normal hormonal pathways. Indole-3-acetic acids are the best characteristics produced by the PGPR and also some other beneficial bacteria (Spaepen et al. 2007). External application of IAA regulates the plant growth and development process. For instance, low IAA can trigger the primary root elongation; however, on the other hand, high level of IAA starts formation of lateral roots. It also reduces the primary root formation and enhances the root hair formation (Patten and Glick 1996; Perrig et al. 2007; Remans et al. 2008). The plant growth-promoting effects of auxin or auxin-like compounds need some signalling molecules in the host plants. The hypothesis can be realised very well by using the *Arabidopsis* as a model plant (Dubrovsky et al. 1994; Alonso et al. 2003). Other hormone such as cytokinin production has also been observed in various species of PGPR like *Arthrobacter giacomelli*, *Azospirillum brasilense*, *Bradyrhizobium japonicum*, *Bacillus licheniformis*, *Pseudomonas fluorescens*, and *Paenibacillus polymyxa* (Glick 1995; Cacciari et al. 1989; Timmusk et al. 1999; García de Salamone et al. 2001; Perrig et al. 2007; Hussain and Hasnain 2009; Ansari et al. 2017). This hormone triggers the cell division of plant cell, controls root meristem differentiation, accelerates root hair proliferations, and, however, inhibits the primary root elongation and root formations (Riefler et al. 2006; Fukaki and Tasaka 2009). Application of cytokinin-producing bacteria enhances the shoot growth and reduces the root to shoot ratio (Arkhipova et al. 2007). PGPR directly enhance the nutrient supply in the rhizosphere and accelerate the ion transportation in the root system. As far as enhanced nutrient supply is concerned, phosphate solubilisation is the key effect of PGPR on plant nutrition. Generally, soil may have phosphorus sink which is due to regular application of fertilisers. Plants are unable to absorb the insoluble forms of phosphorus. PGPR assists rigorously in the mineralisation and solubilisation of the phosphorus (Richardson et al. 2009; Ramaekers et al. 2010). Many PGPR like *Pseudomonas*, *Bacillus*, and *Rhizobium* play significant role in the dissolution of phosphate (Richardson et al. 2009). In addition, PGPR also modulate root structure and other vegetative growth and physiology of the plants. The plant growth and development have long been associated with the production of IAA by various PGPR groups. It has been seen that plant hormones act as regulators for the biosynthesis of many other hormonal pathways. The plant growth and development are accelerated by biological nitrogen fixation,

enhancement in the rhizoplane nutrients, acceleration in root surface area, enhancement in the other beneficial symbiotic relationships, and combination of all modes of action (Ahemad and Kibret 2014). Application of putative strains of PGPR may improve the volume of root and other vegetative parts of the plant and will ultimately improve the plant health. Therefore, research pertaining to PGPR application should strive to design and execute in order to identify the putative strains of PGPR. For example, *Azospirillum brasilense* was found to be effective in promotion of growth and development of plant (Glick et al. 1994; Holguin and Glick 2001; Li et al. 2005; Perrig et al. 2007; Cohen et al. 2008; Cassan et al. 2009; Elias et al. 2018; Malinich and Bauer 2018).

9.3.1 Nitrogen Fixation by PGPR

Moreover, as far as nitrogen fixation is concerned, exploitation of *Allorhizobium, Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* may have considerable influence in the leguminous plants. It is very interesting to note that many PGPR have the ability to fix the nitrogen which ultimately stimulates the plant growth promotion (Sessitsch et al. 2002). PGPR having the nitrogen fixation ability are assumed to be due to the presence of the nitrogenase activity (Hurek and Reinhold-Hurek 2003). For instance, *Beijerinckia* sp. (Baldani et al. 1997), *Klebsiella pneumoniae* (Riggs et al. 2001), *Pantoea agglomerans* (Riggs et al. 2001), and *Rhizobium* sp. (Antoun et al. 1998; Yanni et al. 2001) have shown the nitrogen fixation activity. It is also very important to know that many PGPR are the diazotrophs; however, mechanisms behind improved plant growth are not well understood (Dobbelaere et al. 2003; Ahemad and Kibret 2014). Moreover, inoculation of *Azospirillum brasilense* on nonleguminous crops improves the plant health status. Several mechanisms such as phytohormone production and root morphology may be the substantial reasons behind ameliorated plant health. For example, *Gluconacetobacter diazotrophicus* have been reported to contribute significantly in the nitrogen accumulation of sugarcane (Sevilla et al. 2001). Similarly, other endophytes like *Herbaspirillum seropedicae* have been found to improve the nitrogen status of sugarcane (James et al. 2002).

9.3.2 Augmented Availability of Nutrients

Enough evidences supporting the mode of action of PGPR involving increased availability of nutrients to the plants are available in the literature (Ansari et al. 2017). These mechanisms may involve the solubilisation of unavailable forms of nutrients to the plants and production of siderophores that assist in facilitating the transport of the some nutrients (mainly ferric ion).

9.3.3 Solubilisation of Phosphates

Phosphorus occupies second position only after nitrogen among other minerals that generally limits the growth of terrestrial plants. Generally, soil may contain huge amount of total phosphorus, but the forms that are available to plants constitute a very tiny portion of this phosphorus because most of phosphorus present in the insoluble forms. Plants are able to utilise phosphorus in two forms – the monobasic (H_2PO_4) and diabasic ($HPO4^{2-}$) ions (Glass 1989). PGPR promoting phosphate solubilisation are commonly available in the rhizosphere that promote availability of phosphorus to the plants by the secretion of organic acids as well as phosphatases (Han and Lee 2006). Phosphate solubilisation in the rhizosphere is one of the most common modes of action that might have been implicated in plant growth promotion (Vessey 2003). Moreover, iron is an essential nutrient of plants, but it is relatively insoluble in soil solutions. Plant roots prefer to absorb iron as the more reduced ferrous (Fe^{2+}) ion, but the ferric (Fe^{3+}) ion is more common in well-aerated soil although it is easily precipitated in iron oxide forms (Salisbury and Ross 1992). Plants commonly excrete soluble organic compounds (chelators and phytosiderophores).

In addition, root morphology plays a very important role in the accumulation of essential nutrients. Any alterations in root morphology can lead to altered nutrient availability to the plant parts. PGPR influencing root morphology particularly that increases root surface area improve nutrient uptake potential of roots, thereby positively influencing plant nutrient status and growth. PGPR improving plant health mainly follows the mechanisms of altering root growth and morphology. Improvement in root weight inoculation of PGPR is a most common impact of bacteria (Beneduzi et al. 2012). This may involve the production of some antipathogenic substance or through the induction of resistance against these pathogens in the plants by PGPR. The factor behind increased surface area by PGPR is the production of phytohormone which is well known for their ability to enhance the plant growth and development. These hormones are believed to alter the partitioning assimilation pattern in the plants, thus arranging growth patterns in roots. This in turn resulted in bigger and more branched roots with greater surface area. IAA (indoleacetic acid), a phytohormone, is reported to be responsible for the root interaction as well as cell division and enlargement. PGPR commonly produce this hormone leading to enhanced improvement in root surface area enabling the root to reach to the nutrient sink leading to improved plant health (Ashrafuzzaman et al. 2009).

9.4 Alleviation of Biotic Stress

PGPR influence plant growth promotion and development either indirectly or directly. The direct way of plant growth promotion by PGPR includes either supplying the plants with the compounds produced by the bacterium like growth hormones

or by helping the plants in better uptake of essential nutrients from the plant environment (Saleem et al. 2007; Ansari et al. 2017). While on the other hand, indirect pathways induce the protection of plants from the attack of harmful pests and pathogens (Beneduzi et al. 2012). PGPR can use on or both of these mechanisms to promote the plant growth and development. Some common mechanisms are involved in the plant growth promotion and protection from various pathogens of plant origin. Application of PGPR in the control of plant disease holds a great promise for the sustainable farming. PGPR applications have been investigated in various crops in terms of plant disease management (Gray and Smith 2005).

9.4.1 Microbial Antagonism

Some bacteria able to reduce the disease severity are said to be biocontrol agents (Beneduzi et al. 2012). A wide range of PGPR synthesise some hydrolytic enzymes like chitinase, glucanase, protease, and lipase which may break the fungal cells (Neeraja et al. 2010; Maksimov et al. 2011). In addition, PGPR also create some nutrient and niche competition for plant pathogens, regulation of some plant hormones like ethylene level by ACC deaminase enzyme (Van Loon 2007), and siderophore production (Ansari et al. 2017).

9.4.2 Siderophore, Antibiotic, and Bacteriocin Production as Antagonistic Activities

Siderophores and some other organic molecules greatly contribute in the management of plant pathogens (Maksimov et al. 2011). The siderophores are very closely related to supply and channelisation of iron in various biological processes (Crosa and Walsh 2002). Besides, PGPR can also produce some organic compounds with antimicrobial activity that prevents the entry of plant pathogens. For example, antibiotics, lactic acids, exotoxins, and bacteriocins have bactericidal activity (Riley and Wertz 2002). Siderophores, bacteriocins, and antibiotics are the mechanisms which are found effective in reducing the population of phytopathogens (Ansari et al. 2017).

9.4.2.1 Siderophores

Siderophores are low molecular weight iron chelators that solubilise the iron from surrounding environments. Production of siderophores offers various competitive advantages to PGPR which may colonise the root efficiently and also exclude other microorganisms especially harmful one from the niche (Haas and Défago 2005).

Some bacterial siderophores such as pseudobactin produced by *P. putida* B10 were found to be suppressive to *F. oxysporum* in iron-deficient soil (Kloepper et al. 1980). However, some recent studies have revealed that siderophores producing pseudomonads make iron unavailable to the plant pathogenic organisms (Loper 1988; Paulitz and Loper 1991; Dwivedi and Johri 2003).

9.4.2.2 Antibiotics

In addition to production of siderophores, PGPR like pseudomonads also produce antifungal antibiotics as their biocontrol strategy (Haas and Keel 2003). Production of one or more of such antibiotics makes PGPR able to successfully antagonise a wide scale of phytopathogens (Glick et al. 2007). Antibiotics represent a heterogenous group of low molecular weight organic compounds that are harmful for the growth as well as metabolic activities of other groups of microorganisms (Fernando et al. 2005). Haas and Defago (2005) have categorised antibiotic compounds into six classes – phenazine, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides, and hydrogen cyanide. All of these are better related to biocontrol of root disease, and their mode of action is partially understood (Compant et al. 2005). Moreover, another group of compounds such as lipopeptide biosurfactants released by pseudomonads and *Bacillus* species have been found to be potentially effective against various microorganisms such as bacteria, fungi, oomycetes, protozoa, and nematodes (Raaijmakers et al. 2010). Thus, these can also be implicated in the biocontrol of microorganisms by PGPR. The mechanisms of action behind these antibiotics include the inhibition of the cell wall synthesis of pathogens, effect on cell membrane structure, and also inhibition in the formation of initiation complexes on the ribosome (Reddy et al. 2004). The antibiotic pyrrolnitrin produced by *Pseudomonas fluorescens* BL915 strain was found to be effective against *Rhizoctonia solani* causing damping off in cotton plants (Hill et al. 1994). In addition, 2,4-diacetylphloroglucinol (DAPG) produced by pseudomonads is a largely studied antibiotic which helps in the cell membrane disruptions of *Pythium* spp. and also found effective against oomycetous fungi (de Souza et al. 2003). Another antibiotic, phinazine produced by pseudomonas, has the potential to show redox activity which may damage plant pathogens like *Fusarium oxysporum* and *Gaeumannomyces graminis* (Chin-A Woeng et al. 2003).

Moreover, majority of *Bacillus* spp. are reported to produce antibiotics such as polymyxin, circulin, and colistin which are found to be effective against gram-positive and gram-negative bacteria and are also deleterious to many pathogenic fungi (Katz and Demain 1977). Zwittermicin A (aminopolyol) and kanosamine (aminoglycoside) produced by *Bacillus cereus* UW05 strain were found to restrict the growth of oomycete pathogens, hence contributing in the biocontrol of alfalfa damping off (Silo-Suh et al. 1994; Quagliotto et al. 2009).

9.4.2.3 Bacteriocins

Bacteriocins are the other group of molecules implicated in microbial defence system. Bacteriocins are different from the antibiotics; they generally possess a comparatively lower killing spectrum and are lethal only to bacteria which are closely related to the bacteriocin-producing strain (Riley and Wertz 2002). All the bacteria are able to produce at least type of bacteriocin. Several bacteriocins extracted from gram-negative bacteria seem to have been produced by the recombination between existing bacteriocins (Goh and Philip 2015). The most important representative bacteriocins include the colicins produced by some strains of *E. coli*. Such bacteriocins have been found to be lethal for the related strains. Other examples of bacteriocins from different bacterial strains include pyocins from *P. pyogenes* strains, cloacins from *Enterobacter cloacae*, marcescins from *Serratia marcescens*, and megacins from *B. megaterium* (Cascales et al. 2007). Interestingly, *Bacillus* spp. produces some bacteriocins which sometimes exhibit broader spectrum of inhibition.

9.5 Conclusions

It has been seen through this article that PGPR play a very important role in the amelioration of plant growth and development and protection of the plants from diverse range of plant pathogens. Application of potent PGPR irrespective of mode of application may induce the resistance against various phytopathogens. Exploitation of suitable PGPR might be beneficial in the formulations of new management modules. The bacterial inoculations provide the great antagonists against a wide range of plant pathogens and improve the plant health. Thus, suitable inoculants can induce the resistance and augment the biocontrol activity. Various pseudomonads trigger the production of different phytohormones which ultimately enhance the plant growth and productivity. The siderophores also influence the plant growth and development scale. Conclusively, the PGPR may be considered as good tools in the alleviation of biotic stress especially stresses induced by soil-borne plant pathogens.

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Chapter 10
Harnessing Endophytes as Biocontrol Agents

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Abstract Microbial endophytes represent an endosymbiotic group that colonizes internal plant tissues. Endophytes are one of the least studied and unexplored groups of microbes that need attention, so as to provide comprehensive knowledge regarding beneficial plant-microbe interactions. One of the emerging issues in the area of agriculture is a gradual decrease in productivity (quality and quantity) of agroproducts because of various biotic and abiotic stresses. The problem pertaining to the rise of pesticide resistant phytopathogens and decreased soil fertility is linked with improper use of pesticides. Recent advancement in the area of endophytic microbes working as biocontrol agents could be a potential option to address the aforementioned problems. But the real challenge lies in taking these potential candidates from laboratory to land. In the present chapter, we have discussed different mechanisms through which endophytes suppress microbial diseases in host plant, the major steps involved in developing mechanism-based bioformulations from these endophytes, and their use in advanced agricultural system for future benefits.

Keywords Endophyte · Endorhizae · Plant-Microbe interaction · Bioformulation · Phytopathogens

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10.1 Introduction

Rapid rise in the world's population is becoming a common phenomenon with the head count of this populace predicted to reach around ~8 billion by 2020 (Scherbov et al. 2011). Feeding this gigantic population with limited resources is a big challenge for the world community. About 50 years back, "green revolution" was a much-talked topic, which led to tremendous increase in food production in India. During the time of green revolution, high-yielding varieties and enormous use of chemical fertilizers and pesticides have undeniably contributed to the terrific increase in food production. With time this has led to gradual loss of natural soil microbiota and soil fertility (Nicholson and Hirsch 1998). Incessant use of pesticides gave rise to many pest-resistant species, and huge risk to producers and consumers. In spite of adopting several agricultural advancement strategies, plant pathogens still account for more than 15% losses in the global harvest. Among these, fungal pathogens are a major threat to crops leading to 30% reduction in crop yield. Such a loss translates to nearly 200 billion rupees per annum in global market (Shaikh and Sayyed 2015). Most of the pesticides in use are recalcitrant to biodegradation resulting in long-term environmental concern and health problems. Thus, in order to resolve this burning issue of pest/pathogen control, more eco-friendly, green, and sustainable approach is required. The utilization of biological agents, especially beneficial microbes, is considered as potential alternative and safe way to protect plants from pathogens. Controlling these pathogens by diverse microorganisms acting as natural antagonists has been practiced routinely over the past century.

Biocontrol using antagonistic microbes offers a highly efficient, cost-effective, and eco-friendly substitute to the application of synthetic chemical pesticides. Plant-associated microbes (PAM) are effective competitors, which can establish and persist on diverse crop plants. Extensive literature unfolding possible roles of PAM as plant growth promoters and disease-suppressive agents is available (Singh et al. 2016; Odoh 2017; Shafi et al. 2017). However, one of the least studied and unmapped group of PAM that resides within the plant system and establishes in internal plant environment are known as endophytes. Most of them are able to surpass the endodermal barricade by passing root cortex to the vascular and consequently flourish as endophytes in leaves, tubers, seeds, stem, and other plant organs (Patriquin and Dobereiner 1978; Hallmann et al. 1997). Cryptic life of endophytes states that they are prime colonizers of dead plant tissues and chiefly act as decomposers in the ecosystem (Osono 2006). There are certain validations that suggest that few endophytes play uncommon roles in the ecosystem such as protecting plants from pathogens that cause diseases (Prieto et al. 2011). Also, endophytic colonization within plant system results in development of an intimate relation between the two and offers protection to plants against diverse pathogens. Endophytes have evolved a close relationship with their host plants during the time of evolution thereby affecting physiological route of plants. Additionally, their exclusive ability to persist and

reside within plant system without any competition makes them suitable for biological control (Devi and Momota 2015).

"Harnessing the role of endophytes as biocontrol agent" is an interesting topic that will be covered in this chapter. The reason for selecting endophytes over non-endophytic population in this chapter is due to its innumerable advantages. First, as endophytes reside inside host plants, they can colonize very easily and remain protected throughout their life span. Second, application of endophytes is easy, as it enters the target site and does not require several field applications (booster doses) during crop development (Wani et al. 2015). Third, they have extraordinary capacity to tolerate abiotic and biotic stress factors. Additionally, they also find application in the fields of nanosciences, modern medicine, bioremediation, bio-augmentation, forest management, and industrial perspective (Devi and Momota 2015). In spite of several advantages, endophytes hold some disadvantages too; culture-dependent techniques, used for isolating absolute endophytes, sometimes give false results, and it is difficult to analyze the exact endophytic diversity in plant. Franks et al. (2006) reviewed innumerable molecular tactics for isolating and characterizing endophytic community, which include culture-independent methodologies to gain maximum information on endophytic diversity.

Though different stories of endophytes have been elucidated by different workers, the aim of this chapter is to precisely focus on the biocontrol attributes of endophytes along with their potent mechanisms. The chapter also focuses on the important criteria involved in taking this endophytic system from laboratory to land.

10.2 Endophytes

The term endophytes was first coined by a German botanist, Anton de Bary, in 1886 referring to those organisms that inhabit internal tissues of leaves and stems (Wilson 1995). The existence of endophytes was first documented by Vogl in 1898 revealing a mycelium residing in the seed of *Lolium temulentum* (Guerin 1898; Vogl 1898). Different definitions of endophytes are given by different researchers, but the most widely accepted one is "bacteria or fungi allocated within the plant tissues without causing any harm to the host" (Bressan and Borges 2004).

On the basis of functionality, endophytes are characterized into three main groups, viz., plant growth promoters, biocontrol agents, and plant stress homeoregulating microbes (Bashan and Holguin 1998; Cassan et al. 2009). On the basis of distribution, endophytes have been classified into three main groups: the first group includes obligate endophytes that can proliferate only inside the plant, and they fail to flourish outside; the second group includes facultative endophytes that are usually free-living, but, if opportunity ascends, they can exhibit massive colonization in plant through coordinated infection (Hardoim et al. 2008); and the third group includes passive endophytes, which do not show active colonization but do so as a result of stochastic events like wounds or abrasion in the root curls. Endophytes are

generally host specific. Relationship of endophytes with its host partner could be described in terms of host selectivity, host recurrence, or host preference.

Several authors have elaborated on the microbiome present in pockets of rhizosphere and rhizoplane, but very few have focused on the microbial community residing within the plant root system. Microorganisms present inside plant root significantly differ from those residing in the rhizosphere and rhizoplane (Kloepper and Beauchamp 1992; Gottel et al. 2011). Hence, it is necessary to unravel this hidden and complex zone inside the plant roots, termed as endorhiza, for further exploration of microbial diversity. Endorhiza is broadly defined as root tissues below the epidermal layer including vascular and cortical tissues (Mahaffee and Kloepper 1997).

Rhizobium etli is a well-known endophyte that naturally occurs in maize plant, when maize-bean crops are grown in association with each other (Gutiérrez-Zamora and Martínez-Romero 2001). Sprouts and seeds of alfalfa mainly harbor endophyte *Salmonella enterica* and *Escherichia coli,* which have been detected by green fluorescent proteins (Cooley et al. 2003). *Rhizobium rhizogenes* and *R. leguminosarum* are normal red clover symbiont found in the root nodules of clover plants (Sturz et al. 1997). Xylem vessels and stomatal compartments of *Vitis vinifera* primarily contain endophyte *Burkholderia* within it (Compant et al. 2005a, b). Dong et al. (2003) observed clumping of *Klebsiella* strain Kp342 at lateral root joints of alfalfa and wheat plant. Thus, it seems that the endophytes, whether bacterial or fungal, best adapted for dwelling inside plants are naturally selected and recruited from soil to aboveground plant tissues.

Distribution of endophytes within plant is governed by two main factors: first is colonizing aptitude, and second is resource allocation throughout the plant. Root endophytes often colonize and enter the epidermis from the site of root cracks, lateral root emergence, and below the root hair zone (Compant et al. 2005a, b; Zakria et al. 2007). During initial colonization, few endophytes can enter aboveground plant parts by entering the vascular tissues and scatter systemically (Johnston-Monje and Raizada 2011). Johnston-Monje and Raizada (2011) confirmed the transport of the green fluorescent protein tagged endophytes from seeds into roots, roots into stem, and stems to roots and rhizosphere, suggesting a continuous movement of endophyte throughout the plant system.

The second factor influencing dispersion of endophytes is the allocation of plant resource. Chi et al. (2005) stated that different slices of plant tissues can harbor distinct endophytes, like *Pseudomonas* are more common in the stems than in the roots of potatoes after a month of growth (Garbeva et al. 2001). Higher endophytic population in crown region of carrot was observed compared to metaxylem tissues due to high level of photosynthate (Surette et al. 2003). Fisher et al. (1992) reported significant difference in the distribution of endophytes colonizing maize crop. Leaves of maize disclosed heavy colonization by bacterial endophytes in comparison to stem; however, more fungal endophytes were recovered from core and epidermis of stem in comparison to the leaves. Ji et al. (2010) documented the epiphytic and endophytic lifestyle of rhizobia in tobacco plant and suggested that endophytic rhizobia depart from the leaf interior through stomata and colonize the phyllosphere.

Additionally, rhizobia can also colonize roots and aerial plant tissues of rice, wheat, barley, canola, *Arabidopsis*, and lettuce (Stone et al. 2001; Luby-Phelps et al. 2003). Certain endophytes can colonize fruits, flowers, berries, and seeds. Patil (2013) reported plant growth-promoting endophytic bacteria *Asaia bogorensis* associated with mango fruit. Similarly *Bacillus*, *Acinetobacter*, and *Enterobacter* are common endophytes present in papaya fruit (Krishnan et al. 2012). Fruits belonging to the family Cucurbitaceae *Cucumis melo reticulatus*, commonly known as melon, usually contains endophytes α-, β-, and γ-*Proteobacteria*, *Firmicutes*, and *Actinobacteria* within it (Glassner et al. 2015). Endophytic genera, including *Acinetobacter*, *Methylococcus*, *Bacillus*, *Micrococcus*, and *Planococcus*, residing in rose (*Rosa damascena trigintipetala*) during flowering state, have growth-promoting and biocontrol attributes (El-Deeb et al. 2012). The involvement of these endophytes in development and maturation of reproductive segment of plants and their potential use as biocontrol agent is yet to be elucidated.

Hence, it could be concluded that different endophytes display diverse distribution on associated plants. Several molecular studies have been conducted to observe the distribution of endophytes within plant cell, but the exact mechanisms behind this establishment needs further elucidation. Further investigations related to transcriptomics of endophytes and host plants may serve as promising approaches to discover the drivers of plant–endophyte interactions.

10.2.1 Bacterial Endophytes

Bacterial endophytes have recently been in focus as biocontrol agents, as they provide additional benefits in comparison to rhizospheric colonizer (Hallmann 2001). Bacterial endophytes are recruited from the rhizosphere at the site of wound, cut, or lesion and colonize both vegetative and reproductive parts of plant like tuber, root, stem, leaf, flower, and fruits (Gray and Smith 2005; Compant et al. 2005a, b). Mechanisms by which they protect their host plant are more or less similar as described for PAM in the rhizosphere. Different workers have reviewed the elaborated mechanisms of these endophytes (Kloepper et al. 1999; Hardoim et al. 2015; Chaturvedi et al. 2016). Mechanisms by which endophytes enhance plant growth are categorized as direct and indirect (Long et al. 2008). Direct mechanisms include nitrogen (N_2) fixation, phosphate (P) solubilization, iron (Fe) chelation, 1-aminocy clopropane-1-carboxylate (ACC) deaminase activity, and phytohormone production, whereas indirect modes include pathogen suppression by outcompeting them for macro- and micronutrients, siderophore production, antibiotic production, establishment of the plant's systemic resistance, secretion of lytic enzymes, and secondary metabolite production (Fig. 10.1).

Diseases of bacterial, fungal, and viral origin, and in some cases damage caused by nematodes and insects, can be decreased by endophytic inoculation (Berg and Hallmann 2006; Ryan et al. 2008). Few endophytic microbes elicit the phenomenon of induced systemic resistance (ISR). The role of bacterial endophytes in connection

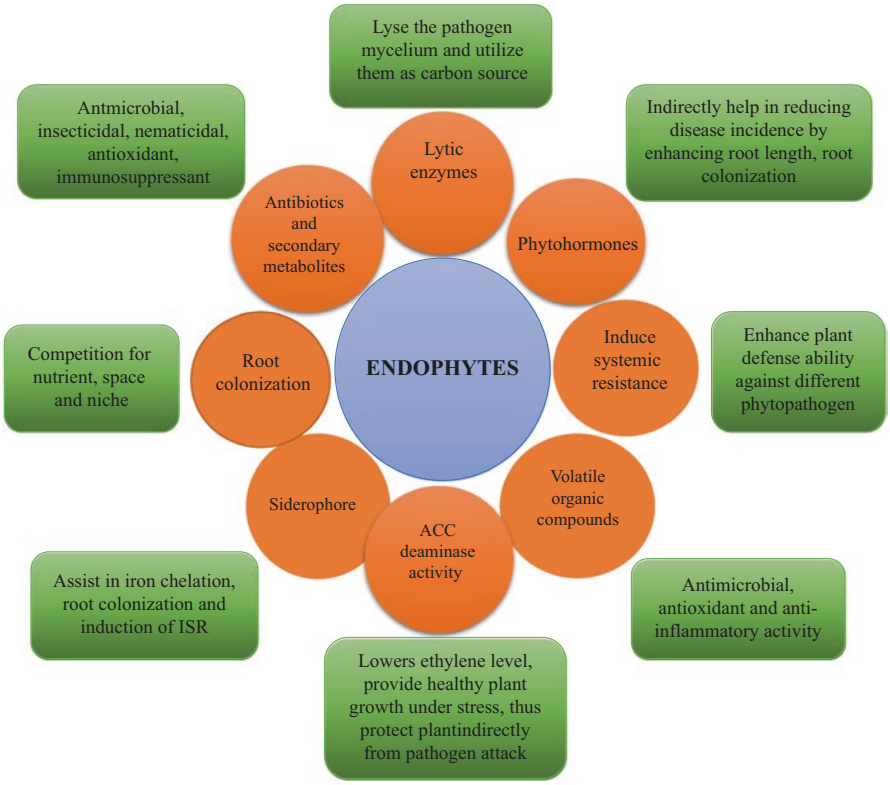

Fig. 10.1 Different mechanisms of disease suppression by endophytes

with ISR has been reviewed by Kloepper and Ryu (2006). Several examples of bacterial endophytes such as *Actinobacter*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Paenibacillus*, *Pantoea*, *Pseudomonas*, *Rhizobium*, *Streptomyces*, etc. are used nowadays as biocontrol agents against plant pathogens; few of them have been discussed below briefly. Table 10.1 summarizes details of endophytic bacteria along with their mechanisms/metabolites that validate their potential role in biocontrol of phytopathogens.

Bacillus pumilus INR7, an endophyte found in the stem of cucumber plant, is capable of suppressing cucurbit wilt disease caused by *Erwinia tracheiphila* under field conditions. There was noteworthy increase in plant growth parameters and disease suppression in sets receiving bacterial treatment in comparison to control sets under field conditions (Wei et al. 1996). *Pseudomonas fluorescens* PICF7 is a native olive (*Olea europaea* L.) root endophyte and active biocontrol agent against *Verticillium* wilt of olive. Strain PICF7 is an active root colonizer, and this rapid invasion not only triggers defense response in root system but also mounts an extensive range of systemic defense responses in aboveground aerial parts of plant like stems and leaves, thus explaining how ISR contributes to biocontrol.

Table 10.1 Diverse mechanisms and metabolites involved in inhibition of growth of phytopathogens by endophytes

Endophyte	Host plant	Biocontrol agent against	Mechanism/bioactive metabolites	References
Bacterial endophytes				
Ampelomyces	*Urospermum picroides*	*Enterococcus*, *Staphylococcus*	3-O-methylalaternin Altersolanol	Aly et al. (2008)
Bacillus cereus BT8	*Solanum lycopersicum*	*Phytophthora capsici*	ISR	Melnick et al. (2008)
Bacillus subtilis MJMP2	*Brassica campestris*	*Xanthomonas oryzae*, *Fusarium oxysporum*, *Rhizoctonia solani*	Iturin A	Cheng et al. (2016)
Bacillus subtilis CEN_3	*Brassica napus*	*Fusarium*, *Magnaporthe*	Siderophores, root colonization	Etesami and Alikhani (2016)
Bacillus amyloliquefaciens CEIZ-11	*Solanum lycopersicum*	*Alternaria alternata*, *Aspergillus niger*, *Botrytis cinerea*, *Fusarium oxysporum*, *Pythium aphanidermatum*	Cyclic lipopeptide	Gao et al. (2015)
Bacillus pumilus INR7	*Cucumis sativus*	*Erwinia tracheiphila*	ISR	Yi et al. (2013)
Enterobacter HA01	*Gossypium*	*Verticillium dahliae*, *Fusarium oxysporum*	Siderophore, protease, root colonization	Li et al. (2012)
Pseudomonas fluorescens PICF7	*Olea europaea*	*Verticillium dahliae*	ISR	Lama Cabanás et al. (2014)
Pseudomonas viridiflava	*Poaceae*	*Cryptococcus neoformans*, *Candida albicans*	Ecomycins	Miller et al. (1998)
Paenibacillus polymyxa M1	*Triticum aestivum*	*Erwinia amylovora*, *Erwinia carotovora*	Polymixin	Niu et al. (2013)
Paenibacillus polymyxa PB71	*Cucurbita*	*Didymella bryoniae*	Unkown soluble and volatile metabolites	Fürnkranz et al. (2012)

(continued)

Table 10.1 (continued)

Endophyte	Host plant	Biocontrol agent against	Mechanism/bioactive metabolites	References
Paenibacillus polymyxa Wb2–3, Mc5Re-14	*Matricaria chamomilla*	*Fusarium culmorum*, *Rhizoctonia solani*, *Verticillium dahliae*	β-1,3-glucanase, siderophores	Köberl et al. (2013)
Rhizobium etli G12	*Solanumtuberosum*, *Arabidopsis*	*Meloidogyne incognita*,	Extensive root colonization, ISR	Hallman (2001)
Serratia plymuthica G3	*Triticum aestivum*	*Botrytis cinerea*, *Cryphonectria parasitica*, *Rhizoctonia cerealis*	Chitinase, protease, pyrrolnitrin, siderophores	Liu et al. (2010)
Stenotrophomonas maltophilia S37, *Bacillus mojavensis*	*Datura stramonium*	*Fusarium oxysporum*, *F. lycopersici*	Lytic enzymes (chitinase, protease, and pectinase) VOC	Abdallah et al. (2016)
Streptomyces somaliensis	*Glycine max*, *Citrus sinensis*	*Guignardia citricarpa*, *Rhizoctonia solani*, *Colletotrichum sublineolum Fusarium oxysporum*, *Phytophthora parasitica*	Chitinase	Quecine et al. 2008
Fungal endophytes				
Acremonium zeae	*Zea mays*	*Aspergillus flavus*, *Fusarium verticillioides*	Pyrrolidines	Wicklow et al. (2005)
Acremonium	*Gossypium herbaceum*	Root-knot nematode disease	Toxin production	Kim et al. (1988) and Goswami et al. (2008)
	Meloidogyne incognita			
Beauveria bassiana ARSEF 3113	*Zea mays*	*Ostrinia nubilalis*	Reduction of larval tunneling	Bing and Lewis (1991)
B. bassiana G41	*Musa balbisiana*	*Cosmopolites sordidus*	Larvicidal	Akello et al. (2008)

(continued)

Table 10.1 (continued)

Endophyte	Host plant	Biocontrol agent against	Mechanism/bioactive metabolites	References
Clonostachys rosea	*Moniliophthora roreri*, *Theobroma gileri*	*Botrytis cinerea*	Antibiotic	Morandi et al. (2000), Berry and Deacon (1992), Evans (1999), and Hajlaoui et al. (2001)
Cladosporium	*Tinospora cordifolia*	*Spodoptera litura*	Larval and pupal mortality	Thakur et al. (2013)
Epicoccum nigrum P16	*Saccharum officinarum*	*Fusarium verticillioides*, *Colletotrichum falcatum*, *Ceratocystis paradoxa*, *Xanthomonas albilineans*	Epicorazines A–B, epirodines A–B, flavipin, epicoccines A–D, pipiridones	Fávaro et al. (2012)
Fusarium oxysporum EF119	*Capsicum*	*Pythium ultimum*, *Phytophthora infestans*, *Phytophthora capsici*	Fungal inhibitors	Benhamou et al. (2002)
Lasiodiplodia pseudotheobromae F2	*Camptotheca acuminate*	*Protozoa*	Lasiodipline 5	Wei et al. (2014)
Lasiodiplodia pseudotheobromae XSZ-3	*C. acuminate*	Human *promyelocytic* *Leukemia* cells	Palmarumycin LP1	Lu et al. (2014)
Lasiodiplodia pseudotheobromae	*Ilex cornuta*	*Blumeria graminis*	Antifungal substances	Xiang et al. (2016)
Leptosphaeria	*Gossypium*	*Arabidopsis thaliana*	Unknown	Yuan et al. (2017)
Nigrospora	*Tinospora cordifolia*	*Spodoptera litura*	Griseofulvin, dechlorogriseofulvin, 8-dihydroramulosin, mellein	Zhao et al. (2012)
Penicillium simplicissimum	*Gossypium*	*Pseudomonas syringae*	Unknown	Hossain et al. (2007)
Phomopsis cassiae	*Cassia spectabilis*	*Cladosporium sphaerospermum*, *Cladosporium cladosporioides*	Cadinane sesquiterpenes	Silva et al. (2006)

As reported, different species of endophytic *Paenibacillus* have been associated with diverse crop plants including *Arabidopsis*, *Coffea arabica*, potato, poplar, pinus, etc. (Rybakova et al. 2016). *Paenibacillus* strain PB71 was obtained from the spermosphere of the Styrian oil pumpkin (SOP), and could efficiently inhibit the phytopathogen *Didymella bryoniae*, causal organisms of SOP under greenhouse conditions (Fürnkranz et al. 2012). *Paenibacillus* is well known for producing large amount of diverse hydrolyzing enzymes that enable plant tissue colonization (El-Deeb et al. 2013). Excellent colonizing ability of *Paenibacillus* results in biofilm formation around the plant roots that act as a protective barrier and restrict the entry of pathogen (Timmusk et al. 2005). Additionally, these endophytes also release certain types of volatile metabolites that hinder the growth of pathogens and induce systemic resistance in plants (Timmusk and Wagner 1999). Currently, few species of *Paenibacillus* can produce antimicrobial compound known as polymyxins, which is active against gram-negative bacteria such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, and *Stenotrophomonas maltophilia* (Landman et al. 2008; Niu et al. 2013).

A very good example of endophytic bacteria is *Rhizobium*. Several reports suggest *Rhizobium* as an efficient plant growth promoter, but limited data is available on its biocontrol potential. An endophytic strain of *R. etli* isolated from potato rhizosphere (and further re-isolated from the root interior) has been shown to be a potent antagonist against potato cyst nematode *Globodera pallida* and root-knot nematode *Meloidogyne incognita*, respectively. There are two mechanisms that have been proposed for this antagonism: first is the massive colonization of internal tissues of plants by *Rhizobium*, thereby suppressing the growth of invading pathogens by niche occupation, nutrient competition, and antibiosis (Hallmann et al. 1997). The second mechanism was believed to be stimulation of general plant defense resistance mechanism (ISR). This defense mechanism is activated due to lipopolysaccharides secreted by the strain (Reitz et al. 2001).

Bacillus amyloliquefaciens recorded strong antagonism against wide range of phytopathogens like *Aspergillus niger*, *Botrytis cinerea*, *Alternaria alternata*, *Fusarium oxysporum*, and *Pythium aphanidermatum,* which causes damping-off disease in tomato. The main mechanism responsible for this inhibition was production of metabolites like cyclic lipopeptide (CLP). In vivo field experiments were also carried out to check the efficacy of the strain in reducing damping-off disease in tomato. Metabolites of CLP were extracted, and active fractions were again tested against *P. aphanidermatum* by well diffusion method. Detailed analysis of CLP by liquid chromatography coupled with mass spectroscopy (LC/MS) showed compounds like iturin, fengycin, and surfactin (Zouari et al. 2016). Similarly another endophytic strain of *Bacillus subtilis* E1R-J proved to be a promising biocontrol agent against *Blumeria graminis*, causal organism of wheat powdery mildew (Gao et al. 2015). An endophytic bacterial strain, *B. subtilis* MJMP2, isolated from fermented *Brassica campestris* displayed strong antimicrobial activity against *Xanthomonas oryzae*, *Rhizoctonia solani*, and *Fusarium oxysporum*, pathogens responsible for causing blight disease, sheath blight, and root rot, respectively, in rice. The metabolite responsible for antagonism was identified as iturin A, which

disturbs fungal cytoplasmic membrane by forming transmembrane channels, resulting in the leakage of K^+ ions from the fungal cells (Hsieh et al. 2009). Crude extract of the supernatant containing iturin A showed antagonistic activity against rice blight disease under in vivo pot assay (Arrebola et al. 2010).

Different strains of endophytic *Streptomyces* sp. including *S. somaliensis*, *S. cyaneus*, *S. purpurascens*, and *S. griseus* isolated from citrus and soybean plant were evaluated for their activity against fungal pathogens, viz., *Guignardia citricarpa*, *Rhizoctonia solani*, *Colletotrichum sublineolum*, *Fusarium oxysporum*, *Pythium* sp., and *Phytophthora parasitica*. High biocontrol activity of the strains was due to the secretion of cell wall-degrading enzyme like chitinase, which was further validated through electron microscopy (Quecine et al. 2008). Shekhar et al. (2006) stated *Streptomyces violaceusniger,* an endophytic bacteria, displayed high chitinase activity and strong antagonism against wood-rotting fungi. Hence, higher chitinase activity has direct corelation with pathogen inhibition. Hastuti et al. (2012) reported other endophytic strains of *Streptomyces*, AB131-1, AB131-2, and LBR02, to be efficient in reducing bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* in rice crop. Effectiveness of the strains was checked both under laboratory and field conditions. Strains AB131-1 and LBR02 displayed strong inhibition of *Xanthomonas* by producing enzymes like phosphatase, chitinase, cellulase, and siderophore. Other endophytes, *Streptomyces griseofuscus* and *S. hygroscopicus*, established 54.5% and 21.8% biocontrol against pathogen *Magnaporthe oryzae* (anamorph *Pyricularia oryzae*), which attacks rice plant and causes disease incidence (Tian et al. 2004). Endophytic strain of *Serratia,* isolated from the stems of *Triticum aestivum,* exhibited antifungal activity against phytopathogens like *Cryphonectria parasitica*, *Rhizoctonia cerealis*, and *Botrytis cinerea*. Diverse mechanisms of biocontrol like chitinase, exoprotease, antibiotic pyrrolnitrin, and siderophore production were displayed by *Serratia* against these pathogens (Liu et al. 2010).

Endophytic strains belonging to the genus *Enterobacter* displayed antagonistic activity against *Verticillium dahliae* causing verticillium wilt in cotton. The strain was phylogenetically affiliated to *Enterobacter cancerogenus*. Its biocontrol efficacy was monitored in pots and further taken to the field with cotton as test crop. Field trials confirmed its antimicrobial activity against *V. dahliae* due to its excellent root-colonizing ability (Berg and Hallmann 2006). *Enterobacter cancerogenus* HA02 displayed extensive colonization and secretion of siderophores and protease that helped in controlling verticillium wilt (Li et al.2010).

10.2.2 Fungal Endophytes

Generally fungal endophytes are found in plant tissues like leaves, stems, and barks asymptomatically. Fungi growing inside vascular tissues play crucial role in protecting host plant by producing different metabolites or toxins that kill many plant pathogens. From the perspective of pest management and control, endophytic

fungus appears to be one of the potential candidates. A list of some important fungal endophytes that have emerged as potent biocontrol agents has been compiled in Table 10.1.

Trichoderma is a filamentous, soilborne fungus that forms mutualistic relationship with different plant species and is capable of colonizing host plant. Different species of *Trichoderma* like *T. viren*, *T. atroviride*, and *T. harzianum* are well known for their biocontrol activity (Abdel-Moity et al. 1982; Elad et al. 1983; Fahim et al. 1989). *Trichoderma* inhibits growth of different phytopathogens like *Macrophomina phaseolina* (Larralde-Corona et al. 2008), *Phytophthora*, *Pythium* (Maisuria and Patel 2009), *Sclerotinia sclerotiorum* (Ibarra-Medina et al. 2010), *Fusarium*, *Sclerotinia rolfsii* (Suraiya et al. 2014), etc. The most common mechanisms for biocontrol by *Trichoderma* are host plant resistance, antibiosis, competition, and parasitism.

Fusarium is a filamentous fungi belonging to the group of hyphomycetes that is widely distributed in soil and plants. Usually it is known as phytopathaogenic fungus that affects majority of crops worldwide. *Fusarium* wilt and *Fusarium* root rots caused by different species of *F.oxysporum* are the most common fungal diseases that affect diverse crop plants. *F. oxysporum* is generally of three types, viz., saprophytic, pathogenic, and parasitic. Although endophytic activity is not well studied in case of *Fusarium*, there are some studies that report its biocontrol potential. Zibbermann et al. (2016) studied the biocontrol activity of *F. oxysporum* f. sp. *strigae* strain "Foxy-2" against parasitic weed *Striga hermonthica* in maize rhizosphere. Since then several workers reported that nonpathogenic *Fusarium* sp. can be used as biocontrol agent against pathogenic *Fusarium* (Park et al. 1988; Biles and Martyn 1989; Kroon et al. 1991; Minuto et al. 1995; Leeman et al. 1996; Fuchs et al. 1997). Mechanisms of action were also studied for the control of *Fusarium* wilt by *F. oxysporum*. ISR was found to be the reason for disease control (Biles and Martyn 1989; Kroon et al. 1991; Fuchs et al. 1997). Few strains of *F. oxysporum* displayed promising nematicidal activity against *Radopholus similis* nematode, causing disease in banana plant (Schuster et al. 1995).

Beauveria bassiana is a fungus that belongs to the family of Clavicipitaceae. It occurs in different forms such as entomopathogens, fungal parasites, plant pathogens, parasites of slime molds, and endophytes of grass (White et al. 2003). For instance, as a fungal parasite, it causes white muscardine disease in many arthopods worldwide. On the other hand, it is also used as a biological insecticide for the control of different pests like white flies, beetles, and bedbugs (Barbarin et al. 2012). *Beauveria bassiana* has a wide host range; however, it differs from strain to strain, which can be categorized into selective or nonselective host range. Members of this family are also known for their toxicogenic secondary metabolite production (White et al. 2003). In addition to this, *B. bassiana* showed their endophytic presence in many plant species helping them to combat different plant pathogens (Vega 2008). Campbell and Coe (1991) reported inhibitory activity of *B. bassiana* against soilborne and foliar plant pathogen *Gaeumannomyces graminis* var. *tritici*. Several studies supported the fact that *B. bassiana* has inhibitory spectrum against wide range of plant pathogens such as *Armillaria mellea*, *Rosellinia necatrix*, *Fusarium*

oxysporum, *Botrytis cinerea*, *Pythium ultimum*, and *Septoria nodorum* due to lysis of cell wall (Vesely and Koubova 1994; Reisenzein and Tiefenbrunner 1997; Lee et al. 1999). Under field conditions, Flori and Roberti (1993) reported that *B. bassiana* not only enhanced plant growth parameters of onion crop but also reduced infection of *Fusarium oxysporum.*

Phoma is a well-known fungal genus that is globally present in soil, plants, air, animals, and human body. *Phoma* is commercially one of the most important fungi, as it produces various pigments and secondary metabolites owing antimicrobial potential. There are certain species of *Phoma* that showed significant biocontrol activity against different plant pathogens, for instance, recently Gupta et al. (2016) reported *P. herbarum* to show inhibitory activity against *C. gloeosporioides*. Endophytic species of *Phoma* are also helpful in controlling weeds by producing secondary metabolites such as anthraquinone and phytotoxin. Hoffman et al. (2008) isolated endophtytic strain of *Phoma*, from *Saurauias caberrinae*, which produced a metabolite called phomodione, an inhibitor of *Staphylococcus aureus*. *Phoma* also produces an antifungal compound known as cytochalasin that is effective against plant pathogens (Wagenaar et al. 2000). Many species of *Phoma* like *P. glomerata*, *P. tracheiphila*, *P. macdonaldii*, *P. sorghina*, *P. proboscis*, *P. herbarum*, *P. macrostoma*, *P. foveata*, and *P. multirostrata,* are well known for their antimicrobial activity against different pathogens, and metabolites from few of them could be used for the production of agrophytochemicals, dyes, and mycopesticides (Rai et al. 2009).

Genus *Cryptosporiopsis* belongs to family Dermateaceae. *Cryptosporiopsis quercina*, a synonym of *Pezicula cinnamomea*, earlier mentioned by Sutton in the 1980s as an imperfect stage of *Pezicula cinnamomea,* was found in association of hardwood species (Sutton 1980). In later year, Tscherter and Dreyfuss (1982) confirmed *Pezicula* sp. as a teleomorph state of the anamorphic fungus *Cryptosporiopsis,* which produces a secondary metabolite that belongs to a group, echinocandin of lipopeptides. After this Fisher et al. (1984) found that endophytic *Cryptosporiopsis* sp. from ericaceous plants showed biological activity against fungi such as *Aspergillus niger*, *Candida albicans*, *Mentagrophytes*, and *Trichophyton*. In further studies on the comparison of fungal endophytes found in xylem and in the whole stem of plants *Fagus sylvatica* and *Pinus sylvestris*, Petrini and Fisher (1988) discovered that fungal *Cryptosporiopsis* species strain P30A was found in the twigs of *Pinus sylvestris*, whereas other endophytic strain P47 of species *Pezicula* was isolated from *Fagus sylvatica*. Noble et al. (1991) reported that fungi P47 and P30 also produce a lipopeptide called L-671,329 which is known as novel antifungal agent. Li et al. (2000) isolated a peptide called cryptocin from endophytic *Cryptosporiopsis* cf. *quercina,* which showed inhibitory activity against pathogens like *Gaeumannomyces graminis*, *Rhizoctonia cerealis*, *Pyricularia oryzae*, and *Phytophthora capsici*. Recently Terhonen et al. (2016) also proclaimed the diversity of metabolites produced by endophytic *Cryptosporiopsis* and its promising biocontrol activity against plant pathogens.

Heteroconium chaetospira is a demantiaceous endophytic fungi. *H. chaetospira* was first reported as an encouraging biocontrol agent by Narisawa et al. (1998). This endophytic fungus was isolated from roots of Chinese cabbage grown in wheat field.

In his experiment, Chinese cabbage seeds were pretreated with an isolate of *H. chaetospira,* which showed reduction in clubroot disease that was caused by soilborne protozoan, *Plasmodiophora brassicae*. Morita et al. (2003) suggested that the isolate of *H. chaetospira* was helpful in suppressing diseases that were caused by *Alternaria brassicae* and *Pseudomonas syringae* due to induced systemic resistance (ISR).

10.3 Bioactive Metabolites from Endophytes

Bioactive metabolites or compounds can be defined as by-products obtained from plants, animals, and microbes (Baker et al. 2000). These bioactive metabolites halt the growth of disease-causing agents especially pathogens causing disease in plants. Few endophytes, which produce bioactive metabolites, belong to the genera *Bacillus*, *Burkholderia*, *Pseudomonas*, *Rhizobium*, *Trichoderma*, *Phoma*, etc. These genera are already known for their secondary metabolite products like antibacterial, antifungal, antiviral, antioxidant, anticancer, insecticidal, immunosuppressants, volatile organic compounds (VOCs), etc. (Strobel 2003). In addition, wide-ranging bioactive metabolites such as alkaloids, aliphatic compounds, benzopyranones, phenols, flavonoids, quinones, steroids, terpenoids, tetralones, xanthones, etc. have been associated with endophytes (Tan and Zou 2001). An endophytic *Pseudomonas viridiflava*, isolated from grass species, produces novel antimicrobial compound ecomycin that is effective against a wide range of microbes (Miller et al. 1998). VOCs obtained from endophytes also possess antibacterial, antifungal, and antiviral properties (Firakova et al. 2007). Group of phenolic acids were extracted from the culture broth of a *Phoma* sp. by Hoffman et al. (2008), displaying antagonistic activities against *Sclerotinia sclerotiorum*, *Pythium ultimum*, and *Rhizoctonia solani*. Further research highlighted the role of another bioactive metabolite pyrrocidines, an alkaloid derivative isolated from endophyte *Acremonium zeae* residing in maize plant, in antagonizing phytopathogen like *Aspergillus flavus* and *Fusarium verticillioides* (Wicklow et al. 2005). An endophyte *Ampelomyces* isolated from the medicinal plant *Urospermum picroides* synthesized quinolone-derived bioactive metabolites known as 3-O-methylalaternin and altersolanol. These compounds presented inhibitory spectrum against a wide range of pathogens such as *Staphylococcus aureus*, *S. epidermidis*, and *Enterococcus faecalis* at minimum inhibitory concentration (MIC) value ranging from 12.5 to 25 mg/ml (Aly et al. 2008). Phenolic compounds, like pestalachloride, were extracted from endophytic fungi *Pestalotiopsis adusta*, which established significant antifungal activity against plant pathogens *Gibberella zeae*, *Verticillium albo-atrum*, and *Fusarium culmorum* (Li et al. 2008). Ethyl 2, 4-dihydroxy-5,6-dimethylbenzoate and phomopsilactone are bioactive metabolites, isolated from an endophytic fungus *Phomopsis cassiae* that showed robust antifungal activity against phytopathogenic fungi *Cladosporium sphaerospermum* and C. *cladosporioides* (Silva et al. 2005).

The aliphatic compound, chaetomugilin, detected in the cell-free culture supernatant of an endophytic fungus *Chaetomium globosum* collected from *Ginkgo*

biloba showed antifungal activity against diverse fungal pathogens (Qin et al. 2009). A unique tetramic acid cryptocin, which possesses biocontrol activity against rice pathogen *Pyricularia oryzae*, was extracted from endophytic fungus *Cryptosporiopsis quercina* (Li et al. 2000). Novel spiroketals, isolated from an endophytic fungi *Edenia gomezpompae*, displayed significant inhibition against *Alternaria solani*, *Fusarium oxysporum*, and *Phytophthora parasitica*. A naphthodianthrone-derived compound hypericin and esmodin revealed antimicrobial activity against *Pseudomonas aeruginosa*, *Salmonella enterica*, *Escherichia coli*, *Aspergillus niger*, *Candida albicans*, etc. (Kusari et al. 2009). Lactone-derived secondary metabolite known as brefeldin, produced by *Cladosporium* sp., demonstrated maximum antifungal activity against phytopathogens. Antifungal bioactive compound pumilacidin produced by *Bacillus pumilus* and compounds like 2-hexyl-3-methyl-butanodioic acid and cytochalasin were synthesized from the endophytic fungus *Xylaria* with strong antifungal activities (Cafeu et al. 2005). Recently, cyclohexanone derivatives have been extracted from endophytic fungus *Pestalotiopsis fici*, which is effective against *Aspergillus fumigatus* (Liu et al. 2009). Antifungal metabolite trichodermin gained from endophytic fungus *Trichoderma harzianum* showed inhibitory spectrum against pathogens causing early blight of tomato and damping-off disease on cucumber plants (Chen et al. 2008). These were the role of few bioactive metabolites that participate in inhibiting pathogens and protecting plant health from diseases.

10.4 Endophytes from Lab to Land

10.4.1 Bioformulations from Endophytes

The delivery of biocontrol agents under field conditions is often hindered by the vulnerability of viable cells due to extremities in environment. Thus, biocontrol agents showing impressive disease-suppressing ability in the laboratory stage or under control conditions like plant growth chamber or glass house study fail to convey similar results in natural field. Several studies have shown that biocontrol agents fail to deliver good results, due to their deprived cell number in the soil, which generally arises due to tough competition with the native microbial community. Formulating suitable bioformulation is an essential criterion for exploiting any microbe-based technology into field. Hence, to certify the viability of endophytic cells, they must be properly shielded and secured. This protection could be offered by formulating them with suitable carriers and developing bioformulations from them (Bashan et al. 2014).

10.4.2 Selecting Right Endophyte

Strain selection is one of the most important steps in bioformulation development. As most of the endophytes are host specific, depending on the type of crop sown, it is necessary to select the correct endophytes for formulation development. Selected endophytes should not be generalized, but it should be specific, so as to give targeted results. Obligate endophytes that colonize plant parts without altering common plant functioning are encouraged for formulation development (Berg et al. 2005). Moreover these obligate endophytes face less competition and remain safe inside plant cells (Hardoim et al. 2008; Gaiero et al. 2013). High temperature-tolerant and endospore-producing strains could also be selected as suitable candidate for developing perfect endophytic bioinoculant (Senthilkumar et al. 2007). Endospore-forming ability makes this strain easy to use, formulate, and commercialize as it has extended shelf life. This distinctive trait has constantly attracted the attention of major research groups attempting to develop biocontrol agents for practical applications as it shows continued existence in soil even when host is not available. Also, while selecting an endophyte, it is of prime importance to state the target disease and the host on which it will be used. Before subjecting them to bioformulation, their mechanism and interaction with plant and pathogen should be established well by using whole or part of endophytic organisms under laboratory, greenhouse, and field conditions.

10.4.3 Selecting Optimized Conditions for Mass Multiplication

Once the right strain has been selected, the next step is to optimize the protocol for its mass multiplication and metabolite production. Optimization of various parameters like concentration of cells, temperature, pH, oxygen, moisture content, and nutrients is considered while mass multiplying the microbes. Zahir et al. (2010) reported that high mass of *Rhizobium* could be obtained by supplementing tryptophan in medium. Formulation containing tryptophan plus *Rhizobium* delivered significant enhancement in improving yield of mung bean crop under field conditions in comparison with untreated sets. For some endophytes applying the same parameters may not work as they are from unique origin; they may require some specialized conditions/nutrients that have not been unraveled. Further to make the process and product economic, cheaper substrates (like egg shells, sawdust, bagasse, hay, soil, peat, charcoal, etc.) at optimum conditions coupled with innovative and competent multistep downstream methods should be explored (Muthusamy et al. 2008). The mass production of cells under optimized state should be cost effective that will not only enhance the applicability of the bioformulation in industries but will also create confidence among the farmers and the production houses.

10.4.4 Formulations and Shelf Life Analysis

Evaluation of different inorganic and organic carriers has been done for the preparation of bioformulations and shelf life analysis of endophytes (Bashan et al. 2014). Bazilah et al. (2011) stated that for commercialization of microbial formulation, it is important to have good viability for certain period of time. Inoculants containing CFU of 10^9 cells and extended shelf life of 1–2 years have successful distribution in fields (Deaker et al. 2004; Schulz and Thelen 2008). Talc-based formulations developed from *Trichoderma* showed growth-promoting effects on cantaloupe plants under greenhouse condition (Vidhyasekaran and Muthamilan 1995). Viability of *Bacillus subtilis* and *Pseudomonas corrugata* in wet alginate beads was recorded to be 3 years (Trivedi and Pandey 2008), whereas, in dry alginate beads, viability of *Azospirillum brasilense* and *Pseudomonas fluorescens* was found to be 14 years (Bashan and Gonzalez 1999). Liquid formulation of *Bradyrhizobium japonicum*, used for enhancing soybean production, could be stored up to 8 years (Bashan et al. 2014).

10.5 Mode of Application

As discussed above, entry point is specific for certain bacteria; hence, the mode of application acts as major detrimental factor in deciding the efficacy of endophytes for disease suppression. Endophytic formulations are available either in powdered form or liquid form and can be inoculated by diverse methods like seed pelleting, seed dressing, soils drench, and foliar spray (Ramyabharti et al. 2016). Seed coating is the most common technique of inoculation as it is very easy and requires small amount of inoculant. Soil drench is generally used while introducing large bacterial cells in the soil. Granules of marble combined with perlite, peat, charcoal, and soil are also in use for soil inoculation as they enhance inoculant to be in contact with plant roots (Bashan et al. 2014). Recently, spraying methods are gaining popularity in case of endophyte inoculation as they can very easily enter inside the plant system and deliver better results. Endophytes that reside within fruits and flowers could just be sprayed or sprinkled to get good results.

Ramyabharathi et al. (2016) observed the utility of liquid formulation developed from endophytic *Bacillus subtilis* strain for enhancing shelf life of strain and reducing wilting symptoms (caused by *Fusarium*) in tomato plant. Formulation of the endophytic fungus *Cladosporium oxysporum* prepared from culture filtrates and conidial suspensions was tested for its inhibitory activity against the black bean aphid *Aphis fabae* by micro-irrigation technique. Results showed that formulation developed from culture filtrate gave much better results in inhibiting aphid population in comparison to conidial suspension, hence suggesting that proteolytic activity plays much important role in inhibition than the chitinolytic activity of the fungus against the aphid (Bensaci et al. 2015). Gao et al. (2015) evaluated different biopreparations of endophytic *B. subtilis* strain using their cells, cell-free culture

supernatant, crude proteins, and non-protein fermentation liquid against *Blumeria graminis* infection in wheat. Application of these formulations demonstrated significant reduction of disease incidence in wheat plant; however, best results were obtained when fermentation liquid of *B. subtilis* was applied on the leaves in comparison with other formulations/treatments. Talc-based bioformulation developed from the combination of rhizobacteria *P. fluorescens* (Pf1) and endophytic bacteria *Bacillus* sp. was quite effective in reducing the incidence of Banana bunchy top virus by 52% in field conditions and also enhancing growth attributes of host plant (Harish et al. 2009).

Apart from direct inhibition of pathogens, endophytes are also known to induce host resistance, which is evidenced by an upsurge in PR proteins, defense-related proteins, and phenolic compounds in host plants. Applications of consortia of beneficial microbes, which can occupy different niches, are considered advantageous over formulations with single microbes. Formulation developed from this combination was not only effective in suppressing banana bunchy top virus but also active in reducing panama wilt of banana caused by *Fusarium oxysporum* (Harish et al. 2009). Talc-based bioformulation developed from rhizobacterial strains of *Pseudomonas fluorescens* and endophytic fungus *B. bassiana* amended with chitin recorded an enhanced biocontrol activity against leaf miner insect and collar rot disease (Senthilraja et al. 2013). Chitin supplement augmented the antagonistic activity of the entomopathogenic fungal and bacterial bioformulation, thus assisting the fact that chitin may induce systemic resistance in plants against insect pests and pathogens (Senthilraja et al. 2010). Muthu and Sharma (2011) reported the potency of talc-based bioformulation developed from *Trichoderma viride* and endophytic *P. fluorescens* (EBL 20-PF) in inhibiting growth of *Pythium aphanidermatum* (causes damping-off disease in chili). Formulation of these co-inoculating bioagents displayed high elicitation of defense-related enzymes, PR proteins, and phenols, in comparison with their sole application (Muthu and Sharma 2011).

Alghuthaymi et al. (2015) reported a special type of formultions developed from nanoparticles (NPs) of different fungi like *Aspergillus*, *Fusarium*, *Verticillium*, and *Penicillium* known as nano-formulations. These diverse fungi have been used to synthesize gold, silver, platinum, tellurium, selenium, silica, quantum, magnetite, and zirconia NPs possessing antifungal activity. Recently, different nano-fungicides, nano-pesticides, and nano-herbicides are being used extensively in the area of agriculture sciences (Alghuthaymi et al. 2015). Park et al. (2006a, b) reported the antimicrobial activity of nano-sized silver particles in suppressing plant diseases. The use of silver NPs (Ag-SiO_2 NPs) as fungicides is safer than using any synthetic fungicides (Oh et al. 2006). Ag-SiO_2 NPs have strong biocontrol activity against *Botrytis cinerea.* Amalgamation of Ag-NPs with fluconazole showed good antifungal activity against *Aspergillus*, *Fusarium*, *Phoma*, *Trichoderma*, *Candida*, etc. (Gajbhiye et al. 2009). Application of nanotechnology in the field of plant pathology is still in its infancy and needs further exploration in the area of nano-delivery systems in natural field conditions.

10.6 Monitoring the Endophytes in Environment

Soil is a composite, heterogeneous, and nutrient-rich habitat where billions of indigenous microbes already exist. After releasing targeted endophytes embellished in a proper formulation, it is somewhat challenging to identify the exact population of bioinoculant, as the added inoculants have to compete with resident soil microorganisms for the nutrient and niche occupation. The probability of finally achieving successful endophytic inoculants is a gruesome task as these microbes enter inside the plant system from roots to stem and further on. So isolating the desired endophytes by crushing or macerating plant tissue is usually opted for to obtain the endophytic load or to check for root colonization. But again only cultivable endophytes could be obtained from plating, baiting, or macerating technique, while unculturable endophytes cannot be obtained. Hence more precise and consistent methods for monitoring the fate of introduced endophyte are required for monitoring its efficacy under field conditions. Conn and Franco (2004) described noteworthy decrease in the population of local actinobacterial endophytes when inoculated with a commercial consortial product. Devi and Momota (2015) reported that successful endophyte colonization can also be visualized by using β-glucuronidase reporter system as shown in case of *Herbaspirillum seropedicae* Z67 when inoculated onto rice seedlings. Apart from that, proteomics, genomics, trancriptomics, or metabolomics could be exploited as an influential tool to comprehend the complex design of genes, proteins, and metabolites with respect to different environmental niches in which the bacteria live (Trivedi et al. 2012).

10.7 Ecological Impact Assessment

It is well established that pesticides and chemicals used in agriculture are highly efficient but their excessive and unregulated use also leads to serious aftereffects on soil, environment, and human health. These concerns are well realized today, in all quarters of scientific community, and are gradually being acknowledged by various social groups and individuals. It must be realized by the policy makers and governments too that the time is ripe, to regulate the use of these chemicals and pesticides and make enabling provisions for replacing them with bioformulations which are more reliable, environment-friendly, and safe. Governments must also promote vigorous research into advanced agricultural systems where the use of chemicals and pesticides shall be completely prohibited and replaced with organic and biological products and compounds. Formulations developed from microbes like endophytes will be purely biological. Apart from their non-toxicity, these formulations will be purely biodegradable, nonpolluting, leaving no carbon footprints (Bashan et al. 2014), and non-disturbing toward the ecology of soil, human, or environmental health, together with helping in carbon sequestration, thereby increasing soil organic carbon. Sharma et al. (2017) performed comparative study by applying

Bradyrhizobium inoculants and chemical fertilizers in pigeonpea in field. The effect of bioinoculant gave promising results not only in terms of plant growth enhancement but in enhancing local microflora residing in the field, thus authenticating the nontarget effects contributing to the overall efficacy of such applications.

10.8 Conclusion and Future Prospects

After analyzing the available scientific literature, it can be concluded that studies on endophytes have opened a new avenue in the area of plant disease management. Endophytes are designated as future "plant probiotics" as they reside inside the plant host and leverage multiple beneficial effects without causing any harm to the host plant. The study of endophytes involves several challenges, the most common being its isolation process. The process of isolating true endophyte by surface sterilization and overlooking the rest of the microbes is somewhat difficult. Further, there are chances of hindering growth of endophytes due to the penetration of surface-sterilizing chemicals in the tissues. Hence, appropriate methods and precautions for isolation should be followed based on the plant type and tissues under consideration. In order to study the diversity of endophytes, more emphasis should be given on culture-independent approaches as they are quick, specific, and time-saving and can find large number of endophytes that could not be easily cultured in laboratory.

Culture-independent approaches concentrate on molecular methods including polymerase chain reaction (PCR) and quantitative PCR (Q-PCR), but they too have their own limitations as no pure culture of endophytes are obtained for field application using this methodology; besides there are some biases that are introduced when performing analysis using cultivation-independent techniques. Various other genomic approaches like denaturing gradient gel electrophoresis (DGGE), and ultrahigh-throughput sequencing methods such as pyrosequencing and microarray are used nowadays to understand endophytic diversity.

Exploring potent endophytes can pave way for rich source of bioactive and novel metabolites, which can find plethora of uses in various agricultural and industrial arenas. Diverse bioactive secondary metabolites produced by endophytes, exhibiting promising biocontrol activities, have been illustrated in the chapter, but much more research is needed to optimize and standardize the protocols for extracting many other unknown and unidentified compounds which might be useful at commercial level.

Though bioformulations derived from endophytic cells (either bacterial or fungal) deliver promising results (as cited with several examples in the chapter) in terms of suppressing disease incidence, efficacy and potency of these formulation can be further enhanced by exogenous application of bioactive secondary metabolites in combination with beneficial endophytes, as these formulations would be more target -specific. Bioformulations derived from pure bioactive ingredients or

combination of endophytes plus metabolites is a novel topic that needs to be researched and worked upon for controlling phytopathogens.

Recently endophytes have been explored for synthesizing nanoparticles like gold and silver, which can treat dreadful diseases in the near future. These innovative technologies suggest boundless role of endophytes in upcoming years for producing more effective and economical nano-formulations that could be used for controlling plant and animal diseases.

Hence, the futuristic approach recommends encouraging research on bioprospecting of endophytes and isolating them from wild, untouched, and unexplored regions. Detailed knowledge on this topic will provide a better understanding of these endophytes and their application in diverse agricultural practices to ensure better food productivity and security in future.

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Chapter 11
Bacillus as Plant Growth Promoting Rhizobacteria (PGPR): A Promising Green Agriculture Technology

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Abstract *Bacillus* is a cosmopolitan bacteria present in all kinds of environments including rhizospheric soil. Root-associated *Bacillus* spp. usually promote plant growth by various means, e.g., production of phytohormone precursor, i.e., indole acetic acid (IAA-auxin), phosphate solubilization, and siderophore production or serve as biocontrol and are thus termed plant growth-promoting rhizobacteria (PGPR). This genus may also be used along with other biocompatible bacteria including nitrogen-fixing species like *Azospirillum* and *Azotobacter* and hence may be called as consortia of bacteria or which can be used as co-inoculant to increase/improve the fertility of soil. This chapter focused on the application of *Bacillus* on different economically important crops.

Keywords *Bacillus* · PGPR · ACC deaminase · Ethylene · IAA · Auxin · Siderophore · Stress · Bio-inoculant

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11.1 Plant Growth Promoting Rhizobacteria (PGPR)

The food demand rapidly increases with asymptomatic growth of population size on the globe. This makes an urgent attention for developing counties like India, where human population is more than 1.3 billion and where farming field area is limited. Even though the productivity of crop per hectare increases sufficiently during the green revolution in the seventh decade of the twentieth century with the use of chemical fertilizer along with genetically improved crop cultivar, this would not be enough to meet the food demand of an increasing population in the future, which focuses to pay an urgent attention and need for next microbial green revolution keeping in the mind for the environment (Ansari and Mahmood 2017).

This could be possible by interventions through biological agents like plant growth-promoting rhizobacteria (PGPR) offering various promising advantages including enhancement in crop yield and decrease in disease occurrence. PGPR are free-living microbes inhabiting in soil that can indirectly or directly facilitate rooting (Nie et al. 2002) and growth of plant (Glick et al. 1998; Kumar et al. 2016; Kumari et al. 2016; Patil and Solanki 2016b). PGPR reside as a microbial community in the rhizospheric zone, interact with other microbes, and stimulate growth of microbes including mycorrhizae which also provide basis of indirect promotion of plant growth (Bhuyan et al. 2015). Indirect enhancement of plant growth involves a mechanism by which the PGPR prevent phytopathogens by inhibiting its growth and development or by diminishing stress-induced plant ethylene levels after breakage of its precursor ACC by secreting ACC deaminase (Mayak et al. 2004). Direct stimulation involves supplementing plants with fixed nitrogen, plant growth hormones, iron (sequestering by bacterial siderophores), and solubilized phosphate. PGPR may also involve a number of different bacteria such as *Pseudomonas*, *Azotobacter*, *Azospirillum*, *Burkholderia*, *Acetobacter*, and *Bacilli* (Glick et al. 1998; Solanki et al. 2017). These PGPR alone or in combination (consortia) may be used as bio-fertilizer to enhance the productivity of crop.

11.2 Mechanism of PGPR

There are various attributes of PGPR which enhance the growth of plant in various ways. The mechanism involved in growth of plant is mentioned below.

11.2.1 Indole Acetic Acid

Indole-3-acetic acid (IAA) is one of the most commonly studied auxins, and most of the scientific paper considers auxin and IAA as interchangeable terms. Tryptophan is an important precursor molecule for IAA altering IAA biosynthesis (Kundan

et al. 2015). Auxin synthesized via IAA either from plant or bacteria differs only in their biosynthesis pathway. More than 80% of bacteria present in the rhizospheric region are able to produce IAA which leads to the formation of auxin growth regulator for the plant affecting root system causing an enhancement in branching number, weight/size, and the surface area in contact with soil. This results in better nutrient exchange through well-developed root (Probanza et al. 1996).

11.2.2 ACC Deaminase

The 1-aminocyclopropane-1-carboxylate (ACC deaminase) is an enzyme present in PGPR which regulates the ethylene production under the stress condition by metabolizing ACC to α-ketobutyrate and ammonia (Fig. 11.1). Ethylene growth regulator effects the growth of plant by initiation of root, ripening of fruit, germination of seed, and inhibition in elongation of root (Kundan and Pant 2015) and responses to various biotic (attack of pathogen) and abiotic stresses of temperature, flooding,

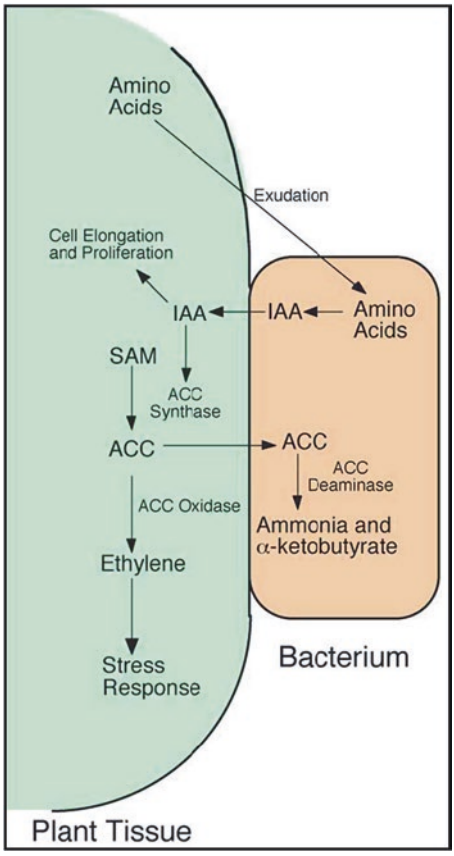

Fig. 11.1 A model describing the role of bacterial ACC deaminase in the promotion of plant root elongation. (Illmer et al. 1995)

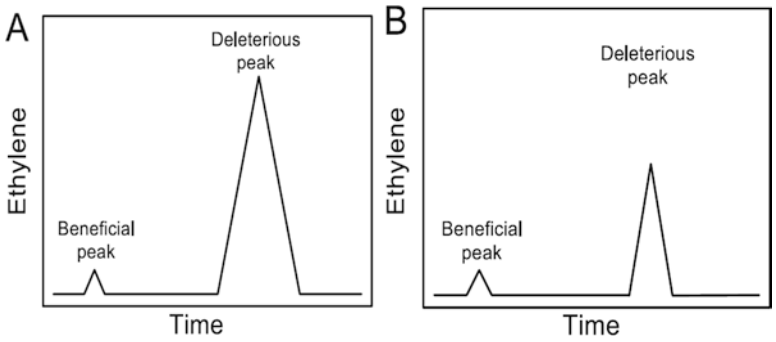

Fig. 11.2 (**a**) Initial small peak of ethylene during stress; a second much larger deleterious peak comes later. (**b**) Use of ACC deaminase-containing PGPR, a selective decrease in the second but not in the first beneficial ethylene peak. (Illmer et al. 1995)

drought, high salt concentration, high toxic metal (Yan-de et al. 2007) content, radiation exposure, etc. During any of these severe conditions, the endogenous level of ethylene production enhanced to deleterious peak (Fig. 11.2) and has a negative impact on root growth. In such case, ACC (an ethylene precursor) may be degraded by ACC deaminase produced by PGPR without affecting the natural impact of ethylene function on plant growth (Ram 2015).

11.2.3 Siderophore

Siderophore is low-molecular-weight hexadentate octahedral compound chelating mainly iron (Fe^{3+}) followed by transportation across the cell membrane (Jha and Saraf 2015). In the soil, iron is present in ferric ion (predominates in nature) form remains sparingly soluble causing too low concentration of iron to support growth of microbes. Siderophore acts as iron chelator which may be released by various PGPR and may be made available for plant growth (Freitas et al. 2015).

11.2.4 Phosphate Solubilization

The major element for plant growth is NPK. Although phosphorous remains present abundantly in rhizospheric soil in insoluble phosphorous complexes and may be made available to the plants for its optimum concentration (30 μmol l^{-1} of phosphorous) for optimum growth of crop by transforming it into solubilized form through phosphate solubilization. The bacteria involved in this type of plant growth promotion are called phosphate-solubilizing bacteria. The content of phosphorous in most of the soil remains in the concentration range of 1 μmol/l and can be made available

to the plant for its optimum growth by solubilization of insoluble phosphorous by PSB through two mechanisms of PGPR (Epstein 1972). This amount of phosphorous availability in soil can be enhanced by two mechanisms: (1) secretion of organic acid which mobilizes phosphorous and (2) secretion of phosphatase which leads to release of phosphate groups (remains bound to organic matter) and thus ability to solubilize the Ca-P complex. Generally, these mechanisms are more efficient in basic soil. Co-inoculating plants with phosphate-solubilizing bacteria (PSB) increase growth and yield directly. Among these, the most efficient PSB belong to the genera *Rhizobium*, *Pseudomonas*, and *Bacillus* (Kundan and Pant 2015).

11.2.5 Induced Systematic Resistance (ISR)

Plant pathogenic fungus causes significant losses in all kinds of agriculture crops, and in the modern agriculture practices, it rises as a major problem, because several strains become resistant to the chemical pesticides (Solanki et al. 2012b; Hyakumachi et al. 2013; Park et al. 2013; Podile and Kishore 2007; Patil and Solanki 2016a). PGPR regulates the plant as defense mechanisms without direct contact with the pathogen via induced systematic resistance (ISR). They enhance the defense-related proteins or enzymes of plants against the pathogens. In this, there is an interaction of ISR bacteria with a plant in localized area, and the response extends to the entire plant as in the case of immunization. This response is not visualized at first glance until the attack of pathogen and mediated by metabolic changes. For effective protection to the plant, a necessary interval is required between the PGPR-plant contact and the pathogen attack in order for the expression of the plant defense genes. This type of ISR mechanism had been developed by *Bacillus amyloliquefaciens* HK34 against *Phytophthora cactorum* in Korean ginseng (*Panax ginseng* Meyer) which causes enhancement in expression of PgPR10, PgPR5, and PgCAT in plant leaves (Lee et al. 2015).

11.2.6 Molecular Basis of the PGPR-Plant Interactions

PGPR produces phytohormones, phosphate mobilization, and other metabolites for plant growth promotion. It has been observed that *Bacillus subtilis* strain FB17, a PGPR, is actively recruited by *Arabidopsis thaliana*. The FB17 strain colonization alters global gene expression in the plant through upregulating and downregulating the important genes. Several genes were upregulated such as auxin-regulated genes, metabolism-associated genes, stress-responsive genes, and plant defense-related genes. However, some other important genes pertaining to defense and genes responsible for modification of cell wall were also downregulated (Lakshmanan et al. 2013). On the other hand, *Bacillus* was found to be associated with plant root exudates to acquire carbon source for their growth. Plant root exudates associated/

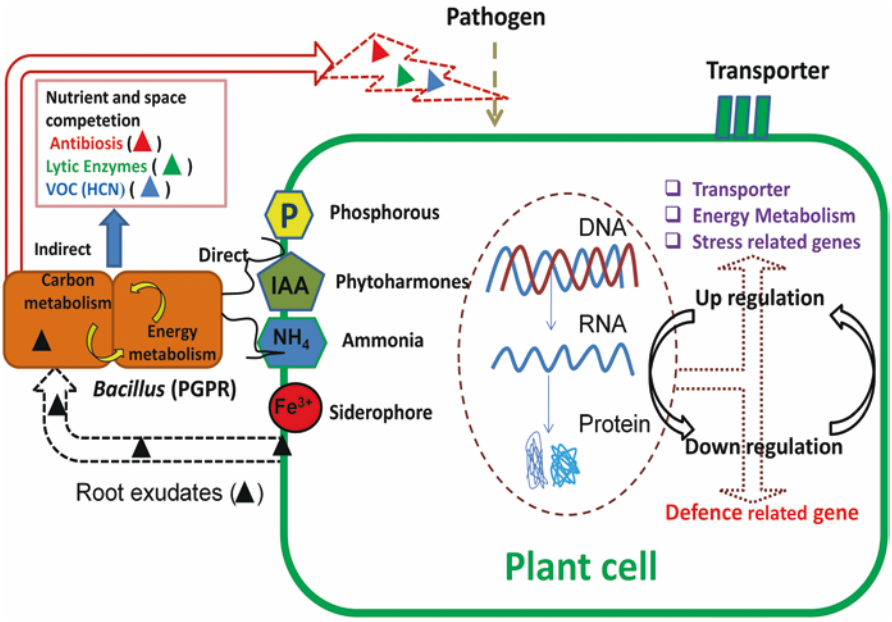

Fig. 11.3 Mechanism of PGPR: interaction of plant cell and PGPR through indirect and direct way causing growth of plant cell

secreted molecules act as signaling molecules and affect the gene expression to promote or repress interaction with beneficial or harmful species. A comparative transcriptomic analysis revealed that interactions lead to the change in global gene expression including oxidative reduction, transmembrane transport, and organic substance metabolism in *Bacillus* (Yi et al. 2017). The various mechanisms of PGPR can be better understood with Fig. 11.3.

11.3 *Bacillus as* Bio-inoculant for Plant Growth

Various PGPR attributes of *Bacillus* species can be introduced to various plant root zones of soil. All over the world, a number of researchers utilize *Bacillus* PGPR for enhancement of growth of various plants as mentioned in Table 11.1.

11.3.1 Tomato

PGPR improve plant growth against various biotic and abiotic stress conditions like high boron content in soil of arid and semiarid regions. Khan et al. (2016) conducted a pot experiment under controlled environment with varying concentration

Table 11.1 List of some *Bacillus* species and their mechanisms which are used to enhance plant growth worldwide

S.No.	PGPR activity	*Bacillus* species (PGPR)	Host plant	Place	Reference
1.	IAA	*B. megaterium*	Peanut	China	Xu et al. (2015)
2.	IAA	*B. amyloliquefaciens* W 19	Banana	–	Wang et al. (2016a)
3.	IAA	*B. subtilis* strain B 4	Cucumber	South Korea	Park et al. (2013)
4.	IAA	*B. licheniformis*	*Suaeda fructicosa*	India	Goswami et al. (2014)
5.	IAA	*B. amyloliquefaciens*	Rice	Germany	Blom et al. (2012)
6.	IAA	*B. amyloliquefaciens* SQR9	Cucumber	–	Shao et al. (2015)
7.	IAA	*B. amyloliquefaciens* W 20	Banana	–	Pindi et al. (2013)
8.	IAA, siderophore, P solubilization, ammonia production, halotolerant (1.0M)	*B. licheniformis* strain A2	*Suaeda fructicosa*	India	Goswami et al. (2014)
9.	IAA, siderophore	*B. amyloliquefaciens* subsp. *plantarum* strain UCMB5113	*Brassica napus* cv. Westar and *A. thaliana*	Ukraine	Niazi et al. (2014)
10.	IAA and siderophores	*B. amyloliquefaciens* strain 9SRTS	*Calendula officinalis* and chickpea	Belgium	Ait Kaki et al. (2013)
11.	Gibberellin	*B. licheniformis* CECT5105	*Pinus pinea*	Madrid, Spain	Probanza et al. (2002)
12.	Phosphate solubilization	*Bacillus* species	*Phaseolus vulgaris*	India	Saxena et al. (2013)
13.	IAA, siderophore, phosphate solubilization, lytic enzyme	*B. subtilis* strain 330-2	Rice and Maize	China	Ahmad et al. (2017)
14.	ACC deaminase	*B. circulans* DUC1, *B. firmus* DUC2, *B. globisporus* DUC3	Canola (*B. campestris*)	Southeastern Wisconsin	Ghosh et al. (2003)
15.	IAA	*Bacillus* sp-PU-7	Cotton Mahyco cultivar	India	Pindi et al. (2013)

(continued)

Table 11.1 (continued)

S.No.	PGPR activity	*Bacillus* species (PGPR)	Host plant	Place	Reference
16.	IAA, ACC deaminase, siderophore	*Bacillus* genera (salt tolerable)	Wheat	India	Upadhyay and Singh (2009)
17.	ABA, IAA, GA	*B. aryabhattai strain* SRB02	Soya bean	South Korea	Park et al. (2017)
18.	IAA, phosphate solubilization, antagonistic	*B. amyloliquefaciens strain* Bac17M11, Bac20M1, Bac20M2	Potato	Andean highlands of Peru	Calvo et al. (2010)
19.	Ammonia, siderophore, phosphate solubilization, IAA, antagonist	*Lysinibacillus fusiformis*, *B. subtilis* (B-CM191, B-CV235, B-CL-122)	Chickpea	India	Singh et al. (2013, 2014)
20.	Ammonia, siderophore, phosphate solubilization, IAA, antagonist	*B. amyloliquefaciens* MB101 and *B. subtilis* MB14	Tomato	India	Solanki et al. (2012a, b, 2015)

of boron (B). The mineral composition analysis after 10 week of growth showed inhibition in fresh weight of shoot, dry weight of shoot, and chlorophyll content of leaf with significant increase in boron and proline concentration along with enhancement in antioxidant enzymes concentration. *Bacillus pumilus* inoculation to the tomato plant resulted in significant improvement in shoot fresh weight (Ram et al. 2013) and dry weight of control (unstressed) as well as B-treated (stressed) plants. In general, there was an enhancement in antioxidation activity of plant with inoculation of *B. pumilus*, specifically catalase (CAT) and superoxide dismutase (SOD) antioxidant enzymes (Khan et al. 2016).

Mena-violante (2007) studied the co-inoculation effect to the root of tomato (*Lycopersicon esculentum* Mill.) with *B. subtilis* BEB-lSbs (BS13). He found that inoculation of PGPR has positive impact on yield per plant, weight of fruit and length as compared to the control treatment (without PGPR inoculation), and enhancement in texture of red fruits which indicates that PGPR have a positive impact on fruit quality of tomato, specifically on size and texture (Mena-violante 2007).

Hyakumachi et al. (2013) studied wilt disease (bacterial)-suppressing activity of *Bacillus thuringiensis* in tomato plants. This bacterium is naturally abundant and Gram-positive with effective bio-insecticidal activity which makes it a potent biocontrol agent for suppression of various plant diseases. Bio-inoculation of tomato roots with *B. thuringiensis* filtrate (cell-free), i.e., pretreatment followed by infection with a pathogen *Ralstonia solanacearum*, showed the decrease in the wilt

symptom development in tomato stem and leaf tissues to less than one third of the control which is caused by induction in defense-related genes expression, β-1, 3-glucanase and acidic chitinase. This study suggests that there was a systematic suppression of bacterial wilt through systemic activation of the plant defense system with co-inoculation of tomato roots with the CF of *B. thuringiensis* (Hyakumachi et al. 2013). The combination of PGPR and plant resistance inducers like acibenzolar-S-methyl (ASM) is supposed to be a good modern crop protection approach for agricultural systems (Myresiotis et al. 2014).

*11.3.2 Canola (*Brassica compretris*)*

Ghosh et al. (2003) isolated three strains of PGPR from southeastern Wisconsin soils which were utilizing ACC compound as sole source of nitrogen. These novel bacteria have been characterized as (1) *B. circulans* DUC1, (2) *B. firmus* DUC2, and (3) *B. globisporus* DUC3. All the three strains expressed the same levels of deaminase activity (EC 4.1.99.4) and were stimulating elongation of canola (*B. campestris*) seedlings. Bio-inoculations of the PGPR bacterial strain to potted rhizospheric soil of canola plant increased the lengths of shoot and root along with dry and fresh weights of plant (Ghosh et al. 2003)

Pindi et al. (2013) isolated seven different *Bacillus* strains from cotton rhizospheric soil of Palamuru University of Deccan Plateau. The Deccan Plateau region of India used to face drought due to irregular rainfall thus presenting poor conditions for farming. The novel *Bacillus* spp. PU-7 (among seven isolates) with Mahyco cultivar (superior cotton cultivars) were grown at field level. The novel strains improved plant growth by increasing the levels of phytohormone production and biochemical analysis. Hence, it is visualized that the isolate which is novel can be used as bio-inoculant or potent bio-fertilizer in the cotton fields.

11.3.3 Wheat

Upadhyay and Singh (2009) isolated 130 rhizobacteria of wheat rhizosphere (saline stressed soil) for screening of PGPR with varying salt (sodium chloride) concentrations (i.e., 2, 4, 6, and 8%). His group found 24 rhizobacterial isolates tolerant at 8% sodium chloride and were producing IAA, 10 isolates capable of solubilizing phosphorus, 8 isolates producing siderophore, and 6 producing gibberellins. Among these 24 isolates, only 3 isolates were able to produce 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Molecular characterization of these isolates with 16S rDNA sequencing revealed dominancy of *Bacillus* and *Bacillus*-derived genera. This study revealed that PGPR attributes under stress condition will be much helpful for increasing the yield of plant.

11.3.4 Saffron

Gupta et al. (2014) isolated *Bacillus spp.* strain W2, a PGPR bacterium, from saffron fields of Kashmir, India. After molecular characterization (16s rDNA sequencing), it was found to be a *B. amyloliquefaciens* strain W2 and was showing 99% identity with *B. amyloliquefaciens* subsp. *plantarum* FZB42, commercially available as bio-fertilizer, i.e., RhizoVital 42 (Gupta et al. 2014). Further, there is a need of utilization of this strain for PGPR attribute characterization and its utilization as bio-inoculants to various plants including saffron.

11.3.5 Soybean

Park et al. (2017) isolated a bacterium as *B. aryabhattai* strain SRB02 from soybean field (rhizospheric region) in Chungcheongbuk-do, South Korea. The growth of soybean was found to be promoted significantly with increase in length of shoots and roots as compared to control plants after bio-inoculation as the significant amounts of abscisic acid, IAA, cytokinin, and different gibberellic acids were produced in culture. Even under heat stress condition, significant amount of ABA was also produced from these treated plants. It can also tolerate oxidative stress due to the expression of enzyme catalase (CAT) and superoxide dismutase (SOD) activities. Because of all the above mentioned PGPR features, *B. aryabhattai* SRB02 may be a promising and valuable asset to be incorporated to bio-fertilizers and other soil reclamations that need to improve productivity of crop (Park et al. 2017).

11.3.6 **Arabidopsis**

B. subtilis (GB03) is a commercially available PGPR bacterium which produces various volatile compounds helping in plant growth, photosynthetic capacity, and iron accumulation at high salt concentration. Xie et al. (2009) studied the interaction of volatile compound (airborne transmission produced by two separate containers) produced by commercially available GB03 strain with *Arabidopsis* plant in vitro way in double Magenta boxes for 3 months in solid MS media. This study suggests that GB03 volatile compounds had positive impact on *Arabidopsis* growth in an in vitro manner; further, there is a need to examine its impact on *Arabidopsis* and other plants in in vivo conditions (Xie et al. 2009).

11.3.7 Banana

The enhanced growth of banana plant had been achieved successfully only after introduction of PGPR with the proper colonization of PGPR around the root which not only promotes growth of plant but also protects it with various pathogens. In PGPR colonization, root exudates of plants play an important role, and these root exudates of banana contained various organic acids which involved oxalic, malic, and fumaric acid confirmed after high-performance liquid chromatography (HPLC) analysis. These organic acids of banana root exudates were involved in both the chemotaxis and in formation of biofilm in *B. amyloliquefaciens* NJN-6 optimally at 50 μM concentration. Among these OAs, the greatest response against chemotaxis was shown by malic acid, while induced biofilm formation was shown significantly by fumaric acid. There are certain transcriptional genes like yqxM and epsD that were also found to be involved in biofilm formation revealed by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) (Yuan et al. 2015). This study reveals that root exudates released by banana root crucially play important roles in attracting, initiating, and making biofilm formation around host roots.

11.3.8 Potato

There is a huge problem in potato crop yield due to many phytopathogens, and nutritional issues in various countries spread all around the world including Andean highlands, Peru. This forces the researcher to find out the PGPR which should not only improve the yield of potato crop by increasing growth but should also protect the plant against various phytopathogens. Calvo et al. (2010) isolated 63 PGPR (*Bacillus* strains) from the rhizospheric region of native potato varieties of Andean highlands, Peru. This was further screened for antagonistic activity against phytopathogens (i.e., *Rhizoctonia solani* and *Fusarium solani*) in an in vitro manner. Sixty-eight percent of *Bacillus* isolates were showing antagonistic activity against *R. solani* and ninety-one percent against *F. solani*. These *Bacillus* isolates were also showing PGPR attributes, i.e., IAA production (81% of *Bacillus* isolates) and solubilized tricalcium phosphate (58% of *Bacillus* isolates). Further, molecular characterization of these isolates confirmed that most of the strains were found to be *B. amyloliquefaciens* spp. (Calvo et al. 2010). This study suggests that the potato rhizospheric region grown in Andean highland, Peru, has various potent species of *Bacillus* strain with high antagonistic activity against various fungal phytopathogens which have an enormous potential to be used as bio-inoculants to improve potato crop yield in the future.

11.3.9 Apple

The *B. methylotrophicus* FKM10 is a PGPR isolate from apple rhizospheric soil region of Shandong, China. This strain was showing PGPR activity (Wang et al. 2016a, b) along with antimicrobial activity. Wang et al. (2016a, b) further elaborated the genomic sequence of PGPR, i.e., *B. methylotrophicus* FKM10, and found various genes related to antimicrobial activity (Wang et al. 2016b). There is further need to utilize this strain using various antimicrobial activities against pathogen along with its PGPR activity in apple and other plants.

11.3.10 Cassava

Cassava (*Manihot esculenta*) is a major staple food eaten as vegetable which is native of South America and grown by small farmer throughout Latin America, South Africa, and Southeast Asia. The tuberous root provides the third largest source of carbohydrate after rice and maize. Low iron content in cassava leads to malnutrition on which more than half a billion people are surviving in tropical region. This makes an urgent attention for the researcher to increase the iron content of cassava though sufficient iron remains present in the soil. Thus, cassava is not providing sufficient iron for humans. This is due to 3–12 times less iron contents in the edible roots of cassava as compared to the other most commonly available food crops including rice, maize, and wheat. Freitas et al. (2015) identified that *B. subtilis* (strain GB03), inhabitant of rhizospheric soil, bacterium is able to activate iron acquisition machinery, apart from siderophore production, for enhancing metal ion assimilation in *Arabidopsis* plant via transcriptionally upregulating factor FIT1 (Fe-deficiency-induced transcription factor 1) required for induction of enzyme ferric reductase (FRO2) and the iron transporter (IRT1) (Zhang et al. 2009). GB03-inoculated cassava over the period of 140 days growth showed subsequent enhancement in accumulation of Fe as determined by microanalysis of X-ray and total foliar iron analysis. This study showed the potential of microbes to increase accumulation of iron to a beneficial agricultural crop, and the idea of plant photosynthesis regulation through microbial signaling can also be applied to other plants by using either this bacterium or other PGPR (Freitas et al. 2015).

11.3.11 Cherry Tree

A number of PGPR had been isolated from rhizospheric zone, but Kim et al. (2012) isolated *Bacillus* spp. strain 5B6 from the leaf region of *Prunus avium* L. (cherry) which showed a leaf-colonizing capacity, promoted plant growth, and also showed various antagonistic activities. The isolate was showing close identity with well-known PGPR, i.e., *B. methylotrophicus* CBMB205 (100%) and *B. amyloliquefaciens* subsp. *plantarum* FZB42 (99.9%) (Kim et al. 2012).

11.3.12 Calendula officinalis

Ait Kaki et al. (2013) reported various *Bacillus* spp. from the rhizospheric region of *Calendula officinalis*, screened for their important antagonistic activity, i.e., antifungal activity against *Aspergillus niger*, *Cladosporium cucumerinum*, *Fusarium oxysporum*, *Alternaria alternata*, and *Botrytis cinerea*. The electro-spray mass spectrometry coupled to liquid chromatography (ESI- LC MS) analysis of *Bacillus* isolates showed that most of it produced the three lipopeptide families. The cellulose was produced by all the tested *Bacillus* isolates, but the protease activity was exhibited only by *B. amyloliquefaciens* species (9SRTS) (Kushwaha et al. 2013). All the screened bacteria were producing indole-3-acetic acid (6–52 µg/ml) through Salkowski colorimetric test and significant zone of siderophores, i.e., more than 10-mm-wide orange zones on Chrome Azurol S. The bio-inoculation of *B. amyloliquefaciens* (9SRTS) strain to chickpea seeds in a naturally infested soil with *Sclerotonia sclerotiorum* in greenhouse experiment played no significant role in in pre-germination of seeds, but the size of chickpea plants was found to be increased and reduced the stem rot disease (P <0.05). This research hypothesized that the isolated *Bacillus* strains may further be utilized in the cropping systems of *C. officinalis* and also various other agricultural crop yields (Ait Kaki et al. 2013).

11.3.13 Tobacco

The wilt disease in tobacco is caused by phytopathogen *Ralstonia solanacearum* (Rsc). This phytopathogen can be controlled by various volatile organic compounds (VOCs) produced by bacteria like *B. amyloliquefaciens* FZB42 and *B. artrophaeus* LSSC22, thus playing important role in enhancement of plant growth after controlling phytopathogens. These VOCs significantly inhibit the viability of cell, colony size, and pathogen motility and influenced chemotaxis negatively. Further, VOCs downregulate expression level of various genes involved in pathogenicity, i.e., type III secretion system (T3SS), type IV secretion system (T4SS), the transcriptional expression level of PhcA, extracellular polysaccharides, and chemotaxis-related genes. VOCs also upregulate the expression of various genes (EDS1 and NPR1) responsible for wilt resistance and pathogen defense (Tahir et al. 2017).

11.3.14 Lonicera japonica

Zhao et al. (2015) screened out 6 strains among 48 endophytic bacteria from tissue (surface sterilized) of *Lonicera japonica* (a medicinal plant) grown in eastern China based on their PGPR attributes (i.e., siderophore and IAA production). These six endophytic bacterial strains were identified through molecular characterization (16S rRNA gene sequencing) (Verma et al. 2018) and were found to be *Paenibacillus* and *Bacillus* strains. Among these six strains, high siderophore production attribute

was expressed by strains 122 and 124, while phosphate solubilization activity and aminocyclopropane-1-carboxylic acid deaminase activity were shown only by strain 122. Highest indoleacetic acid (IAA) production and cellulase and pectinase activities were shown by strain 170. The co-inoculation of the six selected strain showed a strong positive impact on wheat plant growth as compared to control. The bio-inoculation of strain 130 to wheat plant was the most promising among six strains which was found to enhance the growth and yield attribute significantly.

11.4 Conclusion and Future Prospects

PGPR which inhabit near root of plant may enhance plant growth development directly either through assisting in nutrient acquisition (nitrogen, phosphorous, and other essential minerals) to plant via secreting various chemicals nearby plant root or indirectly by protecting the plant root against various pathogens via producing volatile compounds (affect various signaling pathways of plant), by antibiosis, competition for living space and nutrients and induction of systemic resistance (ISR) in plant. Thus, there is improvement in plant health followed by enhancement in crop productivity. There is enormous potential for PGPR to increase crop productivity by utilizing it as bio-inoculant. The PGPR should have enough potential for increasing plant growth and development either by utilizing the mechanism of direct or indirect or both in combinations; the latter is desirable. There is a need for PGPR consortia development in a biocompatible way so that optimum growth of plant could be achieved without any constraints. Further, there is a need for potent PGPR either by isolation or by recombinant DNA technology which may be called as superbug PGPR. This PGPR may have superior gene for P solubilization, siderophore production, IAA production, and various antipathogenic mechanisms which may be commercialized at large scale.

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Chapter 12
Significance of Microbial Agents in Augmentation of Plant Health

R. N. Lakshmipathi, B. Subramanyam, and B. D. Narotham Prasad

Abstract The role of soil microorganisms in establishment of plants is well known. However, it appears that their potential under field conditions is yet to be realized consistently. The main constraint for their ineffectiveness is establishment of introduced microbial populations in soil system, which in native microflora act antagonistically with the introduced ones. Further, use of biofertilizers is limited owing to the factors of reduced shelf life in storage conditions, inconsistent growth responses caused by abiotic stress factors such as higher temperatures during storage, drought, water stagnation in field conditions, etc. An alternative to this could be the development of consortial formulations with beneficial microorganisms having different physiological capabilities to sustain their activity in wide range of field conditions. Entrapment into natural polymers such as alginate and their introduction to soil has been evaluated, and the results have revealed that they protect entrapped organisms from native soil microflora and further enable them to interact synergistically, thus allowing them to finally develop to a stable microbial community in rhizosphere. This could enable them to have higher chances of establishing in soil and cause desirable effect on plant.

Keywords Microflora · Biofertilizers · Consortia · Polymers · Synergistically

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12.1 Introduction

Microbial consortia refer to multiple interacting microbial populations. Most microbes live in heterogeneous groups of surface-bound congregations called biofilms. In many cases microbes in biofilms develop into complex interactive communities called consortia. Members of the consortium (consors) communicate with one another which enable them for the division of labor. The overall output of a consortium rests on combinations of tasks performed by its subpopulations.

Consortia can attend more complex functions and they are robust to environmental fluctuations. This has made synthetic biologists to hone their ability to engineer microbial consortia which are far superior to their constituent mono populations (Brenner et al. 2008).

The concept of using biofilmed biofertilizers (BBs) is considered to be a novel biofertilizing technique. This involves culturing agriculturally beneficial microorganisms (ABMs) together to form biofilms, and they are introduced to soil as biofertilizers (Seneviratne and Jayasinghearachchi 2005). Recent studies demonstrated that biofilms grown in axenic conditions using one, two, or more bacterial species with fungus have better activity than their individual populations (Santaella et al. 2008; Seneviratne et al. 2009). According to their experiments, BBs establish an association with the plant roots, and addition of chemical nutrients increases the microbial biomass of biofilms.

Biofilms containing nitrogen fixers and other organisms act as pseudo nodules which fix nitrogen and release organic acids (Mongiardini et al. 2008). The acidic conditions may suppress microbial pathogens and mobilize nutrients from soil. Also biofilms improve root growth and mycorrhizal growth by producing hormones such as IAA. Thus microbial associations in biofilms help in plant growth and are known to supplement 50 percent of chemical fertilizer requirement of the tested plants.

However, the limitation is identification of combination of microbial isolates with respect to plant growth and their effective formulation to a consortium.

12.2 Benefits of Microbial Associations in Agriculture

Soil microorganisms have been categorized into different groups based on their functionality. Beneficial microorganisms are those that can stimulate plant growth, decompose organic residues, enhance nutrient cycling, detoxify pesticides, and suppress pathogens in general. Using some of these beneficial microorganisms, various microbial inoculants have been prepared for use in crop production in order to cut down the cost and to minimize environmental pollution.

Since use of microbial inoculants is eco-friendly and sustainable, their use in agriculture is gaining importance (Higa and Wididana 1991). These inoculants have been used with considerable success. However, they are not widely accepted due to their inconsistent performance under field conditions.

The interaction effect of microorganisms used in combination is more effective than mono inoculation (Alagawadi and Gaur 1988). Plant growth enhancement due to the inoculation of either single strain or combination of microbial strains has been noticed in several investigations, and the mechanisms of growth improvement are attributed mainly to supply the major nutrients and production of plant hormones and suppression of plant pathogens.

A formulation containing more than one microorganism does not exist despite recorded benefits of dual inoculations. A single formulation containing consortium of agriculturally beneficial microorganisms needs to be developed as they play crucial role in enhancing productivity and maintaining soil health. An ideal microbial consortium formulation is the one that does not allow any negative interactions among the resident microorganisms (Smith 1992). The negative interactions in a formulation may decrease number of viable cells, affecting the effectiveness of consortium, which emphasizes the need for development of ideal formulation (El-yazeid et al. 2007).

12.3 Beneficial Interactions of Microorganisms and Plants

Hiltner (1904) designated the term rhizosphere for the region of soil where he noticed an association of plant root system with soil bacteria. The microflora in rhizosphere differs compared to that in non-rhizosphere; their influence on plant growth is also significantly different (Rangaswami and Vasantharajan 1962; Bowen and Rovira 1976).

High microbial activity in rhizosphere is because of the extra nutrients available from root exudates, sloughed-off root tissues, mucigels, etc. (Curl and Truelove 1986; Darrah 1991; Whipps and Lynch 1985; Dhruvakumar et al. 1992). Microorganisms of different taxonomic and nutritional groups found in rhizosphere have been classified as being harmful, neutral, and beneficial to the plants (Dommergues 1978). Microorganisms that are treated for plant growth promotion are biofertilizers which colonize the root system and enhance nutrient supply by various mechanisms (Rokhzadi et al. 2008).

12.4 Agriculturally Beneficial Microorganisms (ABMs)

Soil harbors a vast array of bacteria, actinomycetes, fungi, and algae which support plant growth, and in general they are referred to as ABMs. The diverse functional groups of microorganisms are mainly categorized as nitrogen fixers, phosphate solubilizers, and other categories.

Most common symbiotic nitrogen fixers are *Rhizobium* and *Bradyrhizobium* that are capable of forming symbiotic association with legumes. The common free living nitrogen fixers are *Azotobacter* and *Azospirillum* which supplement the major plant nutrient.

Several bacterial species improve phosphorous uptake of plants by solubilizing phosphates through production of organic acids (Rodrıguez and Fraga 1999). PSBs are common for all crops and several species of them are present usually in low numbers in soil.

Rhizobacteria enhance plant growth by different mechanisms. Direct mechanism of plant growth improvement is noticed in few studies (Gaskins et al. 1985). The mechanism of offsetting the deleterious effects of phytopathogenic organisms is a secondary effect that improves plant growth (Zehnder et al. 2000). *Pseudomonas* spp. are at present receiving worldwide attention as PGPRs (Kloepper et al. 1989).

Mycorrhizal associations predominate and colonize root system which is one of the most common in terrestrial plants and promote plant growth mainly by enhanced phosphorus absorption (Gerdemann 1968; Bolan 1991).

There are different microbial groups that promote plant growth among which nitrogen fixers, AMFs, and PGPRs are major (Franche et al. 2009; Jeffries et al. 2003; Podile and Kishore 2006).

12.5 Plant Nutrition from Selected ABMs

12.5.1 Nitrogen-Fixing Microorganisms

Nitrogen is the primary nutrient requirement of the plant. Microorganisms that have the capability to source nitrogen from air to plants have been observed and utilized to improve plant growth. Apart from *Rhizobium* spp. which are symbiotically associated with legume plants and fix atmospheric nitrogen in significant quantities, associative and free living nitrogen fixers have been noticed and are equally important to harness atmospheric nitrogen for nonleguminous plants and soil improvement.

Azotobacter chroococcum has recorded satisfactory levels of nitrogen fixation per unit of carbon source used. Inoculation of *Azotobacter* sp. has resulted in higher concentrations of nitrogen in plant tissues and increase in yield parameters of crop plants (De Freitas 2000; Kumar et al. 2001; Konde and Shinde 1986). *Azotobacter* sp. along with *Azospirillum* sp. has shown a positive improvement in plant growth.

Inoculation of *Azotobacter chroococcum* has improved plant growth as well as yield and demonstrated savings on nitrogenous fertilizer in significant quantities (Anjum et al. 2007; Ananthanaik et al. 2007). Further, *Azotobacter* is recommended to substitute inorganic inputs effectively (Rajeshwari et al. 2007) to minimize the use of chemical inputs in agriculture. Thus *Azotobacter chroococcum* has proved to be a promising free living nitrogen fixer and could serve to replenish nitrogen content in soil.

An eco-friendly method of fixing nitrogen for plants has to be considered, for which use of microbial agents is considered to be effective (Gothwal et al. 2007; Bakulin et al. 2007). Different microbial agents that are effective, eco-friendly, and efficient are considered effective to be employed for nitrogen fixation (Gupta 2004).

12.5.2 Phosphate-Solubilizing Bacteria and P-Mobilizers

Source of phosphorus usually present in soil are unavailable for plant uptake. Certain microorganisms have been identified to aid in phosphorus plant nutrition by different mechanisms. There is a positive correlation between the microbial population that solubilizes phosphates and phosphorus that is in available form in soil (Kucey 1983). Many bacteria and fungi have been identified with different mechanisms that aid in phosphorus nutrition for the plants.

Vesicular arbuscular mycorrhiza (VAM) refers to the symbiosis of soil fungus with plant roots that mainly benefit plants by mobilizing phosphorus. Infection with mycorrhizal fungi improves phosphorus nutrition of host plants growing in poor soils (Smith and Gianinazzi-Pearson 1988; Bolan 1991). The role played by them in tropics is of prime agricultural importance as phosphorus availability limits the productivity (Sanchez and Uehera 1980; Bethlenfalvay 1992; Habte and Osorio 2011).

Among bacteria, *Bacillus* sp. has been considered as an efficient phosphate-solubilizing bacteria (PSB), and their formulations have been developed and used. *Acinetobacter* sp. is also an efficient phosphate-solubilizing bacteria (Fan et al. 2011). In addition to phosphate solubilization, *Acinetobacter* sp. is also recorded to be producing different extracellular gibberellins and thus promoting plant growth (Kang et al. 2009).

Hilda and Fraga (1999) described *Acinetobacter* sp. as a PGPR apart from being a phosphate solubilizer. Production of organic acids by *Acinetobacter* sp. and concomitant increase in plant growth by enhanced phosphorus nutrition have been evidenced by Gulati et al. (2010). Thus formulations delivering *Acinetobacter* sp. ensure enhanced nutrition of phosphorus and other benefits.

A selected strain of *Acinetobacter* sp. studied for their plant growth promotion mechanisms have shown different mechanisms including ammonia generation and siderophore production. The study has also demonstrated improved plant growth due to inoculation by selected *Acinetobacter* sp. under controlled conditions and in field conditions. Improved pea growth as well as yield was noticed in a study (Gulati et al. 2009). Microbial role in converting unavailable phosphorus to available form is understood, and different mechanisms such as acid production and consequential effects are studied (Gupta 2004).

12.5.3 Soil Bacteria That Improve Plant Growth

A species of bacteria, *Pseudomonas fluorescens*, has shown nitrogen fixation capability and iron and phosphate mobilization activities (Ansari and Mahmood 2017; Gupta 1995). Inoculation of *Pseudomonas* sp. has controlled the disease incidence and improved plant growth and yield (Haggag and Saber 2000). Biocontrol activity of *Pseudomonas* was evidenced by Moenne-Loccoz et al. (1999). Co-inoculation of two strains of *Pseudomonas* sp. has also showed beneficial impact on vegetative and reproductive growth of plants (Sarma et al. 2009).

Combined inoculation of microbial agents such as *Pseudomonas* sp. and *Rhizobium* sp. significantly improved the plant biomass and nodulation of plants than their individual inoculations, thus ensuring positive interaction effect of *Pseudomonas* sp. with other ABMs (Arora et al. 2008). Significant improvement in yield of commercial rice cultivars has been documented by *Pseudomonas* sp. (Mirza et al. 2006). Improved response of tomato plants due to inoculation with *Pesudomonas* sp. has also been documented (Minorsky 2008).

Improved plant response for combined inoculation of bacteria and fungi than their individual inoculations has been recorded in different studies (Belimov et al. 1995; Barea et al. 2002). Specially, the associations of fungi and bacteria have significant effect on uptake of nutrients and even on their further associations with beneficial bacteria in soil (Barea et al. 2002; Vassilev et al. 2001).

12.6 Formulations of ABMs

The ideal carrier will have to be nontoxic to the microorganism and seeds on which they act. Further, more organic matter and high water-holding capacity along with high surface area are preferred characteristics of the carrier material. To promote ABMs and increase their usage in field, they have to be modulated as per the requirements of transportation, storage, and end usage. The success rate of a formulation is indicated by number of viable cells it could deliver for use (Paau 1988). It is important to maintain the bio-inoculant formulation of microbial cells or spores in their viable condition.

Researchers have attempted to develop formulations of ABMs with garden soil, vermiculite, as well as charcoal (Sparrow and Ham 1983), (Madhok 1934), peat (Iswaran et al. 1969), powder of cellulose (Pugashetti et al. 1971), coffee husk and forest soil, coir dust (Iswaran 1972), husk of rice and sand (Khatri et al. 1973), peat and bagasse (Graham et al. 1974), coal (Dube et al. 1975), sugarcane press mud and coffee waste (Kumar Rao et al. 1982), vermiculite, as well as charcoal for (Sparrow and Ham 1983). Kandasamy and Prasad (1971) reported lignite carrier material as a good substitute for peat.

Though peat is the most popular carrier for bio-inoculant formulations worldwide, the problem of unavailability of good quality peat is the major constraint. As far as India is concerned, lignite and coal are considered to be the better choices as carrier material. But, the formation of hard clumps upon drying and during storage period is an associated problem which reduces the inoculant population in these formulations. Alginate-based formulations are being evaluated and seem to offer substantial practical advantage. They protect microbial inoculants against stress factors of soil and release them to rhizosphere gradually by slow degradation of alginate. Coal, clays, and inorganic soils can also be considered as carriers depending on their availability (Smith 1995).

12.7 Inoculation Effect of ABMs on Plant Growth

Inoculation effect of diverse functional groups of ABMs on plant growth has been investigated by researchers. ABMs might improve plant growth by increased plant nutrition and also by other mechanisms (Bashan and Holguin 1997). Enhanced growth, biomass, and nitrogen content of plants for inoculation of soil with nitrogen fixers have been observed (Ananthanaik et al. 2007). Inoculation with *Acinetobacter*, which acts as P solubilizer and PGPR, resulted in enhanced response of canola and biomass of tomato (Indiragandhi et al. 2008). Field testing of pea with *Acinetobacter* has enhanced the plant response (Gulati et al. 2009). Experiment of Mohammadi et al. (2011) indicated an improved response of chickpea plant.

12.7.1 Dual Inoculations

Several workers have attempted to check the effect of dual inoculations of ABMs on plant growth and have noticed a positive plant growth response. Different combinations of ABMs such as the dual inoculation of symbiotic nitrogen-fixing bacteria with asymbiotic nitrogen fixers and nitrogen fixers with P solubilizers or with PGPRs as well as combinations of strains among asymbiotic N fixers or P solubilizers or VAM fungi have also resulted in positive plant response. Dual inoculation of VAM fungus with symbiotic or asymbiotic N fixer or with a P solubilizer has shown positive plant response. Trials in field conditions also have improved the plant growth (Galal 1997; Rodelas et al. 1999; Ramazon et al. 2004: Yadegari et al. 2008; Askary et al. 2009). Synergistic interaction of microorganisms in dual inoculation seems to be the basic requirement for success of dual inoculations.

Study using wheat plants with combined use of *Azospirillum lipoferum* and *Bacillus megaterium* has shown a balance in N and P nutrition (El-Komy 2005).

12.7.2 Multiple or Mixed Inoculations

Veena (1999) reported the highest plant response of biomass and yield sorghum in treatment receiving consortium of eight organisms comprising bacteria, fungi, and actinomycetes that could substitute major portion of chemical fertilizers dose. Devananda (2000) reported highest plant response when co-inoculated with different biofertilizer agents.

Thus mixed cultures or consortial formulations are more effective than individual microbial inoculations. However, selection of combination of microorganisms having compatibility among them and their synergistic interaction for nutrient management is important to consider in preparation and formulation of consortium.

Mixed inoculation of microbial agents that enhance major nutrients in plants resulted with synergistic interaction effect leading to a significant increase in plant growth (Chanway et al. 1991; Linderman 1997). Combination of symbiotic bioagents is better for co-inoculation and improvement in plant growth (Tambekar et al. 2009).

Continuous studies have led to development of protocols for effective formulations of microbial agents for plant response and sustained use (Podile and Kishore 2006).

12.8 Limitations of Formulations of ABMs

Formulations of ABMs are in general available either as powdered or granular or liquid formulations (Smith 1992). Powdered formulations are usually prepared using peat as carrier material and are coated on seeds using sticker material; however they seem to be unsuccessful always. It is mainly due inefficient initial quorum of inoculants on seed surface and in rhizosphere soil. Though liquid formulations have been improvised by cell protectants to ensure better survival of ABMs, under varied soil conditions, they may not be equally effective (Deaker et al. 2004; Hynes et al. 1995; Hynes et al. 2001). Other limitations of liquid formulations noticed are requirement of cool temperatures for storage, limited shelf life, and increase in handling and end user costs. Formulations of ABMs are perceived to be inconsistent in their effect on plant growth. They have to be popularized emphasizing on their long-term effect on soil health, sustainability, eco-friendliness, and overall benefit to the ecosystem.

Inconsistent response of bio-inoculant formulations owing to varied factors of plant-microbe interactions and their complexity has limited their use (Artursson 2005). Also stringent regulatory procedures and trade restrictions have added to the limitation of their use (Guillon 2006).

12.9 Alginate-Based Formulations of ABMs

Alginate beads can entrap sufficient viable numbers of bioagents, and alginate encapsulation is known to protect inoculants from stress factors and release them gradually, thus serving as viable inoculum source for a long period to facilitate their establishment in rhizosphere (Fenice et al. 2000; Zohar-Perez et al. 2002; Bashan et al. 2002; Vassilev et al. 2001).

Alginate beads are mechanically stable and biodegradable which is a preferable trait of the formulation material. Bashan (1986) succeeded in preparing formulation of alginate with skim milk being a large reservoir of *Azospirillum* or *Pseudomonas* cells. And the release of bacterial cells from beads was at a slow and steady rate.

Interestingly it was noted that entrapment of living bacterial cells is efficient and their survival in alginate beads is better with the moderation of alginate concentration and addition of adjuvant material. Use of additive materials has improved the survivability of bacterial cells (Fages 1990). Strullus and Plenchette (1991) have stated that the physiological properties of mycorrhizal roots and vesicles were stabilized due to their entrapment in alginate beads. Ivanova et al. (2005) studied encapsulation of nitrogen-fixing *Azospirillum* in alginate and found that their application resulted in better yield than application of liquid and powdered formulations in field conditions.

Thus alginate bead formulations can be considered for further development of consortial formulations of ABMs, considering their ability to protect entrapped microbial cells against abiotic stress conditions and their extended shelf life in stored conditions.

12.10 Consortial Formulations of ABMs

Plant requirement is not limited to a single nutrient or to a phytohormone, and that inoculation with various groups of ABMs could benefit the plant most. ABMs certainly improve crop growth and fertility status of agricultural fields. Combinations of these beneficial microorganisms have to be functioning effectively in soil to enhance yields sustainably. Combination of different interactive microorganisms is known as microbial consortia.

Use of ABMs in consortia could act together as a community and interact synergistically resulting in more efficient formulations than their single-strain formulations. Further, identification of strains, compatibility aspects, and their effect on plant are to be considered to constitute consortia (Higa and Wididana 1991).

Biofilms have been considered for constituting effective plant growth helping microbial inocula (Seneviratne et al. 2008). Biofilms are common to most of the soil microbes present with plant root system (Ude et al. 2006). Hence consortial formulations are hypothesized to reduce operational cost and increase chances of improving crop growth and yield.

12.11 Conclusion and Future Prospects

The conclusions can be drawn through this chapter that consortia of microbes is most effective in plant growth promotion and yield maximization. This has attracted root biologist to engineer competent microbial consortia which are able to survive in the harsh environment making the plant more vigour. Application of microbial biofertilizers is considered as one of the important tool to enhance the agricultural productivity. Consortium applications have been found to support the plant health considerably. In addition, microbial associations in biofilms constitute an excellent metabolic cooperation for plant health amelioration and are well known to substitute for major proportion of chemical fertilizer requirement of the tested plants.

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Chapter 13
Plant Growth and Health Promoting Plant-Microbe Interactions

Baby Summuna, Sachin Gupta, and Parveez Ahmed Sheikh

Abstract The interaction of microbes with plants at the molecular biology and molecular genetics level describes a big concern for a broad range of scientific studies. These interactions can be of various types including pathogenic, symbiotic, and associative, all of which have an impact on plant productivity, disease resistance, and stress tolerance. Such plant-microbe interactions determine the plant fitness and soil health. The important functions for the growth of plants are fulfilled by the microorganisms associated with them. Plant fitness depends on the availability of beneficial microbiome and available nutrient status. There are various mechanisms which are either directly or indirectly implicated in the suppression of soilborne pathogens leading to ameliorated plant health. Microorganisms live as complex populations in the soil and not in the form of pure culture. More than one type of organisms is present in every soil particle. Therefore, the sum of abiotic and biotic components of soil comprise the microbial ecosystem of soil. Most of these organisms are dependent upon one another for direct and indirect nutrients. Some organisms are in competition with one another for energy sources and the elements and components used as nutrients. Hence, numerous associations are formed among soil microorganisms. The nature of microbiome is determined by the biological equilibrium which is a result of interaction among the microbial community. The individual microbes may develop various kinds of interactions such as neutral or beneficial or detrimental.

Keywords Plant growth · Plant health · Rhizosphere · Endophytes

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13.1 Introduction

The demand for sound and eco-friendly agriculture is currently increasing as the humankind is being well aware about their health. Application of inorganic fertilizers or pesticides is disastrous causing great impact on agroecology, also not amenable to the survival of microbial community which ultimately provides outstanding services in the soil and plant health ameliorations. Although the current crop cultivation systems, on the one hand, produce good remuneration, on the other hand, they have also offered various plant pathogens to cause drastic damages on the plants. A wide number of plant diseases have been a headache to the grower which have not been so far given any effective treatments. Therefore, this is the high time to apply the beneficial microorganisms or microbial-derived products in the intensification of sustainable agriculture. The quantity and quality of microorganisms may differ and depend on the types of rhizobiome (Morgan et al. 2005; Nihorimbere et al. 2011; Lareen et al. 2016; Poudel et al. 2018). Plant-microbe interactions play a pivotal role in different ecological services such as nutrient recycling and carbon sequestrations (Singh et al. 2004; Bardgett 2018). The important instance of plant-microbe interactions are the relationship of plant-PGPR, endophytes, and some beneficial arbuscular mycorrhizal (AM) fungi (Smith and Read 2008). These interactions have also been very helpful in the management of various soilborne plant pathogens by adopting different mechanisms such as improved nutrient status and ameliorated resistance against pathogens leading to enhanced plant growth and productivity (Morrissey et al. 2004; Berg 2009; Mendes et al. 2011; Selvakumar et al. 2012; Zamioudis and Pieterse 2012; Zolla et al. 2013). In addition, plant releases some root exudates which are later on utilized by the microbes for their proliferations (Bais et al. 2006; Hennion et al. 2018). The microorganisms when applied directly to the plant ameliorate the soil fertility and also improve the plant growth and productivity-related parameters. Application of microorganisms such as PGPR, AM fungi, and biocontrol agents triggers the production of some organic molecules which act as plant growth enhancer and protect the plant from the attack of the several pathogens by improving the tolerance index of the plant. Thus present chapter enlighten the current research done so far on the microorganisms and their beneficial impact on plant health management.

13.2 Rhizosphere and Root Exudates

The rhizosphere is the area which is directly influenced by the root secretion and microorganisms associated with the roots (Arroyave et al. 2018). The rhizosphere may contain billions of bacteria and other microorganisms. These microorganisms may feed upon the root exudates released by the host root organs. The rhizosphere has again been subcategorized as endorhizosphere, rhizoplane, and ectorhizosphere which are the three prominent zones of rhizosphere. In this environment, physical, chemical, and biological properties of soil are greatly influenced as this is the

playground/battlefield for the microorganisms where interaction/cross talk among the microbes-soil-host plant takes place (Nihorimbere et al. 2011). Root exudates are the source of energy for the microorganisms and act as media in the plant-microbe interaction process (Badri et al. 2013a). The root releases about 5–21% of photosynthate as a soluble sugar, secondary metabolites, and building molecules such as amino acids which is eventually utilized by the microbial community for their proliferations (Badri et al. 2013b). Generally, there are two classes of root exudates: (i) organic compounds with low molecular weight such as amino acids, organic acids, phenolic compounds, sugars, and other secondary metabolites and (ii) organic compounds with high molecular weight like proteins and polysaccharides (Badri and Vivanco 2009). However, the quality and quantity of root exudates depend upon several other factors, for instance, plant cultivar, plant species, plant developmental stage, and various abiotic factors, such as soil type, temperature, pH, and biological properties (Badri and Vivanco 2009).

13.2.1 Mechanism of Root Exudation

There are several transport mechanisms which are used by plants during root exudations and depositing it to rhizosphere (Weston et al. 2012; Arroyave et al. 2018). Most of the low molecular weight compounds are secreted through the passive mechanisms; however, permeability of membrane, polarity of compounds, and pH of cytosol are the determining factors (Badri and Vivanco 2009). The root system of plants also secretes a large number of organic compounds, viz., secondary metabolites, polysaccharides, and protein molecules. There are several membrane-binding proteins such as ATP-binding cassette (ABC) proteins (Badri et al. 2008; Weston et al. 2012). Besides, multidrug and toxic compound extrusion (MATE) also assists in the exportation of various proteins across the membrane through electrochemical gradient of other ions (Weston et al. 2012). Moreover, a large number of genes have been so far characterized in various economically important crops such as *Arabidopsis*, sorghum, barley, and rice which play a significant role in the export of a wide range of compounds like plant-derived alkaloids, toxic compounds, antibiotics, citrate anions, and phenolic compounds, from the root cells (Liu et al. 2009; Magalhaes et al. 2007; Ishimaru et al. 2011).

13.3 Rhizospheric Interactions

13.3.1 Root Exudates Are Involved in Plant-Microbe Interactions

Various rhizospheric interactions mediated by root exudates have largely been well researched (Badri et al. 2013a; Verma et al. 2018). A large number of interactions may be of different types such as plant-plant, plant-microbe, and plant-faunal,

varying from neutral to beneficial or deleterious (Raaijmakers et al. 2009). Bio-inoculants or biofertilizers or phytostimulators are known to be as beneficial microorganisms. Application of biofertilizers causes either direct or indirect improvement on plant health status. The spore populations of soilborne pathogens especially fungi are adversely affected due to indirect effect of beneficial microorganisms showing antagonistic activity. Application of microorganisms ameliorates the plant growth by using different kinds of mechanisms such as food and space competition, antibiosis, lysis, and hyperparasitism. Also, initiation of food and space competitions, fast colonization of root surface, and well establishment in the root are the prerequisite for dynamic biocontrol. Bio-inoculants produce different extracellular lytic enzymes which are antagonistic to pathogens. Microorganisms present in the rhizosphere register direct beneficial impact on plant health ameliorations through phytostimulation and biofertilization, involving plant growth hormones, nitrogen fixations, and enhanced availability of phosphate and other important nutrients (Burdman et al. 2000). Moreover, the important section of rhizosphere, i.e., PGPR (plant growth-promoting *Rhizobacteria*), stops the entry and multiplication of soilborne pathogens by producing various toxic metabolites against plant pathogens. The toxic compounds against pathogens, like HCN, phenazines, pyrrolnitrin, and pyoluteorin, as well as enzymes, antibiotics, metabolites, and phytohormones work very effectively in terms of managing the soilborne plant pathogens. The PGPR use different modes for cross talking such as quorum sensing and chemotaxis which are most prominent during the root colonization in the rhizosphere (Jousset et al. 2011). In addition, PGPR also produces iron-chelating agents (siderophores) under the iron-limiting atmosphere. The siderophores are generally low molecular weight compound which sequester the iron and create competition between PGPR and pathogens and this way deprive the growth/proliferations of pathogenic organisms (Pedraza et al. 2007). A systemic response can also be induced in plants by many rhizosphere microorganisms, thus activating plant defense mechanisms. Signaling pathways can be triggered by inoculation of beneficial rhizobacteria leading to the development of higher pathogen resistance, known as induced systemic resistance (ISR). PGPR not only enhance the plant growth but also improve the health of plants growing under nutrient-deficient soil (Hayat et al. 2018). Inoculation of potent PGPR strain also initiates some physical, chemical, or biological changes pertaining to plant defense and also obviates the stress caused by abiotic factors like drought or excess or deficiency of salt and nutrient. PGPR inoculations accelerate the induced systemic tolerance (IST) which are more closely related with the enhancement in tolerance level against various abiotic stresses (Yang et al. 2009). Besides, various metabolic pathways and gene-level activity are involved in the regulations of biotic and abiotic stress (Dimkpa et al. 2009).

Moreover, the environmental factors can also play very important role in the transition of pathogenic fungi to symbiotic microorganisms (Newton et al. 2010). The symbiotic relationship of legume-rhizobia releases more flavones and flavonols under nitrogen-deficient soil (Zhang et al. 2009). Likewise, the symbiotic relationships of mycorrhizae are equal, i.e., exchange of nutrients and photosynthates (Kiers et al. 2011). Kiers et al. (2011) reported that mycorrhizal partner receives more and

more carbon and in return equal amount of nutrients were supplied to the (*Medicago truncatula* Gaertn) host plant. The "fair trade" between host plant and mycorrhizae is beneficial to each partner (Fellbaum et al. 2012). Plant growth and yield improvement directly depend upon the secondary metabolites (quantity and quality), nitrogen fixation, uptake of phosphorus, iron sequestration, and plant growth enhancer molecules (Tortora et al. 2011). On the other hand, biocontrol activity shows some indirect action in the plant health improvement. There are several mechanisms which involve in the inhibition of the entry of plant pathogens such as antibiosis, food and space competition, site colonization, predation and parasitism, and induction of various types of resistance against the pathogens. The microorganisms are also implicated in the plant growth and development by using a wide range of strategies such as the release of growth factors, the enhanced availability and assimilation of the important nutrient, and pathogen suppression.

13.4 Endophytes

The endophytic microbes are defined as those microorganisms living in the plant tissue as endosymbiont with no intention to develop any apparent diseases in the host plant. Bulgarelli et al. (2013) defined endophytes as the set of microbial genomes/communities located inside plant tissues/organs. Endophytic fungi influence the plant health by improving the nutrient status of plant; subsequently the plant growth and developments are greatly enhanced and restrict the pathogen's entry (Yadav 2018). The microbial community outside the host plants greatly influences the plant roots. A large number of endophytes and microbial communities harbor in the root system of different crop plants (Hallman and Berg 2006).

13.4.1 Distribution of Endophytes

The endophytes residing in plants are divided into three categories: (1) obligate endophytes, such microbes are unable to survive outside the host plant; (2) facultative endophytes, free living in nature and start colonization of the roots when the need arises; and (3) passive endophytes, a result of certain determined event such as open wounds (Hardoim et al. 2008).

13.5 Conclusions

The plan-microbe interactions are a complex process in the rhizosphere. The studies have revealed that communication during the plant-microbe interactions are mediated through the root exudates. The root exudates may differ in terms of quantitative

and qualitative assay from plant to plant and species to species. The rhizospheric environments are also a determining factor, although the technology has been now enough to go for next-generation research pertaining to identification of species through sequencing technology at community levels under the different types of environment. The signaling molecules implicated in the plant-microbe interactions are very important molecules and need to be identified in the future research for the generation of good management module. The microbial factors influencing the host root exudation process is still not well understood, and it is high time to touch this area of research for the betterment of sustainable agriculture. The potential microbes/endophytes are to be identified, engineered, and manipulated for root exudation process which in turn improve the colonization ability of the endophytes to the host plant and eventually improve the plant health status.

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