Vivek Kumar · Manoj Kumar
Shivesh Sharma · Ram Prasad *Editors*

Probiotics and Plant Health

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Contents

About the Editors

Dr. Vivek Kumar is a scientist involved in teaching, research, and guidance, with a pledge to enduring knowledge. Dr. Kumar works at the Institute of Microbial Technology at Amity University Uttar Pradesh, Noida, India. He obtained his master's and doctoral degree from CCS Haryana Agricultural University, Hisar, Haryana, India. He serves on the editorial board of reputed international journals, viz., *EnvironmentAsia*, *International Journal of Biological and Chemical Sciences*, *Journal of Advanced Botany and Zoology*, and *Journal of Ecobiotechnology*. He is also reviewer of *Journal of Hazardous Materials*, *Science International*, *Acta Physiologiae Plantarum*, *International Research Journal of Plant Sciences*, *International Journal of Microbiology*, *African Journal of Microbiology Research*, *Journal of Microbiology and Antimicrobials*, *Environmental Science and Pollution Research*, and *Rhizosphere*. He has published 61 research papers, 19 book chapters, 6 review articles, and 2 books. Dr. Kumar has also served as microbiologist for 8 years in the Department of Soil and Water Research, Public Authority of Agricultural Affairs & Fish Resources, Kuwait. Dr. Kumar has organized a number of conferences/workshops as convener/organizing secretary.

Dr. Kumar's research areas are plant-microbe interactions, environmental microbiology, and bioremediation. He has been credited with first time reporting and identification of pink rot inflorescence disease of date palm in Kuwait caused by *Serratia marcescens.* He received the "Young Scientist Award" for the year 2002 in "agricultural microbiology" by the Association of Microbiologists of India (AMI).

Dr. Kumar is establishing an "unearthing and deliverance system," where a balance is being strived between development of drought- and salinity-resistant microbiome for better crop production in rain-fed and saline areas. His teaching interests include general microbiology, environmental microbiology/biotechnology, dairy microbiology, and bacteriology.

Dr. Manoj Kumar is a scientist with sanguine behavior who is devoted to research and development, with a commitment to lifelong learning. He is engaged in high-quality science that contributes broadly to both increasing intellectual knowledge of plant development and the ecological niche. He has a high level of professional desire and intellectual hunt and the potential to fulfill the dream of his high-impact publications and the future recognition of these by academic peers.

Dr. Kumar has pursued his Ph.D. in plant biotechnology from the prestigious Jawaharlal Nehru University and then was awarded two postdoctoral fellowships consecutively DBT-PDF from IISc Bangalore in 2005 and then NRF-PDF from the University of Pretoria.

Dr. Manoj Kumar is a researcher of plant biotechnology in the Division of Microbial Technology at Amity University Uttar Pradesh, India. Until recently, he was a coordinator of the Bio-resource Chapter (Northern India) and served on editorial boards of five international journals. He referees for many more, including the *International Journal of Phytoremediation*, *Journal of Soil Sediments*, and many more. The ultimate functional aim is to adapt crop plants in order to increase productivity and adaptability on such Indian soils, with consequent improvement of sustainability in both developed and developing countries. The ultimate intellectual aim is to understand the metabolic fate of microbial-mediated precursors in whole plant physiology and genetics through processes occurring at the level of metabolism, particularly through rhizosphere communication under in situ and in vitro plants. This aim is being addressed by combining functional genetics and metagenomics approaches with a broad-based understanding of plant-microbe healthy interaction.

Dr. Kumar's research is the integration of microbial genetics with a breadth of plant physiological approaches to enable novel gene discovery and conferring metabolites and the development and use of process parameters for the study and manipulation of specific rare microbial types for suboptimal soil conditions and even in the broader areas of plant cultivation and characterization of its active biomolecules for industry uses.

Dr. Kumar is establishing a "discovery and delivery pipeline," where a balance is being strived between fundamental researches into molecular mechanisms of heavy metal resistance bacterial types on the delivery of those discoveries through the development of susceptible crops with improved abiotic (heavy metals—cobalt, zinc, cadmium) tolerance. In the heavy metal resistance gene research program, metagenomics and functional genomics approaches are being used to discover heavy metal resistance determinant on the transposon that affect traits that are likely to contribute to heavy metal tolerance. These genes, i.e., czc (cobalt, zinc, and cadmium resistance), and others discovered previously by other researchers, are being characterized to elucidate mechanisms of action and natural variation within the target crops/model testing crop (*Arabidopsis thaliana*), to a level necessary to facilitate resistancy. The research focuses on the alteration of genes likely to alter heavy metal tolerance in crop plants and the testing of the effects of these alterations on overall biomass in the field. Work in definition can occur in parallel, as knowledge of some metal resistance-conferring genes is primitive at molecular level, whereas discovery of fundamental processes is still required for such genes and traits.

Dr. Shivesh Sharma is associate professor and head at the Department of Biotechnology at Motilal Nehru National Institute of Technology (MNNIT), Allahabad, Uttar Pradesh, India. Dr. Shivesh Sharma completed his Ph.D. in the field of microbiology from Dr. RML Avadh University, Faizabad, Uttar Pradesh, India, in the year 2001. He obtained his M.Sc. in microbiology from Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya (CSKHPKV), Palampur, Himachal Pradesh, India. His research interests include environmental microbiology/biotechnology, plant-microbe interaction, and bioformulations. Before joining MNNIT, Allahabad, in the year 2009, Dr. Shivesh Sharma has worked at S.B.S (PG) Institute of Biomedical Sciences and Research, Balawala, Dehradun Uttarakhand, during 2002–2009. He has been involved in a number of research projects funded both externally (DBT, UGC, DST, MHRD) and internally in the fields of his research interests. His teaching interests include microbiology, environmental microbiology/biotechnology, food biotechnology, bacteriology, and IPR.

He has successfully supervised 7 Ph.D., 8 M.Tech., and 28 M.Sc. students. He has 72 publications in research journals and 7 book chapters to his name. He has edited/authored a number of books. As convener/coordinator, Dr. Shivesh Sharma has organized a number of conferences and short-term training programs.

Dr. Ram Prasad is assistant professor at the Amity Institute of Microbial Technology, Amity University, Uttar Pradesh, India. Dr. Prasad has completed his Ph.D. from the Department of Microbiology, Chaudhary Charan Singh University, Meerut, UP, India, in collaboration with the School of Life Sciences, Jawaharlal Nehru University (JNU), New Delhi, India. Dr. Prasad received his M.Sc. in life sciences at JNU and also qualified at CSIR-NET, ASRB-NET, and GATE. His research interest includes plant-microbe interactions, sustainable agriculture, and microbial nanobiotechnology. Dr. Prasad has 93 publications to his credit, including research papers and book chapters and five patents issued or pending, and edited or authored several books. Dr. Prasad has 11 years of teaching experience, and he has been awarded the Young Scientist Award (2007) and Prof. J.S. Datta Munshi Gold Medal (2009) by the International Society for Ecological Communications; FSAB fellowship (2010) by the Society for Applied Biotechnology; Outstanding Scientist Award (2015) in the field of microbiology by Venus International Foundation; and the American Cancer Society UICC International Fellowships for Beginning Investigators (USA, 2014). In 2014–2015, Dr. Prasad served as visiting assistant professor in the Department of Mechanical Engineering at Johns Hopkins University, USA.

Management of Tomato Foot and Root Rot (TFRR) by Biocontrol Agents with Emphasis on Factors Affecting Its Effectiveness

1

Bouiz garne Brahim and Y. Ouhdouch

Abstract

Management of tomato foot and root rot (TFRR) caused by *Fusarium oxysporum* f. sp. *radicis-lycopersici* (FORL) could be achieved by biological methods which represent an ecologically friendly strategy for the sustainable crop productivity. Among these biological methods, those using biocontrol agents (BCAs) such as bacteria or fungi able to antagonize soilborne plant pathogens or stimulate plant defenses, leading to plant protection against diseases, are of great promise (de Waard et al. Annu Rev Phytopathol 31:403–421, 1993; El-Tarabily et al. New Phytol 137:495–507, 1997; El-Tarabily. Can J Bot 84:211–222, 2006). They also represent a suitable alternative to the use of chemical pesticides. Some of these antagonistic microorganisms living in association with tomato roots showing also beneficial effects on the plant growth and nutrition are called plant growth-promoting rhizobacteria (PGPR). Consequently, PGPRs could also be used as biofertilizers and are considered as an alternative tool to chemical fertilizers. In tomato, many rhizobacteria were reported to suppress diseases caused by *Fusarium* and/or to lead to growth promotion and tomato yield enhancement. However, BCAs are confronted to ecological parameters that are important to be determined if one wishes to succeed in disease management. The present chapter describes tomato foot and root rot (TFRR) and main mechanisms deployed by BCAs used to suppress the disease (competition by siderophore production, antibiosis, or induced systemic resistance). As the success of biocontrol methods depends largely on biotic and/or abiotic factors, some abiotic factors influencing

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the biocontrol agent's fitness as well as biotic factors represented by BCA interactions with either tomato plants or FORL are discussed in relation to the performance of BCAs either in greenhouse trials and agricultural fields.

1.1 Introduction

Tomato (*Solanum lycopersicum* formerly, *Lycopersicon esculentum* Mill.) is one of the most popular important commercial vegetable crops grown throughout the world. Many soilborne diseases can affect tomato plants in both greenhouse and field-grown tomatoes. Fungi such as *Fusarium oxysporum* f. sp. *lycopersici* (FOL), *F. oxysporum* f. sp. *radicis-lycopersici* (FORL), *Rhizoctonia solani*, *Verticillium albo-atrum*, and *Verticillium dahliae* are among the most destructive phyto pathogens in tomato production areas. *Fusarium* species are responsible of many soilborne plant diseases. *F. oxysporum* includes over 120 different formae speciales. Strains belonging to *F. oxysporum* produce asexual spores called microconidia and macroconidia (Sharma and Nowak 1998). They also produce resting spores called chlamydospores which enable the fungus to survive in soil and plant materials for years.

Each of *F. oxysporum* formae speciales is specific toward a host species. However, *F. oxysporum* f. sp. *radicis-lycopersici* (FORL) causes disease in hosts from several plant families, including tomato (Menzies et al. 1990) for which it is the causal agent of tomato foot and root rot (TFRR) disease, while *F. oxysporum* f. sp. *lycopersici* (FOL) causes vascular wilt disease only in tomato (Rowe 1980). At first, *Fusarium oxysporum* f. sp. *radicis-lycopersici* was identified as a new race (J3) of *F. oxysporum* f. sp. *lycopersici* (Sato and Araki 1974). Later, Jarvis and Shoemaker (1978) pointed out that the pathogen was a new forma specialis of *F. oxysporum*. Morphologically, the two formae speciales are indistinguishable. Attitalla et al. (2004) proposed a molecular method based on mitochondrial DNA (mtDNA) RFLP analysis that enables a rapid differentiation of isolates belonging to the two special forms.

Fusarium oxysporum f. sp. *radicis-lycopersici* (FORL) (Jarvis and Shoemaker 1978) is a devastating fungal soilborne pathogen of tomato crops (Jones et al. 1991; McGovern and Datnoff 1992; Ozbay and Newman 2004). Tomato foot and root rot (TFRR) disease symptoms differ from those of *Fusarium* vascular wilt caused by FOL for which wilted tomato plants show leaf yellowing and wilting that progress upward from the base of the stem. While FORL-infected plants show dry brown lesions in the cortex of roots and often on the surface of the stem at 10–30 cm above the soil line, TFRR symptoms are rather expressed as root and basal stalk rot distinctly different from those of vascular wilt. Other characteristics could distinguish between the two diseases. TFRR occurs at cool (18 °C) soil temperatures (Jarvis and Thorpe 1976), while vascular wilt is most severe at higher soil temperatures (27 °C). In addition, the host range of FORL is larger than FOL (Rowe 1980). Regarding interactions with tomato, tomatinase is also synthesized by FORL (Ito et al. 2004).

1.2 Methods for the Control of TFRR Disease

1.2.1 Use of Resistant Varieties

However, this alternative faces usually many obstacles. One of the most constraints is the breakdown of resistance of high pathogenic disease agents. Thus, resistant cultivars are generally of limited efficiency as they could be grown only for few years.

1.2.2 Prophylaxis

This includes using plant disease-free seeds, transplanting in hot soil (+20 °C), and avoiding to use cool water as cool soil temperatures favor the disease and crop rotation; avoid plants that hold the pathogen such as pepper and eggplant, rotation with lettuce, and use of lettuce residues (Jarvis and Thorpe 1981). However, this strategy has limited effects since the fungi can survive in soil for several years (Ozbay et al. 2001).

1.2.3 Chemical Control

One of the most common strategies to control plant diseases is the use of chemical pesticides. Chemical control including the use of benomyl and captafol (Marois and Mitchell 1981) was reported, and experiments using captafol showed that it succeeded to control the TFRR when it was applied to freshly steamed soil before planting (Rowe and Farley 1978). However, many other pesticides had deleterious effects toward tomato plants, and most of these chemicals are ineffective or showed variable or limited protection effects (Jacobsen and Backman 1993). For example, methyl bromide (MB) does not completely control the fungus. Also, it was reported to cause chlorosis and has the inconvenience of inducing resistance of the pathogen. In addition, most chemical pesticides usually have detrimental effects on the environment. They create imbalances in the microbial communities and are responsible of soil and groundwater pollution. They are also responsible of the accumulation of toxic compounds potentially hazardous to humans. For example, the chemical fungicide methyl bromide (MB) is considered as an ozone-depleting substance (Bell et al. 1996; Duniway et al. 2000), and it was recommended to be removed from use by the Montreal Protocol (Hayes 1994). Since 2005, it was outlawed and banished in several countries.

1.2.4 Biocontrol Methods

Biocontrol by the use of biopesticides and particularly microbial agents could represent a good alternative strategy. This strategy fits well in the worldwide trend to produce vegetable crops in a way to guarantee a healthier human nutrition and a sustainable agriculture (Prasad and Rangeshwaran 2000). However,

one of the major constraints of developing biocontrol agents at field scale is the difficulty to obtain efficient activities as usually observed in vitro experiments. Consequently, microbial-based biocontrol products represent a small portion of the total marketed pesticides. Another fact is that chemical compounds, for which methods of use are easy and well standardized, are still preferable tools though they have multiple negative effects (de Waard et al. 1993). However, its currently thought that microbial-based biocontrol products will be improved in oncoming years thanks to the growing public and government's awareness of these negative effects (Komada 1994).

How microbial biocontrol agents (BCAs), particularly bacteria and rhizobacteria, could suppress diseases caused by *F. oxysporum* f. sp. *radicis-lycopersici* (FORL), what are the mechanisms involved in BCAs-tomato and BCAs-FORL interactions, and how biotic and abiotic factors could affect the efficiency of disease suppression by BCAs are the main questions raised in this chapter.

1.3 Biocontrol by Direct Antagonism

Generally, soil beneficial microorganisms could act either by their abilities to promote plant growth or by protecting plants against phytopathogens. Plant growth-promoting rhizobacteria (PGPR) generally refer to a group of soil and rhizosphere free-living bacteria colonizing roots in a competitive environment and exerting a beneficial effect on plant growth (Kloepper 2003; Bakker et al. 2007; Bouizgarne 2013). Beneficial effects of plant growth-promoting rhizobacteria (PGPR) involve biofertilizing abilities leading to enhanced crop yields through an efficient nutrient uptake and plant growth regulator synthesis. Many bacteria could display those beneficial growth effects on tomato crops (van Peer and Schippers 1989; Gagné et al. 1993; Garcia et al. 2003; Mayak et al. 2004a, b; Gravel et al. 2007; El-Tarabily 2008). Bacterization with selected strains also promoted the tomato growth in harsh environments including drought (Mayak et al. 2004a) and salt stress (Mayak et al. 2004b). Tomato growth and fruit yield enhancement in greenhouse could be due to the ability of PGPRs to produce the phytohormone indole-3-acetic acid (IAA) (Gravel et al. 2007) and 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, which interferes with ethylene biosynthesis, leading to stress reduction (Glick 2006, El-Tarabily et al. 1997) or improving tomato phosphorus nutrition (Khan and Khan 2001). Besides promoting plant growth, PGPRs could also act as BCAs (biopesticides abilities). Indeed, most root-colonizing bacteria could affect the growth of phytopathogens through nutrient or niche competition, production of antibiotics and/or siderophores, or to their ability to trigger induced local or systemic resistance (Larkin and Fravel 2002; Kamilova et al. 2005; Glick et al. 2007; van Loon 2007). Bacteria and nonpathogenic fungi proved their usefulness as biocontrol agents and growth promoters. Consequently, interest in those microorganisms has increased in recent years in a way to make available efficient strains as both biofertilizers and biopesticides. Biocontrol experiments of TFRR in both greenhouse and field level include the use of BCAs belonging to the following groups of fungi and bacteria:

1.3.1 Fungal BCAs

These include *Trichoderma harzianum* (Datnoff et al. 1995; Sivan et al. 1987; Bourbos et al. 1997; Ozbay et al. 2001; Hibar et al. 2005) or nonpathogenic *F. oxysporum* (Louter and Edgington 1990; Horinouchi et al. 2007, 2008) (Table 1.1). Other works used a combination of *T. harzianum* and methyl bromide or soil solarization (Sivan and Chet 1993).

Table 1.1 Selected examples of mechanisms displayed by microbial biocontrol agents against FORL

BC BCAs	Genus/species	Major mechanism involved in the biocontrol	Reference
Fungi	Nonpathogenic *Fusarium*		Louter and Edgington (1990), Horinouchi et al. (2007), and Horinouchi et al. (2008)
	Trichoderma harzianum	Myco-parasitism	Hibar et al. (2005)
	Trichoderma koningiopsis	Induced resistance	Moreno et al. (2009)
	T. harzianum and *Glomus intraradices*		Datnoff et al. (1995)
Bacteria	*Pseudomonas fluorescens* Q2-87	Antibiotic: 2,4-diacetylphloroglucinol	Duffy et al. (2004)
	Pseudomonas fluorescens PCL1751	Competition for nutrients and niches	Kamilova et al. (2005)
	Pseudomonas fluorescens 63-28	Induced resistance	Piga et al. (1997).
	Pseudomonas chlororaphis PCL1391	Antibiotic: phenazine-1-carboxamid	Chin-A-Woeng et al. (1998)
	Pseudomonas putida PCL1760	Competition for nutrients and niches	Validov et al. (2009)
	Pseudomonas putida PCL1758, 1759, 1760 *Pseudomonas chlororaphis* PCL1757 *Pseudomonas rhodesiae* PCL1761 *Paenibacillus amylolyticus* PCL1756 *Delftia tsuruhatensis* PCL1755	Authors suggest the involvement of competition for nutrients and niches	Validov et al. (2007)
	Bacillus subtilis EU07	Induced resistance	Baysal et al. (2008)
	Bacillus subtilis NB22	Antifungal compound	Phae et al. (1992)
	Collimonas fungivorans	Competition for nutrients and niches	Kamilova et al. (2007)

1.3.2 Bacterial BCAs

Several bacteria were used in the biocontrol of TFRR. Strains belonging to *Pseudomonas fluorescens* and *Pseudomonas chlororaphis* (Duffy and Defago 1997; Chin-A-Woeng et al. 2000; Lagopodi et al. 2002; Bolwerk et al. 2003; Kamilova et al. 2005, 2008; Postma et al. 2013), *Pseudomonas putida* (Lee et al. 2005; Validov et al. 2007), and *Bacillus subtilis* (Phae et al. 1992; Baysal et al. 2008) were shown to suppress the disease to various extents, and some experiments used pseudomonads either as seed coating or treatment of tomato seedlings (Chin-A-Woeng et al. 1998; Dekkers et al. 2000). Although *Actinobacteria* are generally effective against *Fusarium* plant diseases (Gopalakrishnan et al. 2011), *Actinobacteria*-based formulation of strain K61 of *Streptomyces griseoviridis* (Mycostop®) was not totally effective against TFRR (Lahdenperä 2000; Minuto et al. 2006).

BCAs could act as competitors to FORL (Table 1.1). Competition is often due to the production of siderophores which are iron-chelating substances that allow PGPRs to compete for iron and consequently to impair the growth of soilborne phytopathogens (Duijff et al. 1994). They represent a biochemically diverse group produced by plants or plant-associated microorganisms (Loper and Buyer 1991). However, they could act by sequestering iron only under iron-limited conditions. Plant rhizosphere ecosystem where low amounts of iron are available can provide such conditions and is considered for this reason a preferable target for BCA applications. Siderophores from fluorescent pseudomonads have been reported to be implicated in iron uptake by tomato plants (Duss et al. 1986) and pyoverdine as the major class of siderophores produced by strains of fluorescent pseudomonad. In addition, competitive BCAs should be rhizosphere competent (with great root-colonizing abilities) (Validov et al. 2007; Lugtenberg and Bloemberg 2004). Making as starting point the principle that candidate strains for biocontrol should be isolated from either rhizospherical soil or plants of the intended crop, Kamilova et al. (2005) and Validov et al. (2007) used an enrichment method to obtain enhanced root-colonizing *Pseudomonas* from the rhizosphere of tomato. Those bacteria able to competitively colonize tomato root tip succeeded to control TFRR by competition. These findings highlight the interest of searching BCAs well adapted to the pathosystem from which they are isolated. Also, antibiosis was shown to be of a major role in TFRR suppressiveness (Table 1.1) as demonstrated by Chin-A-Woeng et al. (1998) who reported production of phenazine-1-carboxamide by *Pseudomonas chlororaphis* PCL1391. The effect of phenazine-producing *P. chlororaphis* on FORL was monitored by Bolwerk et al. (2003) by using laser confocal microscopy. During the first 3 days of interaction between the two microorganisms, no effect of *P. chlororaphis* on the growth and hyphal attachment was registered. However, after 7 days, the hyphal network of FORL was considerably reduced. Moreover, hypha failed to penetrate tomato roots in the vicinity of *P. chlororaphis* (Bolwerk et al. 2003). In addition, *Pseudomonas* was reported to be able not only to colonize the tomato roots (Bloemberg 2007) but also to colonize fungal hyphae and cause stress effects: increase of the diameter of hyphae, curly growth of hyphae, abrupt changes in the growth direction of hyphae, and branching of hyphae that resembles forklike structures (Bolwerk et al. 2003) (Fig. 1.1). This very interesting study raises the interest of studying the interactions between BCAs and FORL in the rhizosphere system.

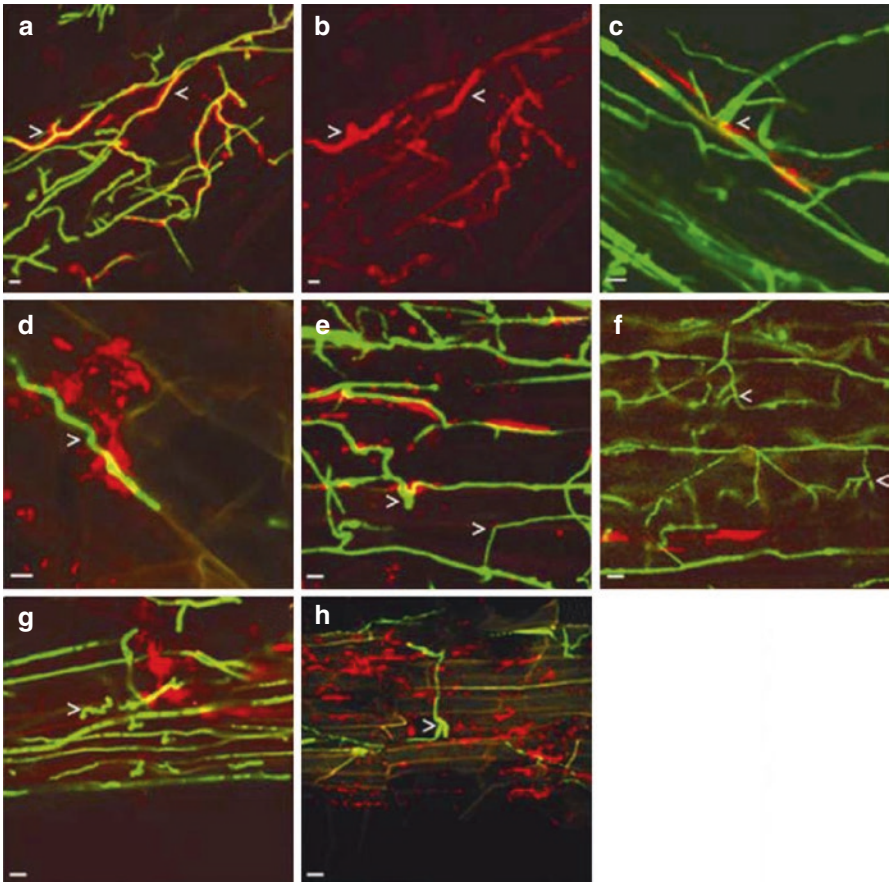

Fig. 1.1 Confocal laser scanning microscope analysis of effects of the presence of *Pseudomonas chlororaphis* PCL1391 and PCL1119 cells on growth of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in the tomato rhizosphere. Two-day-old tomato seedlings were inoculated at time zero with *P. chlororaphis* PCL1391 cells harboring a reporter plasmid expressing the *rfp* gene, which here appear as red cells. Plants were grown in a gnotobiotic sand system containing spores of *F. oxysporum* f. sp. *radicis-lycopersici* harboring a constitutively expressed *gfp* gene. Cell walls of the tomato root appear as red due to autofluorescence. (**a**) *P. chlororaphis* PCL1391 cells concentrating around the hyphae and colonizing *F. oxysporum* f. sp. *radicis-lycopersici* hyphae 10 days after inoculation. (**b**) Same picture as (**a**) without the green fluorescent protein signal showing that all bacterial cells are attached to the fungal hyphae. (**c**) In the presence of strain PCL1391, an increase of the diameter of hyphae (indicated by *arrowheads*) was observed after 7 days. (**d**) Curly growth of hyphae along the cellular junction of the tomato root was observed in close vicinity of PCL1391 cells, 9 days after planting. (**e**) In the presence of strain PCL1391, abrupt changes in the growth direction of hyphae (indicated by *arrowheads*) were observed after 10 days. (**f**) Branching of *F. oxysporum* f. sp. *radicis-lycopersici* hyphae resembles forklike structures (indicated by *arrowheads*) in the presence of strain PCL1391 13 days after inoculation. (**g**) Hyphal growth in the presence of strain PCL1119 in the rhizosphere. (**h**) Branching of *F. oxysporum* f. sp. *radicis-lycopersici* hyphae resembles forklike structures at lower frequency in the presence of strain PCL1119 13 days after inoculation. The *size bar* represents 10 μm in all panels (from Bolwerk et al. 2003)

1.4 Biocontrol of TFRR by Induced Resistance in Tomato

Plant resistance is the ability to exclude or overcome, completely or in some degree, the effect of a pathogen or other damaging factors (Agrios 2005). An example of resistance of tomato to FORL is cell wall reinforcement due to increased phenolic-, lignin-, and suberin-like constituents in the thickened cortical cell walls that were observed by Brammall and Higgins (1988). Besides resistance to pathogens, some rhizobacteria and plant growth-promoting rhizobacteria (PGPR) could indirectly confer protection to plants against pathogen ingress by activating plant defense mechanisms. Such mode of resistance termed "induced systemic resistance" (ISR) is a situation in which the plant's innate defenses stimulated by rhizobacteria confer systemic protection against further pathogen infections (Van Loon et al. 1998; Van Loon 2007). Upon infection with a pathogen, previously treated plants with rhizobacteria show enhanced defensive capacity manifested mainly as a reduction in the rate of disease development. The induced resistance leads generally to a lower number of diseased plants or in lesser disease severity in comparison with untreated plants. This kind of plant defense resulting from inoculation by nonpathogenic microorganisms ISR (Kloepper et al. 1992), first described in 1991 by Van Peer et al. (1991), is mainly characterized by indirect effects of the inducer on the pathogen and by no direct antagonistic effects via antibiotic metabolites (Van Loon et al. 1998). In opposition to antagonism by antibiosis or siderophore production where the population size should be maintained during the biocontrol process, it is sufficient for ISR that the plant and the inducing agent to be in contact for a limited period. Indeed, once induced, ISR is expressed systemically throughout the plant and maintained for prolonged periods (Van Loon et al. 1998). ISR is also characterized by its nonspecificity of protection. Indeed, several rhizobacteria could protect plants against different pathogens, belonging to both root-infecting and leaf-infecting microorganisms (Van Loon et al. 1998).

Rhizobacteria-mediated ISR resembles phenotypically to the classic systemic acquired resistance (SAR) generally induced by the necrotizing pathogens, in which a previously infected plant shows resistance to further infection in its noninfected parts (Ross 1961). To determine whether a microorganism-mediated ISR is involved in plant protection, the inducing rhizobacteria and the challenging pathogens must spatially separate thus excluding any eventual direct antagonism (Van Loon et al. 1998; Van Loon 2007). In the case of root pathogens, ISR is difficult to demonstrate as both the inducer and the pathogen live in the vicinity of the roots. Thus, the indirect effect of the rhizobacterium can be inferred to ISR only when the rhizobacterium has no in vitro activity against the pathogen and produces specific eliciting components able to induce resistance in the plant (Van Loon et al. 1998; Van Loon 2007; Park et al. 2001). The ISR induction in roots was confirmed by works such as those using a separate inoculation system where the lower part of a root is inoculated by a bacterial suspension and the upper part received the challenging pathogen few days after. Another separation method named split-root method (Liu et al. 1995; Ongena et al. 2000; Chen et al. 2000) was also used to establish the ISR nature of root resistance inducing rhizobacteria. Using this method where one part of the root

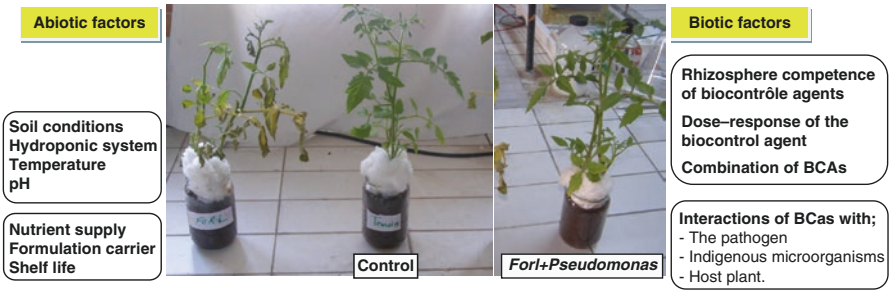

Fig. 1.2 Schematic illustration of main parameters affecting the biocontrol of tomato wilt and TFRR diseases by microorganisms

system of tomato was treated with *P. fluorescens* WCS365 and the other part was challenged with FORL, one week after *P. fluorescens treatment*, Kamilova et al. (2005) obtained a reduction in disease incidence by up to 61%.

Various determinants are involved in ISR phenomenon including bacterial constituents. To induce ISR against FORL, chitosan-based *Bacillus pumilus* was used, and significant effects in tomato were obtained (Benhamou et al. 1998). Under iron-limited conditions, siderophores produced by biocontrol agents could be involved in the antagonism against soilborne pathogens by sequestering iron but also as ISR inducers. Siderophores such as pyochelin (a salicylic substitute cysteinyl peptide) are likely to be the inducing factor produced by *Pseudomonas* in tomato against *Botrytis cinerea* and *Pythium* (Buysens et al. 1996; Audenaert et al. 2002; Meziane et al. 2005). To our knowledge, the role of pyochelin as ISR inducer against FORL is not yet demonstrated in tomato. In addition to pyochelin, *Pseudomonas aeruginosa* 7NSK2 produces also the antibiotic pyocyanin. The two molecules act synergistically to produce active oxygen species that cause cell damage leading to induced resistance (Audenaert et al. 2002). Another mechanism displayed by *Pseudomonas* consists in activating tomato resistance to TFRR by inducing tomato cell wall structural changes (including callose synthesis) that were reported by Piga et al. (1997) (Table 1.1).

1.5 Main Parameters Affecting the Biocontrol of TFRR Diseases by Microorganisms

The success of biocontrol treatments by microorganisms is variable particularly at field scale where it is subjected to various interactions involving biotic and/or abiotic conditions (Fig. 1.2).

1.5.1 Physicochemical Conditions

Highly varying field conditions influence the effectiveness of biological control in comparison with greenhouse and in vitro trials where experimental conditions are

more controlled (Paulitz and Bélanger 2001; van Rij et al. 2004). Varying physico-chemical conditions could affect physiological traits of biocontrol agents. Chin-A-Woeng et al. (1998) observed that at pH less than 5.7, no in vitro antifungal activity of phenazine-1-carboxylic acid (PCA) produced by *Pseudomonas chlororaphis* PCL1391was observed. In addition, availability of nutrients could also play a major role in the success of biocontrol by microorganisms. Soil amendment by Zn improves the biocontrol of TFRR by *P. fluorescens* CHA0 (Duffy and Defago 1997). It is also well known that different carbon and nitrogen sources affect the germination of propagules of *F. oxysporum* (Sneh et al. 1984). Addition of compost to soil increased the population of PGPR in the tomato rhizosphere exhibiting antagonism toward various fungi including FORL (De brito Alvarez et al. 1995). In experiments to control four phytopathogens including FOL and FORL, the effect of biological control by Mycostop® applied separately or in combination with solarization was more pronounced 2 months after transplanting but was less evident at the end of the experiment (6 months after transplanting) suggesting the need for information related to the microorganism's ability to survive in a natural soil (Minuto et al. 2006). In addition, while Mycostop® is ineffective in disinfected soil (Minuto et al. 2006), it showed a satisfactory effect in hydroponic systems suggesting that the effect of the agents could be affected by the type of the system used for the cultivation (Khalil and Alsanius 2010). Indeed, in hydroponic system, the relatively small number of conidia is attached to the root surface compared to colonization along the root system growing in soil system (Turlier et al. 1994).

1.5.2 Rhizosphere Competence of Biocontrol Agents

One of the most important traits involved in the ability of microorganisms to combat phytopathogens is their ability to inhabit the vicinity of roots (the rhizosphere). This is a process by which a microorganism, applied as seed inoculants, colonizes the rhizosphere of developing roots (Baker 1991). Root exudates were found to have influence on the growth and antifungal activity of microbes (Kravchenko et al. 2003) and their rhizosphere competence (Loper and Schroth 1986; Goddard et al. 2001). Major soluble components of tomato root exudates include sugars, organic acids, and amino acids (Lugtenberg and Bloemberg 2004). Spatiotemporal dynamics of bacterial colonization of tomato roots were monitored either indirectly by cultivation of isolated bacteria from the rhizosphere or directly by using microscopy (Chin-A-Woeng et al. 1997; Gamalero et al. 2004, 2005; Bloemberg et al. 2000; Bolwerk et al. 2003) and immunofluorescence technics (Gamalero et al. 2005). Monitoring tomato root colonization by a *P. fluorescens* strain showed that the bacterial population was more constant in the hairy zone, the old hairy zone, and the collar zone than in the apex and the elongation zone. This is probably due to differences in exudate compositions and concentrations along the root (Gamalero et al. 2004). Similar studies using epifluorescence and confocal laser scanning microscopy (CLSM) with autofluorescent proteins showed that antagonistic bacteria to FORL could also colonize fungal hyphae (Bolwerk and Lugtenberg 2005).

In tomato roots, a correlation was found between root colonization by *Pseudomonas* strains and their biocontrol abilities. A method to enrich for biocontrol strains able to compete with FORL was used as an approach to screen for the best tomato root colonizer by Kamilova et al. (2005). In this method, a crude rhizobacterial mixture is applied on a seedling. After plant growth in a gnotobiotic system, bacteria that have reached the root tip are isolated and are subsequently inoculated to new seedlings which were made to grow. The enrichment cycle was repeated three times and allowed to isolate competitive root tip colonizers. Efficient colonization is important as a population threshold of the producing bacteria in the rhizosphere is required to limit the development of the pathogens (Lemanceau et al. 1992). Indeed, it was reported that phenazine-producing strains with weak colonization abilities are ineffective in disease suppression (Chin-A-Woeng et al. 2000, 2003). This enrichment method for competitive colonizers yielded a low percentage of antagonists (Kamilova et al. 2005). However, it has the advantage to provide isolates with competition for nutrient and niches' abilities although with no obvious activities in plate assays (Validov et al. 2007).

The ability to attach to plant root cells was found to be one of the earliest steps involved in the effectiveness in root colonization. *Pili* of the rhizobacteria could be involved in efficient attachment to tomato roots (Camacho 2001). Other constituents could also be involved as proved by the use of mutants (Chin-A-Woeng et al. 2000, 2003). Mutant strains from *Pseudomonas fluorescens* WCS365 lacking in the synthesis of the O-antigenic side chain of the outer membrane of bacterial lipopolysaccharides (LPS) were shown to be impaired in rhizosphere competence (Dekkers et al. 1998). Also, while phenazine-1-carboxamide *P. chlororaphis* PCL1391 is efficient to control TFRR (Chin-A-Woeng et al. 1998), mutant strains of PCL1391 lacking motility, phenylalanine biosynthesis, and a functional site-specific recombinase sss/xerC gene were shown to be impaired in root colonization (Chin-A-Woeng et al. 2000). These mutants also completely lost their ability to suppress TFRR in a gnotobiotic sand system and in potting soil even though they showed normal antifungal activity in vitro as they could produce various antagonistic molecules including chitinase, hydrogen cyanide, and phenazine-1-carboxamide. In a gnotobiotic system and unsterilized potting soil, the mutant bacterium failed to colonize tomato root tips compared to the wild type.

1.5.3 Mutual Interactions Between Soil Microorganisms

Many biotic factors including interactions between the biocontrol agent, tomato plants, and the soilborne pathogens could render the effectiveness of biocontrol by microbial antagonists of limited impact. Dose-response of the biocontrol agent and its survival and activity (or activities) in soil depend largely on various parameters including nature of soil, degree of competition with the pathogen, and/or other microorganisms and interaction with plant material (e.g., stage at which the plants are inoculated). Lemanceau et al. (1995) demonstrated that the populations of fluorescent pseudomonads isolated from uncultivated soils were different from those isolated from the

rhizosphere and tomato root tissue indicating that plant has a selective influence on fluorescent pseudomonad population. Interactions of the antagonist with the pathogens in one hand and of the antagonist with indigenous soil microorganisms in another hand are also of great importance for the efficiency of biocontrol. Since efficient root colonization (rhizosphere competence) is a prerequisite for efficient biocontrol by a BCA, interactions that could render a bacterium to be more rhizosphere competent could be crucial for efficient biocontrol activity. An example of these positive interactions is illustrated by genetic transfer from a rhizosphere-competent *P. fluorescens* to a rhizosphere-incompetent *Pseudomonas* strain which resulted in enhanced ability to colonize root tips (Dekkers et al. 2000; Olivain et al. 2006).

Self-defense mechanisms of the pathogen FORL against antagonistic agents were also reported by Duffy and Defago (1997) as involved in mutual interactions. According to these investigators, fusaric acid could interfere with antibiotic production by fluorescent *Pseudomonas*, and fusaric acid-producing FORL was reported to be able to repress the expression of 2,4-diacetylphloroglucinol (DAPG) genes of *P. fluorescens* CHA0 in a hydroponic tomato production system, thus making the antagonist ineffective in controlling TFRR (Duffy and Defago 1997). Hydrogen cyanide (HCN), which was not repressed by fusaric acid, played a major role in TFRR suppression in *P. fluorescens* strain CHA0. Those results demonstrated the importance of the degree of sensitivity to pathogen self-defense with respect to disease suppression (Duffy et al. 2004). As fusaric acid is a nonspecific toxin produced by most *Fusarium* species including nonpathogenic *F. oxysporum*, a greater negative effect is expected to reign in the rhizosphere (Rowe et al. 1977). The screening for strains able to synthesize the antibiotic 2,4-DAPG in the presence of fusaric acid could be a solution to overcome this pathogen defense (Duffy and Defago 1997). A relevant example is mentioned above where improved ability to control TFRR is shown by *P. fluorescens* CHA0 following Zn addition. Indeed, in this example, control relies on increased DAPG production by the bacterium due to a negative action of Zn on fusaric acid production by the pathogen.

1.5.4 Combination of Two or More Biocontrol Agents

For the biocontrol of *Fusarium* diseases, several studies aimed to use combination of two or more BCAs with other control strategies. A biocontrol agent or bacterial consortia applied either separately or combined to other methods such as solarization, use of resistant cultivars, crop rotation, or even chemical pesticides (on condition that they are used in lower amounts than in conventional control) can be a promising tool in an integrated control point of view. Such combinations are to be encouraged for controlling tomato *Fusarium* diseases. Combinations of two or more antagonistic microorganisms to a phytopathogen may provide improved disease control over the use of single organisms. Indeed, bacterial strains with different disease-suppressive mechanisms could minimize the impact of field fluctuating conditions as some biocontrol mechanism could be effective even if others are unfunctional. It also has the advantage of providing protection against multiple

pathogens. Due to multiple antagonistic mechanisms, failure probabilities in biocontrol are lowered. Combinations offer in addition a good versatility toward environmental condition changes. Bacteria belonging to the genera *Pseudomonas*, *Bacillus*, and *Streptomyces* are of great interest regarding their great adaptation to soil conditions, and nonpathogenic isolates of FORL are in some cases of more interest as they are closely related to the pathogen species and have similar nutrition requirements and responses to varying environmental conditions. For TFRR control, it was demonstrated that mixed inoculation of *P. fluorescens* strain WCS365 and *P. chlororaphis* strain PCL1391 tended to improve biocontrol of TFRR in comparison with single inoculations. Also, Marois and Mitchell (1981) used fumigation combined to *Trichoderma harzianum*, *Aspergillus ochraceus*, and *Penicillium funiculosum*.

However, consortia where some biocontrol agents could display negative interactions with others could be useless. Similarly, synergism between two or more metabolites involved in biocontrol is considered cautiously. Sharifi-Tehrani et al. (1998) compared the biocontrol activity of a collection of 2,4-DAPG-producing fluorescent *Pseudomonas* spp. against *Fusarium* TFRR and *Pythium* damping-off of cucumber and found that strains producing only 2,4-DAPG were more effective than 2,4-DAPG and pyoluteorin-producing strains.

Conclusion

Tomato crops are subjected to diseases caused by *Fusarium oxysporum* species for which several control methods are available. Biocontrol microorganisms offer an attractive alternative to the use of conventional methods for plant disease control as they present the advantage of being of low or no negative impact on the environment. For most of them, mechanisms of action are well studied at least at laboratory scale. However, population dynamics of biocontrol agents intended to be introduced in native soils are very sensitive to variable edaphic and biotic parameters responsible of the frequently observed inconsistencies in biocontrol. Thus, an appropriate understanding of the multiple traits involved in disease suppression by the biocontrol agents should be taken into account especially those involving microbe-microbe and microbe-plant-environment interactions. Those involved in root competence are of great importance for effective biocontrol and are frequently influenced by both roots and competition with other microorganisms.

Finally, in general, development of large-scale screening methods and tools to assess the potential effectiveness of isolated microorganisms in field trials could be very helpful to control *Fusarium* diseases of tomato. Such strategies aiming to select microorganisms that are environmentally versatile and could display a specific action toward fungal diseases or that are multifaceted and displayed diverse mechanisms simultaneously, i.e., antibiosis, competition, induction of resistance mechanisms, and growth promotion of tomato, could contribute to the developing research aiming to control tomato fungal diseases including TFRR. In our laboratory, we selected PGPRs with such traits, and biocontrol experiments of TFRR are ongoing.

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References

Agrios GN (2005) Plant pathology, 5th edn. Elsevier Academic Press, San Diego. 922 pp

Attitalla IH, Fatehi J, Levenfors J, Brishammar S (2004) A rapid molecular method for differentiating two special forms (*lycopersici* and *radicis -lycopersici*) of *Fusarium oxysporum*. Mycol Res 108:787–794

Audenaert K, Pattery T, Cornelis P, Höfte M (2002) Induction of systemic resistance to Botrytis cinerea in tomato by *Pseudomonas aeruginosa* 7NSK2: role of salicylic acid, pyochelin, and pyocyanin. Mol Plant Microbe Interact 15:1147–1156

Baker R (1991) Induction of rhizosphere competence in the biocontrol fungus *Trichoderma* In: Keister DL, Cregan PB (eds) The Rhizosphere and plant growth. Kluwer Acadamic Publishers, Dordrecht, pp 221–228

Bakker PAHM, Raaijmakers JM, Bloemberg GV, Hofte M, Lemanceau P, Cooke M (2007) New perspectives and approaches in plant growth-promoting rhizobacteria research. Eur J Plant Pathol 119:241–242

Baysal Ö, Çalıskan M, Yesilova Ö (2008) An inhibitory effect of a new Bacillus subtilis strain (EU07) against *Fusarium oxysporum* f sp *radicis -lycopersici*. Physiol Mol Plant Pathol 73:25–32

Bell CH, Price N, Chakrabarti B (1996) The methyl bromide issue. Wiley, New York. 400 pp

Benhamou N, Kloepper JW, Tuzun S (1998) Induction of resistance against *Fusarium* wilt of tomato by combination of chitosan with an endophytic bacterial strain: ultrastructure and cytochemistry of the host response. Planta 204:153–168

Bloemberg GV (2007) Microscopic analysis of plant-bacterium interactions using auto fluorescent proteins. Eur J Plant Pathol 119:301–309

Bloemberg GV, Wijfjes AHM, Lamers GEM, Stuurman N, Lugtenberg BJJ (2000) Simultaneous imaging of *Pseudomonas fluorescens* WCS365 populations expressing autofluorescent proteins in the rhizosphere: new perspectives for studying microbial communities. Mol Plant Microbe Interact 13:1170–1176

Bolwerk A, Lugtenberg BJJ (2005) Visualization of interactions of microbial biocontrol agents and phytopathogenic fungus *Fusarium oxysporum* f.sp *radicis lycopersici* on tomato roots. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 217–231

Bolwerk A, Lagopodi AL, Wijfjes AHM, Lamers GEM, Chin-A-Woeng TFC, Lugtenberg BJJ, Bloemberg GV (2003) Interactions in the tomato rhizosphere of two *Pseudomonas* biocontrol strains with the phytopathogenic fungus *Fusarium oxysporum* f. sp. *radicis -lycopersici*. Mol Plant Microbe Interact 16:983–993

Bouizgarne B (2013) Bacteria for plant growth promotion and disease management. In Maheshwari DK (ed) Bacteria in agrobiology: disease management. Springer, Berlin, pp 15–47

Bourbos VA, Michalopoulos G, Skoudriakis MT (1997) Lutte biologique contre *Fusarium* f. sp. *radicis -lycopersici* chez la tomate en serre non-chauffée. IOBC/WPRS Bull 20:58–62

Brammall RA, Higgins VJ (1988) A histological comparison of fungal colonization in tomato seedlings susceptible or resistant to *Fusarium* crown and root rot disease. Can J Bot 66:915–925

Buysens S, Heungens K, Poppe J, Hofte M (1996) Involvement of pyochelin and pyoverdine in suppression of *Pythium*-induced damping-off of tomato by *Pseudomonas aeruginosa* 7NSK2. Appl Environ Microbiol 62:865–871

Camacho MM (2001) Molecular characterization of the role of type 4 pili, NDH-I and PyrR in rhizosphere colonization of *Pseudomonas fluorescens* WCS365. PhD thesis, Leiden University, Leiden

Chen C, Belanger RR, Benhamou N, Paulitz TC (2000) Defense enzymes induced in cucumber roots by treatment with plant growth promoting rhizobacteria (PGPR). Physiol Mol Plant Pathol 56:13–23

Chin-A-Woeng TFC, de Priester W, van der Bij AJ, Lugtenberg BJJ (1997) Description of the colonization of a gnotobiotic tomato rhizosphere by *Pseudomonas fluorescens* biocontrol strain WCS365, using scanning electron microscopy. Mol Plant Microbe Interact 10:79–86

Chin-A-Woeng TFC, Bloemberg GV, van der Bij AJ, van der Drift KMGM, Schripsema J, Kroon B, Scheffer RJ, Keel C, Bakker PAHM, Tichy HV, de Bruijn FJ, Thomas-Oates JE, Lugtenberg BJJ (1998) Biocontrol by phenazine-1-carboxamide-producing *Pseudomonas chlororaphis* PCL1391 of tomato root rot caused by *Fusarium oxysporum* f. sp. *radicis -lycopersici*. Mol Plant Microbe Interact 1:1069–1077

Chin-A-Woeng TFC, Bloemberg GV, Mulders IHM, Dekkers LC, Lugtenberg BJJ (2000) Root colonization by phenazine-1-carboxamide-producing bacterium *Pseudomonas chlororaphis* PCL1391 is essential for biocontrol of tomato foot and root rot. Mol Plant Microbe Interact 13:1340–1345

Chin-A-Woeng TFC, Bloemberg GV, Lugtenberg BJJ (2003) Phenazines and their role in biocontrol by *Pseudomonas* bacteria. New Phytol 157:503–523

Datnoff L, Nemec S, Pernezny K (1995) Biological control of *Fusarium* crown and root rot of tomato in Florida using *Trichoderma harzianum* and *Glomus intraradices*. Biol Control 5:427–431

De Brito Alvarez MA, Gagne S, Antoun H (1995) Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth-promoting rhizobacteria. Appl Environ Microbiol 61:194–199

de Waard MA, Georgopoulos SG, Hollomon DW, Ishii H, Leroux P, Ragsdale NN, Schwinn FJ (1993) Chemical control of plant diseases: problems and prospects. Annu Rev Phytopathol 31:403–421

Dekkers LC, van der Bij AJ, Mulders IHM, Phoelich CC, Wentwoord RAR, Glandorf DCM, Wijffelman CA, Lugtenberg BJJ (1998) Role of the O-antigen of lipopolysaccharide, and possible roles of growth rate and NADH: ubiquinone oxidoreductase (nuo) in competitive tomato root-tip colonization by *Pseudomonas fluorescens* WCS365. Mol Plant Microbe Interact 11:763–771

Dekkers LC, Mulders IHM, Phoelich CC, Chin-A-Woeng TFC, Wijfjes AHM, Lugtenberg BJJ (2000) Interactions in the tomato rhizosphere of two *Pseudomonas* biocontrol strains with the phytopathogenic fungus *Fusarium oxysporum* f sp *radicis -lycopersici*. Mol Plant Microbe Interact 13:1177

Duffy BK, Defago G (1997) Zinc improves biocontrol of *Fusarium* crown and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. Phytopathology 87:1250–1257

Duffy B, Christoph K, Défago G (2004) Potential role of pathogen signaling in multitrophic plant-microbe interactions involved in disease protection. Appl Environ Microbiol 70:1836–1842

Duijff BJ, Bakker PAHM, Schippers B (1994) Suppression of *Fusarium* wilt of carnation by *Pseudomonas putida* WCS358 at different levels of disease incidence and iron availability. Biocontrol Sci Technol 4:279–288

Duniway JM, Hao JJ, Dopkins DM, Ajwa H, Browne GT (2000) Some chemical, cultural, and-biological alternatives to methyl bromide fumigation of soil for Strawberry. In: Proceedings of the annual international research conference on methyl bromide alternatives and emissions reductions, Orlando, p 9

Duss F, Moazfar A, Oertli JJ, Jaeggi W (1986) Effect of bacteria on the iron uptake by axenically-cultured roots of Fe-efficient and Fe-inefficient tomatoes (*Lycopersicon esculentum* Mill.) J Plant Nutr 9:587–598

El-Tarabily K (2006) Rhizosphere-competent isolates of streptomycete and non-streptomycete actinomycetes capable of producing cell-wall-degrading enzymes to control *Pythium aphanidermatum* damping-off disease of cucumber. Can J Bot 84:211–222

El-Tarabily KA (2008) Promotion of tomato (*Lycopersicon esculentum* Mill.) plant growth by rhizosphere competent 1-aminocyclopropane-1-carboxylic acid deaminase-producing streptomycete actinomycetes. Plant Soil 308:161–174

El-Tarabily KA, Hardy GESJ, Sivasithamparam K, Hussein AM, Kurtböke ID (1997) The potential for the biological control of cavity spot disease of carrots caused by *Pythium coloratum* by Streptomycete and non-Streptomycete actinomycetes in Western Australia. New Phytol 137:495–507

Gagné S, Dehbi L, Le Quéré D, Cayer F, Morin J-L, Lemay R, Fournier N (1993) Increase of greenhouse tomato fruit yields by plant growth-promoting rhizobacteria (PGPR) inoculated into the peat-based growing media. Soil Biol Biochem 25:269–272

Gamalero E, Lingua G, Caprì FG, Fusconi A, Graziella B, Lemanceau P (2004) Colonization pattern of primary tomato roots by *Pseudomonas fluorescens* A6RI characterized by dilution plating, flow cytometry, fluorescence, confocal and scanning electron microscopy. FEMS Microbiol Ecol 48:79–87

Gamalero E, Lingua G, Tombolini R, Avidano L, Pivato B, Berta G (2005) Colonization of tomato root seedling by *Pseudomonas fluorescens* 92 rkG5: Spatio-temporal dynamics, localization, organization, viability, and culturability. Microb Ecol 50:289–297

Garcia JL, Probanza A, Ramos B, Manero FG (2003) Arch Agron Soil Sci 49:119

Glick BR (2006) 7th international workshop on plant growth promoting rhizobacteria, 28 May–2 June 2006 Noordwijkerhout, p 30

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242

Goddard VJ, Bailey MJ, Darrah P, Lilley AK, Thompson IP (2001) Monitoring temporal and spatial variation in rhizosphere bacterial population diversity: a community approach for the improved selection of rhizosphere competent bacteria. Plant Soil 232:181–193

Gopalakrishnan S, Pande S, Sharma M, Humayun P, Kiran BK, Sandeep D, Vidya MS, Deepthi K, Rupela O (2011) Evaluation of actinomycete isolates obtained from herbal vermicompost for the biological control of *Fusarium wilt* of chickpea. Crop Prot 30:1070–1078

Gravel V, Antoun H, Tweddell RJ (2007) Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biol Biochem 39:1968–1977

Hayes W (1994) Methyl bromide: the Montreal protocol and the clean air act. In: Vavrina CS (eds) Proceedings of the Florida Tomato Institute. University of Florida, pp 34–44

Hibar K, Daami-Remadi M, Khiareddine H, El Mahjoub M (2005) Effet inhibiteur in vitro et in vivo du *Trichoderma harzianum* sur *Fusarium oxysporum* f.sp. *radicis* -lycopersici. Biotechnol Agron Soc Environ 9:163–171

Horinouchi H, Muslim A, Suzuki T, Hyakumachi M (2007) *Fusarium* equiseti GF191 as an effective biocontrol agent against *Fusarium* crown and root rot of tomato in rock wool systems. Crop Prot 26:1514–1523

Horinouchi H, Katsuyama N, Taguchi Y, Hyakumachi M (2008) Control of *Fusarium* crown and root rot of tomato in a soil system by combination of a plant growth-promoting fungus, *Fusarium* equiseti, and biodegradable pots. Crop Prot 27:859–864

Ito S, Kawaguchi T, Nagata A, Tamura H, Matsushita H, Takahara H, Tanaka S, Ikeda T (2004) Distribution of the FoToml gene encoding tomatinase in formae speciales of *Fusarium oxysporum* and identification of a novel tomatinase from *F. oxysporum* f.sp. *radicis -lycopersici*, the causal agent of *Fusarium* crown and root rot of tomato. J Gen Plant Pathol 70:195–201

Jacobsen BJ, Backman PA (1993) Biological and cultural plant disease controls: alternatives and supplements to chemicals in IPM systems. Plant Dis 77:311–315

Jarvis WR, Shoemaker RA (1978) Toxonomic status of *Fusarium oxysporum* causing foot and root rot of tomato. Phytopathology 68:1679–1680

Jarvis WR, Thorpe HJ (1976) Susceptibility of Lycopersicon species and hybrids to the foot and root rot pathogen *Fusarium oxysporum*. Plant Dis Rep 60:1027–1031

Jarvis WR, Thorpe HJ (1981) Control of *Fusarium* foot and root rot of tomato by soil amendment with lettuce residues. Can J Plant Pathol 3:159–162

Jones JB, Jones JP, Stall RE, Zitter TA (eds) (1991) *Compendium* of tomato diseases. American Phytopathological Society, St. Paul

Kamilova F, Validov S, Azarova T, Mulders I, Lugtenberg B (2005) Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. Environ Microbiol 7:1809–1817

Kamilova F, Leveau JHJ, Lugtenberg B (2007) Collimonas fungivorans, an unpredicted in vitro but efficient in vivo biocontrol agent for the suppression of tomato foot and root rot. Environ Microbiol 9:1597–1603

Kamilova F, Lamers G, Lugtenberg B (2008) Biocontrol strain *Pseudomonas fluorescens* WCS365 inhibits germination of *Fusarium oxysporum* spores in tomato root exudate as well as subsequent formation of new spores. Environ Microbiol 10:2455–2461

Khalil S, Alsanius BW (2010) Evaluation of biocontrol agents for managing root diseases on hydroponically grown tomato. J Plant Dis Prot 117:214–219

Khan MR, Khan SM (2001) Biomanagement of *Fusarium* wilt of tomato by the soil application of certain phosphate-solubilizing microorganisms. Int J Pest Manag 47:227–231

Kloepper JW (2003) In: Reddy MS, Anandaraj M, Eapen SJ, Sarma YR, Kloepper JW (eds) Abstracts and short papers 6th international PGPR workshop, 5–10 October 2003, Indian Institute of Spices Research, Calicut pp 81–92

Kloepper JW, Tuzun S, Ku DJA (1992) Proposed definitions related to induced disease resistance. Biocontrol Sci Technol 2:349–351

Komada H (1994) Strategies and prospects on biocontrol of soilborne diseases in soilless culture. Plant Prot 48:413–417

Kravchenko LV, Azarova TS, Leonova-Erko EI, Shaposhnikov AI, Makarova NM, Tikhonovich IA (2003) Root exudates of tomato plants and their effect on the growth and antifungal activity of *Pseudomonas* strains. Microbiology 72:37–41

Lagopodi AL, Ram AFJ, Lamers GEM, Punt PJ, Van den Hondel CAMJJ, Lugtenberg BJJ, Bloemberg GV (2002) Novel aspects of tomato root colonization and infection by *Fusarium oxysporum* f.sp. *radicis -lycopersici* revealed by confocal laser scanning microscopic analysis using the green fluorescent protein as a marker. Mol Plant Microbe Interact 15:172–179

Lahdenperä ML (2000) Mycostop® in the control of *Fusarium oxysporum* on vegetables. Biocontrol Infoletter. Kemira Agro Oy, Espoo Research Centre, Espoo

Larkin RP, Fravel DR (2002) Effects of varying environmental conditions on biological control of *Fusarium* wilt of tomato by nonpathogenic *Fusarium* spp. Phytopathology 92:1160–1166

Lee S-W, Ahn I-P, Lim J-W, Lee Y-H (2005) Pseudomonas putida strain 17 isolated from replant soil promotes tomato growth and inhibits conidial germination of soilborne plant pathogens. Plant Pathol J 21:244–251

Lemanceau P, Bakker PAHM, de Kogel J, Alabouvette C, Schippers B (1992) Effect of pseudobactin 358 production by *Pseudomonas putida* WCS358 on suppression of fusarium wilt of carnations by nonpathogenic *Fusarium oxysporum.* Fo47. Appl Environ Microbiol 58:2978–2982

Lemanceau P, Corberand T, Gardan L, Latour X, Laguerre G, Boeufgras J-M, Alabouvette C (1995) Effect of two plant species, flax (*Linum usitatissimum* L.) and tomato (*Lycopersicon esculentum* Mill.), on the diversity of soilborne populations of fluorescent pseudomonads. Environ Microbiol 61:1004–1012

Liu L, Kloepper JW, Tuzun S (1995) Induction of systemic resistance in cucumber against *Fusarium* wilt by plant growth-promoting rhizobacteria. Phytopathology 85:695–698

Loper JE, Buyer JS (1991) Siderophores in microbial interactions on plant surfaces. Mol Plant Microbe Interact 4:5–13

Loper JE, Schroth MN (1986) Importance of siderophores in microbial interactions in the rhizosphere. In: Swinburne TR (ed) Iron siderophores and plant disease. Plenum Press, New York, pp 85–89

Louter JH, Edgington LV (1990) Indications of cross-protection against *Fusarium* crown and root rot of tomato. Can J Plant Pathol 12:283–288

Lugtenberg BJJ, Bloemberg GV (2004) Life in the rhizosphere. In: Ramos JL (ed) The pseudomonads, vol I: Genomics, life style and molecular architecture. Kluwer Academic/Plenum, New York, pp 403–430

Marois JJ, Mitchell DJ (1981) Effects of fumigation and fungal antagonists on the relationships of inoculum density to infection incidence and disease severity in *Fusarium* crown rot of tomato. Phytopathology 71:167–170

Marois JJ, Mitchell DJ, Sonoda RM (1981) Biological control of *Fusarium* crown rot of tomato under field condition. Phytopathology 71:1257–1260

Mayak S, Tirosh T, Glick BR (2004a) Plant growth-promoting bacteria that confer resistance to water stress in tomato and pepper. Plant Sci 166:525–530

Mayak S, Tirosh T, Glick BR (2004b) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572

McGovern RJ, Datnoff LE (1992) *Fusarium* crown and root rot: reevaluation of management strategies. In: Vavrina CS (ed) 'Proceedings of Florida Tomato Institute' Vegetable Crops Special Series SS-HOS-1, Vegetable Crops Department, University of Florida-IFAS, Gainesville, FL 32611, pp 75–86

Menzies JG, Koch C, Seywerd F (1990) Additions to the host range of *Fusarium oxysporum* f. sp. *radicis -lycopersici*. Plant Dis 74:569–572

Meziane H, Van der Sluis I, Van Loon LC, Hofte M, Bakker PAHM (2005) Determinants of *Pseudomonas putida* WCS358 involved in inducing systemic resistance in plants. Mol Plant Pathol 6:177–185

Minuto A, Spadaro D, Garibaldi A, Gullino ML (2006) Control of soilborne pathogens of tomato using a commercial formulation of *Streptomyces griseoviridis* and solarization. Crop Prot 25:468–475

Moreno CA, Castillo F, Gonzàlez A, Bernal D, Jaimes Y, Chaparro M, Gonzàlez C, Rodriguez F, Restrepo S, Cotes AM (2009) Biological and molecular characterization of the response of tomato plants treated with *Trichoderma koningiopsis*. Physiol Mol Plant Pathol 74:111–120

Olivain C, Humbert C, Nahalkova J, Fatehi J, L'Haridon F, Alabouvette C (2006) Colonization of tomato root by pathogenic and nonpathogenic *Fusarium oxysporum* strains inoculated together and separately into the soil. Appl Environ Microbiol 72:1523–1531

Ongena M, Daayf F, Jacques P, Thonart P, Banhamou N, Paulitz TC, Belanger RR (2000) Systemic induction of phytoalexins in cucumber in response to treatment with fluorescent Pseudomonads. Plant Pathol 49:523–530

Ozbay N, Newman S (2004) *Fusarium* crown and root rot of Tomato and control methods. Plant Pathol J 3:9–18

Ozbay N, Newman SE, Basham CW, Brown WM (2001) Biological control of *Fusarium* crown and root rot of tomato with *Trichoderma harzianum*. Hortscience 36:554

Park KS, Ahn IP, Kim CH (2001) Systemic resistance and expression of the pathogenesis-related genes mediated by the plant growth-promoting rhizobacterium Bacillus amyloliquefaciens EXTN-1 against anthracnose disease in cucumber. Mycobiology 29:48–53

Paulitz TC, Bélanger RR (2001) Biological control in greenhouse systems. Annu Rev Phytopathol 39:103–133

Phae CG, Shoda M, Kita N, Nakano M, Ushiyama K (1992) Biological control of crown and root rot and bacterial wilt of tomato by *Bacillus subtilis* NB22. Ann Phytopathol Soc Jpn 58:329–339

Piga PM, Belanger RR, Paulitz TC, Benhamou N (1997) Increased resistance to *Fusarium oxysporum* f. sp. *radicis -lycopersici* in tomato plants treated with the endophytic bacterium *Pseudomonas fluorescens* strain 63-28. Physiol Mol Plant Pathol 50:301–320

Postma J, Clematis F, Nijhuis EH, Someus E (2013) Efficacy of four phosphate-mobilizing bacteria applied with an animal bone charcoal formulation in controlling *Pythium aphanidermatum* and *Fusarium oxysporum* f. sp. *radicis lycopersici* in tomato. Biol Control 67:284–291

Prasad RD, Rangeshwaran R (2000) Effect of soil application of a granular formulation of *Trichoderma harzianum* on *Rhizoctonia solani* incited seed rot and damping-off of chickpea. J Mycol Plant Pathol 30:216–220

Ross AF (1961) Systemic acquired resistance induced by localized virus infection in plants. Virology 14:340–358

Rowe RC (1980) Comparative pathogenicity and host ranges of *Fusarium oxysporum* isolates causing crown and root rot of greenhouse and field-grown tomatoes in North America and Japan. Phytopathology 70:1143–1148

Rowe RC, Farley JD (1978) Control of *Fusarium* crown and root rot of greenhouse tomatoes by inhibiting recolonization of steam-disinfested soil with a captafol drench. Phytopathology 68:1221–1224

Rowe RC, Farely JD, Coplin DL (1977) Air-borne spore dispersal and recolonization of steamed soil by *Fusarium oxysporum* in tomato greenhouse. Phytopathology 67:1513–1517

Sato R, Araki T (1974) On the tomato root-rot disease occurring under vinyl-house conditions in southern Hokkaido. Ann Rep Soc Plant Prot North Jpn 25:5–13

Sharifi-Tehrani A, Zala M, Natsch A, Moënne-Loccoz Y, Défago G (1998) Biocontrol of soil-borne fungal plant diseases by 2, 4-diacetylphloroglucinol producing fluorescent pseudomonads with different restriction profiles of amplified 16S rDNA. Eur J Plant Pathol 104:631–643

Sharma VK, Nowak J (1998) Enhancement of *Verticillium* wilt resistance in tomato transplants by in vitro co-culture of seedlings with a plant growth promoting rhizobacterium (*Pseudomonas* sp. strain PsJN). Can J Microbiol 44:528–536

Sivan A, Chet I (1993) Integrated control of *Fusarium* crown and root rot of tomato with *Trichoderma harzianum* in combination with methyl bromide or soil solarization. Crop Prot 12:380–386

Sivan A, Ucko O, Chet I (1987) Biological control of *Fusarium* Crown Rot of tomato by *Trichoderma harzianum* under field conditions. Plant Dis 71:587–592

Sneh B, Dupler M, Elad Y, Baker R (1984) Chlamydospore germination of *Fusarium oxysporum* f.sp. *cucumerinum* as affected by fluorescent and lytic bacteria from a *Fusarium*-suppressive soil. Phytopathology 14:1115–1124

Turlier MF, Eparvier A, Alabouvette C (1994) Early dynamic interactions between *Fusarium oxysporum* f sp lini and the roots of *Linum usitatissimum* as revealed by transgenic GUS-marked hyphae. Can J Bot 72:1605–1612

Validov S, Kamilova F, Qi S, Stephan D, Wang JJ, Makarova N, Lugtenberg B (2007) Selection of bacteria able to control *Fusarium oxysporum* f.sp. *radicis lycopersici* in stonewool substrate. J Appl Microbiol 102:461–471

Validov SZ, Kamilova F, Lugtenberg BJJ (2009) Pseudomonas putida strain PCL1760 controls tomato foot and root rot in stonewool under industrial conditions in a certified greenhouse. Biol Control 48:6–11

Van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119:243–254

Van Loon LC, Bakker PAHM, Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483

Van Peer R, Schippers B (1989) Plant growth responses to bacterization and rhizosphere microbial development in hydroponic cultures. Can J Microbiol 35:456–463

Van Peer R, Niemann GJ, Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. Strain WCS417r. Phytopathology 81:728–734

Van Rij ET, Wesselink M, Chin-A-Woeng TFC, Bloemberg GV, Lugtenberg BJJ (2004) Influence of environmental conditions on the production of phenazine-1-carboxamide by *Pseudomonas chlororaphis* PCL1391. Mol Plant Microbe Interact 17:557–566

Microbial Inoculants for Optimized Plant Nutrient Use in Integrated Pest and Input Management Systems

2

Anthony Oyegoke Adesemoye, Gary Yuen, and Dexter Brown Watts

Abstract

The use of fertilizers and pesticides has greatly increased agricultural productivity over the past few decades. However, there is still an ongoing search for additional or alternate tools that can proffer agricultural sustainability and meet the needs of profitability and greater food production for the growing world population. This review examines the enhancement of plant nutrient use efficiency derived from interactions of the diverse microorganisms that live in and around plants such as plant growth-promoting rhizobacteria (PGPR) and mycorrhizal fungi. These microorganisms form the major bases of the biorational sector of the agriculture industry which has exploded in the last few years with the production of many new microbial inoculant products and the improvement of existing products. Microbial inoculants cannot replace chemical fertilizers now or in the immediate future; thus this review discusses the concept of integrated pest and input management (IPIM), compatibility of inoculants with existing chemicals, and efficacy issues associated with biologicals. Also discussed are inoculant products, the conditions that may affect their success, the untapped potentials for agriculture, and the possible impacts on greenhouse gas emissions and global warming.

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2.1 Introduction

The progress that was made in increased crop production since the middle of the twenty-first century can be attributed in part to developments in plant breeding and genetic engineering and to changes in irrigation and tillage practices. The intensive use of fertilizers and pesticides is another factor that greatly enhanced crop productivity. There is concern, however, that increasing and continuous use of agrochemicals is not sustainable as it leads to the pollution of the environment, especially surface water and groundwater. In addition, applications of agrochemicals may leave residues in foods, thus generating public concern about the impact of agrochemicals on food quality and safety.

The search for additional or alternate tools that will enhance agricultural sustainability while allowing for profitability and more food production for the growing world population is pivotal to agricultural production worldwide. Understanding the diversity of microbes that live in and around plants in different natural environments and modulating their activities offer prospective tools. Microbes interact with plants and support plant health in many different ways such as enhancing plant growth and yield, controlling diseases, and contributing to survival and recovery from adverse environmental conditions, including drought (Cook 2002; Adesemoye and Kloepper 2009; Reid and Greene 2012).

One group of beneficial microbes termed plant growth-promoting rhizobacteria (PGPR) (Kloepper et al. 1989) has been defined in the literature for their ability to live freely in the rhizosphere, stimulate plant growth, enhance root development and architecture, help plants in nutrient acquisition, and provide control of plant pathogens (Kloepper et al. 1991; Canbolat et al. 2006; Adesemoye et al. 2009; Figueiredo et al. 2010). The major genera of PGPR that have been studied or used as inoculants include *Azotobacter*, *Azospirillum*, *Pseudomonas*, *Acetobacter*, *Herbaspirillum*, *Burkholderia*, and *Bacillus* (Glick 1995; Probanza et al. 1996; Artursson et al. 2006; Adesemoye and Kloepper 2009). The direct and indirect mechanisms involved in PGPR activities have been discussed by many authors (Vessey 2003; Glick et al. 2007). Other important groups of plant beneficial microbes include mycorrhizal fungi and *Trichoderma*, a root-colonizing fungus. The role of PGPR and *Trichoderma* as antagonists of soilborne pathogens is well documented. Their use as alternative tools when development of resistance by pathogens to chemical fungicides is possible or where chemical fungicides are not available is one reason why the interest in biological products continues to increase.

The use of PGPR and beneficial fungi to enhance nutrient use is a newer concept than plant growth promotion and biocontrol and comparatively has not been extensively studied. While many of the benefits are known, the potentials for agriculture are only starting to be tapped (Barea et al. 2002; Reid and Greene 2012). Interest in commercial production of microbial inoculants for agricultural use has increased within the last few years. This is important for agricultural sustainability where there is a need to produce more food for the increasing population and limit the amount of fertilizers and pesticides being used.

This review will focus on how PGPR, mainly *Bacillus* spp., and beneficial fungi, particularly mycorrhizae and *Trichoderma* species, help plants in nutrient

acquisition. The beneficial roles of rhizosphere microbial populations in soil nutrient availability and how microorganisms applied as inoculants can help plants to improve nutrient use efficiency will be examined. We will explain how microbial inoculants fit within the concept of integrated pest and input management and also look at some of the inoculants which are currently on the market that are based on bacteria and/or fungi. Interactions between PGPR and beneficial fungi used as inoculants will be examined. Also, the conditions that may affect the success of microbial inoculants as well as the untapped potentials for agriculture will be examined.

2.2 Soil Microbial Diversity, Nutrient Dynamics, and Integrated Input and Pest Management

Degradation processes, decline in soil nutrition and productivity, nutrient runoff, leaching, erosion, organic matter depletion, and the negative impacts on groundwater and surface waters are major public concerns. The diversity of microbes in the soil could be used in a sustainable way in agriculture to solve or reduce these problems.

Integrated pest management (IPM) is an important component of sustainable agriculture, and the definition may vary among scientific disciplines, but the concept is very similar. The Office of Technology (1979) defined IPM in the broad sense as "the optimization of pest/pathogen control in an economically and ecologically sound manner, accomplished by the coordinated use of multiple tactics to assure stable crop production and to maintain pest/pathogen damage below the economic injury level while minimizing hazards to humans, wildlife, and the environment." Integrated nutrient management (INM) is another term that has gained momentum in agricultural sustainability circles. The INM system promotes low chemical input but improved nutrient use efficiency through using natural and manmade sources of plant nutrients for crop production in an efficient and environmentally prudent manner that preserves resources for the future without sacrificing current productivity (Gruhn et al. 2000; Adesemoye et al. 2008b). In recent years, the idea of combining IPM and INM together in a systems approach to become integrated pest and input management (IPIM) continues to emerge and its relevance is expanding. The reasons for this are not farfetched, if we look at the intricate connectivity of soil quality and health to IPM, INM, soil productivity, food quality and safety, and environmental soundness. The functionality of this relationship is better seen from the perspective of a cycle.

Parr et al. (1992) opined that the maintenance or restoration of soil quality is highly dependent on organic matter and the diversity of beneficial macroorganisms and microorganisms that it supports. Their suggestion that reduced input of chemical fertilizers and pesticides and the use of alternative practices that enhance organic matter and soil microbial diversity will improve soil quality and productivity aptly fits the concept of IPIM.

The biorational sector will continue to expand, and the discussion of IPIM will consequently be more relevant. The word biorational will be used through this

review, and it is important to define what it connotes as there is no universally accepted definition. Biorationals in agriculture refer to substances or products derived from natural or biological origins that are used in crop production. According to information from the American Society for Testing and Materials (ASTM) International and ATTRA—Sustainable Agriculture, biorationals include biopesticides and nonpesticidal products, such as, but not limited to, those used for crop stress management, enhanced plant physiology benefits, root growth management, enhanced nutrient use, postharvest, or as control agents to pesticides and antimicrobials. Biorationals should not be equated with biologicals or microbial inoculants because microbial inoculant is a component of biorationals as "biologicals" include living organisms. While an extract from a living source, such as neem, is a biorational, it is not a biological. Additional definition for microbial inoculant and some related terms such as biostimulant can be found in Calvo et al. (2014).

There are no scientific data to suggest that biological products will replace chemical fertilizers or pesticides now or in the immediate future, but there is interest in using them to supplement and reduce the amount of chemical products that growers are applying. The concept of IPIM system supposes that biorationals including microbial inoculants will reduce the need for agrochemicals such as fertilizers and pesticides. If this would happen, microbial inoculants and most biorationals must be developed to be compatible with existing agrochemicals.

The biological products in the IPIM systems will have to be locally adaptable and beneficial to the soil-plant systems in terms of the resident beneficial microbial community, overall soil biodiversity, soil structure and health, and enhanced nutrient use efficiency. The improved system should lead to or confer resiliency and sustainability to the agroecosystems in the face of challenges of rapidly changing environmental conditions.

The concept of how microbial inoculants (PGPR and mycorrhizae) can interact with crop roots and be used compatibly with fertilizer in an IPIM leading to a more efficient use of nutrients can be explained by the schematic in Fig. 2.1. Currently in agricultural systems, excess fertilizers (A) more than needed by crops are applied. The amount of nutrient taken up by the crop (B) is far less than applied. Thus, "B" as a percentage of "A" or use efficiency may vary from 10% to 50%. Significant parts of the chemical fertilizer are lost or not available to the crop, and this includes portions lost through runoff (C), immobilization, denitrification (D), leaching (E), greenhouse gas emissions (F), and volatilization (G). How to improve plant available nutrients (I) and make more nutrients available through processes such as mineralization and solubilization (J) and thus maximize the amount of nutrient that is eventually taken up (B) is paramount to IPIM or INM. Part of the goal is to use less fertilizer (i.e., reduce "A") and reduce the parameters C, D, E, F, and G. All these seem possible in a carefully designed IPIM system where microbial inoculants (H) are better understood and combined with chemicals and appropriate cultural practices, including moisture.

In the diagram, fertilizers (A) refer to different kinds of fertilizers, and microbial inoculants (H) might include the combination of PGPR, mycorrhizae, and/or *Trichoderma* species.

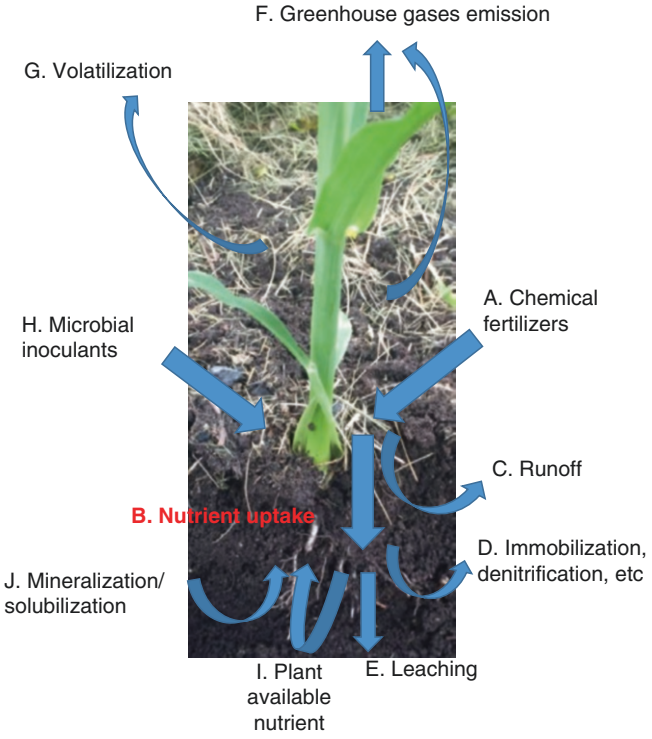

Fig. 2.1 Schematic of the possibilities from the interaction of crop roots and inoculants containing PGPR and mycorrhizae for improved efficient use of nutrients

2.3 Activity of Specific Microbial Groups in Enhanced Plant Nutrient Use

Natural beneficial symbiotic relationships formed by different groups of organisms such as mycorrhizae have been known for years (Barea et al. 1993, 2002). Many reports have shown that free-living plant growth-promoting rhizobacteria (PGPR) can form mutualistic relationships with plants (Kloepper et al. 1991; Bashan and Holguin 1998; Compant et al. 2005). When these organisms are introduced into the rhizosphere, they have the potential to alter microbial populations in the rhizosphere and influence nutrient transformation, availability, and uptake by plants (Adesemoye et al. 2008b; Shen et al. 2012). Regardless of whether PGPR and mycorrhizae populations in a location are indigenous or result from purposeful inoculation, neither group will exist or function in isolation. Better understanding of the interaction of PGPR and mycorrhizae as well as other relevant beneficial microorganisms such as *Trichoderma* and their joint influence on crop growth, development and physiology of the plant (Glick and Bashan 1997; Volpin and Phillips 1998; Barea et al. 2002), morphological characteristics of inoculated roots

(Yanni et al. 1997; Biswas et al. 2000), and how improved nutrient uptake occurs (Okon and Kapulnik 1986; Biswas et al. 2000; Adesemoye et al. 2008b) is crucial.

2.3.1 Plant Growth-Promoting Bacillus Species

Diverse effects and mechanisms of plant growth-promoting rhizobacteria (PGPR) have been reported, including phytohormone production (Tien et al. 1979; Hussain et al. 1987; Chabot et al. 1996), production and secretion of siderophores (Joo et al. 2004), N_2 fixation and efficient use of N sources (Yanni et al. 1997) and use of other nutrients (Chabot et al. 1996), and inhibition of plant pathogens (Compant et al. 2005; Haas and Defago 2005; Someya and Akutsu 2006).

Bacillus species are the most widely used PGPR in agricultural products, mainly because of their long-term survival as spores (Yildirim et al. 2006; Adesemoye et al. 2008a). *Bacillus* spp. have wide metabolic capabilities allowing them to play important roles in soil ecosystem functions and processes. Due to their heterotrophic nature, *Bacillus* spp. are also important in soil carbon, nitrogen, and sulfur cycling, as well as the transformation of other soil nutrients (Mandic-Mulec and Prosser 2011). The capacity for survival in constantly changing environments gave *Bacillus* spp. an edge in the potential to alter soil microbial community composition (Compant et al. 2005). Capacity to form stress-resistant endospores, secretion of peptide antibiotics and signal molecules, multilayered cell wall, and extracellular enzymes are characteristics that contribute to their survival and longevity (Kumar et al. 2011).

Bacillus megaterium and *B. muciaraglaginous* co-inoculated with AMF were reported to improve nutritional assimilation of plant total N, P, and K in maize (*Zea mays*) (Wu et al. 2005). *Bacillus polymyxa* was reported with the capacity to fix atmospheric nitrogen (N_2) (Omar et al. 1996), and different *Bacillus* spp. have been identified as phosphorus solubilizers (de Freitas et al. 1997; Rodriguez and Fraga 1999). *Bacillus amyloliquefaciens* FZB45 was shown to contribute to plant growth promotion and produce phytases, the enzymes that solubilize P from phytate, an organic phosphate (Idriss et al. 2002), while *Bacillus licheniformis* and *B. amyloliquefaciens* were found to produce mixtures of lactic, isovaleric, isobutyric, and acetic acids which are organic acids responsible for decreasing the pH of the surrounding soil, thereby releasing phosphate ions (Rodriguez and Fraga 1999). *Bacillus* spp. have also been reported in enhanced K uptake (Sheng and He 2006) and increase uptake of micronutrients (Kohler et al. 2008).

Bacillus PGPR have been shown to help plants in tolerance and survival of abiotic stresses such as drought and salt (Arshad et al. 2008; Vardharajula et al. 2011; Lim and Kim 2013; Egamberdieva and Adesemoye 2016). Drought conditions can elicit various biochemical and physiological reactions in crops, hinder crop growth and productivity, and may lead to death. Lim and Kim (2013) reported growth promotion of pepper plants under drought stress through the inoculation of PGPR *B. licheniformis* K11 and suggested that the strain was able to produce ACC deaminase which reduced the ethylene concentration of the plants by cleaving the

precursor ACC (1-aminocyclopropane-1-carboxylate) under drought stress, thereby increasing plant growth. Adaptation and PGPR-induced salt tolerance has been attributed to improved water use efficiency and more efficient overall metabolic processes (Arshad et al. 2008; Yildirim et al. 2006; Egamberdieva and Adesemoye 2016). Though gene expression under these conditions has been studied and drought-responsive gene characterized (Vardharajula et al. 2011), the molecular basis and the detailed physiological changes have not been well understood.

The *Bacillus* genus is very important as an inoculant and is widely used as active ingredients in many biological products that are available in the United States and many parts of the world. For example, *Bacillus* spp. are components of the commercial inoculants. Accomplish LM and QuickRoots as well as many products are used for biological control of plant diseases. This explains why the genus *Bacillus* is a main interest in this review. Though there is a volume of work on the genus, there is a need for more extensive studies as biology-based products are now more important than ever.

2.3.2 Mycorrhizal Fungi and Interactions with PGPR

Mycorrhizal fungi are key components of the soil microbiota, and in addition to the beneficial relationships with plant roots, they also interact with other microorganisms in the rhizosphere. Mycorrhiza formation changes several aspects of plant physiology and some nutritional and physical properties of the rhizosphere soil, which in turn affects how other soil microorganisms colonize plant roots (Barea et al. 2002).

Mycorrhizal fungi are recognized beneficial organisms that can help improve plant establishment, better nutrient use, biological control of pathogens, and protection against cultural and environmental stresses. The mutually beneficial associations formed naturally with plant roots by ectomycorrhizae and/or endomycorrhizae are known to affect plant physiology including chemical composition of root exudates, better use of nutrient, hormonal balance, and carbon allocation patterns (Schenck 1981; Barea et al. 2002), but these potentials have not been well explored. The dense layer of hyphae (mantle) formed by ectomycorrhizal fungi and the vesicles formed by arbuscular mycorrhizal fungi (the most common among endomycorrhizae) are able to play significant roles in better uptake of nutrients. Other benefits of mycorrhizae listed by Schenck (1981) include enhancement of water transport in plants, decrease transplant injury, and plants' ability to survive extreme temperatures.

In the complex soil ecosystem, it will be impossible for the interaction of mycorrhizae and plant to occur in isolation. The interaction of mycorrhizae and beneficial bacteria especially PGPR in the plant rhizosphere is crucial in understanding the overall effects of microbes in nutrient uptake; however, very little is known about these interactions. It has been shown that bacteria can directly affect the germination and growth rate of mycorrhizal fungi. On the other hand, mycorrhizal fungi affect bacteria community compositions directly or indirectly through plants. The development of the mycorrhizal fungal mycelium can serve as a carbon source to PGPR as well as other rhizosphere microbial communities and introduce physical modifications into the environment surrounding the roots (Barea et al. 2002).

Bacteria and mycorrhizae through these interactions (and some of them may be very specific among strains) have been shown to jointly enhance the growth of plants and better root branching and architecture, thus improving nutrient acquisition (Artursson et al. 2006; Adesemoye et al. 2008b). The functional mechanisms behind the interactions of mycorrhiza and PGPR are not yet clear, but a better understanding is needed to achieve effectiveness for practical application in sustainable crop production.

Applications of single pure cultures of microbial inoculants have recorded little success in the past due in part to low knowledge about the organisms, their colonization and adaptation capabilities, and their interactions with other organisms that are present during the interaction with plant roots. The co-inoculation of PGPR and mycorrhizae and their use as inoculants in optimizing plant nutrient uptake are promising (Adesemoye et al. 2008b). There are indications that the biological sector of the agricultural industry is increasingly interested in combining or exploring multiple organisms or strains in products. This should improve efficacy because overall there is relatively more success in research with joint application of multiple inoculants involving multiple bacteria or bacteria and mycorrhizae.

The benefits of co-inoculation of phosphate-solubilizing PGPR and/or nitrogen-fixing PGPR with mycorrhizae to plants have been demonstrated (Rodriguez and Fraga 1999; Barea et al. 1993, 2002), but the interactions have to be managed to improve on the enhancement of plant nutrient use efficiency. The available knowledge on the tripartite interactions of root, mycorrhiza, and PGPR interactions is still little (Requena et al. 1997; Adesemoye et al. 2008b). In a field study with corn, Adesemoye et al. (2008b) showed improved uptake of nitrogen and phosphorus through co-inoculation of *Bacillus* PGPR and mycorrhizae. In a study involving mycorrhiza, *Rhizobium*, and PGPR, Requena et al. (1997) demonstrated effectiveness of the interactions in improving plant development, nutrient uptake, and root system quality and recommended the use of local isolates due to physiological and genetic adaptation of microbes to the environment. There is a need for more understanding.

It is important that challenges associated with co-inoculation are tackled in research studies or as part of the product developmental process. One of such challenges is compatibility of potential co-inoculants. Stephens and Rask (2000) reported a study where among seven different ways of combining four strains and 16 comparisons made in only one situation was the population of the mixed culture similar to the monoculture. Thus, compatibility of strains must be well tested as there is a possibility that individual strains in a mixed culture may be antagonistic against one another or may find it difficult to reach desired populations.

2.3.3 Other Select Microbial Inoculants

Many organism groups have been well reported in the literature as possible inoculants including bacteria in the genera *Pseudomonas*, *Burkholderia*,

Bradyrhizobium, *Rhizobium*, *Azospirillum*, and *Lysobacter* and fungi in the genera *Trichoderma* and *Penicillium*. Some of these have been used as active ingredients in biological products currently on the market, and the products are available for use as biopesticide or microbial inoculants in plant growth promotion or nutrition enhancement.

Species of *Bradyrhizobium* and *Rhizobium* are important microbial inoculants but will not be discussed in this review as they are not free living and, therefore, not considered as PGPR. *Azospirillum* species have been well reported to enhance the growth of legumes and nonlegumes and capable of interacting with other PGPR and mycorrhizae (Bashan 1999). The beneficial impacts of *Pseudomonas* species on growth promotion, drought tolerance, and plant nutrient uptake have been demonstrated (Arshad et al. 2008; Kohler et al. 2008; Sharma et al. 2013). However, the inability of *Pseudomonas* spp. to form durable resistant endospores makes it less attractive compared to *Bacillus* spp. (Adesemoye et al. 2008a) in product formulation, especially products in nonliquid forms. Species of *Trichoderma* and *Penicillium* are common in many of the commercially available inoculants on the market. For example, as shown in Table 2.1, Graph-Ex SA, QuickRoots, and SabrEx are examples of inoculants containing *Trichoderma* sp., while JumpStart and TagTeam LCO contained *Penicillium* sp.

Table 2.1 Some examples of inoculants for crop growth and nutrient use enhancement registered in the United States

Microbial inoculant	Active ingredients	Registered crop	Manufacturer
Accomplish LM	*Acidovorax facilis*, *Bacillus subtilis*, *B. licheniformis*, *B. megaterium*, *B. oleronius*, *B. marinus*, and *Rhodococcus rhodochrous*	Corn, soybean	Loveland Products
AGTIV	*Glomus intraradices*, *Rhizobium leguminosarum* bv. viciae	Field crops, potato, peas, lentils, and faba beans	Premier Tech
Bioboost	*Delftia acidovorans*, *Bradyrhizobium japonicum*	Soybean, pea, and lentil	BrettYoung
Cell-Tec	*Bradyrhizobium japonicum*	Soybean, chickpea, pea and lentil, peanut	Monsanto
Dyna-Start	*Bradyrhizobium japonicum*	Soybean, peanut	Loveland Products
Graph-Ex SA	*Bradyrhizobium japonicum*, *Trichoderma* sp.	Soybean, dry beans	Advanced Biological Marketing
HiStick N/T	*Bacillus subtilis*	Soybean, bean (dry/snap)	BASF

(continued)

Table 2.1 (continued)

Microbial inoculant	Active ingredients	Registered crop	Manufacturer
JumpStart	*Penicillium bilaii*	Chickpea, corn, dry bean, sorghum, soybean, sugar beet, sunflower, wheat	Novozymes
Optimize liquid soybean	*Bradyrhizobium japonicum*	Soybean	Novozymes
QuickRoots	*Bacillus amyloliquefaciens*, *Trichoderma virens*	Alfalfa, corn, sorghum, soybean, sugar beet, sunflower, wheat	Monsanto
Regalia Rx	*Reynoutria* spp.	Corn, soybean	Marrone Bio Innovations
Rhizo-Flo	*Bradyrhizobium japonicum*	Soybean	BASF
SabrEx	*Trichoderma* sp.	Corn, wheat, sorghum, rye, and oats	Advanced Biological Marketing
TagTeam LCO	*Bradyrhizobium japonicum, Penicillium bilaii*	Pea and lentil, soybean, dry bean	Monsanto
Vault SP	*Bradyrhizobium japonicum*	Soybean, peanut	BASF

Note: Products shown on this table are specific examples of inoculants in the market, and only products registered in the United States mostly in Nebraska and/or Alabama are shown
Warning: Authors or publishers are not endorsing or approving any product on this table, and, similarly, the nonappearance of any product does not imply disapproval

There is a volume of knowledge on biological control by many beneficial microbes (Zehnder et al. 2001; Buensanteai et al. 2008; Zhang et al. 2010; Zhou et al. 2016), but scientific information is evolving on the role of microbes in helping plants in nutrient uptake (Adesemoye and Kloepper 2009). One common observation from these studies is that one strain of a microbial species may have biocontrol properties and other strains may be effective in enhancing nutrient uptake, but it is less common to find strains that perform effectively in both capacities. These properties may be related but evidently they are separate. Along the path of IPIM, it would be great to have products with strains that are very effective in both properties or combo products with each component having different organisms that have each of these properties. The process to identify high-performance strains for product development and formulation is dependent on the collection and screening procedure. We concur with Fravel (2005) that there is no single correct way for strain collection and screening procedures as the decision is affected by multiple factors, especially cropping system of interest, but the flowchart presented as Fig. 11.1 by Egamberdieva and Adesemoye (2016) is adaptable to a lot of screening programs with different purposes.

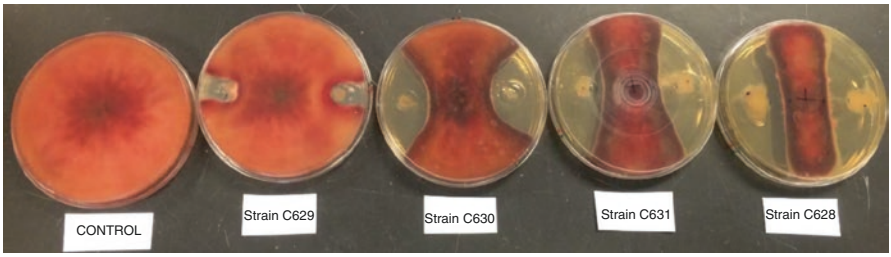

Fig. 2.2 *Burkholderia* sp. antibiosis against *Fusarium graminearum*

It should be added, however, that no screening program should be viewed strictly as a linear process because a precedent level may not correlate with the next level. An example can be seen in Fig. 2.2, where inhibition screening was conducted for four different strains of *Burkholderia* species against one *Fusarium graminearum* by the first author in this review. Based on the inhibition test only, it is correct to conclude with a bigger zone of inhibition, for instance, that strain #4 is more effective than strain #3. However, this trend did not hold true for all the strains after screening with plants in the greenhouse. Therefore, for any screening program to be successful, it should use a collection of data from different screening levels in the flowchart to make a decision of the best strains. One crucial trait that every potential strain to be used in the development of biologicals for nutrient use efficiency or biological control must have though is plant colonization ability. The correlation between root colonization and effectiveness as a PGPR and performance in the field is well established (Zehnder et al. 2001; Vessey 2003; Nelson 2004; Adesemoye and Kloepper 2009). Possible host plant specificity, adaptation to soil types, climatic conditions, and competitive edge against other organisms are some other crucial factors to be considered for any screening process to be effective (Nelson 2004).

Strain C628 has the highest effectiveness and largest zone of inhibition followed by strain C631 and strain C630, while strain C629 has the least effect and barely has any zone of inhibition.

2.4 Potentials of Microbial Inoculants to Reduce Greenhouse Gas Emissions from Fertilizers

Concerns for rising atmospheric concentrations of greenhouse gases (GHG) resulting from human-induced activities continue to be a major issue in the United States and worldwide. Carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) have been implicated as the most significant gases of concern because their radiative forcing potential could impact global climate change. While debate exists within the scientific community, as to the extent these emissions have contributed to global climate change, it is a fact that the Earth's surface temperature has increased about 0.8 °C since 1880, with more than two-thirds of this warming occurring since 1975 (Hansen et al. 2010).

Agricultural activities including crop and soil management practices have been identified as a potential source of GHG emissions. Specifically, N fertilization practices have been noted as the greatest contributor via N loss in the form of N_2O flux. Agricultural N_2O emissions are more than twice that of pre-1940 management and about six times greater than native vegetation (Del Grosso et al. 2005). It is estimated that N-fertilization practices account for approximately 75% of the anthropogenic N_2O flux in the United States (US EPA 2012) making it the largest non-fossil fuel contributor, with crop (~51%) and grazing lands (~21%) being the major contributors. Given that most of the world's population depends on crops supplemented with N fertilizer for food, it is crucial to identify alternative N sources and management practices that reduce GHG emissions (Watts et al. 2015).

Recently, there has been evidence showing that PGPR may have a GHG emission reduction effect (Calvo et al. 2013), which has led to a US Patent (US Patent 9,266,786). These GHG emission reductions have been observed under both laboratory (Calvo et al. 2013, 2016a) and greenhouse conditions (Calvo et al. 2016b). This research evaluated the influence of SoilBuilder, a metabolite extract of SoilBuilder, and a mixture of four strains of PGPR *Bacillus* strains [*Bacillus safensis* T4 (previously called *B. pumilus* T4), *Bacillus pumilus* INR7, *Bacillus subtilis* ssp. subtilis IN937a (previously called *B. amyloliquefaciens* IN937a), and *Lysinibacillus xylanilyticus* SE56 (previously called *B. sphaericus* SE56)]. Nitrous oxide reductions of up to 80% were observed when PGPR inoculants were applied to a N-fertilized soil and sand mixture without plants under laboratory conditions (Calvo et al. 2013, 2016a). Similarly, these microbial inoculants exhibited N_2O reductions under greenhouse conditions with corn (*Zea mays* L.) planted in a N-fertilized soil-sand mixture, reducing N_2O flux up to 50% (Calvo et al. 2016b). Moreover, not only did these microbial inoculants decrease N_2O emissions under greenhouse conditions, improvements in plant growth (roots and aboveground biomass) and nutrient uptake were also observed, implying that nutrient use efficiency was also improved (Calvo et al. 2016b).

The exact mechanism involved in N_2O reduction by the microbial inoculants has not been discerned. However, possible mechanisms involved in the reductions are (1) the production or presence of nitrification inhibitors and inhibition of nitrifying and/or denitrifying microorganisms, (3) competition of applied microbial inoculants with native nitrifiers and/or denitrifying community, and (4) immobilization of fertilizer N and root exudates by microbes (when plants are present). It is important to mention that N_2O emissions were also observed when only the microbial metabolite portion of SoilBuilder product was applied. Calvo et al. (2013) suggested that the microbial metabolite portion of SoilBuilder may contain phenolic compounds that inhibited soil nitrifying and/or denitrifying bacterial communities.

Previous research have shown that N_2O emissions can be impacted differently depending on the N fertilizer source applied. Results from Calvo et al. (2013, 2016a, 2016b) showed that microbial inoculant effects on N_2O emissions are also impacted differently by N fertilizer source. For instance, urea-ammonium nitrate and calcium-ammonium nitrate reduced N_2O emissions, while no effect was observed with urea, and ammonium nitrate increased emissions (Calvo et al. 2013, 2016a, 2016b).

These previous observations from Calvo et al. work suggest that microbial inoculants have promise for reducing agriculture's GHG emission footprint; however, its impact may be dependent on the N fertilizer type used. It is believed that adaption and adoption of microbial inoculants into commercialized products as a N_2O-reducing agent will increase in the coming years once research and development for this new technology's use is refined.

2.5 Better Use of Nitrogen from Fertilizers and the Impact on Nitrous Oxide

Nitrogen is a limiting nutrient which is essential to optimize yield of most cropping systems, and this has resulted in the consumption of nitrogen increasing faster than that of any other plant nutrient source since the 1960s (USDA ERS 2012). This is contributing to rising atmospheric N_2O emissions as nitrous oxide emissions have been correlated with increasing N rates (Halvorson et al. 2014; Snyder et al. 2009), but impacts can vary depending on fertilizer source (Halvorson et al. 2010; Venterea et al. 2010; Sistani et al. 2011). Although N_2O levels reaching the atmosphere are minuscule compared to CO_2, its radioactive forcing is 298 times greater (Myhre et al. 2013) making it a major player in the total GHG emission budget. Abatement strategies to minimize and mitigate N input effects on global warming potential are thus essential.

Soil N_2O production primarily occurs through nitrification and denitrification processes (Firestone and Davidson 1989). Nitrification has been identified as the primary source of N_2O in many aerobic soils and denitrification under anaerobic conditions (Bremner 1997; Dell et al. 2014). Denitrification occurs in anaerobic microsites within partially aerated soils which contribute to the N_2O loss budget (Parkin 1987; Parkin and Kaspar 2006). N-management practices are needed that can better synchronize N supply with crop demand, govern nitrification and denitrification processes, and increase plant N uptake to reduce the potential for environmental loss.

Current fertilizer management practices often exceed plant N needs, where an excess is applied as insurance for crop production (Fig. 2.1). As a result, estimated worldwide N-use efficiency is 20–50% in most agricultural systems with the excess being susceptible to loss through runoff, leaching, volatilization, and N_2O emissions. Consequently, recent fertilizer advancements have facilitated enhanced formulation development for reducing N release rates to soil in efforts to minimize N loss. These enhanced efficiency nitrogen fertilizers (EENFs) are categorized as slow-release, control-release, and/or stabilized N fertilizers (Halvorson et al. 2014) that minimize early season N availability when crop uptake is slow, thereby reducing the loss potential (Akiyama et al. 2010). Some of these EENFs contain chemical formations with nitrification inhibitors to reduce the potential for N_2O emissions.

Recently, there has also been a great interest in the development and implementation of agricultural greenhouse gas (GHG) reduction offset protocols that can be included in cap and trade markets (Millar et al. 2010). Direct strategies or technologies for N_2O reduction are limited, but are expected to include nitrification inhibitors

and slow-release fertilizers (Mosier et al. 1998; Singh and Verma 2007) which have had mixed or inconsistent results. However, none of these strategies have included the application of microorganisms, which could play an important role in N_2O reduction by interacting with the native N-cycle microbes. Soil microorganisms are responsible for the mineralization, immobilization, nitrification, and denitrification processes.

Hence, manipulating native soil microbial communities by application of selected inoculants with specific microorganisms can potentially alter N_2O emissions from the soil. Bacteria can transform gaseous nitrogen into ammonia, which can directly be used by plants. It has been well demonstrated that co-inoculation of *Bacillus* PGPR and mycorrhizae or their individual inoculations could enhance nitrogen and phosphorus uptakes in the field (Adesemoye et al. 2008b). The success of inoculants to stimulate uptake of nutrient is affected by many factors, one of which is soil type. Egamberdieva (2007) demonstrated that inoculant did not show significant effects in loamy soil whereas the strains had significant impacts on nutrient uptake in a nutrient-deficient calcisol soil.

2.6 Better Use of Phosphorus from Fertilizers

Soils contain large reserves of P but significant parts of it are not soluble or available for plant uptake (Watts et al. 2010). There are many bacteria in the soil and plant rhizosphere reported to have the capacity to solubilize inorganic phosphates (dicalcium phosphate, tricalcium phosphate, hydroxyapatite, and rock phosphate) or to mineralize organic phosphates, thus making P available to plants; *Bacillus* has been reported as one of the most active groups (Rodriguez and Fraga 1999). Hydrolysis or mineralization of organic phosphates to inorganic forms is carried out through phosphatases. Organic acids produced by bacteria are able to bind P and extracellular phosphatases and release P from organophosphates, making it available to plants. Synergistic interactions of phosphate-solubilizing PGPR and mycorrhizal fungi have been demonstrated (Rodriguez and Fraga 1999; Barea et al. 2002) where mycorrhiza plays a role in increasing the population of phosphate-solubilizing PGPR and the extraradical mycelium acts as a bridge for making phosphorus that was solubilized from nonsoluble inorganic and organic P compounds by the PGPR available.

Vassileva et al. (2010) explained that mineralization of lignocellulosic agro-industrial wastes by microbial processes and simultaneous solubilization of inorganic insoluble phosphates will provide the plant with an organic amendment rich in polysaccharide compounds and make P and nutrients available to plants but could also enhance soil enzyme activities and quality. In this system, more efficiency was reported with association of arbuscular mycorrhizal (AM) fungi with P-solubilizer/agro-waste-amended treatments. In the effort to understand how inoculants can affect the use of phosphorus by alfalfa, Piccini and Azcon (1987) inoculated three different endomycorrhizal fungi—*Glomus mosseae*, *G. fasciculatum*, and *Glomus* sp.—with or without co-inoculation of phosphate-solubilizing bacteria (PSB) in the presence or absence of Bayovar rock phosphate. The researchers reported that in the presence of Bayovar rock phosphate, PSB increased the dry weight of alfalfa in all

inoculated treatments but dual inoculation of PSB and mycorrhizal fungi stimulated alfalfa dry weight more than either organism alone. The results also showed that alfalfa plants reached the maximum yield in the presence of rock phosphate plus PSB and mycorrhizal colonization.

There is substantial evidence that P-solubilizing bacteria have great potentials for the inoculant sector. What is needed is further investigation to improve their performance and develop them for compatibility with other bacteria and/or mycorrhizae for effective co-inoculation and practical application to enhance nutrient uptake in agricultural systems. For this to happen, each lead P-solubilizing strain must be well studied in terms of their survival and establishment, stability of their P-solubilization trait following inoculation, and their competitiveness and resilience in the soil system. Adequate phosphorus (P) availability for plants stimulates early plant growth, proper maturity, and consequently good yield. As a limiting nutrient, too little P will hinder plant development, and too much will contribute to agro-environmental pollution through leaching or runoff with surface water and contribute to eutrophication (Adesemoye and Kloepper 2009). Effective use of inoculants in an IPIM would help ensure application of appropriate amounts of P, which will be less than current recommended amounts, and ensure availability of adequate amount to plants.

2.7 Examples of Microbial Products on the Market for Optimized Nutrient Use

The interest of consumers, farmers, and research information in recent years has been driving the evolution and development of the biorational sector in the agriculture industry. The factors, among others, include (1) growers/farmers demand for information on alternative strategies and products that can help ensure better and sustainable use of soil resources, (2) how to reduce the dependence on chemical inputs, (3) development of resistance by pathogens and pests against pest control chemicals, and (4) there is increasing consumers' interest in foods that are free from chemical residues. The agricultural industry has exploded in the last few years with many new inoculant products or fortification of existing products. There has been a lot of investment going into the sector, and this includes the big six pesticide companies—Bayer, BASF, Dow Chemical Company, DuPont, Monsanto, and Syngenta. Some specific examples of inoculant products on the market that are registered nationally or in certain states in the United States are provided in Table 2.1. Readers should note that the appearance of any product on this list does not imply endorsement by the authors or the publishers, and, similarly, the nonappearance of any product does not imply disapproval.

Conclusion

There are indications that the inoculant market will continue to grow and expand. There is a correlation between inoculant and organic production and demand of customers for organic products.

Progress is being made in understanding the role of microbes in nutrient use, but how to translate the volume of scientific information into practical field applications is still elusive. One of the reasons for this is that most studies were based on the impact of individual strains on plants, but field situations are far more complex, with many organisms interacting with the plant at the same time. Future studies need to address the interactions of multiple beneficial microorganisms with plants concurrently to provide better understanding of the complexity of microbial interactions in plant nutrient utilization and make findings more applicable in the field.

One of the challenges with inoculants is the inconsistent results under different conditions. Acceptance of these products by farmers is increasing, but there are concerns from farmers that the efficacy of many of the products currently available on the market is not consistent. Results from university studies have confirmed this concern on many occasions. Efficacy is improving and there is optimism that improvement will continue with more investments in related research. Molecular technology, especially metagenomics, has been evolving and helping to understand the complex interactions that occur in the soil-plant systems, especially the root microbiome.

Another concern is that products may not be compatible with existing chemicals and/or farming practices. What is the worth of a new agricultural product that is not compatible with common agricultural practices or cannot be delivered with equipment that are commonly used by farmers? Compatibility and efficacy are determined in part by the form in which the product is made available and applied. Is the product going to be coated onto the seed before going to the market or mixed with the seed shortly before planting? Is the inoculum to be delivered in-furrow onto the seed during planting or later onto the seedling? These are considerations that should be paramount in the formulation processes for a new product or improvement of an existing product.

Recent major investments in the biological sector are an indication of the role that these products will play in crop production going forward. Following many acquisitions, realignments, new ventures, and launch of new product platforms in this sector, market watchers have projected that the biological sector should experience double-digit growth between now and 2020 from the current estimated $2 billion market, and inoculants and biostimulants will be a crucial part of this growth. The investment in research is increasing and no doubt, this will improve efficacy. The interests in inoculants and overall biology-based technology will continue to expand as it is good for conventional agriculture as well as organic farming.

References

Adesemoye AO, Kloepper JW (2009) Plant–microbes interactions in enhanced fertilizer use efficiency. Appl Microbiol Biotechnol 85:1–12

Adesemoye AO, Obini M, Ugoji EO (2008a) Comparison of plant growth-promotion with *Pseudomonas aeruginosa* and *Bacillus subtilis* in three vegetables. Braz J Microbiol 39:423–426

Adesemoye AO, Torbert HA, Kloepper JW (2008b) Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Can J Microbiol 54:876–886
Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929
Akiyama H, Yan X, Yagi K (2010) Evaluation of effectiveness of enhanced-efficiency fertilizers as mitigation options for N2O and NO emissions from agricultural soils: meta-analysis. Glob Chang Biol 16:1837–1846
Arshad M, Shaharoona B, Mahmood T (2008) Inoculation with *Pseudomonas* spp. containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (*Pisum sativum* L.) Pedosphere 18:611–620
Artursson V, Finlay RD, Jansson JK (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ Microbiol 8(1):1–10
Barea JM, Azcón R, Azcón-Aguilar C (1993) Mycorrhiza and crops. In: Tommerup I (ed) Advances in plant pathology, vol 9: Mycorrhiza: a synthesis. Academic, London, pp 167–189. ISBN: 84-604-7996-X
Barea JM, Azcon R, Azcon-Aguilar C (2002) Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie van Leeuwenkoek 81:343–351
Bashan Y (1999) Interactions of *Azospirillum* spp. in soils: a review. Biol Fertil Soils 29:246–256
Bashan Y, Holguin G (1998) Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. Soil Biol Biochem 30:1225–1228
Biswas JC, Ladha JK, Dazzo FB (2000) Rhizobia inoculation improves nutrient uptake and growth of lowland rice. Soil Sci Soc Am J 64:1644–1650
Bremner JM (1997) Sources of nitrous oxide in soils. Nutr Cycl Agroecosyst 49:7–16
Buensanteai N, Yuen GY, Prathuangwong S (2008) The biocontrol bacterium *Bacillus amyloliquefaciens* KPS46 produces auxin, surfactin and extracellular proteins for enhanced growth of soybean plant. Thai J Agric Sci 41:101–116
Calvo P, Watts DB, Ames RN, Kloepper JW, Torbert HA (2013) Microbial-based inoculants impact nitrous oxide emissions from an incubated soil medium containing urea fertilizers. J Environ Qual 42:704–712
Calvo P, Nelson L, Kloepper JW (2014) Agricultural uses of plant biostimulants. Plant Soil 383:3–41
Calvo P, Watts DB Kloepper JW, Torbert HA (2016a) Application of microbial-based inoculants for reducing N_2O emissions from soil under two difference ammonium nitrate-based fertilizers. Soil Science. 181:427–434
Calvo P, Watts DB, Kloepper JW, Torbert HA (2016b) Microbial-based inoculants influence on N_2O emissions from soil planted to corn (*Zea mays* L.) under greenhouse conditions with different nitrogen fertilizer regimens. Can J Microbiol 62:1041–1056
Canbolat M, Bilen S, Çakmak R, Şahin F, Aydın A (2006) Effect of plant growth-promoting bacteria and soil compaction on barley seedling growth, nutrient uptake, soil properties and rhizosphere microflora. Biol Fert Soils 42:350–357
Chabot R, Antoun H, Cescas M (1996) Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar *phaseoli*. Plant Soil 184:311–321
Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospect. Appl Environ Microbiol 71:4951–4959
Cook RJ (2002) Advances in plant health management in the twentieth century. Ann Rev Phytopathol 38:95–116
de Freitas JR, Banerjee MR, Germida JJ (1997) Phosphatesolubilizing rhizobacteria enhance the growth and yield but not phosphorus uptake of canola (*Brassica napus*). Biol Fertil Soils 36:842–855
Del Grosso SJ, Mosier AR, Parton WJ, Ojima DS (2005) DAYCENT model analysis of past and contemporary soil N2O and net greenhouse gas flux for major crops in the USA. Soil Tillage Res 83:9–24
Dell CJ, Han K, Bryant RB, Schmidt JP (2014) Nitrous oxide emissions with enhanced efficiency nitrogen fertilizers in a rainfed system. Agron J 106:723–731

Egamberdieva D (2007) The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl Soil Ecol 36:184–189
Egamberdieva D, Adesemoye AO (2016) Improvement of crop protection and yield in hostile agroecological conditions with PGPR-based biopesticide and biofertilizer formulations. In: Arora NK, Mehnaz S, Balestrini R (eds) Bioformulations for sustainable agriculture. Springer, pp 199–211. doi:10.1007/978-81-322-2779-3_11
Figueiredo M, Seldin L, Araujo F, Mariano R (2010) Plant growth promoting rhizobacteria: fundamentals and applications. In: Maheshwari DK (ed) Plant growth and health promoting bacteria. Springer, Berlin, pp 21–43
Firestone MK, Davidson EA (1989) Microbial basis of NO and N2O production and consumption in soil. In: Andreae MO, Schimel DS (eds) Exchange of trace gases between terrestrial ecosystems and the atmosphere. Wiley, New York, pp 7–21
Fravel DR (2005) Commercialization and implementation of biocontrol. Annu Rev Phytopathol 43:337–359
Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117
Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnol Adv 15:353–378
Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007) Promotion of plant growth by bacterial ACC deaminase. Crit Rev Plant Sci 26:227–242
Gruhn P, Golletti F, Yudelman M (2000) Integrated nutrient management, soil fertility, and sustainable agriculture: current issues and future challenges. A 2020 vision for food, agriculture, and the environment, discussion paper 32. International Food Policy Research Institute, Washington, pp 15–16
Haas D, Defago G (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319
Halvorson AD, Del Grosso SJ, Alluvione F (2010) Nitrogen source effects on nitrous oxide emissions from irrigated no-till corn. J Environ Qual 39:1554–1562
Halvorson AD, Snyder CS, Blaylock AD, Del Grosso SJ (2014) Enhanced-efficiency nitrogen fertilizers: potential role in nitrous oxide emission mitigation. Agron J 106:715–722
Hansen J, Ruedy R, Sato M, Lo K (2010) Global surface temperature change. Rev Geophys 48:4. doi:10.1029/2010RG000345
Hussain A, Arshad M, Hussain E (1987) Response of maize (Zea mays) to Azotobacter inoculation under fertilized and unfertilized conditions. Biol Fert Soils 4:73–77
Idriss EE, Makarewicz O, Farouk A, Rosner K, Greiner R, Bochow H, Richter T, Borriss R (2002) Extracellular phytase activity of Bacillus amyloliquefaciens FZB45 contributes to its plant-growth-promoting effect. Microbiology 148:2097–2109
Joo G-J, Kim Y-M, Lee IJ, Song KS, Rhee IK (2004) Growth promotion of red pepper plug seedlings and the production of gibberellins by *Bacillus cereus*, *Bacillus macroides*, and *Bacillus pumilus*. Biotechnol Lett 26:487–491
Kloepper JW, Lifshitz R, Zablotowicz RM (1989) Free living bacterial inocula for enhancing crop productivity. Trends Biotechnol 7:39–44
Kloepper JW, Zablotowick RM, Tipping EM, Lifshitz R (1991) Plant growth promotion mediated by bacterial rhizosphere colonizers. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer Academic Press, Dordrecht
Kohler J, Hernandez JA, Caravaca F, Roldan A (2008) Plant growth-promoting rhizobacteria and arbuscular mycorrhizal fungi modify alleviation biochemical mechanisms in waterstressed plants. Funct Plant Biol 35:141–151
Kumar KVK, Reddy MS, Kloepper JW, Lawrence KS, Yellareddygari SKR, Zhou XG, Sudini H, Surrendranatha Reddy EC, Groth DE, Miller ME (2011) Screening and selection of elite plant growth promoting rhizobacteria (PGPR) for suppression of *Rhizoctonia solani* and enhancement of rice seedling vigor. J Pure Appl Microbiol 5(2):641–651
Lim J-H, Kim S-D (2013) Induction of drought stress resistance by multi-functional PGPR *Bacillus licheniformis* K11 in pepper. Plant Pathol J 29:201–208
Mandic-Mulec I, Prosser JI (2011) Diversity of endospore-forming bacteria in soil: characterization and driving mechanisms. In: Logan NA, Vos PD (eds) Endospore-forming soil bacteria. Springer, London

Millar N, Robertson G, Grace P, Gehl R, Hoben J (2010) Nitrogen fertilizer management for nitrous oxide (N2O) mitigation in intensive corn (Maize) production: an emissions reduction protocol for US Midwest agriculture. Mitig Adapt Strateg Global Change 15:185–204

Mosier AR, Delgado JA, Keller M (1998) Methane and nitrous oxide fluxes in an acid Oxisol in western Puerto Rico: effects of tillage, liming and fertilization. Soil Biol Biochem 30:2087–2098

Myhre G, Shindell D, Bréon F-M, Collins W, Fuglestvedt J, Huang J, Koch D, Lamarque J-F, Lee D, Mendoza B, Nakajima T, Robock A, Stephens G, Takemura T, Zhang H (2013) Anthropogenic and natural radiative forcing. In: Stocker TF, Qin D, Plattner G-K, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds) Climate change 2013: the physical science basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Nelson LM (2004) Plant growth promoting rhizobacteria (PGPR): prospects for new inoculants. Online Crop Manag. doi:10.1094/CM-2004-0301-05-RV

Okon Y, Kapulnik Y (1986) Development and function of Azospirillum-inoculated roots. Plant Soil 90:3–16

Omar MNA, Mahrous NM, Hamouda AM (1996) Evaluating the efficiency of inoculating some diazotrophs on yield and protein content of 3 wheat cultivars under graded levels of nitrogen fertilization. Ann Agric Sci 41:579–590

Parkin TB (1987) Soil microsites as a source of denitrification variability. Soil Sci Soc Am J 51:1194–1199

Parkin TB, Kaspar TC (2006) Nitrous oxide emissions from corn-soybean systems in the Midwest. J Environ Qual 35:1496–1506

Parr JF, Papendick RI, Hornick SB, Meyer RE (1992) Soil quality: attributes and relationship to alternative and sustainable agriculture. Am J Altern Agric 7:5–11

Piccini D, Azcon R (1987) Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizal fungi on the utilization of Bayovar rock phosphate by alfalfa plants using a sand-vermiculite medium. Plant Soil 101:45–50

Probanza A, Lucas JA, Acero N, Gutierrez Mañero FJ (1996) The influence of native rhizobacteria on european alder (*Alnus glutinosa* (L.) Gaertn.) growth. Part I. Characterization of growth promoting and inhibiting bacterial strains. Plant Soil 182:59–66

Reid A, Greene SE (2012) How microbes can help feed the world. Report of an American Academy of Microbiology Colloquium, p 3

Requena N, Jimenez I, Toro M, Barea JM (1997) Interactions between plant-growth-promoting rhizobacteria (PGPR), arbuscular mycorrhizal fungi and *Rhizobium* spp. in the rhizosphere of *Anthyllis cytisoides*, a model legume for revegetation in mediterranean semi-arid ecosystems. New Phytol 136:667–677

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Schenck NC (1981) Can mycorrhizae control root diseases? Plant Dis 65:230–234

Sharma A, Shankhdar D, Shankhdhar SC (2013) Enhancing grain iron content of rice by the application of plant growth promoting rhizobacteria. Plant Soil Environ 59:89–94

Shen J, Li C, Mi G, Li L, Yuan L, Jiang R, Fuso F (2012) Maximizing root/rhizosphere efficiency to improve crop productivity and nutrient use efficiency in intensive agriculture of China. J Exp Bot 64:1181–1192

Sheng XF, He LY (2006) Solubilization of potassium-bearing minerals by a wild-type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. Can J Microbiol 52:66–72

Singh SN, Verma A (2007) Environmental review: the potential of nitrification inhibitors to manage the pollution effect of nitrogen fertilizers in agricultural and other soils: a review. Environ Pract 9:266–279

Sistani KR, Jn-Baptiste M, Lovanh N, Cook KL (2011) Atmospheric emissions of nitrous oxide, methane, and carbon dioxide from different nitrogen fertilizers. J Environ Qual 40:1797–1805

Snyder CS, Bruulsema TW, Jensen TL, Fixen PE (2009) Review of greenhouse gas emissions from crop production systems and fertilizer management effects. Agric Ecosyst Environ 133:247–266

Someya N, Akutsu K (2006) Biocontrol of plant diseases by genetically modified microorganisms: current status and future prospects. In: Siddiqui Z (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 297–312

Stephens JHG, Rask HM (2000) Inoculant production and formulation. Field Crop Res 65:249–258

The Office of Technology (1979) Pest management strategies, vols 1 and 2. U.S. Government Printing Office, Washington

Tien TM, Gaskins MH, Hubbell DH (1979) Plant growth substances produced by *Azospirillum brasilense* and their effect on the growth of pearl millet (*Pennisetum americanum* L.) Appl Environ Microbiol 37:1016–1024

US Environmental Protection Agency (2012) Inventory of U.S. greenhouse gas emissions and sinks: 1990–2010. EPA 430-R-12-001. U.S. Environmental Protection Agency, Washington. http://www.epa.gov/climatechange/ghgemissions/usinventoryreport.html

USDA Economic Research Service (2012) Fertilizer use and markets: 1960–2011. USDA Economic Research Service, Washington. http://www.ers.usda.gov/topics/farm-practices-management/chemical-inputs/fertilizer-use-markets.aspx

Vardharajula S, Ali SZ, Grover M, Reddy G, Bandi V (2011) Drought-tolerant plant growth promoting Bacillus spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. J Plant Interact 6:1–14

Vassileva M, Serrano M, Bravo V, Jurado E, Nikolaeva I, Martos V, Vassilev N, (2010) Multifunctional properties of phosphate-solubilizing microorganisms grown on agro-industrial wastes in fermentation and soil conditions. Applied Microbiology and Biotechnology 85(5):1287–1299

Venterea RT, Dolan MS, Ochsner TE (2010) Urea decreases nitrous oxide emissions compared with anhydrous ammonia in a Minnesota corn cropping systems. Soil Sci Soc Am J 74:407–418

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Volpin H, Phillips DA (1998) Respiratory elicitors from *Rhizobium meliloti* affect intact alfalfa roots. Plant Physiol 116:777–783

Watts DB, Torbert HA, Prior SA, Huluka G (2010) Long-term tillage and poultry litter impacts soil carbon and nitrogen mineralization and fertility. Soil Sci Soc Am J 74:1239–1247

Watts DB, Runion GB, Smith Nannenga KW, Torbert HA (2015) Impact of enhanced efficiency nitrogen fertilizer on greenhouse gas emissions in a coastal plain soil under cotton. J Environ Qual 44(6):1699–1710

Wu SC, Caob ZH, Lib ZG, Cheunga KC, Wonga MH (2005) Effects of biofertilizer containing N-fixer, P and K solubilizers and AM fungi on maize growth: a greenhouse trial. Geoderma 125:155–166

Yanni YG, Rizk RY, Corich V, Squartini A, Ninke K, Philip-Hollingsworth S, Orgambide F, de Bruijn F, Stoltzfus J, Buckley D, Schmidt TM, Mateos PF, Ladha JK, Dazzo FB (1997) Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. Plant Soil 194:99–114

Yildirim E, Taylor AG, Spittler TD (2006) Ameliorative effects of biological treatments on growth of squash plants under salt stress. Sci Hortic 111:1–6

Zehnder GW, Murphy JF, Sikora EJ, Kloepper JW (2001) Application of rhizobacteria for induced resistance. Eur J Plant Pathol 107:39–50

Zhang S, White KL, Martinez MC, McInroy JA, Kloepper JW, Klassen W (2010) Evaluation of plant growth-promoting rhizobacteria for control of Phytophthora blight on squash under greenhouse conditions. Biol Control 53:129–135

Zhou L, Yuen G, Wang Y, Wei L, Ji G (2016) Evaluation of bacterial biological control agents for control of rootknot nematode disease on tomato. Crop Prot 84:8–13

The Legume Nodule Microbiome: A Source of Plant Growth-Promoting Bacteria

3

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Abstract

Legume nodules harbour two types of bacteria, the rhizobia, responsible for their formation, and other endophytic bacteria whose role in the nodule is still poorly known. These bacteria constitute the nodule microbiome from which the rhizobia have been widely studied for decades, whereas the nodule endophytes have been started to be studied in the last years. These studies showed a more complex

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bacterial composition than previously thought, including bacteria from very different phylogenetic groups. Unlike other plant microbiomes, which have been widely studied by metagenomic techniques, the nodule microbiomes have been basically studied by culture-dependent methods because the main objective of the legume nodule studies is the selection of plant growth-promoting bacteria to be used in agronomic practices in a sustainable agriculture context. In this chapter we revise the groups of bacteria found to date in legume nodules that present in vitro mechanisms of plant growth promotion, with special emphasis in those that are able to promote the plant growth in plant assays.

3.1 Introduction: The Legume Nodule Microbiome

Legumes constitute a large group of plants included in a wide family named Fabaceae or Leguminosae, which contains three subfamilies Faboideae (or Papilionoideae), Mimosoideae and Caesalpinioideae. The legumes have been used as foods since ancient times, and the benefits of including legumes in the human diet are due to their rich content in proteins, some of them of high nutritional quality (Tharanathan and Mahadevamma 2003; Williams et al. 2008; Rebello et al. 2014), and also to their content in bioactive compounds (Silva et al. 2016). After cereals, legumes constitute the second most important food for humans, and, combined, they may contain all the necessary amino acids for a healthy human nutrition (Ejigui et al. 2007; Paul et al. 2008) and are used in crop rotation and intercropping practices (Bedoussac et al. 2015). The benefit of the use of legumes in rotation or intercropping schemes is due to their low dependence of N supply for their ability to obtain it directly from the atmosphere (Remigi et al. 2016).

The presence of nodules in roots of legumes is known since the seventeenth century through the draws of Malpighi, who thought that they were insect galls. Hellriegel and Wilfarth at the end of the nineteenth century established that legume nodules were responsible for nitrogen fixation suggesting that their formation should be related with some soil agents, since nodules only were formed when peas (*Pisum*) were cultivated in substrates containing raw soil (revised by Leigh 2004). Beijerinck in 1888 obtained for the first time a bacterium from nodules of *Vicia*, which was initially named *Bacillus radicicola* (Beijerinck 1888) and later renamed as *Rhizobium leguminosarum* (Frank 1889), and since then, the bacteria nodulating legumes were generically called rhizobia. Currently, we know that rhizobia are able to induce the formation of nodules through a complex process involving several symbiotic genes (Remigi et al. 2016) carrying out the fixation of atmospheric nitrogen after their conversion in bacteroids (Haag et al. 2013; Ren et al. 2011).

Since the description of *R. leguminosarum*, many genera and species of bacteria have been isolated from root or stem legume nodules (revised by Peix et al. 2015b). Those described before year 2000 are commonly considered as classic rhizobia and were placed in the alpha subdivision (class) of *Proteobacteria* (Woese et al. 1984) being currently distributed in several families and genera in the Bergey's

Manual of Systematic Bacteriology (Kuykendall et al. 2005). In the first years of the twenty-first century, it was discovered that other *Alphaproteobacteria*, different to the classic rhizobial genera, and that some bacterial species belonging to *Betaproteobacteria* are also able to nodulate different legumes (revised by Peix et al. 2015b).

During more than one century, only the bacteria able to induce the nodules were studied, and all bacteria not having the macroscopic morphology of rhizobia were discarded as external contaminants; however, in the last decade, the researchers have been paying attention to other bacteria living within the nodules, which are named nodule endophytes. They can enter the inner of nodules together with the rhizobia as has been recently observed in *Vigna* nodules by confocal microscopy (Pandya et al. 2013). To date, the role of these bacteria has been poorly studied, but it has been recently reported that endophyte accommodation within legume nodules is also under host genetic control (Zgadzaj et al. 2015).

Both endosymbionts and endophytes constitute the legume nodule microbiome which can be analysed by culture-dependent and by metagenomic techniques, which to date have been mostly used to detect rhizobia in nodules for which they were not isolated (Muresu et al. 2008) or to confirm the ability of rhizobia to nodulate a legume that is not its common partenaire, as occurs in the case of *R. leguminosarum* symbiovar trifolii, which is able to nodulate *Cicer canariense* (Martínez-Hidalgo et al. 2015a). Nevertheless, it is predictable that in the future, the metagenomic techniques will be widely used to analyse legume nodule microbiomes, and they should be based not only in sequencing of core genes, such as the 16S rRNA gene, but also in that of the nodulation genes allowing us to know what bacteria from the set of nodule microbiome was responsible for the nodule formation, which we named endosymbionts, and what are nodule endophytes.

Currently, the legume nodule microbiomes are analysed by culture-dependent techniques because the analysis of legume nodules bacteria is usually focused on the selection of rhizobia and plant growth-promoting endophytes to design biofertilizers. The culture-dependent techniques (culturomics) have allowed the description of many new species of both rhizobia and endophytes as well as the knowledge of their ability to promote the growth of legumes and nonlegumes. In this chapter we review the most relevant milestones and the current advances in the study of the bacteria constituting the legume nodule microbiomes with special emphasis in their abilities to promote the plant growth of both legumes and nonlegumes.

3.2 Mechanisms of Plant Growth Promotion

Bacteria can promote plant growth through different direct and indirect mechanisms (Lugtenberg and Kamilova 2009) and are currently called plant probiotics (Berlec 2012). Several of them are found inside legume nodules as endosymbionts or as endophytes, and they presented different in vitro mechanisms of plant growth promotion, including the production of phytohormones, such as indoleacetic acid, ACC

deaminase and siderophores, the nitrogen fixation and the phosphate solubilization (García-Fraile et al. 2012; Velázquez et al. 2013).

The indoleacetic acid (IAA) is a phytohormone involved in plant growth promotion produced by diverse organisms that modulates plant growth and development (Duca et al. 2014). The production of this auxin has been reported for classic rhizobia of genera *Rhizobium* (Datta and Basu 2000; Bhattacharjee et al. 2012; García-Fraile et al. 2012; Flores-Félix et al. 2013b; Kumar and Ram 2012), *Ensifer* (formerly *Sinorhizobium*) (Bianco and Defez 2010; Dubey et al. 2010), *Mesorhizobium* (Wdowiak-Wróbel and Małek 2016), *Bradyrhizobium* (Boiero et al. 2007; Valdez et al. 2016) and *Allorhizobium* (Ghosh et al. 2015), for *Burkholderia* nodulating *Mimosa* (Pandey et al. 2005) and for legume nodule endophytes (Palaniappan et al. 2010; Aserse et al. 2013; Lin et al. 2013; Saïdi et al. 2013; Tariq et al. 2014; Flores-Félix et al. 2015b; Khalifa and Almalki 2015; Pandya et al. 2015; Subramanian et al. 2015; de Almeida Lopes et al. 2016).

The ACC deaminase cleaves 1-aminocyclopropane-1-carboxylate (ACC), precursor of ethylene, into ammonia and α-ketobutyrate lowering the levels of ethylene and allowing an increase of the plant resistance to environmental stresses (Glick 2005). The production of ACC deaminase has been reported for classic rhizobia (Othman and Tamimi 2016; Valdez et al. 2016), for *Burkholderia* nodulating *Mimosa* (Pandey et al. 2005) and for several legume nodule endophytes (Palaniappan et al. 2010; Lin et al. 2013; Schwartz et al. 2013; Tariq et al. 2014; Subramanian et al. 2015). Thus, endophytic bacteria contribute positively to prevent plant stresses and to promote plant growth (Glick 2014; Santoyo et al. 2016), particularly in the case of legumes (Nascimento et al. 2016).

Nutrient mobilization involves, among other mechanisms, atmospheric nitrogen fixation, which is carried out by symbiotic and free-living bacteria through a family of enzymes named nitrogenases (Hoffman et al. 2014). Classic and new rhizobia are legume endosymbionts specialized in the symbiotic nitrogen fixation within legume nodules (Remigi et al. 2016), but free-living nitrogen-fixing bacteria have been reported as nodule endophytes (Zakhia et al. 2006; Li et al. 2008; Aeron et al. 2015; Flores-Félix et al. 2015b; Subramanian et al. 2015).

Phosphate solubilization is one of the most studied mechanisms of plant growth promotion, and the ability of rhizobia to solubilize phosphate is well known since the past century (Rodríguez and Fraga 1999), and within them, *Mesorhizobium* species are the most active phosphate solubilizers in vitro (Peix et al. 2001; Rivas et al. 2006; Verma et al. 2013; Imen et al. 2015; Wdowiak-Wróbel and Małek 2016). Nevertheless, there are several works reporting this ability for strains belonging to other classic rhizobial genera, such as *Rhizobium* (Chabot et al. 1996a; Antoun et al. 1998; Yanni et al. 2001; Alikhani et al. 2006; Abril et al. 2007; Sridevi et al. 2007; Flores-Félix et al. 2013b; Ramírez-Bahena et al. 2015; Othman and Tamimi 2016), *Ensifer* (formerly *Sinorhizobium*) (Ormeño et al. 2007; Villar-Igea et al. 2007), and for endosymbiotic *Burkholderia* (Angus et al. 2013). Also, the ability to solubilize phosphate has been widely found in legume nodule endophytes (Palaniappan et al. 2010; Rajendran et al. 2012; Aserse et al. 2013; Saïdi et al.

2013; Tariq et al. 2014; Flores-Félix et al. 2015b; Khalifa and Almalki 2015; Pandya et al. 2015; Saini et al. 2015).

Siderophore production is a plant growth mechanism present in several bacteria which enhances the Fe uptake by plants and facilitates the biocontrol against phytopathogens (Saha et al. 2013). This plant growth mechanism has been studied in *Rhizobium* (Chabot et al. 1996a; García-Fraile et al. 2012; Flores-Félix et al. 2013b; Wright et al. 2013), *Mesorhizobium* (Datta and Chakrabartty 2014), *Bradyrhizobium* (Antoun et al. 1998; Valdez et al. 2016), *Ensifer* (formerly *Sinorhizobium*) (Lynch et al. 2001) and the endosymbiont *Burkholderia* (Pandey et al. 2005; Angus et al. 2013). Some endophytic bacteria have been reported as siderophore producers (Palaniappan et al. 2010; Deng et al. 2011; Rajendran et al. 2012; Aserse et al. 2013; Saïdi et al. 2013; Flores-Félix et al. 2015b; Pandya et al. 2015).

All these studies showed that both rhizobia and nodule endophytic bacteria exhibit several in vitro plant growth promotion mechanisms rendering them as good candidates to be used as biofertilizers in agricultural crops. Nevertheless, not all strains having in vitro plant growth promotion mechanisms are able to promote the growth of plants (Montañez et al. 2012), and conversely, strains without such mechanisms are able to enhance the plant growth (Smyth et al. 2011). Therefore, the final selection of bacterial strains for biofertilization schemes must be done in plant assays and only including strains safe for humans, animals, plants and the environment (García-Fraile et al. 2012).

3.3 The Legume Nodule Endosymbionts

As was previously mentioned, it was the microbiologist Beijerinck in 1888 who isolated a bacterium from a *Vicia* nodule which was later named *Rhizobium leguminosarum* (Frank 1889). This discovery started the research on bacteria responsible for the nodule formation in legumes with the main interest focusing on their ability to fix atmospheric nitrogen. The first works studying the ability of the rhizobia to nodulate different legumes (Nobbe et al. 1895; Wilson 1939) concluded that both rhizobia and legumes present different degree of promiscuity. These cross-inoculation assays were the basis of the description of new species within the genus *Rhizobium* according to the legume they nodulated, leading Baldwin and Fred (1929) to establish the rhizobial species nomenclature based on the cross-nodulation groups.

In 1984 the concept of rhizobial species changed drastically when the 16S rRNA gene analysis placed the rhizobia within the alpha subdivision of *Proteobacteria*, and this gene became the basis of species classification (Woese et al. 1984). In the same year, Jordan (1984) also introduced the concept of biovar (currently symbiovar) linked to the ability of strains to nodulate concrete legumes regardless of the species to which they belong. From this year onwards, the criteria for species and symbiovar definition have suffered several changes, and currently, the species definition in rhizobia is based on the analysis of core genes and that of symbiovars on the analysis of symbiotic genes (Rogel et al. 2011; Peix et al. 2015b).

The first core gene analysed was the 16S rRNA gene, included in the minimal standards for rhizobial species description in 1991 (Graham et al. 1991) and whose analysis has been used to distribute the rhizobia into several genera and families (Kuykendall et al. 2005). Later, the core housekeeping genes *recA* and *atpD* were analysed in the members of family *Rhizobiaceae* (Gaunt et al. 2001). Currently, the analysis of multilocus sequences (MLSA or MLST) including three or more housekeeping genes is commonly used for description of new genera and species, as occurred with the recently described new genera *Neorhizobium* (Mousavi et al. 2014) and *Pararhizobium* (Mousavi et al. 2015). Nevertheless, the last trend is the whole genome analysis which has already been used for definition of new legume-nodulating species within the genera *Rhizobium* (Rashid et al. 2015) and *Ensifer* (Yan et al. 2016b).

The analysis of core genes allowed the most relevant discovery of the twenty-first century in the field of legume-bacteria symbiosis, since in year 2001 non-rhizobial bacteria able to nodulate legumes were reported, one of them belonging to *Alphaproteobacteria* (Sy et al. 2001) and the other to *Betaproteobacteria* (Moulin et al. 2001). From year 2001 onwards, several new species and genera from these two classes of *Proteobacteria* belonging to different families have been reported as responsible for nodule formation in different legumes (revised by Peix et al. 2015b). Particularly in the case of *Betaproteobacteria*, which are widespread in nodules of legumes from several tribes (Barrett and Parker 2005; Lemaire et al. 2015), the number of new species nodulating legumes has considerably increased in the last years (Table 3.1).

On the other hand, the analysis of symbiotic genes allowed the definition of symbiovars, initially named biovars (Jordan 1984). The reiteration of *nifH* gene has been used to identify the biovar phaseoli (Aguilar et al. 1998; Amarger et al. 1997), and this gene has been analysed for the recent definition of new symbiovars (Rincón-Rosales et al. 2013). Nevertheless, for this purpose the nodulation genes are the most used because they are related with rhizobia host range and legume promiscuity (Perret et al. 2000). The *nodA* gene has been used to define some symbiovars (Villegas et al. 2006; Nandasena et al. 2007), but most of them have been defined on the basis of the *nodC* gene analysis which has been proposed as the most adequate to delineate symbiovars in rhizobia by Peix et al. (2015b). The rhizobial symbiovars have been revised by Peix et al. (2015b); thus, only the recently described symbiovars are recorded in Table 3.2.

In the last years of the twentieth century, with the application of the 16S rRNA gene sequencing to the identification of nodule isolates, the studies of biodiversity and biogeography of rhizobia isolated from diverse legumes in different countries and continents started. These works continued in the present century with the addition of the housekeeping gene analysis, and currently a huge amount of data is available showing the high phylogenetic diversity of both classic rhizobia and new rhizobia. From these studies many new species have been added to all previously known genera, and also several new genera have been described, being the most recent ones *Neorhizobium* (Mousavi et al. 2014) and *Pararhizobium* (Mousavi et al. 2015). The classical and new rhizobial species have been recently revised by

Table 3.1 Recent new species of classic and new rhizobia isolated from legume nodules

Species	Isolation source	Reference
Family *Rhizobiaceae*		
Genus *Rhizobium*		
R. acidisoli	*Phaseolus vulgaris*	Román-Ponce et al. (2016)
R. aegyptiacum	*Trifolium alexandrinum*	Shamseldin et al. (2016)
R. anhuiense	*Vicia faba*, *Pisum sativum*	Zhang et al. (2015)
R. bangladeshense	*Lens culinaris*	Rashid et al. (2015)
R. binae	*Lens culinaris*	Rashid et al. (2015)
R. ecuadorense	*Phaseolus vulgaris*	Ribeiro et al. (2015)
R. lentis	*Lens culinaris*	Rashid et al. (2015)
R. pakistanensis	*Arachis hypogaea*	Khalid et al. (2015)
R. paranaense	*Phaseolus vulgaris*	Dall'Agnol et al. (2014)
R. puerariae	*Pueraria candollei*	Boonsnongcheep et al. (2015)
R. sophorae	*Sophora flavescens*	Jiao et al. (2015b)
R. sophoriradicis	*Sophora flavescens*	Jiao et al. (2015b)
Genus *Ensifer* **(formerly** *Sinorhizobium***)**		
E. glycinis	*Glycine max*, *Astragalus mongholicus*	Yan et al. (2016b)
Genus *Neorhizobium*		
N. alkalisoli	*Caragana intermedia*	Lu et al. (2009) and Mousavi et al. (2014)
N. galegae	*Galega officinalis*	Lindström (1989) and Mousavi et al. (2014)
N. huautlense	*Sesbania herbacea*	Wang et al. (1998) and Mousavi et al. (2014)
Genus *Allorhizobium*		
A. taibaishanense	*Kummerowia striata*	Yao et al. (2012) and Mousavi et al. (2015)
A. undicola	*Neptunia natans*	de Lajudie et al. (2002), Young et al. (2001) and Mousavi et al. (2015)
Genus *Pararhizobium*		
P. giardinii	*Phaseolus vulgaris*	Amarger et al. (1997) and Mousavi et al. (2015)
P. herbae	*Astragalus membranaceus*, *Oxytropis cashemiriana*	Mousavi et al. (2015)
P. sphaerophysae	*Sphaerophysa salsula*	Xu et al. (2011) and Mousavi et al. (2015)
Family *Phyllobacteriaceae*		
Genus *Mesorhizobium*		
M. acaciae	*Acacia melanoxylon*	Zhu et al. (2015)
M. calcicola	*Sophora* spp.	De Meyer et al. (2015)
M. cantuariense	*Sophora microphylla*	De Meyer et al. (2015)
M. erdmanii	*Lotus* spp.	Martínez-Hidalgo et al. (2015c)
M. jarvisii	*Lotus* spp.	Martínez-Hidalgo et al. (2015c)
M. kowhaii	*Sophora* spp.	De Meyer et al. (2016)
M. loti	*Lotus* spp.	Jarvis et al. (1982), Jarvis et al. (1997), and Martínez-Hidalgo et al. (2015c)

(continued)

Table 3.1 (continued)

Species	Isolation source	Reference
M. newzealandense	*Sophora* spp.	De Meyer et al. (2016)
M. sophorae	*Sophora* spp.	De Meyer et al. (2016)
M. waimense	*Sophora longicarinata*	De Meyer et al. (2015)
M. waitakense	*Sophora* spp.	De Meyer et al. (2016)
Family *Nitrobacteriaceae* (*Bradyrhizobiaceae*)		
Genus *Bradyrhizobium*		
B. americanum	*Centrosema macrocarpum*	Ramírez-Bahena et al. (2016)
B. centrosemae	*Centrosema molle*	Ramírez-Bahena et al. (2016)
B. embrapense	*Neonotonia wightii*, *Desmodium heterocarpon*	Delamuta et al. (2015)
B. erythrophlei	*Erythrophleum fordii*	Yao et al. (2015)
B. ferriligni	*Erythrophleum fordii*	Yao et al. (2015)
B. ganzhouense	*Acacia melanoxylon*	Lu et al. (2014)
B. guangdongense	*Arachis hypogaea*	Li et al. (2015)
B. guangxiense	*Arachis hypogaea*	Li et al. (2015)
B. icense	*Phaseolus lunatus*	Durán et al. (2014a)
B. ingae	*Inga laurina*	da Silva et al. (2014)
B. kavangense	*Vigna subterranea*, *Arachis hypogaea*	Grönemeyer et al. (2015b)
B. lupini	*Lupinus* spp.	Eckhardt et al. (1931) and Peix et al. (2015a)
B. manausense	*Vigna unguiculata*	Silva et al. (2014a)
B. neotropicale	*Centrolobium paraense*	Zilli et al. (2014)
B. ottawaense	*Glycine max*	Yu et al. (2014)
B. paxllaeri	*Phaseolus lunatus*	Durán et al. (2014a)
B. subterraneum	*Vigna subterranea*, *Arachis hypogaea*	Grönemeyer et al. (2015a)
B. stylosanthis	*Stylosanthes*	Delamuta et al. (2016)
B. tropiciagri	*Neonotonia wightii*, *Desmodium heterocarpon*	Delamuta et al. (2015)
B. valentinum	*Lupinus mariae-josephae*	Durán et al. (2014b)
B. vignae	*Vigna subterranea*, *Arachis hypogaea*	Grönemeyer et al. (2016)
B. viridifuturi	*Centrosema pubescens*	Helene et al. (2015)
Family *Burkholderiaceae*		
Genus *Paraburkholderia* (**formerly** *Burkholderia*)		
P. kirstenboschensis	*Hypocalyptus* spp., *Virgilia oroboides*	Steenkamp et al. (2015) and Dobritsa and Samadpour (2016)

Peix et al. (2015b); thus, only the recent changes in the rhizobial names and the new species described in the last years have been included in Table 3.1. The complete list of valid species of rhizobia is constantly updated and recorded in the List of Prokaryotic names with Standing in Nomenclature (http:// www.bacterio.cict.fr).

Table 3.2 Recently described symbiovars of rhizobial species

Species	Symbiovar	Isolation legume	References
Family *Rhizobiaceae*			
Genus *Rhizobium*			
R. aegyptiacum	Trifolii	*Trifolium alexandrinum*	Shamseldin et al. (2016)
R. bangladeshense	Trifolii	*Trifolium alexandrinum*	Shamseldin et al. (2016)
	Viciae	*Lens culinaris*	Rashid et al. (2015)
R. binae	Viciae	*Lens culinaris*	Rashid et al. (2015)
R. lentis	Viciae	*Lens culinaris*	Rashid et al. (2015)
Family *Phyllobacteriaceae*			
Genus *Mesorhizobium*			
M. erdmanii	Loti	*Lotus* spp.	Martínez-Hidalgo et al. (2015c)
M. jarvisii	Loti	*Lotus* spp.	Martínez-Hidalgo et al. (2015c)
M. loti	Loti	*Lotus* spp.	Martínez-Hidalgo et al. (2015c)
Family *Nitrobacteriaceae* (*Bradyrhizobiaceae*)			
Genus *Bradyrhizobium*			
B. americanum	Phaseolarum	*Centrosema macrocarpum*	Ramírez-Bahena et al. (2016)
B. centrosemae	Centrosemae	*Centrosema molle*	Ramírez-Bahena et al. (2016)
B. embrapense	Tropici	*Desmodium heterocarpon*	Ramírez-Bahena et al. (2016)
B. tropiciagri	Tropici	*Neonotonia wightii*	Ramírez-Bahena et al. (2016)
B. viridifuturi	Tropici	*Centrosema* spp.	Ramírez-Bahena et al. (2016)
Bradyrhizobium sp.	Vignae	*Vigna unguiculata*	Bejarano et al. (2014)

3.3.1 Legume Nodule Endosymbionts as Plant Growth Promoters

The biological nitrogen fixation was the first studied mechanism of plant growth, particularly in the case of rhizobia-legume symbiosis. From ancient times legumes were cultivated in rotation with cereals in all cultures, but it was Boussingault in the nineteenth century who first reported an increase in the soil nitrogen subsequently to the legume cultivation. Later, as we mentioned in the Introduction of this chapter, Hellriegel and Wilfarth related the legume nodules with the atmospheric nitrogen fixation, and finally, in 1888, Beijerinck achieved the isolation of the first bacterium responsible for this process. Since then a huge amount of works has been performed in microcosms and field conditions to test the nodulation efficacy and nitrogen fixation effectiveness of rhizobia inoculated in different legumes as well as the influence of different factors in plant yield.

In field conditions, the success of the nodulation and nitrogen fixation depends on the successful survival ability, competitiveness against the resident rhizobial population and efficiency of the rhizobial strain involved (Mehboob et al. 2013). The resident rhizobial populations are mainly correlated with pH, salinity and mineral nutrient content of soils but also with the promiscuity degree of legumes (Li et al. 2016; Lira et al. 2015; Yan et al. 2016a). The abundance of resident species determines the success in the rhizobial inoculation, and several long-term studies have been performed to analyse the evolution of rhizobial population nodulating legumes when a legume or a rhizobial species was introduced in specific soils. The results showed significant increases in *Rhizobium* population densities in response to planting *Acacia* spp. in Senegal (Sene et al. 2013) and the survival of *Bradyrhizobium japonicum* strains 20 years after their introduction in Poland (Narozna et al. 2015). In some cases the ability of resident rhizobia to nodulate a legume in an ineffective way can preclude the introduction of a legume crop in some regions as has been observed for *Lessertia* spp. (African legume) in Western Australian soils (Gerding et al. 2013).

Other important factors in the success of the legume inoculation in field conditions are the land use, crop management and soil conditions, since they are determinants of the rhizobial population dynamics, as has been recently showed for soybean crops (Yan et al. 2014). It has been reported that fertilization leads to a decrease in the rhizobial diversity indexes, although it does not change the species composition of soybean rhizobial communities (Yan et al. 2014). Also the cropping system, maize residues application and N fertilization on soybean determinate nodulation and yield (Herrmann et al. 2014). In common bean, the tillage system determines the success of the rhizobial inoculants in field experiments, since the grain yield observed with seed inoculation was significantly higher than that obtained without inoculation under conventional tillage and cover crops, whereas under no tillage inoculation had no effect (Mulas et al. 2015). Likewise, the nodulation and growth of *Senegalia senegal* was dependent on rhizobial strain inoculation, plant provenance and soil type (Bakhoum et al. 2016).

The ability of rhizobia to improve the legume productivity has been studied under different stresses, such as cold temperatures, drought and saline conditions. For example, a positive effect of rhizobial inoculation on *Galega officinalis* has been reported in field experiments carried out in different soils and agroclimatic conditions in Spain (González-Andrés et al. 2004). Increases in seed and total biomass yields were also demonstrated in chickpea in cold highland areas in Erzurum (Turkey) under field conditions (Elkoca et al. 2008). Likewise, Stambulska and Lushchak (2015) reported that an effective *Rhizobium*-legume symbiosis gives an increase in harvest in pea plants and enhances the harvest of pea crops under local climatic and soil conditions. Drought and salt stress are the major constrains to plant productivity in desert environment; nevertheless, rhizobia isolated from desert soils are able to survive, grow and effectively nodulate legumes, so the inoculation of legume crops in these environments could be an important strategy to improve their productivity (Faghire et al. 2012; Sharma et al. 2013). The use of salt-tolerant cultivars could also be an effective selection technology to overcome

the problem of soil salinity, as was shown under saline conditions for some chickpea cultivars, which after inoculation with *M. ciceri* showed a significant increase in the number of nodules, shoot and root dry weight, pod number and yield (Egamberdieva et al. 2014).

The ultimate goal of the most recent studies is the substitution of chemical nitrogen fertilizers by biofertilizers in legume crops in order to avoid the problems caused by the use of chemical nitrogen fertilizers for health and environment. For example, in field assays in Northern Spain, Mulas et al. (2011) showed that *Rhizobium leguminosarum* can completely replace the chemical fertilization in *Phaseolus vulgaris*. In Dominican Republic, the inoculation of several slow growing rhizobial nodulating *Cajanus cajan* L. produced the same or even higher yield than the fertilization with mineral nitrogen (Araujo et al. 2015). In Cameroon, *Pueraria phaseoloides* inoculated with *Bradyrhizobium* strains increased in nitrogen fixation and soil nitrogen uptake (Sarr et al. 2016). Therefore, legume biofertilization with rhizobia could substitute the nitrogen fertilizers in legumes by exploiting the symbiotic nitrogen fixation carried out by these bacteria.

In addition to the ability to fix atmospheric nitrogen in symbiosis with legumes, rhizobial strains presented other plant growth promotion mechanisms more recently studied which enable them to promote the plant growth of legumes and nonlegumes (revised by García-Fraile et al. 2012). The production of indoleacetic, siderophores and/or ACC deaminase has been reported in several strains of *R. leguminosarum* (Chabot et al. 1996a; Bhattacharjee et al. 2012; García-Fraile et al. 2012; Flores-Félix et al. 2013b). For some strains of *R. leguminosarum*, the ability to solubilize phosphate was also reported (Chabot et al. 1996a; Yanni et al. 2001; Flores-Félix et al. 2013b). Nevertheless, the species from genus *Mesorhizobium* are the most active phosphate solubilizers within rhizobia (Peix et al. 2001; Rivas et al. 2006), being able to increase the yields and nutrient uptake of legumes, such as chickpea (Peix et al. 2001; Valverde et al. 2006; Imen et al. 2015).

Besides having different in vitro mechanisms of plant growth promotion, the rhizobia are able to colonize the roots of nonlegumes, which is an important step for obtaining beneficial effects on plant growth (Compant et al. 2010). In 1996 it was already shown that bioluminescent strains of *R. leguminosarum* were able to colonize the roots of canola and lettuce (Chabot et al. 1996b), and later the colonization of rice and tobacco using GFP-tagged rhizobia was reported (Chi et al. 2005; Ji et al. 2010). Using GFP-tagged rhizobia and confocal microscopy, we have shown that *Rhizobium* strains are able to colonize the roots of different vegetables, such as tomato and pepper (García-Fraile et al. 2012), lettuce and carrots (Flores-Félix et al. 2013b), strawberries (Flores-Félix et al. 2015a) and spinach (Jiménez-Gómez et al. 2016).

The ability of *Rhizobium* to promote the growth of nonlegumes was reported by several authors from the last years of the twentieth century when *Rhizobium* was found as cereal endophyte on roots of rice (Yanni et al. 1997), maize (Gutiérrez-Zamora and Martínez-Romero 2001), wheat (Lupwayi et al. 2004; Yanni et al. 2016), barley and canola (Lupwayi et al. 2004). The ability to promote plant growth has been mainly analysed in *R. leguminosarum* and rice (Yanni et al. 2001; Yanni

and Dazzo 2010; Bhattacharjee et al. 2012; Granada et al. 2014), and it has been related with an enhancement of phenolics involved in plant defence of this cereal (Mishra et al. 2006) and with an increase in the phytohormone levels in its tissues after inoculation with rhizobia (Chi et al. 2005). In addition to rice, *R. leguminosarum* inoculation enhances the growth of other cereals, such as maize (Chabot et al. 1996a; Singh et al. 2013) and wheat (Yanni et al. 2016). *Rhizobium* strains have also been reported as growth promoters of other extensive crops such as canola (Noel et al. 1996) and sunflowers (Alami et al. 2000), and *Mesorhizobium* strains have been reported as growth promoters for barley (Peix et al. 2001).

In addition, due to the safety of rhizobia for human, animal and plant health, these bacteria are particularly interesting for biofertilization of vegetables that are commonly consumed fresh (García-Fraile et al. 2012). The first works showing the potential of *Rhizobium* to promote the plant growth of vegetables were carried out in the 1990s of the past century. They showed that *Rhizobium* strains are able to promote the plant growth of lettuce (Noel et al. 1996) and that *Rhizobium* and *Bradyrhizobium* strains are able to promote the growth of radishes (Antoun et al. 1998). More recently several works showed that *Rhizobium* strains are able to promote the growth of tomato and pepper (García-Fraile et al. 2012), lettuce and carrots (Flores-Félix et al. 2013b), strawberries (Flores-Félix et al. 2015a) and arugula (Rubio-Canalejas et al. 2016).

All these works showed that classic rhizobia are good biofertilizers for legumes and nonlegumes, but also the new rhizobia have been reported as plant growth promoters. For example, *Burkholderia tuberum* (currently named *Paraburkholderia tuberum*) solubilizes phosphate and produces siderophores (Angus et al. 2013), and one strain of *Methylobacterium* nodulating *Sesbania rostrata* produces indoleacetic acid, colonizes the rice roots and promotes the growth of this cereal (Senthilkumar et al. 2009). Nevertheless, this is a field still poorly exploited, and considering that several non-rhizobial genera are able not only to carry out the nitrogen fixation in symbiosis but also in free life such as *Phyllobacterium* (Rojas et al. 2001) and *Burkholderia* (Caballero-Mellado et al. 2007), this group of bacteria are promising for future agronomic applications, particularly taking into account that several species of *Burkholderia* have been separated from the pathogen *Burkholderia cepacia* and currently belong to the new genus *Paraburkholderia* that contains only non-pathogenic species (Sawana et al. 2014).

3.4 The Legume Nodule Endophytic Bacteria

Legume nodules harbour rhizobia together with other endophytic bacteria (Peix et al. 2015b; Velázquez et al. 2013) whose role in legume symbiosis is poorly known. Nevertheless, they could have synergistic effects with rhizobia, since many of these endophytes presented mechanisms of plant growth promotion (Velázquez et al. 2013). Although, the mode of entry of endophytes in legume nodules remains unknown, by using confocal microscopy, it has been shown that endophytic bacteria can enter the inner of nodules of *Vigna* together with rhizobia (Pandya et al. 2013).

Also it has been reported that the legume *Lotus japonicus* selectively regulate the access and accommodation of rhizobia and endophytes inside the root nodules (Zgadzaj et al. 2015).

The fact that the nodule endophytes were discarded for decades led to a lack of knowledge of their diversity, and possible essential role in plant growth has been occurred. Nevertheless, this practice prevented to erroneously assign the ability to nodulate legumes to endophytic bacteria. Now, we can fall in the opposite scene, since a failure in the rhizobial isolation from nodules can lead researchers to wrongly think that the non-rhizobial isolated strains are responsible for the nodule formation. This firstly occurred with strains of *Gammaproteobacteria* that were suggested as possible nodule-inducing bacteria in *Hedysarum* species because the authors were not able to isolate rhizobia from nodules of those legumes (Benhizia et al. 2004).

Later, it has been reported the nodulation of *Robinia pseudoacacia* by a strain of the *Gammaproteobacteria Pseudomonas* (Shiraishi et al. 2010) and that of legumes from tribe *Trifoliae*, *Lotus* and *Anthyllis* by strains of the sporulating Gram-positive genera *Paenibacillus* and *Geobacillus* (Ampomah and Huss-Danell 2011; Latif et al. 2013). Although the authors stated that these strains are able to induce legume nodules, it is necessary to be very cautious before concluding that a "non-rhizobia" is responsible for legume nodulation, because, using metagenomic techniques, unculturable rhizobia belonging to rhizobial species probably responsible for the nodule formation were recently found in the *Hedysarum* nodules from which previously only endophytic bacteria were isolated (Muresu et al. 2008). Therefore, the nodulation ability of strains that do not belong to classic rhizobia must be confirmed by using strains labelled with fluorescent proteins or by metagenomic techniques to avoid confusing nodule endophytes with the endosymbionts responsible for the legume nodule formation.

The first studies about nodule endophytes were performed in the last years of the past decade showing the existence of bacteria from different phyla in the inner of legume nodules, including rhizobia (Sturz et al. 1997). Nevertheless, most of them have been performed in the present century and allowed the description of several new species firstly found in legume nodules (Table 3.3). These studies have been carried out in nodules of different legumes showing they harboured bacteria from different phyla including *Proteobacteria* (classes *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria*), *Bacteroidetes*, *Sphingobacteria*, *Actinobacteria* and *Firmicutes*, which have been extensively revised by Velázquez et al. (2013).

In the recent years, several works have been carried out in different legumes such as *Hedysarum* (Torche et al. 2014), *Pisum sativum* (Tariq et al. 2014), *Clitoria ternatea* (Aeron et al. 2015), *Cicer arietinum* (chikpea) (Saini et al. 2015), *Astragalus* spp. (Chen et al. 2015), *Acacia* (Boukhatem et al. 2016), *Lespedeza* (Busby et al. 2016) and several wild legumes (Xu et al. 2014; De Meyer et al. 2015). Although most of these works have been carried out using culture-dependent techniques, in some of them they have been combined with metagenomic techniques (Torche et al. 2014), which are the only used approaches in some recent works (Busby et al. 2016).

Table 3.3 New taxa of non-nodulating endophytic bacteria isolated from legume nodules

Endophytic species	Legume	Reference
Gram negative		
Family *Acetobacteraceae*		
Endobacter medicaginis	*Medicago sativa*	Ramírez-Bahena et al. (2013)
Family *Brucellaceae*		
Ochrobactrum ciceri	*Cicer arietinum*	Imran et al. (2010)
Family *Burkholderiaceae*		
Burkholderia aspalathi	*Aspalathus abietina*	Mavengere et al. (2014)
Burkholderia dipogonis	*Dipogon lignosus*	Sheu et al. (2015)
Family *Comamonadaceae*		
Diaphorobacter ruginosibacter	*Glycine max*	Wei et al. (2015a)
Family *Nitrobacteriaceae* (*Bradyrhizobiaceae*)		
Bosea lathyri	*Lathyrus latifolius*	de Meyer and Willems (2012)
Bosea lupini	*Lupinus polyphyllus*	de Meyer and Willems (2012)
Bosea robiniae	*Robinia pseudoacacia*	de Meyer et al. (2012)
Bosea vaviloviae	*Vavilovia formosa*	Safronova et al. (2015)
Tardiphaga robiniae	*Robinia pseudoacacia*	de Meyer et al. (2012)
Family *Oxalobacteraceae*		
Herbaspirillum lusitanum	*Phaseolus vulgaris*	Valverde et al. (2003)
Family *Phyllobacteriaceae*		
Phyllobacterium endophyticum	*Phaseolus vulgaris*	Flores-Félix et al. (2013a)
Phyllobacterium loti	*Lotus corniculatus*	Sánchez et al. (2014)
Phyllobacterium sophorae	*Sophora flavescens*	Jiao et al. (2015a)
Family *Rhizobiaceae*		
Rhizobium pongamiae	*Pongamia pinnata* (*Millettia pinnata*)	Kesari et al. (2013)
Rhizobium puerariae	*Pueraria candollei*	Boonsnongcheep et al. (2015)
Gram positive		
Family *Xanthobacteraceae*		
Labrys neptuniae	*Neptunia oleracea*	Chou et al. (2007)
Family *Bacillaceae*		
Bacillus radicibacter	*Oxytropis ochrocephala*	Wei et al. (2015b)
Family *Paenibacillaceae*		
Cohnella lupini	*Lupinus albus*	Flores-Félix et al. (2014a)
Cohnella phaseoli	*Phaseolus vulgaris*	García-Fraile et al. (2008)
Fontibacillus phaseoli	*Phaseolus vulgaris*	Flores-Félix et al. (2014b)
Paenibacillus endophyticus	*Cicer arietinum*	Carro et al. (2013)
Paenibacillus enshidis	*Robinia pseudoacacia*	Yin et al. (2015)
Paenibacillus lupini	*Lupinus albus*	Carro et al. (2014)
Paenibacillus medicaginis	*Medicago sativa*	Lai et al. (2015)

(continued)

Table 3.3 (continued)

Endophytic species	Legume	Reference
Paenibacillus periandrae	*Periandra mediterranea*	Menéndez et al. (2016)
Paenibacillus prosopidis	*Prosopis farcta*	Valverde et al. (2010)
Family *Micromonosporaceae*		
Micromonospora lupini	*Lupinus angustifolius*	Trujillo et al. (2007)
Micromonospora luteifusca	*Pisum sativum*	Carro et al. (2016a)
Micromonospora noduli	*Pisum sativum*	Carro et al. (2016b)
Micromonospora pisi	*Pisum sativum*	García et al. (2010)
Micromonospora saelicesensis	*Lupinus angustifolius*	Trujillo et al. (2007)
Micromonospora ureilytica	*Pisum sativum*	Carro et al. (2016b)
Micromonospora vinacea	*Pisum sativum*	Carro et al. (2016b)
Xiangella phaseoli	*Phaseolus vulgaris*	Wang et al. (2013)
Family *Rhodobacteraceae*		
Paracoccus sphaerophysae	*Sphaerophysa salsula*	Deng et al. (2011)

3.4.1 Legume Nodule Endophytic Bacteria as Plant Growth Promoters

Several studies carried out in nodule endophytes have focused on their ability to promote the plant growth of legumes, and the first ones were carried out with rhizobial strains isolated from legume nodules which are not able to reinfect the legume from which they were isolated (Chen et al. 2003). For example, strains of *R. leguminosarum* bv. phaseoli isolated from nodules of *Trifolium pratense* but unable to nodulate this host were inoculated together with *R. leguminosarum* bv. *trifolii* resulting in the promotion of clover growth (Sturz et al. 1997).

More recently several studies of legume nodule endophytes have focused in the analysis of their in vitro plant growth promotion mechanisms. The ability to produce indoleacetic acid, to solubilize phosphate, to produce siderophores, to fix nitrogen and/or to produce ACC deaminase has been reported for several endophytes isolated from nodules of *Glycine max* (Kuklinsky-Sobral et al. 2004; Li et al. 2008; Subramanian et al. 2015), *Pueraria thunbergiana* (Selvakumar et al. 2008), *Lespedeza* (Palaniappan et al. 2010), *Glycyrrhiza* (Li et al. 2012), *Vicia faba* (Saïdi et al. 2013), *Pisum* (Tariq et al. 2014) and *Medicago sativa* (Martínez-Hidalgo et al. 2015b).

Many legume nodule endophytes are able to promote the growth of the legume from which they were isolated as occurred in the case of *Lespedeza* (Palaniappan et al. 2010) and *Arachis hypogaea* (Ibáñez et al. 2014). Strains of the Gram-positive genus *Bacillus* isolated from soybean increased the nodule number, nodule weight, shoot weight, root weight, total biomass, total nitrogen and grain yield (Bai et al. 2002, 2003). Also, legume nodule endophytic strains of Gram-negative genera are able to promote the plant growth of the hosts from which they were isolated, such as *Pantoea* nodules which increased the biomass of peanuts (Taurian et al. 2009); *Pseudomonas*, which increased the shoot and seed dry weights of *Vicia faba* (Saïdi

et al. 2013); *Agrobacterium*, which promotes the growth of common bean (Chihaoui et al. 2015); and *Serratia* which increased the grain yield of chickpea (Zaheer et al. 2016). Nevertheless, the most common approach is to analyse the growth promotion in coinoculation assays together with the endosymbionts responsible for the legume nodulation.

Coinoculation of nodule endophytic strains from *Bacillus megaterium* together with *Bradyrhizobium japonicum* in soybean promoted the nodulation, nitrogen fixation and leghaemoglobin content (Subramanian et al. 2015) and together with *Ensifer meliloti* promoted the nodulation and root development in alfalfa (Khalifa and Almalki 2015). Also in coinoculation with *E. meliloti*, an endophytic strain of *Exiguobacterium* isolated from *Trigonella foenum-graecum* (fenugreek) promotes the growth of this legume. Nodule endophytic strains of *Bacillus thuringiensis*, isolated from *Pueraria thunbergiana* and soybean, were responsible for an enhancement of plant growth and nodulation in pea and lentil in coinoculation with *Rhizobium leguminosarum* (Mishra et al. 2009a). The same results were found in soybean when *B. japonicum* was coinoculated with *B. thuringiensis* (Bai et al. 2002; Mishra et al. 2009b) and with *Bacillus pumilus* (Li et al. 2008). The aerial biomass and the nitrogen content in this legume were also increased after the coinoculation of endophytic strains from *Micromonospora* and *Streptomyces* in combination with *Ensifer meliloti* (Martínez-Hidalgo et al. 2015b; Le et al. 2015). The coinoculation of an endophytic strain of *Pseudomonas* and *Rhizobium* also increased the yield of *Vicia faba* (Saïdi et al. 2013).

The studies of the effect of legume nodule endophytes on the growth of non-legumes are starting with good results. In this sense, it has been reported that endophytic strains of *Pseudomonas*, *Enterobacter* and *Klebsiella* isolated from peanut nodules were able to promote its growth (Ibáñez et al. 2008) and also promote the growth of maize used in rotation with peanuts (Ibáñez et al. 2014). Also, the type strain of *Phyllobacterium endophyticum* isolated from common bean nodules (Flores-Félix et al. 2013a) is able to promote the plant growth of strawberries increasing stolons and fruit yield (Flores-Félix et al. 2015b). Most of these studies were performed under greenhouse or microcosm conditions, but also some works have been carried out in field conditions. Colás Sánchez et al. (2014) showed that the coinoculation of common bean with *Rhizobium* sp. isolated from these crops with a nodule endophyte strain of *Pseudomonas* sp. enhanced the nodulation and the crop yield. Pandya et al. (2015) isolated non-rhizobial species from nodules of *Vigna radiata*, which coinoculated with *Ensifer adhaerens*-related strains increased the values of several yield components in this crop.

The most recent advance in the research of benefits from plant probiotics is the finding of increases in diverse bioactive compounds in different plants. For example, after the inoculation of *Bradyrhizobium japonicum* in *Glycine max*, an increase of organic and fatty acids in soybean seeds was found (Silva et al. 2013). Also, an increase in fatty acids was also found in fruits of the nonlegume *Capsicum annuum* after the inoculation of *Rhizobium* strains (Silva et al. 2014b). Moreover, increases in the vitamin C content of strawberry fruits after the inoculation with the nodule

endophyte *Phyllobacterium endophyticum* have been reported (Flores-Félix et al. 2015b). This is a promising research field since currently there is an increasing interest on the bioactive compounds present in legumes and other vegetables (Shashirekha et al. 2015; Silva et al. 2016).

Conclusions

The legume nodule is a complex ecosystem whose microbiome is constituted by rhizobia, which are responsible for the nodule formation, and by other nodule endophytic bacteria whose role in the nodule is still few known. The diversity of both types of bacteria is much higher than initially believed, and several new bacterial species firstly isolated from legume nodules have been described in the last years, although the bacterial endophytes have been studied much more recently than rhizobia. Therefore, it is expected that the number of bacterial species inhabiting nodules will increase when more legumes are studied using metagenomic and culturomic approaches, the latter being necessary for the analysis of their plant growth promotion abilities. Both rhizobia and nodule endophytes have been reported as good plant probiotics since they presented several direct and indirect mechanisms of plant growth promotion. Plant assays carried out in microcosms and field conditions have shown that alone or in coinoculation schemes, rhizobia and nodule endophytes are good plant probiotics for legumes and nonlegumes constituting an ecological and healthy alternative to chemical fertilizers.

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References

Abril A, Zurdo-Piñeiro JL, Peix A, Rivas R, Velázquez E (2007) Solubilization of phosphate by a strain of *Rhizobium leguminosarum* bv. trifolii isolated from *Phaseolus vulgaris* in El Chaco Arido soil (Argentina). In: Velázquez E, Rodríguez-Barrueco C (eds) First international meeting on microbial phosphate solubilization. Springer, Germany, pp 135–138

Aeron A, Singh Chauhan P, Dubey RC, Maheshwari DK, Bajpaid VK (2015) Root nodule bacteria from *Clitoria ternatea* L. are putative invasive nonrhizobial endophytes. Can J Microbiol 61:131–142

Aguilar OM, Grasso DH, Riccillo PM, López MV, Szafer E (1998) Rapid identification of bean *Rhizobium* isolates by a *nifH* gene-pcr assay. Soil Biol Biochem 30:1655–1661

Alami Y, Achouak W, Marol C, Heulin T (2000) Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. strain isolated from sunflower roots. Appl Environ Microbiol 66:3393–3398

Alikhani HA, Saleh-Rastin N, Antoun H (2006) Phosphate solubilization activity of rhizobia native to Iranian soils. Plant Soil 287:35–41

Amarger N, Macheret V, Laguerre G (1997) *Rhizobium gallicum* sp. nov. and *Rhizobium giardinii sp*. nov., from *Phaseolus vulgaris* nodules. Int J Syst Bacteriol 47:996–1006

Ampomah OY, Huss-Danell K (2011) Genetic diversity of root nodule bacteria nodulating *Lotus corniculatus* and *Anthyllis vulneraria* in Sweden. Syst Appl Microbiol 34:267–275

Angus AA, Lee A, Lum MR, Shehayeb M, Hessabi R, Fujishige NA, Yerrapragada S, Kano S, Song N, Yang P, Estrada de los Santos P, de Faria SM, Dakora FD, Weinstock G, Hirsch AM (2013) Nodulation and effective nitrogen fixation of Macroptilium atropurpureum (siratro) by Burkholderia tuberum, a nodulating and plant growth promoting beta-proteobacterium, are influenced by environmental factors. Plant Soil 369:543–562

Antoun H, Beauchamp CJ, Goussard N, Chabot R, Lalande R (1998) Potential of *Rhizobium* and *Bradyrhizobium* species as growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.) Plant Soil 204:57–67

Araujo J, Díaz-Alcántara CA, Velázquez E, Urbano B, González-Andrés F (2015) *Bradyrhizobium yuanmingense* related strains form nitrogen-fixing symbiosis with *Cajanus cajan* L. in Dominican Republic and are efficient biofertilizers to replace N fertilization. Sci Hortic 192:421–428

Aserse AA, Räsänen LA, Aseffa F, Hailemariam A, Lindström K (2013) Diversity of sporadic symbionts and nonsymbiotic endophytic bacteria isolated from nodules of woody, shrub, and food legumes in Ethiopia. Appl Microbiol Biotechnol 97:10117–10134

Bai Y, D'Aoust F, Smith DL, Driscoll BT (2002) Isolation of plant-growth-promoting *Bacillus* strains from soybean root nodules. Can J Microbiol 48:230–238

Bai Y, Zhou X, Smith DL (2003) Enhanced soybean plant growth resulting from coinoculation of *Bacillus* strains with *Bradyrhizobium japonicum*. Crop Sci 43:1774–1781

Bakhoum N, Odee DW, Fall D, Ndoye F, Kane A, Kimiti JM, Zoubeirou AM, Sylla SN, Noba K, Diouf D (2016) *Senegalia Senegal* response to inoculation with rhizobial strains vary in relation to seed provenance and soil type. Plant Soil 398:181–193

Baldwin IL, Fred EB (1929) Nomenclature of the root nodule bacteria of the Leguminosae. J Bacteriol 17:141–150

Barrett CF, Parker MA (2005) Prevalence of *Burkholderia* sp. nodule symbionts on four mimosoid legumes from Barro Colorado Island, Panama. Syst Appl Microbiol 28:57–65

Bedoussac L, Journet EP, Hauggaard-Nielsen H, Naudin C, Corre-Hellou G, Steen Jensen E, Prieur L, Justes E (2015) Ecological principles underlying the increase of productivity achieved by cereal-grain legume intercrops in organic farming. A review. Agron Sustain Dev 35:911–935

Beijerinck MW (1888) Cultur des Bacillus radicicola aus den Knöllchen. Bot Ztg 46:740–750

Bejarano A, Ramírez-Bahena MH, Velázquez E, Peix A (2014) *Vigna unguiculata* is nodulated in Spain by endosymbionts of Genisteae legumes and by a new symbiovar (vignae) of the genus *Bradyrhizobium*. Syst Appl Microbiol 37:533–540

Benhizia Y, Benhizia H, Benguedouar A, Muresu R, Giacomini A, Squartini A (2004) Gamma proteobacteria can nodulate legumes of the genus *Hedysarum*. Syst Appl Microbiol 27:462–468

Berlec A (2012) Novel techniques and findings in the study of plant microbiota: search for plant probiotics. Plant Sci 193-194:96–102

Bhattacharjee RB, Jourand P, Chaintreuil C, Dreyfus B, Singh A, Mukhopadhyay SN (2012) Indole acetic acid and ACC deaminase-producing *Rhizobium leguminosarum* bv. trifolii SN10 promote rice growth, and in the process undergo colonization and chemotaxis. Biol Fertil Soils 48:173–182

Bianco C, Defez R (2010) Improvement of phosphate solubilization and *Medicago* plant yield by an indole-3-Acetic acid-overproducing strain of *Sinorhizobium meliloti*. Appl Environ Microbiol 76:4626–4632

Boiero L, Perrig D, Masciarelli O, Penna C, Cassán F, Luna V, Castellano-Hinojosa A, Correa-Galeote D, Bedmar EJ (2007) Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. Appl Microbiol Biotechnol 74:874–880

Boonsnongcheep P, Prathanturarug S, Takahashi Y, Matsumoto A (2015) *Rhizobium puerariae* sp. nov., an endophytic bacterium from the root nodules of medicinal plant *Pueraria candollei* var. candollei. Int J Syst Evol Microbiol. doi:10.1099/ijsem.0.000863

Boukhatem ZF, Merabet C, Bekki A, Sekkour S, Domergue O, Dupponois R (2016) Nodular bacterial endophyte diversity associated with native *Acacia* spp. in desert region of Algeria. Afr J Microbiol Res 10:634–645

Busby RR, Rodriguez G, Gebhart DL, Yannarell AC (2016) Native *Lespedeza* species harbor greater non-rhizobial bacterial diversity in root nodules compared to the coexisting invader, *L. cuneata*. Plant Soil 401:427–436

Caballero-Mellado J, Onofre-Lemus J, Estrada de los Santos P, Martínez-Aguilar L (2007) The tomato rhizosphere, an environment rich in nitrogen-fixing *Burkholderia* species with capabilities of interest for agriculture and bioremediation. Appl Environ Microbiol 73:5308–5319

Carro L, Flores-Félix JD, Cerda-Castillo E, Ramírez-Bahena MH, Igual JM, Tejedor C, Velázquez E, Peix A (2013) *Paenibacillus endophyticus* sp. nov., isolated from nodules of *Cicer arietinum*. Int J Syst Evol Microbiol 63:4433–4438

Carro L, Flores-Félix JD, Ramírez-Bahena MH, García-Fraile P, Martínez-Hidalgo P, Igual JM, Tejedor C, Peix A, Velázquez E (2014) *Paenibacillus lupini* sp. nov., isolated from nodules of *Lupinus albus*. Int J Syst Evol Microbiol 64:3028–3033

Carro L, Riesco R, Spröer C, Trujillo ME (2016a) *Micromonospora luteifusca* sp. nov. isolated from cultivated *Pisum sativum*. Syst Appl Microbiol 39:237–242

Carro L, Riesco R, Spröer C, Trujillo ME (2016b) Three new species of *Micromonospora* isolated from *Pisum sativum* nodules: *Micromonospora ureilytica* sp. nov., *Micromonospora noduli* sp. nov., and *Micromonospora vinacea* sp. nov. Int J Syst Evol Microbiol. doi:10.1099/ijsem.0.001231.

Chabot R, Antoun H, Cescas MP (1996a) Growth promotion of maize and lettuce by phosphate-solubilizing *Rhizobium leguminosarum* biovar phaseoli. Plant Soil 184:311–321

Chabot R, Antoun H, Kloepper JW, Beauchamp C (1996b) Root colonization of maize and lettuce by bioluminescent *Rhizobium leguminosarum* biovar phaseoli. Appl Environ Microbiol 62:2767–2772

Chen WM, Moulin L, Bontemps C, Vandamme P, Béna G, Boivin-Masson C (2003) Legume symbiotic nitrogen fixation by beta-proteobacteria is widespread in nature. J Bacteriol 185:7266–7272

Chen W, Sun L, Lu J, Bi L, Wang E, Wei G (2015) Diverse nodule bacteria were associated with *Astragalus* species in arid region of northwestern China. J Basic Microbiol 55:121–128

Chi F, Shen SH, Cheng HP, Jing YX, Yanni YG, Dazzo FB (2005) Ascending migration of endophytic rhizobia, from roots to leaves, inside rice plants and assessment of benefits to rice growth physiology. Appl Environ Microbiol 71:7271–7278

Chihaoui SA, Trabelsi D, Jdey A, Mhadhbi H, Mhamdi R (2015) Inoculation of *Phaseolus vulgaris* with the nodule-endophyte *Agrobacterium* sp. 10C2 affects richness and structure of rhizosphere bacterial communities and enhances nodulation and growth. Arch Microbiol 197:805–813

Chou YJ, Elliott GN, James EK, Lin KY, Chou JH, Sheu SY, Sheu DS, Sprent JI, Chen WM (2007) *Labrys neptuniae* sp. nov., isolated from root nodules of the aquatic legume *Neptunia oleracea*. Int J Syst Evol Microbiol 57:577–581

Colás Sánchez A, Torres Gutiérrez R, Cupull-Santana R, Rodríguez-Urrutia A, Fauvart M, Michiels J, Vanderleyden J (2014) Effects of co-inoculation of native *Rhizobium* and *Pseudomonas* strains on growth parameters and yield of two contrasting *Phaseolus vulgaris* L. genotypes under Cuban soil conditions. Eur J Soil Biol 62:105–112

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678

da Silva K, De Meyer SE, Rouws LF, Farias EN, dos Santos MA, O'Hara G, Ardley JK, Willems A, Pitard RM, Zilli JE (2014) *Bradyrhizobium ingae* sp. nov., isolated from effective nodules of *Inga laurina* grown in Cerrado soil. Int J Syst Evol Microbiol 64:3395–3401

Dall'Agnol RF, Ribeiro RA, Delamuta JR, Ormeño-Orrillo E, Rogel MA, Andrade DS, Martínez-Romero E, Hungria M (2014) *Rhizobium paranaense* sp. nov., an effective N2-fixing symbiont of common bean (*Phaseolus vulgaris* L.) with broad geographical distribution in Brazil. Int J Syst Evol Microbiol 64:3222–3229

Datta C, Basu PS (2000) Indole acetic acid production by a *Rhizobium* species from root nodules of a leguminous shrub, *Cajanus cajan*. Microbiol Res 155:123–127

Datta B, Chakrabartty PK (2014) Siderophore biosynthesis genes of *Rhizobium* sp. isolated from *Cicer arietinum* L. 3 Biotech 4:391–401

de Almeida Lopes KB, Carpentieri-Pipolo V, Oro TH, Stefani Pagliosa E, Degrassi G (2016) Culturable endophytic bacterial communities associated with field-grown soybean. J Appl Microbiol 120:740–755

de Lajudie P, Laurent-Fulele E, Willems A, Torck U, Coopman R, Collins MD, Kersters K, Dreyfus B, Gillis M (1992) *Allorhizobium undicola* gen. nov., sp. nov., nitrogen-fixing bacteria that efficiently nodulate *Neptunia natans* in Senegal. Int J Syst Bacteriol 42:93–96

de Meyer SE, Willems A (2012) Multilocus sequence analysis of *Bosea* species and description of *Bosea lupini* sp. nov., *Bosea lathyri* sp. nov. and *Bosea robiniae* sp. nov., isolated from legumes. Int J Syst Evol Microbiol 62:2505–2510

de Meyer SE, Coorevits A, Willems A (2012) *Tardiphaga robiniae* gen. nov., sp. nov., a new genus in the family *Bradyrhizobiaceae* isolated from *Robinia pseudoacacia* in Flanders (Belgium). Syst Appl Microbiol 35:205–214

de Meyer SE, Tan HW, Heenan PB, Andrews M, Willems A (2015) *Mesorhizobium waimense* sp. nov. isolated from *Sophora longicarinata* root nodules and *Mesorhizobium cantuariense* sp. nov. isolated from *Sophora microphylla* root nodules. Int J Syst Evol Microbiol 65:3419–3426

de Meyer SE, Tan HW, Andrews M, Heenan PB, Willems A (2016) *Mesorhizobium calcicola* sp. nov., *Mesorhizobium waitakense* sp. nov., *Mesorhizobium sophorae* sp. nov., *Mesorhizobium newzealandense* sp. nov. and *Mesorhizobium kowhaii* sp. nov. isolated from *Sophora* root nodules in New Zealand. Int J Syst Evol Microbiol. doi:10.1099/ijsem.0.000796

Delamuta JR, Ribeiro RA, Ormeño-Orrillo E, Parma MM, Melo IS, Martínez-Romero E, Hungria M (2015) *Bradyrhizobium tropiciagri* sp. nov. and *Bradyrhizobium embrapense* sp. nov, nitrogen-fixing symbionts of tropical forage legumes. Int J Syst Evol Microbiol 65:4424–4433

Delamuta JR, Ribeiro RA, Araújo JL, Rouws LF, Zilli JÉ, Parma MM, Melo IS, Hungria M (2016) *Bradyrhizobium stylosanthis* sp. nov., comprising nitrogen-fixing symbionts isolated from nodules of the tropical forage legume *Stylosanthes* spp. Int J Syst Evol Microbiol. doi:10.1099/ijsem.0.001148

Deng ZS, Zhao LF, Xu L, Kong ZY, Zhao P, Qin W, Chang JL, Wei GH (2011) *Paracoccus sphaerophysae* sp. nov., a siderophore-producing, endophytic bacterium isolated from root nodules of *Sphaerophysa salsula*. Int J Syst Evol Microbiol 61:665–669

Dobritsa AP, Samadpour M (2016) Transfer of eleven *Burkholderia* species to the genus *Paraburkholderia* and proposal of *Caballeronia* gen. nov., a new genus to accommodate twelve species of *Burkholderia* and *Paraburkholderia*. Int J Syst Evol Microbiol. doi:10.1099/ijsem.0.001065

Dubey RC, Maheshwari DK, Kumar H, Choure K (2010) Assessment of diversity and plant growth promoting attributes of rhizobia isolated from *Cajanus cajan* L. Afr J Biotechnol 9:8619–8629

Duca D, Lorv J, Patten CL, Rose D, Glick BR (2014) Indole-3-acetic acid in plant–microbe interactions. Antonie Van Leeuwenhoek 106:85–125

Durán D, Rey L, Mayo J, Zúñiga-Dávila D, Imperial J, Ruiz-Argüeso T, Martínez-Romero E, Ormeño-Orrillo E (2014a) *Bradyrhizobium paxllaeri* sp. nov. and *Bradyrhizobium icense* sp. nov, nitrogen-fixing rhizobial symbionts of Lima bean (*Phaseolus lunatus* L.) in Peru. Int J Syst Evol Microbiol 64:2072–2078

Durán D, Rey L, Navarro A, Busquets A, Imperial J, Ruiz-Argüeso T (2014b) *Bradyrhizobium valentinum* sp. nov, isolated from effective nodules of *Lupinus mariae-josephae*, a lupine endemic of basic-lime soils in Eastern Spain. Syst Appl Microbiol 37:336–341

Eckhardt MM, Baldwin IR, Fred EB (1931) Studies on the root-nodule bacteria of *Lupinus*. J Bacteriol 21:273–285

Egamberdieva D, Shurigin V, Gopalakrishnan S, Sharma R (2014) Growth and symbiotic performance of chickpea (*Cicer arietinum*) cultivars under saline soil conditions. J Biol Chem Res 31:333–341

Ejigui J, Savoie L, Marin J, Desrosiers T (2007) Improvement of the nutritional quality of a traditional complementary porridge made of fermented yellow maize (*Zea mays*): effect of maize-legume combinations and traditional processing methods. Food Nutr Bull 28:23–34

Elkoca E, Kantar F, Sahin F (2008) Influence of nitrogen fixing and phosphorus solubilising bacteria on the nodulation, plant growth and yield of chickpea. J Plant Nutr 31:157–171

Faghire M, Mandri B, Oufdou K, Bargaz A, Ghoulam C, Ramírez-Bahena MH, Velázquez E, Peix A (2012) Identification at the species and symbiovar levels of strains nodulating *Phaseolus vulgaris* in saline soils of the Marrakech region (Morocco) and analysis of the *otsA* gene putatively involved in osmotolerance. Syst Appl Microbiol 35:156–164

Flores-Félix JD, Carro L, Velázquez E, Valverde Á, Cerda-Castillo E, García-Fraile P, Rivas R (2013a) *Phyllobacterium endophyticum* sp. nov., isolated from nodules of *Phaseolus vulgaris*. Int J Syst Evol Microbiol 63:821–826

Flores-Félix JD, Menéndez E, Rivera LP, Marcos-García M, Martínez-Hidalgo P, Mateos PF, Martínez-Molina E, Velázquez E, García-Fraile P, Rivas R (2013b) Use of *Rhizobium leguminosarum* as a potential biofertilizer for *Lactuca sativa* and *Daucus carota* crops. J Plant Nutr Soil Sci 176:876–882

Flores-Félix JD, Carro L, Ramírez-Bahena MH, Tejedor C, Igual JM, Peix A, Velázquez E (2014a) *Cohnella lupini* sp. nov., an endophytic bacterium isolated from root nodules of *Lupinus albus*. Int J Syst Evol Microbiol 64:83–87

Flores-Félix JD, Mulas R, Ramírez-Bahena MH, Cuesta MJ, Rivas R, Brañas J, Mulas D, González-Andrés F, Peix A, Velázquez E (2014b) *Fontibacillus phaseoli* sp. nov. isolated from *Phaseolus vulgaris* nodules. Antonie Van Leeuwenhoek 105:23–28

Flores-Félix JD, Marcos-García M, Silva LR, Menéndez E, Martínez-Molina E, Mateos PF, Velázquez E, García-Fraile P, Andrade P, Rivas R (2015a) *Rhizobium* as plant probiotic for strawberry production under microcosm conditions. Symbiosis 67:25–32

Flores-Félix JD, Silva LR, Rivera LP, Marcos-García M, García-Fraile P, Martínez-Molina E, Mateos PF, Velázquez E, Andrade P, Rivas R (2015b) Plants probiotics as a tool to produce highly functional fruits: the case of *Phyllobacterium* and vitamin C in strawberries. PLoS One 10:e0122281

Frank B (1889) Ueber die Pilzsymbiose der Leguminosen. Bet Dtsch Bot Ges 7:332–346

García LC, Martínez-Molina E, Trujillo ME (2010) *Micromonospora pisi* sp. nov., isolated from root nodules of *Pisum sativum*. Int J Syst Evol Microbiol 60:331–337

García-Fraile P, Velázquez E, Mateos PF, Martínez-Molina E, Rivas R (2008) *Cohnella phaseoli* sp. nov., isolated from root nodules of *Phaseolus coccineus* in Spain, and emended description of the genus *Cohnella*. Int J Syst Evol Microbiol 58:1855–1859

García-Fraile P, Carro L, Robledo M, Ramírez-Bahena MH, Flores-Félix JD, Fernández MT, Mateos PF, Rivas R, Igual JM, Martínez-Molina E, Peix Á, Velázquez E (2012) *Rhizobium* promotes non-legumes growth and quality in several production steps: towards a biofertilization of edible raw vegetables healthy for humans. PLoS One 7:e38122

Gaunt MW, Turner SL, Rigottier-Gois L, Lloyd-Macgilp SA, Young JPW (2001) Phylogenies of *atpD* and *recA* support the small subunit rRNA-based classification of rhizobia. Int J Syst Evol Microbiol 51:2037–2048

Gerding M, Howieson JG, O'Hara GW, Real D, Bräu L (2013) Establishment and survival of the South African legume *Lessertia* spp. and rhizobia in Western Australian agricultural systems. Plant Soil 370:235–249

Ghosh PK, De TK, Maiti TK (2015) Production and metabolism of indole acetic acid in root nodules and symbiont (*Rhizobium undicola*) isolated from root nodule of aquatic medicinal legume *Neptunia oleracea* Lour. J Bot 2015:ID 575067

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:1–7

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169:30–39

González-Andrés F, Redondo P, Pescador R, Urbano B (2004) Management of *Galega officinalis* L. and preliminary results on its potential for milk production improvement in sheep. N Z J Agric Res 47:233–245

Graham PH, Sadowsky MJ, Keyser HH, Barnet YM, Bradley RS, Cooper JE, De Ley J, Jarvis BDW, Roslycky EB, Strijdom BW, Young JPW (1991) Proposed minimal standards for the description of new genera and species of root- and stem-nodulation bacteria. Int J Syst Bacteriol 41:582–587

Granada CE, Arruda L, Brito Lisboa B, Passaglia LMP, Vargas LK (2014) Diversity of native rhizobia isolated in south Brazil and their growth promotion effect on white clover (*Trifolium repens*) and rice (*Oryza sativa*) plants. Biol Fertil Soils 50:123–132

Grönemeyer JL, Chimwamurombe P, Reinhold-Hurek B (2015a) *Bradyrhizobium subterraneum* sp. nov, a symbiotic nitrogen-fixing bacterium from root nodules of groundnuts. Int J Syst Evol Microbiol 65:3241–3247

Grönemeyer JL, Hurek T, Reinhold-Hurek B (2015b) *Bradyrhizobium kavangense* sp. nov, a symbiotic nitrogen-fixing bacterium from root nodules of traditional Namibian pulses. Int J Syst Evol Microbiol 65:4886–4894

Grönemeyer JL, Hurek T, Bünger W, Reinhold-Hurek B (2016) *Bradyrhizobium vignae* sp. nov, a nitrogen-fixing symbiont isolated from effective nodules of *Vigna* and *Arachis*. Int J Syst Evol Microbiol 66:62–69

Gutiérrez-Zamora ML, Martínez-Romero E (2001) Natural endophytic association between *Rhizobium etli* and maize (*Zea mays* L.) J Biotechnol 91:117–126

Haag AF, Arnold MFF, Myka KK, Kerscher B, Dall'Angelo S, Zanda M, Mergaert P, Ferguson GP (2013) Molecular insights into bacteroid development during *Rhizobium*-legume symbiosis. FEMS Microbiol Rev 37:364–383

Helene LC, Marçon Delamuta JR, Augusto Ribeiro R, Ormeño-Orrillo E, Antonio Rogel M, Martínez-Romero E, Hungria M (2015) *Bradyrhizobium viridifuturi* sp. nov, encompassing nitrogen-fixing symbionts of legumes used for green manure and environmental services. Int J Syst Evol Microbiol 65:4441–4448

Herrmann L, Chotte JL, Thuita M, Lesueur D (2014) Effects of cropping systems, maize residues application and N fertilization on promiscuous soybean yields and diversity of native rhizobia in Central Kenya. Pedobiologia 57:75–85

Hoffman BM, Lukoyanov D, Yang ZY, Dean DR, Seefeldt LC (2014) Mechanism of nitrogen fixation by nitrogenase: the next stage. Chem Rev 114:4041–4062

Ibáñez F, Angelini J, Taurian T, Tonelli ML, Fabra A (2008) Endophytic occupation of peanut root nodules by opportunistic Gammaproteobacteria. Syst Appl Microbiol 32:49–55

Ibáñez F, Arroyo ME, Angelini J, Tonelli ML, Muñoz V, Ludueña L, Valetti L, Fabra A (2014) Non-rhizobial peanut nodule bacteria promote maize (*Zea mays* L.) and peanut (*Arachis hypogaea* L.) growth in a simulated crop rotation system. Appl Soil Ecol 84:208–212

Imen H, Neila A, Adnane B, Manel B, Mabrouk Y, Saidi M, Bouaziz S (2015) Inoculation with phosphate solubilizing *Mesorhizobium* strains improves the performance of chickpea (*Cicer arietinum* L.) under phosphorus deficiency. J Plant Nutr 38:1656–1671

Imran A, Hafeez FY, Frühling A, Schumann P, Malik KA, Stackebrandt E (2010) *Ochrobactrum ciceri* sp. nov., isolated from nodules of *Cicer arietinum*. Int J Syst Evol Microbiol 60:1548–1553

Jarvis BDW, Pankhurst CE, Patel JJ (1982) *Rhizobium loti*, a new species of legume root nodule bacteria. Int J Syst Bacteriol 32:378–380

Jarvis BDW, van Berkum P, Chen WX, Nour SM, Fernandez MP, Cleyet-Marel JC, Gillis M (1997) Transfer of *Rhizobium loti, Rhizobium huakuii, Rhizobium ciceri, Rhizobium mediterraneum,* and *Rhizobium tianshanense* to *Mesorhizobium* gen. nov. Int J Syst Bacteriol 47:895–898

Ji KX, Chi F, Yang MF, Shen SH, Jing YX, Dazzo FB, Cheng HP (2010) Movement of rhizobia inside tobacco and lifestyle alternation from endophytes to free-living rhizobia on leaves. J Microbiol Biotechnol 20:238–244

Jiao YS, Yan H, Ji ZJ, Liu YH, Sui XH, Zhang XX, Wang ET, Chen WX, Chen WF (2015a) *Phyllobacterium sophorae* sp. nov., a symbiotic bacterium isolated from root nodules of *Sophora flavescens*. Int J Syst Evol Microbiol 65:399–406

Jiao YS, Yan H, Ji ZJ, Liu YH, Sui XH, Wang ET, Guo BL, Chen WX, Chen WF (2015b) *Rhizobium sophorae* sp. nov. and *Rhizobium sophoriradicis* sp. nov., nitrogen-fixing rhizobial symbionts of the medicinal legume *Sophora flavescens*. Int J Syst Evol Microbiol 65:497–503

Jiménez-Gómez A, Menéndez E, Flores-Félix JD, García-Fraile P, Mateos PF, Rivas R (2016) Effective colonization of spinach root surface by *Rhizobium*. In: González-Andrés F, James E (eds) Biological nitrogen fixation and beneficial plant-microbe interaction. Springer, Germany, pp 109–122

Jordan DC (1984) Family III *Rhizobiaceae.* In: Krieg NR, Holt JG (eds) Bergeys manual of systematic bacteriology, vol I. Williams and Wilkins Co, Baltimore, pp 234–242

Kesari V, Ramesh AM, Rangan L (2013) *Rhizobium pongamiae* sp. nov. from root nodules of *Pongamia pinnata*. Biomed Res Int 2013:165198

Khalid R, Zhang YJ, Ali S, Sui XH, Zhang XX, Amara U, Chen WX, Hayat R (2015) *Rhizobium pakistanensis* sp. nov., isolated from groundnut (*Arachis hypogaea*) nodules grown in rainfed Pothwar, Pakistan. Antonie Van Leeuwenhoek 107:281–290

Khalifa AYZ, Almalki MA (2015) Isolation and characterization of an endophytic bacterium, *Bacillus megaterium* BMN1, associated with root-nodules of *Medicago sativa* L. growing in Al-Ahsaa region, Saudi Arabia. Ann Microbiol 65:1017–1026

Kuklinsky-Sobral J, Araújo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1251

Kumar PR, Ram MR (2012) Production of indole acetic acid by *Rhizobium* isolates from *Vigna trilobata* (L) Verdc. Afr J Microbiol Res 6:5536–5541

Kuykendall LD, Young JM, Martínez-Romero E, Kerr A, Sawada H (2005) Order *Rhizobiales* (new) family *Rhizobiaceae* genus *Rhizobium*. In: Brenner DJ, Krieg NR, Staley JT, Garrity GM (eds) The alpha-, beta-, delta- and epsilonproteobacteria, the proteobacteria, part C, Bergey's manual of systematic bacteriology, vol 2, 2nd edn. Springer, New York, pp 324–340

Lai WA, Hameed A, Lin SY, Hung MH, Hsu YH, Liu YC, Shahina M, Shen FT, Young CC (2015) *Paenibacillus medicaginis* sp. nov. a chitinolytic endophyte isolated from the root nodule of alfalfa (*Medicago sativa* L.). Int J Syst Evol Microbiol doi:10.1099/ijsem.0.000505

Latif S, Khan S, Naveed M, Mustafa G, Bashir T, Mumtaz AS (2013) The diversity of Rhizobia, Sinorhizobia and novel non-Rhizobial *Paenibacillus* nodulating wild herbaceous legumes. Arch Microbiol 195:647–653

Le XH, Franco CMM, Ballard RA, Drew EA (2015) Isolation and characterisation of endophytic actinobacteria and their effect on the early growth and nodulation of lucerne (*Medicago sativa* L.). doi:10.1007/s11104-015-2652-9

Leigh GJ (2004) The world's greatest fix: a history of nitrogen and agriculture. Oxford University Press, New York

Lemaire B, Dlodlo O, Chimphango S, Stirton C, Schrire B, Boatwright JS, Honnay O, Smets E, Sprent J, James EK, Muasya AM (2015) Symbiotic diversity, specificity and distribution of rhizobia in native legumes of the Core Cape subregion (South Africa). FEMS Microbiol Ecol 91:1–17

Li JH, Wang ET, Chen WF, Chen WX (2008) Genetic diversity and potential for promotion of plant growth detected in nodule endophytic bacteria of soybean grown in Heilongjiang province of China. Soil Biol Biochem 40:238–246

Li L, Sinkko H, Montonen L, Wei G, Lindström K, Räsänen LA (2012) Biogeography of symbiotic and other endophytic bacteria isolated from medicinal *Glycyrrhiza* species in China. FEMS Microbiol Ecol 79:46–68

Li YH, Wang R, Zhang XX, Young JP, Wang ET, Sui XH, Chen WX (2015) *Bradyrhizobium guangdongense* sp. nov. and *Bradyrhizobium guangxiense* sp. nov, isolated from effective nodules of peanut. Int J Syst Evol Microbiol 65:4655–4661

Li Y, Li X, Liu Y, Wang ET, Ren C, Liu W, Xu H, Wu H, Jiang N, Li Y, Zhang X, Xie Z (2016) Genetic diversity and community structure of rhizobia nodulating *Sesbania cannabina* in saline-alkaline soils. Syst Appl Microbiol 39:195–202

Lin T, Zhao L, Yang Y, Guan Q, Gong M (2013) Potential of endophytic bacteria isolated from *Sophora alopecuroides* nodule in biological control against *Verticillium* wilt disease. Aust J Crop Sci 7:139–146

Lindström K (1989) *Rhizobium galegae*, a new species of legume root nodule bacteria. Int J Syst Bacteriol 39:365–367

Lira MA, Nascimento LRS, Fracetto GGM (2015) Legume-rhizobia signal exchange: promiscuity and environmental effects. Front Microbiol 6:945

Lu YL, Chen WF, Han LL, Wang ET, Chen WX (2009) *Rhizobium alkalisoli* sp. nov., isolated from the legume *Caragana intermedia* growing in saline-alkaline soils. Int J Syst Evol Microbiol 59:3006–3011

Lu JK, Dou YJ, Zhu YJ, Wang SK, Sui XH, Kang LH (2014) *Bradyrhizobium ganzhouense* sp. nov, an effective symbiotic bacterium isolated from *Acacia melanoxylon* R. Br. nodules. Int J Syst Evol Microbiol 64:1900–1905

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556

Lupwayi NZ, Clayton GW, Hanson KG, Rice WA, Biederbeck VO (2004) Endophytic rhizobia in barley, wheat and canola roots. Can J Plant Sci 84:37–45

Lynch D, O'Brien J, Welch T, Clarke P, Ócuív P, Crosa JH, O'Connell M (2001) Genetic organization of the region encoding regulation, biosynthesis, and transport of rhizobactin 1021, a siderophore produced by *Sinorhizobium meliloti*. J Bacteriol 183:2576–2585

Martínez-Hidalgo P, Flores-Félix JD, Menéndez E, Rivas R, Carro L, Mateos PF, Martínez-Molina E, León-Barrios M, Velázquez E (2015a) *Cicer canariense*, an endemic legume to the Canary Islands, is nodulated in mainland Spain by fast-growing strains from symbiovar trifolii phylogenetically related to *Rhizobium leguminosarum*. Syst Appl Microbiol 38:346–350

Martínez-Hidalgo P, Galindo-Villardón P, Trujillo ME, Igual JM, Martínez-Molina E (2015b) *Micromonospora* from nitrogen fixing nodules of alfalfa (*Medicago sativa* L.). A new promising plant probiotic bacteria. Sci Rep 5:8271

Martínez-Hidalgo P, Ramírez-Bahena MH, Flores-Félix JD, Rivas R, Igual JM, Mateos PF, Martínez-Molina E, León-Barrios M, Peix Á, Velázquez E (2015c) Revision of the taxonomic status of type strains of *Mesorhizobium loti* and reclassification of strain USDA 3471^T as the type strain of *Mesorhizobium erdmanii* sp. nov. and ATCC 33669^T as the type strain of *Mesorhizobium jarvisii* sp. nov. Int J Syst Evol Microbiol 65:1703–1708

Mavengere NR, Ellis AG, Le Roux JJ (2014) *Burkholderia aspalathi* sp. nov., isolated from root nodules of the South African legume *Aspalathus abietina* Thunb. Int J Syst Evol Microbiol 64:1906–1912

Mehboob I, Naveed M, Zahir ZA, Sessitch A (2013) Potential or rhizosphere bacteria for improving *Rhizobium*-legume symbiosis. In: Arora NK (ed) Plant microbe symbiosis: fundamentals and advances, Springer, India, pp 305–349

Menéndez E, Ramírez-Bahena MH, Carro L, Fernández-Pascual M, Peter Klenk H, Velázquez E, Mateos PF, Peix A, Rita Scotti M (2016) *Paenibacillus periandrae* sp. nov., isolated from nodules of *Periandra mediterranea*. Int J Syst Evol Microbiol 66:1838–1843

Mishra RPN, Singh RK, Jaiswal HK, Kumar V, Maurya S (2006) *Rhizobium*-mediated induction of phenolics and plant growth promotion in rice (*Oryza sativa* L.) Curr Microbiol 52:383–389

Mishra PJ, Mishra S, Selvakumar G, Bisht JK, Kundu S, Gupta HS (2009a) Coinoculation of *Bacillus thuringeinsis*-KR1 with *Rhizobium leguminosarum* enhances plant growth and nodulation of pea (*Pisum sativum* L.) and lentil (*Lens culinaris* L.) World J Microbiol Biotechnol 25:753–761

Mishra PJ, Mishra S, Selvakumar G, Kundub S, Gupta HS (2009b) Enhanced soybean (Glycine max L.) plant growth and nodulation by *Bradyrhizobium japonicum*-SB1 in presence of *Bacillus thuringiensis*-KR1. Acta Agric Scand Sect B Soil Plant Sci 59:189–196

Montañez A, Blanco AR, Barlocco C, Beracochea M, Sicardi M (2012) Characterization of cultivable putative endophytic plant growth promoting bacteria associated with maize cultivars (*Zea mays* L.) and their inoculation effects *in vitro*. Appl Soil Ecol 58:21–28

Moulin L, Munive A, Dreyfus B, Boivin-Masson C (2001) Nodulation of legumes by members of the beta-subclass of Proteobacteria. Nature 411:948–950. Erratum in: Nature 412:926

Mousavi SA, Österman J, Wahlberg N, Nesme X, Lavire C, Vial L, Paulin L, de Lajudie P, Lindström K (2014) Phylogeny of the *Rhizobium-Allorhizobium-Agrobacterium* clade supports the delineation of *Neorhizobium* gen. nov. Syst Appl Microbiol 37:208–215

Mousavi SA, Willems A, Nesme X, de Lajudie P, Lindström K (2015) Revised phylogeny of *Rhizobiaceae*: proposal of the delineation of *Pararhizobium* gen. nov., and 13 new species combinations. Syst Appl Microbiol 38:84–90

Mulas D, García-Fraile P, Carro L, Ramírez-Bahena MH, Casquero P, Velázquez E, González-Andrés F (2011) Distribution and efficiency of *Rhizobium leguminosarum* strains nodulating *Phaseolus vulgaris* in Northern Spanish soils: selection of native strains that replace conventional N fertilization. Soil Biol Biochem 43:2283–2293

Mulas D, Seco V, Casquero P, Velázquez E, González-Andrés F (2015) Inoculation with indigenous Rhizobium strains increase yields of common bean (*Phaseolus vulgaris* L.) in norther Spain, although its efficiency is affected by the tillage system. Symbiosis 67:113–124

Muresu R, Polone E, Sulas L, Baldan B, Tondello A, Delogu G, Cappuccinelli P, Alberghini S, Benhizia Y, Benhizia H, Benguedouar A, Mori B, Calamassi R, Dazzo FB, Squartini A (2008) Coexistence of predominantly nonculturable rhizobia with diverse, endophytic bacterial taxa within nodules of wild legumes. FEMS Microbiol Ecol 63:383–400

Nandasena KG, O'Hara GW, Tiwari RP, Willlems A, Howieson JG (2007) *Mesorhizobium ciceri* biovar biserrulae, a novel biovar nodulating the pasture legume *Biserrula pelecinus* L. Int J Syst Evol Microbiol 57:1041–1045

Narozna D, Pudelko K, Kroliczak J, Golinska B, Sugawara M, Madrzak C, Sadowsky MJ (2015) Survival and competitiveness of *Bradyrhizobium japonicum* strains 20 years after introduction into field locations in Poland. Appl Environ Microbiol 81:5552–5559

Nascimento FX, Rossi MJ, Glick BR (2016) Role of ACC deaminase in stress control of leguminous plants. In: Subramaniam G, Arumugam S, Rajendran V (eds) Plant growth promoting actinobacteria. Springer, Germany, pp 179–192

Nobbe F, Hiltner L, Schmid E (1895) Versuche über die Biologie der Knöllchenbakterien der Leguminosen, insbesondere über die Frage der Arteinheit derselben. Landwirtsch Versuchstat Dresden 45:1–27

Noel TC, Sheng C, Yost CK, Pharis RP, Hynes MF (1996) *Rhizobium leguminosarum* as a plant growth-promoting rhizobacterium: direct growth promotion of canola and lettuce. Can J Microbiol 42:279–283

Ormeño E, Torres R, Mayo J, Rivas R, Peix A, Velázquez E, Zúñiga D (2007) *Phaseolus lunatus* is nodulated by a phosphate solubilizing strain of *Sinorhizobium meliloti* in a Peruvian soil. In: Velázquez E, Rodríguez-Barrueco C (eds) First international meeting on microbial phosphate solubilization. Springer, Germany, pp 143–147

Othman H, Tamimi SM (2016) Characterization of rhizobia nodulating faba bean plants isolated from soils of Jordan for plant growth promoting activities and N2 fixation potential. Int J Adv Res Biol Sci 3:20–27

Palaniappan P, Chauhan PS, Saravanan VS, Anandham R, Sa T (2010) Isolation and characterization of plant growth promoting endophytic bacterial isolates from root nodule of *Lespedeza* sp. Biol Fertil Soils 46:807–816

Pandey P, Kang SC, Maheshwari DK (2005) Isolation of endophytic plant growth promoting *Burkholderia* sp. MSSP from root nodules of *Mimosa pudica*. Curr Sci 89:177–180

Pandya M, Kumar GN, Rajkumar S (2013) Invasion of rhizobial infection thread by non rhizobia for colonization of *Vigna radiata* root nodules. FEMS Microbiol Lett 348:58–65

Pandya M, Rajput M, Rajkumar S (2015) Exploring plant growth promoting potential of non rhizobial root nodules endophytes of *Vigna radiata*. Microbiology 84:80–89

Paul KH, Dickin KL, Ali NS, Monterrosa EC, Stoltzfus RJ (2008) Soy and rice-based processed complementary food increases nutrient intakes in infants and is equally acceptable with or without added milk powder. J Nutr 138:1963–1968

Peix A, Rivas-Boyero AA, Mateos PF, Rodríguez-Barrueco C, Martínez-Molina E, Velázquez E (2001) Growth promotion of chickpea and barley by a phosphate solubilizing strain of *Mesorhizobium mediterraneum* under growth chamber conditions. Soil Biol Biochem 33:103–110

Peix A, Ramírez-Bahena MH, Flores-Félix JD, Alonso de la Vega P, Rivas R, Mateos PF, Igual JM, Martínez-Molina E, Trujillo ME, Velázquez E (2015a) Revision of the taxonomic status of the species *Rhizobium lupini* and reclassification as *Bradyrhizobium lupini* comb. nov. Int J Syst Evol Microbiol 65:1213–1219

Peix A, Ramírez-Bahena MH, Velázquez E, Bedmard EJ (2015b) Bacterial associations with legumes. Crit Rev Plant Sci 34:17–42

Perret X, Staehelin C, Broughton WJ (2000) Molecular basis of symbiotic promiscuity. Microbiol Mol Biol Rev 64:180–201

Rajendran G, Patel MH, Joshi SJ (2012) Isolation and characterization of nodule-associated *Exiguobacterium* sp. from the root nodules of fenugreek (*Trigonella foenum-graecum*) and their possible role in plant growth promotion. Int J Microbiol 2012:693982

Ramírez-Bahena MH, Flores Félix JD, Chahboune R, Toro M, Velázquez E, Peix A (2016) *Bradyrhizobium centrosemae* (symbiovar centrosemae) sp. nov., *Bradyrhizobium americanum* (symbiovar phaseolarum) sp. nov. and a new symbiovar (tropici) of *Bradyrhizobium viridifuturi* establish symbiosis with *Centrosema* species native to America. Syst Appl Microbiol 39:378–383

Ramírez-Bahena MH, Tejedor C, Martín I, Velázquez E, Peix A (2013) *Endobacter medicaginis* gen. nov., sp. nov., isolated from alfalfa nodules in an acidic soil. Int J Syst Evol Microbiol 63:1760–1765

Ramírez-Bahena MH, Salazar S, Velázquez E, Laguerre G, Peix A (2015) Characterization of phosphate solubilizing rhizobacteria associated with pea (*Pisum sativum* L.) isolated from two agricultural soils. Symbiosis 67:33–41

Rashid MH, Young JP, Everall I, Clercx P, Willems A, Santhosh Braun M, Wink M (2015) Average nucleotide identity of genome sequences supports the description of *Rhizobium lentis* sp. nov., *Rhizobium bangladeshense* sp. nov. and *Rhizobium binae* sp. nov. from lentil (*Lens culinaris*) nodules. Int J Syst Evol Microbiol 65:3037–3045

Rebello CJ, Greenway FL, Finley JW (2014) A review of the nutritional value of legumes and their effects on obesity and its related co-morbidities. Obes Rev 15:392–407

Remigi P, Zhu J, Young JPW, Masson-Boivin C (2016) Symbiosis within symbiosis: evolving nitrogen-fixing legume symbionts. Trends Microbiol 24:63–75

Ren d W, Wang ET, Chen WF, Sui XH, Zhang XX, Liu HC, Chen WX (2011) *Rhizobium herbae* sp. nov. and *Rhizobium giardinii*-related bacteria, minor microsymbionts of various wild legumes in China. Int J Syst Evol Microbiol 61:1912–1920

Ribeiro RA, Martins TB, Ormeño-Orrillo E, Marçon Delamuta JR, Rogel MA, Martínez-Romero E, Hungria M (2015) *Rhizobium ecuadorense* sp. nov., an indigenous N2-fixing symbiont of the Ecuadorian common bean (*Phaseolus vulgaris* L.) genetic pool. Int J Syst Evol Microbiol 65:3162–3169

Rincón-Rosales R, Villalobos-Escobedo JM, Rogel MA, Martinez J, Ormeño-Orrillo E, Martínez-Romero E (2013) *Rhizobium calliandrae* sp. nov, *Rhizobium mayense* sp. nov. and *Rhizobium jaguaris* sp. nov. rhizobial species nodulating the medicinal legume *Calliandra grandiflora*. Int J Syst Evol Microbiol 63:3423–3429

Rivas R, Peix A, Mateos PF, Trujillo ME, Martínez-Molina E, Velázquez E (2006) Biodiversity of populations of phosphate solubilizing rhizobia that nodulates chickpea in different Spanish soils. Plant Soil 287:23–33

Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rogel MA, Ormeño-Orrillo E, Martinez Romero E (2011) Symbiovars in rhizobia reflect bacterial adaptation to legumes. Syst Appl Microbiol 34:96–104

Rojas A, Holguin G, Glick BR, Bashan Y (2001) Synergism between *Phyllobacterium* sp. (N2-fixer) and *Bacillus licheniformis* (P-solubilizer), both from a semiarid mangrove rhizosphere. FEMS Microbiol Ecol 35:181–187

Román-Ponce B, Jing Zhang Y, Soledad Vásquez-Murrieta M, Hua Sui X, Feng Chen W, Carlos Alberto Padilla J, Wu Guo X, Lian Gao J, Yan J, Hong Wei G, Tao Wang E (2016) *Rhizobium acidisoli* sp. nov., isolated from root nodules of *Phaseolus vulgaris* in acid soils. Int J Syst Evol Microbiol 66:398–406

Rubio-Canalejas A, Celador-Lera L, Cruz-González X, Menéndez E, Rivas R (2016) *Rhizobium* as potential biofertilizer of *Eruca Sativa*. In: González-Andrés F, James E (eds) Biological nitrogen fixation and beneficial plant-microbe interaction. Springer, Germany, pp 213–220

Safronova VI, Kuznetsova IG, Sazanova AL, Kimeklis AK, Belimov AA, Andronov EE, Pinaev AG, Chizhevskaya EP, Pukhaev AR, Popov KP, Willems A, Tikhonovich IA (2015) *Bosea vaviloviae* sp. nov., a new species of slow-growing rhizobia isolated from nodules of the relict species *Vavilovia formosa* (Stev.) Fed. Antonie Van Leeuwenhoek 107:911–920

Saha R, Saha N, Donofrio RS, Bestervelt LL (2013) Microbial siderophores: a mini review. J Basic Microbiol 53:303–317

Saïdi S, Chebil S, Gtari M, Mhamdi R (2013) Characterization of root-nodule bacteria isolated from Vicia faba and selection of plant growth promoting isolates. World J Microbiol Biotechnol 29:1099–1106

Saini R, Dudeja SS, Giri R, Kumar V (2015) Isolation, characterization, and evaluation of bacterial root and nodule endophytes from chickpea cultivated in Northern India. J Basic Microbiol 55:74–81

Sánchez M, Ramírez-Bahena MH, Peix A, Lorite MJ, Sanjuán J, Velázquez E, Monza J (2014) *Phyllobacterium loti* sp. nov. isolated from nodules of *Lotus corniculatus*. Int J Syst Evol Microbiol 64:781–786

Santoyo G, Moreno-Hagelsieb G, Orozco-Mosqueda Mdel C, Glick BR (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99

Sarr PS, Okon JW, Begoude DAB, Araki S, Ambang Z, Shibata M, Funakawa S (2016) Symbiotic N2-fixation estimated by the 15n tracer technique and growth of *Pueraria phaseoloides* (Roxb.) Benth. inoculated with *Bradyrhizobium* strain in field conditions. Scientifica 2016:ID 7026859

Sawana A, Adeolu M, Gupta RS (2014) Molecular signatures and phylogenomic analysis of the genus *Burkholderia*: proposal for division of this genus into the emended genus *Burkholderia* containing pathogenic organisms and a new genus *Paraburkholderia* gen. nov. harboring environmental species. Front Genet 5:429

Schwartz AR, Ortiz I, Maymon M, Herbold CW, Fujishige NA, Vijanderan JA, Villella W, Hanamoto K, Diener A, Sanders ER, DeMason DA, Hirsch AM (2013) *Bacillus simplex* – a little known pgpb with anti-fungal activity – alters pea legume root architecture and nodule morphology when coinoculated with *Rhizobium leguminosarum* bv. viciae. Agronomy 3:595–620

Selvakumar G, Kundu S, Gupta AD, Shouche YS, Gupta HS (2008) Isolation and characterization of nonrhizobial plant growth promoting bacteria from nodules of Kudzu (*Pueraria thunbergiana*) and their effect on wheat seedling growth. Curr Microbiol 56:134–139

Sene G, Thiao M, Samba-Mbaye R, Khasa D, Kane A, Samba Mbaye M, Beaulieu ME, Manga A, Ndao Sylla S (2013) The abundance and diversity of legume-nodulating rhizobia in 28-year-old plantations of tropical, subtropical, and exotic tree species: a case study from the forest reserve of Bandia, Senegal. Microb Ecol 65:128–144

Senthilkumar M, Madhaiyan M, Sundaram SP, Kannaiyan S (2009) Intercellular colonization and growth promoting effects of *Methylobacterium* sp. with plant-growth regulators on rice (*Oryza sativa* L. Cv CO-43). Microbiol Res 164:92–104

Shamseldin A, Carro L, Peix A, Velázquez E, Moawad H, Sadowsky MJ (2016) The symbiovar trifolii of *Rhizobium bangladeshense* and *Rhizobium aegyptiacum* sp. nov. nodulate *Trifolium alexandrinum* in Egypt. Syst Appl Microbiol 39:275–279

Sharma SR, Rao NK, Gokhale TS, Ismail S (2013) Isolation and characterization of salt-tolerant rhizobia native to the desert soils of United Arab Emirates. Emirates J Food Agric 25:102–108

Shashirekha MN, Mallikarjuna SE, Rajarathnam S (2015) Status of bioactive compounds in foods, with focus on fruits and vegetables. Crit Rev Food Sci Nutr 55:1324–1339

Sheu SY, Chen MH, Liu WY, Andrews M, James EK, Ardley JK, De Meyer SE, James TK, Howieson JG, Coutinho BG, Chen WM (2015) *Burkholderia dipogonis* sp. nov., isolated from root nodules of *Dipogon lignosus* in New Zealand and Western Australia. Int J Syst Evol Microbiol 65:4716–4723

Shiraishi A, Matsushita N, Hougetsu T (2010) Nodulation in black locust by the Gammaproteobacteria *Pseudomonas* sp. and the Betaproteobacteria *Burkholderia* sp. Syst Appl Microbiol 33:269–274

Silva LR, Pereira MJ, Azevedo J, Mulas R, Velazquez E, González-Andrés F, Valentão P, Andrade PB (2013) Inoculation with *Bradyrhizobium japonicum* enhances the organic and fatty acids content of soybean (*Glycine max* (L.) *Merrill*) seeds. Food Chem 141:3636–3648

Silva FV, De Meyer SE, Simões-Araújo JL, Barbé Tda C, Xavier GR, O'Hara G, Ardley JK, Rumjanek NG, Willems A, Zilli JE (2014a) *Bradyrhizobium manausense* sp. nov, isolated from effective nodules of *Vigna unguiculata* grown in Brazilian Amazonian rainforest soils. Int J Syst Evol Microbiol 64:2358–2363

Silva LR, Azevedo J, Pereira MJ, Carro L, Velazquez E, Peix A, Valentão P, Andrade PB (2014b) Inoculation of the nonlegume *Capsicum annuum* L. with *Rhizobium* strains. 2. Changes in sterols, triterpenes, fatty acids, and volatile compounds. J Agric Food Chem 62:565-573

Silva LR, Peix A, Albuquerque C, Velàzquez E (2016) Bioactive compounds of legumes as health promoters. In: da Silva LR, Silva BM (eds) Natural bioactive compounds from fruits and vegetables. Bentham Science Publishers, Sharjah, pp 3–26

Singh RK, Malik N, Singh S (2013) Impact of rhizobial inoculation and nitrogen utilization in plant growth promotion of maize (Zea mays L.) Bioscience 5:8–14

Smyth EM, McCarthy J, Nevin R, Khan MR, Dow JM, O'Gara F, Doohan FM (2011) In vitro analyses are not reliable predictors of the plant growth promotion capability of bacteria; a *Pseudomonas fluorescens* strain that promotes the growth and yield of wheat. J Appl Microbiol 111:683–692

Sridevi M, Mallaiah KV, Yadav NCS (2007) Phosphate solubilization by *Rhizobium* isolates from *Crotalaria* species. J Plant Sci 2:635–639

Stambulska UY, Lushchak VI (2015) Efficacy of symbiosis formation by pea plants with local Western Ukrainian strains of *Rhizobium*. J Microbiol Biotechnol Food Sci 5:92–98

Steenkamp ET, van Zyl E, Beukes CW, Avontuur JR, Chan WY, Palmer M, Mthombeni LS, Phalane FL, Sereme TK, Venter SN (2015) *Burkholderia kirstenboschensis* sp. nov. nodulates papilionoid legumes indigenous to South Africa. Syst Appl Microbiol 38:545–554

Sturz AV, Christie BR, Matheson BG, Nowak J (1997) Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. Biol Fertil Soils 25:13–19

Subramanian P, Kim K, Krishnamoorthy R, Sundaram S, Sa T (2015) Endophytic bacteria improve nodule function and plant nitrogen in soybean on co-inoculation with *Bradyrhizobium japonicum* MN110. Plant Growth Regul 76:327–332

Sy A, Giraud E, Jourand P, Garcia N, Willems A, de Lajudie P, Prin Y, Neyra M, Gillis M, Boivin-Masson C, Dreyfus B (2001) Methylotrophic *Methylobacterium* bacteria nodulate and fix nitrogen in symbiosis with legumes. J Bacteriol 183:214–220

Tariq M, Hameed S, Yasmeen T, Zahid M, Zafar M (2014) Molecular characterization and identification of plant growth promoting endophytic bacteria isolated from the root nodules of pea (*Pisum sativum* L.) World J Microbiol Biotechnol 30:719–725

Taurian T, Anzuay MS, Angelini JG, Tonelli ML, Ludueña L, Pena D, Ibáñez F, Fabra A (2009) Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting activities. Plant Soil 329:421–431

Tharanathan RN, Mahadevamma S (2003) Grain legumes-a boon to human nutrition. Trends Food Sci Technol 14:507–518

Torche A, Benhizia H, Rosselli R, Romoli O, Zanardo M, Baldan E, Alberghini S, Tondello A, Baldan B, Benguedouar A, Squartini A, Benhizia Y (2014) Characterization of bacteria associated with nodules of two endemic legumes of Algeria, *Hedysarum naudinianum* and *H. perrauderianum*. Ann Microbiol 64:1065–1071

Trujillo ME, Kroppenstedt RM, Fernández-Molinero C, Schumann P, Martínez-Molina E (2007) *Micromonospora lupini* sp. nov. and *Micromonospora saelicesensis* sp. nov., isolated from root nodules of *Lupinus angustifolius*. Int J Syst Evol Microbiol 57:2799–2804

Valdez RA, Soriano B, Prado G, Zavaleta D, Matsubara M, Zúñiga D, Dion P, Valladolid A, Ruesta A, Castellano-Hinojosa A, Correa-Galeote D, Bedmar EJ (2016) Symbiotic and agronomic characterization of bradyrhizobial strains nodulating cowpea in Northern Peru. In: González-Andrés F, James E (eds) Biological nitrogen fixation and beneficial plant-microbe interaction. Springer, Germany, pp 195–212

Valverde A, Velázquez E, Gutiérrez C, Cervantes E, Ventosa A, Igual JM (2003) *Herbaspirillum lusitanum* sp. nov., a novel nitrogen-fixing bacterium associated with root nodules of *Phaseolus vulgaris*. Int J Syst Evol Microbiol 53:1979–1983

Valverde A, Burgos A, Fiscella T, Rivas R, Velázquez E, Rodríguez-Barrueco C, Cervantes E, Chamber M, Igual MJ (2006) Differential effects of coinoculations with *Pseudomonas jessenii* PS06 (a phosphate-solubilizing bacterium) and *Mesorhizobium ciceri* C-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. Plant Soil 287:43–50

Valverde A, Fterich A, Mahdhi M, Ramírez-Bahena MH, Caviedes MA, Mars M, Velázquez E, Rodriguez-Llorente ID (2010) *Paenibacillus prosopidis* sp. nov., isolated from the nodules of *Prosopis farcta*. Int J Syst Evol Microbiol 60:2182–2186

Velázquez E, Martínez-Hidalgo P, Carro L, Alonso P, Peix A, Trujillo M, Martínez-Molina E (2013) Nodular endophytes: an untapped diversity. In: Rodelas-González MB, González-López J (eds) Beneficial plant-microbe interactions: ecology and applications. CRC Press, USA, pp 214–236

Verma JP, Yadav J, Tiwaric KN, Kumarb A (2013) Effect of indigenous *Mesorhizobium* spp. and plant growth promoting rhizobacteria on yields and nutrients uptake of chickpea (*Cicer arietinum* L.) under sustainable agriculture. Ecol Eng 51:282–286

Villar-Igea M, Velázquez E, Rivas R, Willems A, van Berkum P, Trujillo ME, Mateos PF, Gillis M, Martínez-Molina E (2007) Phosphate solubilizing rhizobia originating from *Medicago*, *Melilotus* and *Trigonella* grown in a Spanish soil. In: Velázquez E, Rodríguez-Barrueco C (eds) First international meeting on microbial phosphate solubilization. Springer, Germany, pp 149–156

Villegas MC, Rome S, Mauré L, Domergue O, Gardan L, Bailly X, Cleyet-Marel JC, Brunel B (2006) Nitrogen-fixing sinorhizobia with *Medicago laciniata* constitute a novel biovar (bv. medicaginis) of *S. meliloti*. Syst Appl Microbiol 29:526–538

Wang ET, van Berkum P, Beyene D, Sui XH, Dorado O, Chen WX, Martínez-Romero E (1998) *Rhizobium huautlense* sp. nov., a symbiont of *Sesbania herbacea* that has a close phylogenetic relationship with *Rhizobium galegae*. Int J Syst Bacteriol 48:687–699

Wang X, Jia F, Liu C, Zhao J, Wang L, Shen Y, Wang J, Zhang J, Li C, Xiang W (2013) *Xiangella phaseoli* gen. nov., sp. nov., a member of the family *Micromonosporaceae*. Int J Syst Evol Microbiol 63:2138–2145

Wdowiak-Wróbel S, Małek W (2016) Properties of *Astragalus* sp. microsymbionts and their putative role in plant growth promotion. Arch Microbiol. doi:10.1007/s00203-016-1243-3

Wei XL, Han MS, Xia CC, Ding SL, Xu L, Lin YB, Wang L, Wei GH (2015a) *Diaphorobacter ruginosibacter* sp. nov., isolated from soybean root nodule, and emended description of the genus *Diaphorobacter*. Arch Microbiol 197:683–692

Wei XL, Lin YB, Xu L, Han MS, Dong DH, Chen WM, Wang L, Wei GH (2015b) *Bacillus radicibacter* sp. nov., a new bacterium isolated from root nodule of *Oxytropis ochrocephala* Bunge. J Basic Microbiol 55:1212–1218

Williams PG, Grafenauer SJ, O'Shea JE (2008) Cereal grains, legumes, and weight management: a comprehensive review of the scientific evidence. Nutr Rev 66:171–182

Wilson JK (1939) Leguminous plants and their associated organisms. Cornell University Agricultural Experiment Station memoir 221. Cornell University Press, Ithaca

Woese CR, Stackebrandt E, Weisburg WG, Paster BJ, Madigan MT, Fowler VJ, Hahn CM, Blanz P, Gupta R, Nealson KH, Fox GE (1984) The phylogeny of purple bacteria: the alpha subdivision. Syst Appl Microbiol 5:315–326

Wright W, Little J, Liu F, Chakraborty R (2013) Isolation and structural identification of the trihydroxamate siderophore vicibactin and its degradative products from *Rhizobium leguminosarum* ATCC 14479 bv. trifolii. Biometals 26:271–283

Xu L, Shi JF, Zhao P, Chen WM, Qin W, Tang M, Wei GH (2011) *Rhizobium sphaerophysae* sp. nov., a novel species isolated from root nodules of *Sphaerophysa salsula* in China. Antonie Van Leeuwenhoek 99:845–854

Xu L, Zhang Y, Wang L, Chen W, Wei G (2014) Diversity of endophytic bacteria associated with nodules of two indigenous legumes at different altitudes of the Qilian Mountains in China. Syst Appl Microbiol 37:457–465

Yan J, Han XZ, Ji ZJ, Li Y, Wang ET, Xie ZH, Chen WF (2014) Abundance and diversity of soybean-nodulating rhizobia in black soil are impacted by land use and crop management. Appl Environ Microbiol 80:5394–5402

Yan H, Ji ZJ, Jiao YS, Wang ET, Chen WF, Guo BL, Chen WX (2016a) Genetic diversity and distribution of rhizobia associated with the medicinal legumes *Astragalus* spp. and *Hedysarum polybotrys* in agricultural soils. Syst Appl Microbiol 39:141–149

Yan H, Yan J, Sui XH, Wang ET, Chen WX, Zhang XX, Chen WF (2016b) *Ensifer glycinis* sp. nov, an novel rhizobial species associated with *Glycine* spp. Int J Syst Evol Microbiol. doi:10.1099/ijsem.0.001120

Yanni YG, Dazzo FB (2010) Enhancement of rice production using endophytic strains of *Rhizobium leguminosarum* bv. trifolii in extensive field inoculation trials within the Egypt Nile delta. Plant Soil 336:129–142

Yanni YG, Rizk RY, Corich V, Squartini A, Ninke K, Philip-Hollingsworth S, Orgambide G, de Bruijn F, Stoltzfus J, Buckley D, Schmidt TM, Mateos PF, Ladha JK, Dazzo FB (1997) Natural endophytic association between *Rhizobium leguminosarum* bv. trifolii and rice roots and assessment of its potential to promote rice growth. Plant Soil 194:99–114

Yanni YG, Rizk RY, Abd El-Fattah FK, Squartini A, Corich V, Giacomini A, de Bruijn F, Rademaker J, Maya-Flores J, Ostrom P, Vega-Hernandez M, Hollingsworth RI, Martínez-Molina E, Mateos P, Velázquez E, Wopereis J, Triplett E, Umali-García M, Anarna JA, Rolfe BG, Ladha JK, Hill J, Mujoo R, Ng PK, Dazzo FB (2001) The beneficial plant growth-promoting association of *Rhizobium leguminosarum* bv. trifolii with rice roots. Aust J Plant Physiol 28:845–870

Yanni YG, Dazzo FB, Squartini A, Zanardo M, Zidan MI, Elsadany AEY (2016) Assessment of the natural endophytic association between *Rhizobium* and wheat and its ability to increase wheat production in the Nile delta. Plant Soil. doi:10.1007/s11104-016-2895-0

Yao LJ, Shen YY, Zhan JP, Xu W, Cui GL, Wei GH (2012) *Rhizobium taibaishanense* sp. nov., isolated from a root nodule of *Kummerowia striata*. Int J Syst Evol Microbiol 62:335–341

Yao Y, Sui XH, Zhang XX, Wang ET, Chen WX (2015) *Bradyrhizobium erythrophlei* sp. nov. and *Bradyrhizobium ferriligni* sp. nov, isolated from effective nodules of *Erythrophleum fordii*. Int J Syst Evol Microbiol 65:1831–1837

Yin J, He D, Li X, Zeng X, Tian M, Cheng G (2015) *Paenibacillus enshidis* sp. nov., isolated from the Nodules of *Robinia pseudoacacia* L. Curr Microbiol 71:321–325

Young JM, Kuykendall LD, Martínez -Romero E, Kerr A, Sawada H (2001) A revision of *Rhizobium* Frank 1889, with an emended description of the genus, and the inclusion of all species of *Agrobacterium* Conn 1942 and *Allorhizobium undicola* de Lajudie et al. 1998 as new combinations: *Rhizobium radiobacter*, *R. rhizogenes, R. rubi, R. undicola* and *R. vitis*. Int J Syst Evol Microbiol 51:89–103

Yu X, Cloutier S, Tambong JT, Bromfield ES (2014) *Bradyrhizobium ottawaense* sp. nov, a symbiotic nitrogen fixing bacterium from root nodules of soybeans in Canada. Int J Syst Evol Microbiol 64:3202–3207

Zaheer A, Mirza BS, Mclean JE, Yasmin S, Shah TM, Malik KA, Mirza MS (2016) Association of plant growth-promoting *Serratia* spp. with the root nodules of chickpea. Res Microbiol. doi:10.1016/j.resmic.2016.04.001

Zakhia F, Jeder H, Willems A, Gillis M, Dreyfus B, Lajudie P (2006) Diverse bacteria associated with root nodules of spontaneous legumes in Tunisia and first report for *nifH*-like gene within the genera *Microbacterium* and *Starkeya*. Microb Ecol 51:375–393

Zgadzaj R, James EK, Kelly S, Kawaharada Y, de Jonge N, Jensen DB, Madsen LH, Radutoiu S (2015) A legume genetic framework controls infection of nodules by symbiotic and endophytic bacteria. PLoS Genet 11:e1005280

Zhang YJ, Zheng WT, Everall I, Young JP, Zhang XX, Tian CF, Sui XH, Wang ET, Chen WX (2015) *Rhizobium anhuiense* sp. nov., isolated from effective nodules of *Vicia faba* and *Pisum sativum*. Int J Syst Evol Microbiol 65:2960–2967

Zhu YJ, Lu JK, Chen YL, Wang SK, Sui XH, Kang H (2015) Mesorhizobium acaciae sp. nov., isolated from root nodules of Acacia melanoxylon R. Br. Int J Syst Evol Microbiol 65(10):3558–3563

Zilli JE, Baraúna AC, da Silva K, De Meyer SE, Farias EN, Kaminski PE, da Costa IB, Ardley JK, Willems A, Camacho NN, Dourado Fdos S, O'Hara G (2014) *Bradyrhizobium neotropicale* sp. nov., isolated from effective nodules of Centrolobium paraense. Int J Syst Evol Microbiol 64(12):3950–3957

Plant Growth-Promoting Microbes: Diverse Roles in Agriculture and Environmental Sustainability

4

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Abstract

The need for environmental sustainability to create a balance between the future's need and resources available is a key issue at the global level. The world's population is increasing day by day, and natural resources are being exploited rapidly. In this situation, enhancement of agricultural productivity for feeding expanding population is a matter of concern. Conventional agricultural practices for enhancing productivity pose a threat to agroecosystems. Experience with the indiscriminate use of chemical fertilizers and pesticide is bitter. Similarly, the impact of anthropogenic activities and global climate change on the environment is detrimental and created irreversible changes in the agroecosystems. In this scenario, a major focus on plant growth-promoting microbes (PGPM) for restoring the agroecosystems to their original shape is gaining the attention of agronomists and environmentalists. Work on rhizospheric bacteria and fungi has already shown potential in the management of various agricultural problems, and especially their use in the form of biofertilizers and biopesticides has resulted in lesser reliance on synthetic agrochemicals. However, a fresh perspective suggests the role of PGPM in the remediation of ecosystems through removal of recalcitrant compounds and as alleviators of abiotic stresses, thus also helping to combat the impact of climate change. Although PGPM are proving promising tools for environmental sustainability, yet more work needs to be carried out for establishing their firm position to manage agroecosystems in a sustainable manner. Greater knowledge and revelation of the secret of plant–microbe interactions will provide a state-of-the-art solution for food security in terms of quality, quantity, and environmental sustainability.

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4.1 Introduction

Food requirement in the form of nutrients and balanced diet is a major challenge for every nation, particularly in circumstances when there is accelerated rate of urbanization and industrialization resulting in the loss of agricultural lands. Global Land Assessment of Soil Degradation (GLASOD) mapping system (Oldeman 1998) estimated that 8.7 billion hectares of agricultural land, pasture, and forest and 2 billion hectares of woodland have been degraded since mid-century, the mapping system also shows that 3.5% of the total has been degraded severely and 10% moderately and another 9% is lightly degraded. Another striking outcome of this study reveals that the degradation of cropland appears to be most extensive in Africa, affecting 65% of cropland area, compared with 51% in Latin America and 38% in Asia. Presently, 1.5 billion hectares, i.e., about 12% of the world's land area, is used for agriculture, whereas 38% of this has been degraded due to poor natural resource management practices (FAO 2013). Data also suggests that if corrective measures are not taken to prevent further degradation of agroecosystems, then it will be impossible to maintain even current levels of production (IFPRI 2000). The growing world population, which is projected to be around 9.38 billion by 2050 (USCB 2015) also entails that the quantity and quality of food produced globally must be increased significantly, otherwise, it will be impossible to feed an additional 1.16 billion mouths (Meyers and Kalaitzandonakes 2012). In the last few decades, growers' trust in sustainable agriculture has increased even in developing countries as they have been the prime victims of using unsustainable approaches for increasing productivity (Kesavan and Swaminathan 2008). Although the major goal is to increase the productivity without harming the agroecosystems, in the absence of sustainable approach, the current measures being taken are insufficient. The conventional agriculture, which involves high-yielding plants, mechanized tillage, inorganic fertilizers, and chemical pesticides, has only raised the problems (Horrigan et al. 2002). Enormous amounts of chemical fertilizers used for increasing productivity remain inaccessible to crops (Bhandari 2014). Similarly, chemical pesticides for disease eradication have also caused effects on soil biota and health (Aktar et al. 2009). According to Hole et al. (2005), agricultural practices using too much pesticides result in a loss of biodiversity. In a study, Pelosi et al. (2013) stated that even earthworms are at huge risk due to use of pesticides. Additionally, a study by Lu et al. (2008) reported that exposure to organophosphate pesticides or their residues may occur via consumption of conventionally grown fruits and vegetables that lead to negative effects on human health.

Another important issue, which has gained the global attention, is related to environmental sustainability. It is well understood that anthropogenic activities are the cause of environmental degradation, increasingly polluting air and water, altering earth's climate, eroding and negatively impacting the soil, fragmenting and eliminating the habitat of plants and animals and depleting the natural bank of nonrenewable resources (Harte 2007).

For the past few years, the role of PGPM in sustainable agriculture and environmental management got attention, and their use in agroecosystems and solving key

environmental problems has shown remarkable results. In the agricultural sector, their use enabled the conversion of low-input systems to more sustainable high-output systems. Similarly, multifunctional roles of PGPM have shown good results in bioremediation of different types of wastes generated by anthropogenic activities. However, a detailed understanding of the various functions and applications of PGPM in agroecosystems for maintaining sustainability is of prime importance. This chapter is aimed to report the potential use of PGPM in maintaining the quality of the soil and enhancing crop productivity in a sustainable manner.

4.2 Plant Growth-Promoting Microbes

PGPM are soil and rhizosphere-inhabiting microorganisms that can colonize plant roots in significant numbers (10^5–10^7 CFU per gram of fresh root) and influence plant growth in a positive manner (Spaepen et al. 2009). These soil microorganisms with beneficial activities assist in plant growth and health (Antoun and Prevost 2005). In general, the rhizospheric region is a hot spot for microbial activities contributed mainly by indigenous bacteria and fungi (Pinton et al. 2001; Nelson 2004). PGPM can be divided into two main groups: plant growth-promoting rhizobacteria (PGPR) and plant growth-promoting fungi (PGPF). PGPR were first defined by Kloepper and Schroth (1978) to describe soil bacteria that colonize the roots of plants and in a mutualistic manner enhance the plant growth. Research shows that their broad application in the agricultural system is gaining the faith of growers (Reddy et al. 2014). Microbial activities such as solubilization of inorganic compounds, degradation and mineralization of organic compounds, and secretion of biologically active substances such as phytohormones, chelators and antibiotics help a lot in plant growth enhancement (Kapulnik and Okon 2002).

4.2.1 Plant Growth-Promoting Rhizobacteria

PGPR are soil bacteria that stimulate plant growth by various means, often in association with plant roots, sometimes on leaves and/or within plant tissues (Glick 2012). A vast array of PGPR including species of *Pseudomonas*, *Rhizobium*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus*, and *Serratia* have been reported to enhance plant growth (Beneduzi et al. 2012; Ahemad and Kibret 2014). These microbes directly assist in several fundamental processes required for plant growth, for example, fixation of atmospheric nitrogen (N) (Hirel et al. 2011), solubilization of inorganic phosphate (Sharma et al. 2013), sequestration of iron (Fe) (Sayyed et al. 2013), and synthesis of phytohormones (Maheshwari et al. 2015). PGPR indirectly help in plant growth promotion by preventing it from the deleterious phytopathogens (Fernando et al. 2005; Fatima et al. 2009; Mishra and Arora 2012a). Most recognized mechanisms of indirect growth promotion mediated by PGPR are competition for ecological niche or a substrate, production of inhibitory allelochemicals, and induction of

systemic resistance (ISR) in host plants to a broad spectrum of pathogens and/or abiotic stresses (Compant et al. 2005).

Biological nitrogen fixation (BNF) is the conversion of atmospheric N into ammonia by symbiotic, associative, and free-living bacteria and being considered as relevant to the environment and to world agriculture (Dixon and Kahn 2004). BNF is very well understood in many diazotrophs, but more recent research has demonstrated that a vast array of microbes are capable of it, including archaea as well as many previously undiscovered bacteria (Vitousek et al. 2013). Among all N-fixing microbes, bacteria-forming root nodules, commonly known as rhizobia, are of most importance and show obligate symbiotic association with legumes. These bacteria colonize the host plant's root system and cause the roots to form nodules, which are a storehouse of fixed nitrogen (for more details on N-fixation process in legume, see review by Bruijn 2015). The symbiotic association of rhizobia and host legume is so intricate that a particular *Rhizobium* will only modulate a select number of plant genera (Wagner 2012). A number of studies show that symbiotic N-fixing PGPR (*Rhizobium*, *Bradyrhizobium*, *Sinorhizobium*, *Mesorhizobium*, and *Azorhizobium*) increase N content in legumes in field conditions (Bruijn 2015). Rhizobial biofertilizers are reported to be used worldwide for many of the legume crops including pulses.

Phosphate-solubilizing bacteria (PSB), and their use as inoculants, are known to increase phosphate uptake by plants (Zaidi et al. 2009). *Pseudomonas*, *Bacillus*, and *Rhizobium* are dominant PSB used for commercial application (Rodríguez and Fraga 1999; Bossis et al. 2000). PGPR also play a significant role in Fe bioavailability to plants by secretion of siderophores. Siderophores are Fe-binding extracellular compounds with low molecular weight (2 kDa) and high affinity for ferric (Fe^{3+}) form of Fe (Krewulak and Vogel 2008). Siderophores chelate Fe in a reversible manner (Budzikiewicz 2010). Siderophores first bind with Fe^{+3} tightly and then the siderophore-Fe complex moves into the cell through the cell membrane receptors (Ahmed and Holmstrom 2014). There are 500 different types of siderophores reported, of which 270 are well characterized (Boukhalfa et al. 2003), while the functions of the rest are yet to be determined (Ali and Vidhale 2013). Apart from this siderophore production confers a competitive advantage to PGPR that can colonize roots and exclude other microorganisms from the ecological niche (Kannahi and Senbagam 2014).

Other mechanisms adopted by PGPB are the production of hydrogen cyanide (HCN), a broad-spectrum antimicrobial compound involved in biological control of root diseases by many plant-associated fluorescent pseudomonads (Ramette et al. 2003). Selected strains of beneficial PGPR also trigger a plant-mediated ISR response that is effective against a broad spectrum of plant pathogens (Ramos et al. 2008). ISR is a plant-mediated mechanism. It resembles classic pathogen-induced resistance, in which noninfected parts of previously pathogen-infected plants become more resistant to further infection (Pieterse et al. 2001). Antagonistic activities of PGPR may also involve the synthesis of hydrolytic enzymes, such as chitinases, glucanases, proteases, and lipases, which can lyse hyphae of pathogenic fungi (Maksimov et al. 2011).

Since a century, members of the genus *Bacillus* are being exploited as microbial pesticides, fungicides, or fertilizers (Sivasakthi et al. 2014), and still in the agricultural sector *Bacillus*-based products represent the most important class of microbial products (Fravel 2005). More detail on Bt is also given in other section of this chapter. Another very important PGPR of the genus *Pseudomonas* are known for their wider biocontrol and PGP activities (Saravanakumar and Samiyappan 2007; Arora et al. 2008; Tewari and Arora 2015). Several studies have confirmed their biocontrol activity against plant pathogenic fungi (Ganeshan and Kumar 2005; Weller 2007; Khare and Arora 2011a; Tewari and Arora 2014a) Numerous types of metabolites have been reported from diverse strains of *Pseudomonas* inhabiting in the rhizospheric region of the plants (Spago et al. 2014). Antibiotics including 2,4-diacetylphloroglucinol, phenazine-1-carboxyclic acid, phenazine-1-carboxamide, pyoluteorin, pyrrolnitrin, oomycin A, viscosinamide, butyrolactones, kanosamine, zwittermycin-A, aerugine, rhamnolipids, cepaciamide A, ecomycins, pseudomonic acid, azomycin, antitumor antibiotics, cepafungins, and antiviral antibiotics are known to be produced by pseudomonads having roles in controlling phytopathogens (Fernando et al. 2005).

Some studies show the potential of actinomycetes in PGP activities (Merzaeva and Shirokikh 2006; Verma et al. 2011; Kaur et al. 2013). Actinomycetes strains like *Streptomyces* spp., *Micromonospora* spp., *Streptosporangium* spp., and *Thermobifida* spp. are recorded as biocontrol agents against a range of root pathogenic fungi (Kaur et al. 2013; Sreevidya et al. 2016). Actinomycetes benefit plants by various activities such as production of phytohormones (Solans et al. 2011), fungal cell wall-degrading enzymes (Anitha and Rabeeth 2010), and production of antibiotics (de Lima Procópio et al. 2012).

4.2.2 Plant Growth-Promoting Fungi

Various workers have also studied PGP attributes of rhizospheric fungi (Salas-Marina 2011; Murali et al. 2012). Among the PGPF, species of *Phoma*, *Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma*, and arbuscular mycorrhizal fungus (AMF) have gained attention due to their effective role in plant growth activities and disease suppression (Table 4.1).

Mechanisms, stimulating plant growth by PGPF, involve production of plant hormones (Khan et al. 2012), decomposing organic matter (Magdoff and Weil 2004), solubilization of unavailable soil bound nutrient elements (Khan et al. 2010), and protection of plants from biotic and abiotic stresses (Khan et al. 2012). Indirect growth promotion by PGPF occurs via niche exclusion, antibiosis, predation, mycoparasitism, and ISR (Whipps 2001; Benhamou et al. 2002; Bent 2006). Sometimes more than one mechanism is used to promote growth (Benhamou et al. 2002).

The role of AMF in growth promotion and disease suppression in plants is reported since the very old times (Brundrett 2002). The ability of AMF to promote plant growth is due to nutrient uptake, particularly phosphorus (P) (Smith et al. 2010). AMF-colonized crop shows increased growth and yield (Ibibijen et al. 1996

Table 4.1 Various roles of PGPF in sustainable agriculture

PGPF	Effect on plant	Reference
Penicillium simplicissimum GP17-2	Resistance to Pseudomonas *syringae* pv. tomato DC3000 (Pst)	Hossain et al. (2007)
Penicillium spp. GP15-1	Growth enhancement and increased systemic resistance against leaf infection by the anthracnose pathogen *Colletotrichum orbiculare*	Hossain et al. (2014)
Penicillium citrinum strain BHUPC01 and *Aspergillus niger* strain BHUAS01	In vitro phosphate-solubilizing and IAA production	Yadav et al. (2011)
Penicillium sp. (UOM PGPF 27)	Seed quality enhancement of pearl millet and induce resistance to downy mildew disease	Murali et al. (2012)
Penicillium oxalicum	Plant growth and induces resistance in pearl millet against Downy mildew disease	Murali and Amruthesh (2015)
Trichoderma virens and *T. atroviride*	Biomass production and stimulated lateral root development by the production of auxin-related compounds: indole-3-acetic acid, indole-3-acetaldehyde, and indole-3-ethanol	Contreras-Cornejo et al. (2009)
Trichoderma koningi	Biosynthesis of the isoflavonoid phytoalexin vestitol, a major defensive response of leguminous plant	Masunaka et al. (2011)
Trichoderma sp. (UOM PGPF 37)	Induce resistance against downy mildew disease in pearl millet	Murali et al. (2012)
Fusarium equiseti GF18-3	Biocontrol of Fusarium wilt of spinach caused by *Fusarium oxysporum* f. sp. spinaciae	Horinouchi et al. (2010)
	Cucumber growth and the biocontrol of the yellow strain of cucumber mosaic virus (CMV-Y)	Elsharkawy et al. (2012)
F. equiseti GF18-3 and GF19-1	Root and rhizosphere colonization and biocontrol of anthracnose (*C. orbiculare*) and damping-off (*Rhizoctonia solani* AG-4) disease	Saldajeno and Hyakumachi (2011)
Cladosporium sp. MH-6	Gibberellin production and plant growth promotion	Hamayun et al. (2010)
Aspergillus ustus	Phytohormone production and induced systemic resistance against the necrotrophic fungus *Botrytis cinerea* and the hemibiotrophic bacterium *Pseudomonas syringae* DC3000	Salas-Marina (2011)
Phoma glomerata LWL2 and *Penicillium* sp. LWL3	Growth promotion by gibberellins and IAA	Waqas et al. (2012)
Phoma sp. GS8-1	Systemic resistance to bacterial leaf speck pathogen *P. syringae* pv. tomato DC3000 (Pst)	Hossain et al. (2008)
Phoma sp. (GS6-2 and GS7-3)	Systemic resistance to bacterial leaf speck pathogen *P. syringae* pv. tomato DC3000 (Pst)	Sultana et al. (2008)
Phoma sp. GS8-3	Growth promotion in tobacco in vitro by the emission of volatile organic compounds (VOCs)	Naznin et al. (2013)

and Koide et al. 2000). Now researchers recognize that AMF are multifunctional (Sikes 2010). Smith and Smith (2012) discussed the nutritional and nonnutritional advantages of AMF symbiosis. Studies indicate that AMF in the family *Gigasporaceae* are more efficient in enhancing plant P, while AMF in the *Glomeraceae* protect plants from root pathogens (Maherali and Klironomos 2007).

4.3 Roles of PGPM in Agriculture Sustainability

Tilman et al. (2002) defined agricultural sustainability as practices that meet the current and future society needs for food and feed, ecosystem services, and human health, maximizing the net benefit for people. Siddiqui and Pichtel (2008) also stated that sustainable agriculture should be ecologically sound, economically viable, and socially responsible. A long-standing recognition of microbial use and application for making agriculture sustainable is a matter of interest. Research has proved that the biology of the rhizosphere could be exploited by manipulating root and microbial interactions to improve the productivity and sustainability of agricultural systems (McNear 2013). Using PGPM as biofertilizers in place of synthetic N, P, and K (Maheshwari et al. 2012) and also as biological control agents in the form of biopesticides to control plant pests and pathogens (Mishra et al. 2015) have been considered as best practices for sustaining agroecosystems. Here in this section, we explore the different roles of PGPM that can be used in making agriculture more sustainable and free of harmful chemicals.

4.4 Improving Soil Fertility

Soil is supposed to be fertile if it provides physical, chemical, and biological needs for the growth of plants (Abbott and Murphy 2007). The use of excessive fertilization, intensive deep tillage, and luxury irrigation in agricultural systems leads to deterioration and misbalancing of chemical and biological properties of soil (Diacono et al. 2012; Liang et al. 2013). Soil under repeated cultivation loses the original soil organic matter (SOM) levels, and as a consequence, crumb structure is lost, bulk density rises, soil porosity suffers, and biological activity also decreases (Wander 2004). SOM is an important indicator of soil quality (Islam and Weil 2000). PGPM play a range of different functions that make soil fertile. The direct benefit given to soil by PGPM is their contribution in the formation of SOM (Trabelsi and Mhamdi 2013). SOM also plays a major role in long-term soil conservation and restoration (Sequi 1989). Dissolved organic compounds (DOM) are considered to have a major role in the supply of soil-derived ammonium (NH4+), regulate microbial-mediated N transformations (Jones et al. 2004a), and help in balancing carbon (C) to N ratio in soil (Michalzik et al. 2001). Research also showed that soil having low level of SOM if inoculated with PGPM gives better yields (Cakmakci et al. 2006). Soil aggregation is a major physical factor of soil fertility, contributing to retention and movement of water. Exopolysaccharides (EPS) production by some PGPR is found to increase soil

aggregation (Sandhya et al. 2009) and fertility. Application of EPS-producing PGPR also helps in increasing crop yield of soil affected by drought and salinity stresses (Tewari and Arora 2014a, b).

In agricultural soils, there are at least 25,000 fungal species (Carlile and Watkinson 1996) which contribute 70% of the microbial biomass (Paul and Clark 1996), whereas the contribution of AMF accounts for 5–50% of the total microbial biomass (Olsson et al. 1999). AMF, by different mechanisms, affect C, P, and N dynamics (Correa et al. 2015). The mycelia of AMF go deep inside the soil and help in nutrient recycling of C and also improve soil texture (Olsson et al. 1999). C cost to the plant is balanced by access to a greater volume of soil through fungal hyphae having larger surface area to volume ratio than do root hairs and goes up to 8 cm beyond nutrient depletion zones around roots (Millner and Wright 2002). Smith et al. (2011) found that AMF utilize "AM pathway" by which P is scavenged from large volumes of soil and rapidly delivered to cortical cells within the root. Study also shows that they produce glomalin protein (acts as glue), hydrophobin protein (provides mycelium attachment to surfaces, alteration of biotic or abiotic surface properties, and lowering water tension), and mucilage (provides attachment, nutrient capture, and desiccation resistance) that help in better aggregation of soil (Rillig and Mummey 2006). AMF also influence bacterial communities via rhizodeposition that serve as substrates for bacterial growth (Jones et al. 2004b). In a study, Filion et al. (1999) found that AMF exudates influence the abundance and activities of specific fungal and bacterial species. Further studies also suggest that AMF colonization plays an essential role in N uptake and increases amino acids and organic acids in shoots and roots (Bucking and Kafle 2015).

Mostly in agriculture perspective, soil fertility deals more with soil chemicals and physical fertility across diverse soils of different origins and climatic zones (Cass et al. 1996; Merry 1996) and the role of soil biological activity is ignored. The reason for this discrepancy is the lacking fundamental understanding of how soil biological activity and chemical and physical attributes are interrelated and how they are affected by agronomic management practices (for a comprehensive detail on soil biological fertility review by Lynette and Murphy 2007 can be seen). Efforts are required for assessing biological components of soil involved in sustainable crop production in relation to microbe–microbe, plant–microbe, and physical–chemical–biological interactions for a better understanding of the soil system.

4.5 Macro- and Micronutrient facilitators

Despite several environmental threats, nearly half of the world population is supplied with food produced using synthetic fertilizers (Skowronska and Filipek 2013). At the global level, three countries that are using highest amount of fertilizers are China, India, and the USA, consuming 50.15, 21.65, and 20.83 million tons of N, P, and K fertilizers, respectively (http://www.fertilizer.org/ifa). By 2017, fertilizer demand is forecasted to expand by 1.9%. The direct consequence of increased demand for fertilizers may lead to environmental problems such as eutrophication (Yang et al. 2008). Hence, there is urgent need to use PGP microbes to facilitate the availability of macro- and micronutrient to the plants.

4.5.1 Macronutrients

Macronutrients, i.e., N, P, K, magnesium (Mg), calcium (Ca), and sulfur (S), that are absorbed by the plant in significant amounts from the soil. Although each element has its own physiological role in plant growth (Marschner 2012), N, P, K, and S are appreciably required. Proteins and nucleic acids are vital stores of N, P for nucleic acids, and K is most abundant cation, playing essential roles in enzyme activation, protein synthesis, photosynthesis, osmoregulation, stomatal movement, energy transfer, phloem transport, cation–anion balance, and stress resistance (Marschner 2012). S is a constituent of cysteine (Cys) and methionine (Met) and takes part in essential plant metabolism and development (Droux 2004). Microbes play a significant role in nutrient uptake from soil. In the last few years, the use of microbial inoculants for providing macronutrients to plants has not only got attention but also resulted in substantial reduction in the use of chemical fertilizers (Trabelsi and Mhamdi 2013). Hence, there are an increasing number of biofertilizers produced commercially for various crops (Mishra and Arora 2016).

After C, N is the element required in largest amounts by plants (about 1–5% of total plant dry matter) (Marschner 2012). However, soils normally contain between 0.1% and 0.6% N, which represents 2000 to 12,000 kg N ha^{-1} depending on the soil type (Cameron et al. 2013). Plants prefer nitrate (NO_3^-) and ammonium (NH_4^+) types of soil N and retrieve it from indigenous organic and inorganic forms. As the atmospheric N is only available to plants that are capable of forming symbiosis with N-fixing soil bacteria by the process of BNF, the rest of the plants depend on other N sources. This is why for enhancing crop production, application of inorganic form of N fertilizers has always been in demand (Maheshwari et al. 2012). According to an estimate, N fertilizer demand is expected to reach 119.2 metric tons in 2020 (Heffer and Prud'homme 2015).

In context to agricultural system, legumes are very important and cover up to 50% of the global area (Vance 2001). According to Herridge et al. (2008), N fixed by all crop legumes is 16.4 Tg annually, representing 77% of the N. PGPM, having the capability of BNF, are considered as the best alternatives to N fertilizers at least for legume crops (Gothwal et al. 2009). BNF produces roughly 200 million tons of N annually (Peoples et al. 2009). Although the maximum of the N input in soil is provided by symbiotic fixation, free-living or asymbiotic fixation also contributes a critical N input to most terrestrial ecosystems, particularly those lacking large numbers of symbiotic N-fixing plants (Sasha et al. 2011). For example, BNF by free-living heterotrophs such as *Azotobacter, Bacillus, Clostridium*, and *Klebsiella* fix 20 kg N ha^{-1} $year^{-1}$ (Vadakattu and Paterson 2006). Associative N fixation by species of *Azospirillum* was calculated in tune of 52 mg N g^{-1} malate (Stephan et al. 1979). Symbiotic N fixation by a cyanobacterium *Anabaena azollae* in association with water fern *Azolla* is in use since at least 1000 years as a biofertilizer and fix up to 600 kg N ha^{-1} $year^{-1}$ during the growing season (Fattah 2005).

The phosphorus content in average soils is only about 0.05% (w/w) of which only 0.1% is available to plants (Achal et al. 2007) due to its inorganic fixation and formation of organic complexes (Eswaran et al. 1997). The world phosphate fertilizer demand increased from 41.7 million tons in 2013 to 42.7 million tons in 2014 (FAO 2015). Data

indicates that about 5.7 billion hectares of land worldwide is phosphate deficient (Vassilev and Vassileva 2006). As in the case of nitrogen, a large proportion (nearly 80%) of phosphate fertilizers applied to soil remain unavailable to plants (Holford 1997). PSB and AMF are renowned for their involvement in the conversion of insoluble forms of P to accessible forms (Khan et al. 2007). PSB of the genus *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Rhodococcus*, *Serratia*, *Gordonia*, *Phyllobacterium*, *Delftia*, *Azotobacter*, *Xanthomonas*, *Chryseobacterium*, *Enterobacter*, *Pantoea*, *Klebsiella*, *Xanthobacter*, and *Rhizobium* are marketed as inoculant in various countries (Mishra and Arora 2016). Among PGPF, genera *Aspergillus* and *Penicillium* (Khan and Khan 2002) dominate whereas, some strains of *Trichoderma* and *Rhizoctonia* (Altomare et al. 1999; Jacobs et al. 2002) have also been reported as P solubilizers. It has been observed that the major input of P in soil is of organic P compounds accounting for 30–65% of total soil P. Studies also indicate that these are not easily mineralized in soil. For example, one such compound myo-inositol hexakisphosphate (phytate) which may constitute up to 80% of organic P remains unavailable to plants (Turner et al. 2002). Phytase is reported to be produced by various rhizospheric microbes. These enzymes use P from phytate and have been demonstrated to be useful in phosphate solubilization and availability to plants (Singh et al. 2014).

K takes part in enzyme activation of several physiological reactions (Rehm and Schmitt 2002). Although 2.5% of the lithosphere is of K, its actual soil concentrations vary widely, ranging from 0.04 to 3% (Sparks and Huang 1985). According to Sheldrick et al. (2002) on a global basis, it is not P supply that is of most concern but the supply of K, with an annual global deficit of 20 kg K ha^{-1}. Soil K exists in four forms in soils: solution, exchangeable, fixed or non-exchangeable, and structural or mineral. The order of its availability to plants and microbes are solution > exchangeable > fixed (non-exchangeable) > mineral (Sparks 2000). K fertilizer demand rose from 30.06 million tons in 2013 to 31.04 million tons in 2014, which is further expected to be 34.50 million tons in 2018 (FAO 2015). The "non-exchangeable" form of potassium in soils is solubilized by the release of organic acids by some rhizospheric bacteria (Meena et al. 2014). A large number of bacteria of the genus *Pseudomonas*, *Burkholderia*, *Acidithiobacillus*, *Bacillus*, and *Paenibacillus* solubilize K-bearing minerals such as micas, illite, and orthoclase in soil and provide soluble K to plants (Lian et al. 2002; Sheng and He 2006; Liu et al. 2012). In a study, Han et al. (2006) showed that co-inoculation of PSB and KSB resulted in consistently higher P and K availability to plants by using rock materials as source of P and K. K-solubilizing fungi (KSF) such as *Aspergillus terreus* and *Aspergillus niger* were also isolated from various K-rich soil (Prajapati et al. 2012). In a similar study, Lopes-Assad et al. (2010) showed the potential of *A. niger* as a potassic biofertilizer.

Sulfate (SO_4^{2-}) is the most important source of S for plant and taken up by the roots; however, aerial parts can also utilize atmospheric sulfur dioxide (SO_2) (Marschner 2012). SO_4^{2-} generally accounts for less than 5% of total S in soil (Autry and Fitzgerald 1990), and the remaining 95% is bound to organic molecules and is therefore not directly available (Kertesz and Mirleau 2004). SO_4^{2-} can occur as water-soluble SO_4^{2-}, adsorbed on inorganic colloids and as insoluble SO_4^{2-} (Freney 1967). Its concentration varies continuously and at any time depends on the

balance between plant uptake, S-fertilizer input, mineralization, and immobilization (McLaren and Cameron 2004). The main physiological factor governing adsorption of SO_4^{2-} in soil is pH (Prietzel et al. 2001). Generally, acidic pH favors more adsorption and as the pH rises adsorption falls (Kamprath et al. 1956). Biological oxidation of reduced sulfur compounds to sulfate is reported in phylogenetically diverse group of sulfur-oxidizing prokaryotes including domain archaea and bacteria (Bruser et al. 2000). Sulfur-oxidizing bacteria are aerobic lithotrophs or anaerobic phototrophs. In the last few years, their role in the utilization of elemental S to meet the plant's requirement has been assessed by several workers (Gahan and Schmalenberger 2014). However, still they have not gained recognition in the form of PGPR or biofertilizers. Recently, their role and mechanisms have been studied in agriculture perspective, and role in uptake of sulfate to plant was confirmed (Salimpour et al. 2010; Khatibi 2011; Anandham et al. 2014). S uptake with the help of AMF is reported by Allen and Shachar-Hill (2009) and Sieh et al. (2013).

4.5.2 Micronutrients

Although all macronutrients are present in relatively high concentrations in plants, the micronutrients although required in lesser amount are also very essential. However, regarding micronutrient uptake by PGPM, most of the research has been carried out on two elements: Fe and zinc (Zn). Fe is required in ample amounts and it functions in physiological processes, including photosynthesis, respiration, and chlorophyll biosynthesis, and is a component in heme, the Fe–sulfur cluster, and other Fe-binding sites (Kobayashi and Nishizawa 2012). In soil, the insoluble Fe^{3+} (ferric) form of Fe dominates, and its concentration ranges from 7000 to 500,000 mg kg^{-1}, but plant uptake of Fe occurs in ferrous (II) form and depends on pH and oxygen level in soil (Fageria et al. 1990). Only at physiological pH (7.35–7.40) the ferrous form (Fe^{2+}) of Fe is soluble (Bou-Abdallah 2010). Plants and microorganisms require approximately 1–10 μM soluble Fe to meet the average demand (Crowley 2006). According to an estimate, roughly one-third of earth's soil can be considered as Fe deficient (Yi et al. 1994). Hence, Fe-deficient conditions and low availability to plants are a serious threat to agroecosystems. Since then, foliar and root delivery of Fe in inorganic form ($FeSO_4$) or as synthetic or non-synthetic Fe chelates is a common method (Fernández et al. 2005; Godsey et al. 2003), but their indiscriminate use causes an adverse impact on plant growth (Adesemoye et al. 2009). In Fe-starved conditions, siderophores produced by rhizospheric microbes play an important role in plant health management (Ahmed and Holmstrom 2014). Fluorescent pseudomonads (Takase et al. 2000), *Bacillus* spp. (Yu et al. 2011), *Azotobacter* spp. (Baars et al. 2016), *Acinetobacter* spp., and *Rhizobium* spp. (Datta and Chakrabartty 2014) are regarded as efficient siderophore-producing PGPR. In a study, Radzki et al. (2013) showed that siderophore produced by the *Chryseobacterium* C138 bacterium significantly increased plant yield and chlorophyll content of tomato. Recently, microbial siderophores and their potential applications, including PGP attributes, were reviewed by Saha et al. (2013, 2016).

Many important metabolic reactions of the plants are Zn-dependent. Soils low in Zn content result in lower yields and quality of crops. Nearly half of the world's population is being considered as Zn deficient (Cakmak 2009). About 30% of the cultivable soils of the world contain low levels of available plant Zn (Sillanpaa 1990). Application of inorganic Zn does not fulfill the plants need as 96–99% of it is converted into different insoluble forms depending upon the soil types and physicochemical reactions, within seven days of application (Saravanan et al. 2004). Secretion of organic acids by rhizospheric microbes facilitates the metal solubilization. Recently, the use of PGPR for Zn uptake particularly in Zn-deficient soil has been suggested as a sustainable way to fulfill plant needs (Saravanan et al. 2007; Wang et al. 2013; Sunithakumari et al. 2016). AMF have an increased absorption surface and may be considered as more important for Zn uptake. Recently, Kangwankraiphaisan et al. (2013) showed the role of AMF in enhancing Zn availability in the rhizosphere of indigenous plants.

4.6 Suppressing Phytopathogens

Almost 10–16% of the global harvest is lost by plant diseases each year costing loss of an estimated US$220 billion (https://www.sciencedaily.com/releases/2011/04/110411194819.htm). All crop pests (pathogens, arthropods, and weeds) cause preharvest losses of 42% and an additional 10% loss after harvest (Fletcher et al. 2006). The use of pesticides for controlling plant diseases has always been associated with various ecological issues. Biocontrol agents such as PGPR and PGPF offer the advantages of higher selectivity and lower or no toxicity in comparison to conventional chemical pesticides (MacGregor 2006; Mishra et al. 2015). PGPR belonging to the genus *Bacillus* have got recognition for wider biocontrol activity against pests. *Bacillus thuringiensis* (Bt) covers 90% of the biopesticide market in the USA (Chattopadhyay et al. 2004). This bacterium is essentially used for insect pest control and also as "Bt genetically modified (GM) crops" (Cawoy et al. 2011). Biocontrol activity of *Bacillus* is due to insecticidal crystal proteins (ICPs), and it has been proved that their high specificity and safety in the environment is a sustainable alternative to chemical pesticides for the control of insect pests (Kumar et al. 2012). Besides *Bacillus*, genus *Pseudomonas*, *Serratia*, and *Arthrobacter* have also been reported as BCA (Joseph et al. 2007). Strains of *Pseudomonas* are known to produce a variety of antibiotics or antifungal metabolites directly involved in the suppression of diseases (Weller 2007; Khare and Arora 2011b; Mishra and Arora 2012b).

At least 750 species of fungi are known to be entomopathogenic (Copping 2009). Among them, several were used as BCA against phytopathogens. *Metarhizium anisopliae* and *Beauveria bassiana* have been mainly developed for commercial applications (McCoy 1990). Currently, *Trichoderma harzianum*, *Trichoderma asperellum*, *Trichoderma gamsii*, *Coniothyrium minitans*, *Aspergillus flavus*, and *Chondrostereum purpureum* (Auld 2002) are among the most studied fungal biocontrol agents, and their commercialization is increasing day by day (Vinale et al.

2008). For further details on the use of PGPM as biocontrol agents or biopesticides, see review by Mishra et al. (2015).

4.7 Improving Food Quality: Biofortification

According to FAO (2015), one among nine people in the world is suffering from hunger. From a human nutrition perspective, Welch and Graham (2004) stated that over three billion of the world's population is malnourished vis-à-vis nutrient elements and vitamins. Although the total number of undernourished people has fallen in the past 2 years, the problem of undernourishment is a big challenge at the global level. Data indicates that elements such as Fe, Zn, iodine (I), selenium (Se), calcium (Ca), Mg, and Cu have commonly been deficient in diets (Stein 2014). Crop productivity and nutritional quality of plants are closely related to mineral nutrition. Most growers select for high-yield varieties of the crops (usually 80–90% of the dry weight yield is carbohydrate) and don't consider about other nutrients. Research and programs are underway to enrich nutrient content of the major food crops. HarvestPlus was a pioneer biofortification program started in 2002, funded by Bill and Melinda Gates Foundation. In 2012, similarly, BioCassava Plus (BC Plus), an innovative project funded by Bill and Melinda Gates Foundation, is also focused to increase the nutritional value of cassava. Currently, biofortification can be attempted genetically or through agronomic or soil management practices to combat nutrient deficiency by increasing micronutrient contents in staple food crops such as rice, wheat, maize, pearl millet, and others (Prasanna et al. 2016). However, the strategy of using PGPM to enrich amounts of minerals and vitamins in major food crops has also gained consideration of workers. Use of PGPM may reduce substantially the recurrent costs that are associated with various fortification schemes. Rana et al. (2012) showed that by the application of PGPR consortia, 28–60% micronutrient content can be increased in wheat. Recently in a similar study, Rana et al. (2015) have shown that combined inoculation of cyanobacterium (*Anabaena oscillarioides* CR3) and PGPR (*Brevundimonas diminuta* PR7; *Ochrobactrum anthropi* PR10) significantly increased N, P, and K content and micronutrient concentrations. Biari et al. (2008) observed the effect of *Azospirillum* and *Azotobacter* on the growth and nutrient uptake of maize (*Zea mays*) in the field conditions. Biofortification of Fe in chickpea by using PGPR is also reported by Khalid et al. (2015). Rana et al. (2015) prospected the role of PGPR for enrichment of macro- and micronutrients in rice and wheat. In the future, PGPM can be explored as biofortification agents in an eco-friendly and economic manner.

4.8 Roles in Environmental Sustainability

Indiscriminate use of natural resources is continuously transforming our ecosystems and putting the environment at risk. Anthropogenic activities such as rapid industrialization, deforestation, and emission of greenhouse gases are making earth

more and more unsustainable. At the global level, researchers are trying to find a possible solution to these environmental issues. The microbial world, due to their high survival rate and fast adaptation capabilities to changing environmental conditions, holds great potential to mitigate the negative impact of climate change (Milosevic et al. 2012). Moreover, a beneficial relationship between microbial diversity, soil, and plant quality and their role in ecosystem sustainability has been well established. Various workers have extensively studied PGPR roles in the soil and their responses to overcome stresses (Yang et al. 2009; Grover et al. 2011; Tewari and Arora 2016). Soil microbial dynamics is crucial for proper functioning of ecosystems where PGPM are thought to be significantly helpful and realized as strong candidates for the restoration of the degraded agroecosystems (Ramos Solana et al. 2006) and managing them in changing climatic conditions.

4.9 Rhizoremediation

The term "rhizoremediation" involves the elimination of the contaminants by the microbes present in the rhizosphere (Segura et al. 2009). The technique is developing as a prominent method of removing pollutants from contaminated sites by utilizing the combined degradative potential of plants and their rhizospheric microorganisms (Kuiper et al. 2004; Chaudhry et al. 2005; Zhuang et al. 2007). Rhizospheric microorganisms accelerate the degradation process by producing a wide range of hydrolytic enzymes and help in ecorestoration of polluted sites (Brazil et al. 1995; Daane et al. 2001). Various processes are involved in biotransformation, degradation, and removal of the pollutants (Mejare and Bulow 2001; Prasad 2011) from the soil by plants associated with rhizospheric microbes (Box 4.1).

Box 4.1 (Adapted from Speight, 2017)

- **Phytostabilisation** is an immobilization process where plants in combination with soil additives to assist plant installation, to mechanically stabilize the site and to reduce pollutant transfer to other ecosystem compartments and the food chain.
- **Phytoextraction** is a removal process where plants uptake and accumulate metals/metalloids in their tissues and transform the pollutants into harvestable biomass.
- **Phytovolatilisation/Rhizovolatilisation** is a removal process employing metabolic capabilities of plants and associated rhizospheric microorganisms which transform pollutants into volatile compounds and release to the atmosphere through leaves by evapotranspiration.
- **Phytodegradation/Rhizodegradation** utilizes the combined metabolic capabilities of plants and rhizosphere microorganisms to degrade organic pollutants.

The success of rhizoremediation chiefly relies on the survival and establishment of plants with rhizospheric microbes. Root exudates, secreted by plants, support flourishing microbial consortium which assists in rhizoremediation. In turn, a healthy microbial consortium can benefit the plants by performing a number of PGP activities such as N_2 fixation, phosphate solubilization, siderophore production, phytohormones production, and protection against plant diseases (Glick 1995; Lee et al. 2012). The role of plant–microbe interactions in accelerated degradation process of organic pollutants in rhizosphere has been extensively reviewed (Siciliano and Germida 1999; Kuiper et al. 2004; Newman and Reynolds 2004; Dzantor 2007). Earlier studies on rhizoremediation were mainly focused on degradation of pesticides which suggested the reduced toxicity of these compounds for plants in the presence of degrading rhizospheric microorganisms (Kuiper et al. 2004). ACC deaminase-producing bacteria assist plants in root growth and proliferation in polluted sites (Arshad et al. 2007). Studies on ACC-deaminase-producing bacteria suggest their role in enhanced metal resistance for plants. Enhanced tolerance for metal toxicity of *Brassica napus*, *Brassica compestris* (Burd et al. 1998; Belimov et al. 2001), and nickel hyperaccumulator plant *Thlaspi goesingense* (Idris et al. 2004) was observed to be attributed to ACC deaminase-producing rhizobacteria, associated with these plants. Cairney (2000) provided an overview of the potential role of ectomycorrhizal associations in rhizosphere remediation of persistent organic pollutants. Ectomycorrhizal fungi protect plant roots from direct exposure to toxic pollutants by covering roots with densely packed mycelial sheath with the phenolic inter-hyphal material (Ashford et al. 1988). This mycelial covering of ectomycorrhizae provides extended surface area to reduce bioavailability and to enhance degradation of pollutant in the mycorrhizosphere (Wenzel 2009).

Pollutant toxicity, adverse soil conditions, water stress, and nutrient deficiency are typical problems challenging the establishment of vegetation on contaminated sites (Tordoff et al. 2000; Bradshaw and Johnson 1992), and PGPRs help their host plants to overcome these limitations. Solubility and bioavailability of pollutants in soil are largely dependent upon soil properties, i.e., pH, redox potential, clay content, mineral composition, organic matter, etc. Pollutants are adsorbed by organic matter and minerals in the soil, leading to their entrapment and less bioavailability (Semple et al. 2003; Mohan et al. 2006). Plants and microorganisms work in a coordinated manner to increase the bioavailability of these entrapped pollutants where plant roots improve diffusivity of soil and induce transpiration-driven pumping of water-soluble pollutants toward the rhizosphere (Erickson 1997) and expose them for microbial degradation (Ferro et al. 1994). Similarly, secretion of biosurfactants by plants as well as rhizospheric microbes enhances the mobilization of entrapped hydrophobic pollutants to the site of higher microbial activity (Wenzel 2009). Enhanced degradation of pollutants by a combined effort of plants and associated microorganisms (in rhizosphere) has been demonstrated by several studies listed in Table 4.2.

Among rhizospheric microbial communities, saprotrophic fungi (e.g., species of *Mucor*, *Cunninghamella*, *Rhizopus*, and other *Zygomycota*) are highly active in the degradation process utilizing sugars and other simple soluble nutrients secreted by

Table 4.2 Studies on enhanced degradation of recalcitrant pollutants by rhizoremediation

Plant	Associated microorganisms	Degraded pollutants	Reference
Triticum aestivum	*Pseudomonas putida* strains	2,4-D	Kingsley et al. (1994)
Beta vulgaris	*P. fluorescens*	Polychlorinated biphenyls (PCBs)	Brazil et al. (1995)
Hordeum vulgare	*Burkholderia cepacia*	2,4-D	Jacobsen (1997)
T. aestivum	*P. fluorescens*	Trichloroethylene (TCE)	Yee et al. (1998)
Populus spp.	Actinomycetes	1,4-Dioxane	Schnoor et al. (1998)
Zea mays	*P. putida*	3-Methylbenzoate	Ronchel and Ramos (2001)
Populus sp.	Actinomycete *Amycolata* sp. CB1190	1,4-Dioxane	Kelley et al. (2001)
Astragalus sinicus	*Mesorhizobium huakuii*	Cd	Sriprang et al. (2002)
Brassica napus	Cd-resistant rhizospheric bacterial strains	Cd-polluted soil	Sheng and Xia (2006)
Brassica juncea	PGPR consortium of N2-fixing *Azotobacter chroococcum* HKN5, P-solubilizing *Bacillus megaterium* HKP-1, K-solubilizing *Bacillus mucilaginous* HKK-1	Pb–Zn mine	Wu et al. (2006)
Brassica juncea	*Bacillus subtilis* strain SJ-101	Nickel	Zaidi et al. (2006)
Pityrogramma calomelanos	Uncharacterized rhizobacteria	Arsenic	Jankong et al. (2007)
Populus deltoids	*Bacillus circulans* SBA12, *Kurthia* sp. SBA4, *Micrococcus varians* SBA8	Anthracene and naphthalene	Bisht et al. (2010)
P. deltoids	*Bacillus* sp. SBER3	Polyaromatic hydrocarbon-contaminated soil	Bisht et al. (2014)

plant roots. Fungi have a short exploitative phase and a high competitive ability (Anastasi et al. 2013) as well as possess high tolerance to toxic pollutants (Tigini et al. 2009). Several genera, e.g., *Trichoderma*, *Fusarium*, *Penicillium*, *Aspergillus*, etc., have been widely studied and reported as highly tolerant groups to pollutants such as PCBs, chlorobenzoic acids (CBA), and endosulfan (Garon et al. 2000; Tigini et al. 2009; Pinedo-Rivilla et al. 2009). Fungi can accumulate heavy metals as their cell wall components containing free amino, hydroxyl, and carboxyl groups, which bind heavy metals very efficiently (Gaddie 1993; Morley and Gadd 1995). Their ability to chelate heavy metals makes them commercial biosorbents. Mycorrhizal fungi can also play an important role in rhizoremediation. Mycorrhizae due to the small diameter of their hyphae can efficiently explore the soil volume and

even the microsites that are not accessible for plant roots. By forming an ectomycorrhizal sheath around roots, these fungi protect the roots from direct interaction with toxic pollutants and enhance their degradation (Meharg et al. 1997; Hassan et al. 2010). Mycorrhizal fungi are known to have great potential in the accumulation of heavy metals. In addition to the cell wall components, glomalin proteins produced by mycorrhizal fungi also seem to be very efficient in sequestering metals such as Pb, Mn, Fe, Cu, Cd, and Zn (Gonzales-Chavez et al. 2004; Carnejo et al. 2008). Rhizospheric bacteria belonging to genus *Pseudomonas*, *Bacillus*, *Burkholderia*, *Arthrobacter*, *Flavobacterium*, *Alcaligenes*, and *Sphingomonas* have been successfully studied for their degradation potential of recalcitrant compounds (Hoagland et al. 1994; Kuiper et al. 2004). Similarly, actinomycetes are explored widely for their ability to degrade recalcitrant and toxic compounds, i.e., organochlorine pesticides in the rhizosphere. Actinomycetes have the ability to oxidize, partially dechlorinate, and dealkylate some of highly recalcitrant pesticides, e.g., aldrin, metolachlor, atrazine, DDT, etc. (Ferguson and Korte 1977; Radosevich et al. 1995). Moreover, actinomycetes are well suited for soil inoculation due to their mycelial growth, rapid growth rate, and easy genetic manipulations (Shelton et al. 1996). Soil microorganisms are also known to produce biosurfactant compounds that may further facilitate the removal/degradation of organic pollutants by increasing their availability to plants (Lafrance and Lapointe 1998). Organic acids, one of the principal components of root exudates, exist in anionic forms, e.g., citrate, oxalate, malate, malonate, fumarate, and acetate, which chelate metal ions and decrease their toxicity for plants and rhizospheric microorganisms (Ryan et al. 2001; Ling et al. 2015). Similarly, phenolic compounds exuded by plant roots provide carbon source for certain rhizospheric microbes. To catabolize these phenolic compounds, enzymes are produced by microorganisms that can co-metabolize pesticides with similar structures (Chaudhry et al. 2005; Rohrbachen and St-Arnaud 2016).

4.10 Combating Climate Change and Abiotic Stresses

Conventional agricultural system is already facing reduced production due to imbalanced practices for increasing productivity. Apart from these, altered climatic conditions due to global warming have further aggravated the problem. Climate change can be considered as the biggest threat to the whole planet due to continuous acceleration in global temperature and CO_2 concentration. Although change has always been a part of our world, human activities are increasingly influencing the atmosphere, terrestrial, and marine biospheres, which together constitute the global climate system (Chakraborty et al. 2000). One of the most significant effects of climate change can be seen in the changes of the frequency and magnitude of extreme events such as floods and droughts altering the soil fertility and thus impacting the crop production. A minor modification in climate can have a significant impact on all living organisms. In agroecosystems, decomposition of soil organic matter, soil respiration, and growth of microbial biomass are the main events that are significantly influenced by altered climatic conditions (Bradford et al. 2008). In recent past, some

model studies have been performed to observe the effect of altered climatic conditions on crop productivity (Lobell et al. 2008), and the findings suggest that in extreme conditions rhizospheric microbial community can provide substantial help (Dimkpa et al. 2009; Fahad et al. 2015). The emerging body of knowledge strongly suggests that climate conditions such as prolonged drought, intense rains (flooding), high temperatures, frost, and low temperatures are forcing agricultural systems to adapt mitigation strategies and PGP microbes can help to minimize negative impacts of climate change.

4.10.1 Abiotic Stress Tolerance

Abiotic stress can be defined as any factor exerted by the environment on the optimal functioning of a plant (Bohnert 2007). Abiotic stress affects the productivity of crops as well as the microbial activity in soil (Milosevic et al. 2012). At the global level, crop loss due to abiotic stresses is reported to up to 50% (Rasool et al. 2013; Rodziewicz et al. 2014). In recent years, PGPR-mediated tolerance to abiotic stresses has been extensively studied at molecular, physiological, and morphological level (Dimkpa et al. 2009; Lim and Kim 2013) which strengthens our understanding of enhancing crop productivity under harsh environmental conditions (Yang et al. 2009; Tewari and Arora 2015; Tewari and Arora 2016). The impact of abiotic stresses resulting to anthropogenic activities and climate change such as drought, flooding, and salinity has been also studied by several workers (Belimov et al. 2009). Studies suggest that it would be preferable to use PGP microbes for providing protection against such stresses. Recent investigations show that PGPR help plants to tolerate abiotic stresses by various mechanisms (Yang et al. 2009). Among them, the production of osmoprotectors (K+, glutamate, trehalose, proline, glycine, and polysaccharates), stress-induced production of phytohormones (IAA and gibberellins), and stimulation of induced systemic tolerance (IST) are of importance (Yuwono et al. 2005; Saleem et al. 2007; Sziderics et al. 2007; Barriuso et al. 2008). The most important mechanism reported in several PGPRs under stress conditions is the production of enzyme ACC deaminase. Under stress conditions, this enzyme facilitates the growth of plants by decomposing plant ACC (ethylene precursor in plants) (Saleem et al. 2007). By reducing the level of ethylene, the plant becomes more resistant to stress conditions in the environment (Glick 2005).

4.10.2 Drought Stress

There is a projection that by the year 2050, the land area affected by drought will increase twofold and water resources will decline by 30% (Falkenmark 2013). Under such conditions, crop growth and productivity will be limited, especially in arid and semiarid regions. PGPR use different mechanisms to mitigate adverse effects of drought. According to Grover et al. (2011), certain PGPR may alleviate the impact of soil drought through the production of exopolysaccharides (EPS),

increased circulation of water in the plant, and the synthesis of ACC deaminase, IAA, and proline. EPS production tends to improve soil structure by facilitating the formation of macroaggregates and increase in plant resistance to drought stress (Tewari and Arora 2015). Macroaggregates facilitate nutrients uptake by influencing root-adhering soil/root tissue (RAS/RT) ratio (Alami et al. 2000). PGPR possess tremendous potential for modulating the physiological response to water deprivation (Bresson et al. 2013). In water-deficit conditions, PGPR-mediated reproductive delay and changes in transpiration rate have been found to assist plant growth (Marasco et al. 2012). In a study, Bresson et al. (2013) also reported that strain *Phyllobacterium brassicacearum* STM196 induces a reproductive delay and physiological changes responsible for improved drought tolerance in *Arabidopsis thaliana.*

PGPR can also mitigate the impact of drought on plants through induced systemic tolerance (IST) which includes (a) production of cytokinins, (b) production of antioxidants, and (c) degradation of the ethylene precursor ACC by ACC deaminase. The production of cytokinins causes the accumulation of abscisic acid (ABA) in leaves, which in turn results in the closing of stomata (Figueiredo et al. 2008) which restricts potentially damaging foliar water loss (Weyens et al. 2009). Thus, cytokinin originating from PGPR could confer drought resistance. In a study, Arkhipova et al. (2005) also showed that cytokinin-producing bacteria enhance plant growth in drying soil. The production of antioxidants (e.g., the enzyme catalase) causes the degradation of reactive forms of oxygen. Under drought conditions, plant inoculation with ACC deaminase-producing rhizobacteria causes root elongation and water uptake from deeper soil (Zahir et al. 2008). Grover et al. (2011) reported that when exposed to drought stress, some rhizobacteria produce antioxidants which neutralize the toxic effects of reactive oxygen species (ROS) in plant cells, reducing damage to cells and biomolecules. Vurukonda et al. (2016) provide an insight on adaptations and mitigation strategies of PGPR required to cope with drought stress. Recently, Kaushal and Wani (2016) also discussed the role of PGPR in drought stress alleviation in drylands and provided an update on the mechanisms involved in stress tolerance. In a study, Kang et al. (2014) also showed that PGPR applied to cucumber (*Cucumis sativus* L.) crop increased their productivity and reduced adverse impacts of salinity and drought.

AMF are known to alleviate drought stress by improving soil texture (Schreiner et al. 1997; Akema and Futai 2005). Under water stress conditions, AMF contribute in improving physiological conditions such as regulation of water absorption, transpiration, and photosynthesis of plants (Auge 2001). It has been observed that AMF-colonized plants show different transpiration rates and stomatal conductance compared to non-AMF plants (Marschner and Dell 1994). Boyer et al. (2015) showed that the addition of AMF inocula to plants subjected to reduced irrigation restored plant growth to the same or higher values as the non-mycorrhizal, fully watered plants. Zhang et al. (2013) showed that AMF colonization may enhance the drought tolerance of *Cyclobalanopsis glauca* (a hard woody evergreen oak) seedlings by improving growth performance, nutrient content, the quantity of osmotic adjustment compounds, and antioxidant enzyme activity. They also suggested

potential use of AMF for the restoration of vegetation in the Karst region which is an ecologically fragile system that covers about 12% of the global landmass (Chen et al. 2013).

4.10.3 Temperature Stress

Among the changing climatic conditions, constantly rising ambient temperature is considered one of the most detrimental stresses (Hasanuzzaman et al. 2013). All aspects of plant processes like germination, growth, development, reproduction, and yield are affected by elevated temperatures (McClung and Davis 2010). Heat stress is considered as a short-term elevation in temperature, about 10–15 °C above the normal temperature, depending upon the intensity (temperature in degrees), duration, and rate of increase (Wahid 2007). High temperatures can affect plants by causing several morpho-anatomical responses such as burning of leaves and twigs, leaf senescence and abscission, shoot and root growth inhibition, fruit discoloration, and yield loss (Guilioni et al. 1997; Vollenweider and Gunthardt-Goerg 2005). High temperature also induces hormonal changes in plants, resulting into increased levels of stress hormones such as abscisic acid (ABA) and ethylene (Larkindale and Huang 2005). Heat stress may also induce oxidative stress by inducing the generation and reactions of ROS which cause autocatalytic peroxidation of membrane lipids and pigments and affect membrane permeability (Xu et al. 2006).

Although plants have various biochemical and molecular mechanisms for combating heat stress, decreasing crop productivity under this stress is a serious threat to food security, and its eradication is essential. Microbes have evolved the adaptive capability of survival under altered climatic conditions. Some bacterial species and strains help in plant tolerance to high temperature (Grover et al. 2011). Redman et al. (2002) found that plant–microbe symbiosis increases the thermotolerance of both the symbiotic partners. They observed that the colonization of *Dichanthelium lanuginosum* by an endophytic fungus *Curvularia* sp. enhanced the survival of all endophyte-treated plants at 65 °C (8 h/day incubation for 10 days) whereas all endophyte-free plants died. According to Allison and Martiny (2008), microbial communities respond to warming and other perturbations through resistance, enabled by microbial trait plasticity, or resilience as the community returns to an initial composition after the stress has passed. Bradford (2013) also stated that the elevated temperature affects microbial metabolism. A thermotolerant bacterial strain *Pseudomonas putida* NBR10987 isolated from drought-stressed rhizosphere of chickpea exhibited thermotolerance due to overexpression of stress sigma factor σs and enhanced biofilm formation at high temperatures (Srivastava et al. 2008). Ali et al. (2009) investigated thermotolerance of rhizospheric bacteria *Pseudomonas* AKM-P6 and its role in alleviating heat stress in sorghum seedlings. Studies revealed that heat tolerance in sorghum seedlings was attributed to biosynthesis of high-molecular weight proteins in leaves, reduced membrane injury, and enhanced levels of cellular metabolites like proline, chlorophyll, sugars, amino acids, and

proteins under elevated temperatures. The protein profiling of inoculated and uninoculated sorghum seedlings at ambient and elevated temperature revealed the presence of three additional polypeptides in the seedlings of treated plants (Selvakumar et al. 2012).

4.10.4 Salinity Stress

It has been estimated that worldwide, approximately 900 million ha of land is affected by salinity, accounting for total 6% of global land mass (Flowers 2004) and rapidly changing climatic conditions will result into loss of arable land up to 50% by the year 2050 (Munns 2002). Salinity adversely affects plant growth and development by reducing osmotic potential and creating an ionic imbalance, which causes Na^+ and Cl^- toxicity, production of stress hormone ethylene, plasmolysis, decrease of photosynthetic capacity due to the osmotic stress, and partial closure of stomata (Drew et al. 1990). Osmotic and ionic imbalance also causes various oxidative damages in plants and formation of ROS in chloroplast (Asada 2000). ROS are highly reactive and can cause widespread damage to membranes, proteins, and deoxyribonucleic acid (DNA) (Stepien and Klobus 2005). Overall salinity may cause reduced growth, smaller leaves and shorter stature, early senescence, decreased photosynthesis, respiratory changes, loss of cellular integrity, and tissue necrosis in plants (Cheeseman 1988). The intimate relationship of PGPR with plants is well established, and their use to enhance crop productivity by mitigating salt stress is gaining momentum (Table 4.3).

Table 4.3 Different roles of PGPM in alleviating salinity stress

Crop	Bacterial species	Effect	References
Zea mays	*Azospirillum*	Osmoprotection	Hamdia et al. (2004)
Lactuca sativa	*Azospirillum*	Enhanced seed germination	Barassi et al. (2006)
Brassica napus	*Pseudomonas putida* UW 4	The bacterium promoted plant growth at 1 mol/L and 150 mol/L at 10 and 20 °C, respectively	Cheng et al. (2007)
Arachis hypogaea	*Pseudomonas fluorescens*	Enhanced ACC deaminase activity	Saravanakumar and Samiyappan (2007)
Z. mays	*Pseudomonas syringae*, *P. fluorescens*, *Enterobacter aerogenes*	ACC deaminase activity	Nadeem et al. (2007)
Phaseolus vulgaris	*Azospirillum brasilense*	Promoted root branching in bean seedling roots and increased secretion of nod-gene-inducing flavonoid species	Dardanelli et al. (2008)

(continued)

Table 4.1 (continued)

Crop	Bacterial species	Effect	References
Z. mays	*Rhizobium, Pseudomonas*	Decreased electrolyte leakage and increase in proline production, maintenance of relative water content of leaves, and selective uptake of K ions	Bano and Fatima (2009)
T. aestivum	*Pseudomonas* sp., *Serratia* sp.	ACC deaminase activity	Zahir et al. (2007)
Gossypium sp.	*P. putida* Rs-198	Increase the absorption of the Mg2+, K2+, and Ca2+ and decrease the uptake of the Na2+ from the soil	Yao et al. (2010)
Vigna radiata	*P. syringae*, *P. fluorescens*, and *Rhizobium phaseoli*	ACC deaminase activity	Ahmad et al. (2011)
Puccinellia tenuiflora	*Bacillus subtilis*	Selective absorption capacity for K^+ over Na^+ and reduced Na^+ transport from root to shoot as well as Na^+ uptake in roots	Niu et al. (2015)

It has been studied that interaction of PGPR with crops in saline conditions reduced the extent of poor growth and improved performance in adverse conditions (Dimkpa et al. 2009) by enhancing nutrient availability to plants. To date, many bacterial genera such as *Alcaligenes*, *Azospirillum*, *Bacillus*, *Clostridium*, *Klebsiella*, *Pseudomonas*, *Rhizobium*, *Thiobacillus*, *Serratia*, and *Streptomyces* have been studied for their salt tolerance and plant growth-promoting ability under saline conditions (Whipps 2001). Mayak et al. (2004) studied the growth-promoting ability of a salt-tolerant bacterium *Achromobacter piechaudii* on tomato at 172 mM NaCl concentration and found a significant increase in fresh and dry weights of tomato seedling. A PGP bacterium *Chryseobacterium balustinum* has been reported to promote seed germination, increase root surface and total N content in *Lupinus albus* seedlings in saline conditions (Gutierrez-Manero et al. 2003). In another study, *C. balustinum*, when co-inoculated with *Sinorhizobium fredii*, increased nodulation and root growth of soybean plants under saline conditions (Estevez et al. 2009). The combined use of salt-tolerant microbes is effective to combat salt effect on plant growth. In a study, Estevez et al. (2009) co-inoculated salt-tolerant *Bradyrhizobium japonicum* with two PGPR strains, *Bacillus subtilis* and *Sinorhizobium proteamaculans* in soybean under saline conditions and found an increase in dry weight of plant by 10%. Tewari and Arora (2014a, b) reported enhancement in yield of sunflower crop under saline conditions in field trials when inoculated with fluorescent pseudomonads. Various PGPRs can maintain their PGP ability even at high saline conditions (Arora et al. 2012). By producing osmoprotectants or compatible solutes under highly saline conditions, bacteria stabilize their enzymes and cellular machinery

from osmotic imbalance (Yancey et al. 1982; Galinski and Truper 1994; Miller and Wood 1996; Talibart et al. 1997; Arora et al. 2006). Various species of *Azospirillum*, *Pseudomonas*, *Bacillus*, and *Rhizobium* have been reported to produce osmoprotectants in saline habitats (Egamberdieva and Lugtenberg 2014). EPS production is an important mechanism by which PGPRs mitigate the harmful effects of salt stress (Tewari and Arora 2015, 2016). By producing EPS, PGPRs bind cations including Na^+ and decrease the content of Na^+ available for plant uptake and, thus, help to alleviate salt stress in plants growing in saline environments (Gaddie 1993). EPS also possess water holding and cementing properties, thus play a vital role in maintaining soil moisture and in the formation of soil aggregates. Ethylene, a stress hormone, produced in plants under salt stress, is also lowered by PGPRs (Glick 2005).

4.10.5 Flood Stress

According to a report of United Nations Framework Convention on Climate Change (UNFCCC), millions of people all over the world could be exposed to greater risk of floods by 2020 (UNFCCC 2007). Increasing frequency and intensity of large-scale flood disasters considerably hamper agricultural production in highly productive alluvial soils (FAO 2011). In response to waterlogging, plant's permeability of roots is reduced, water absorption, and nutrients uptake is affected which lessens the growth of aboveground parts and roots. Flood stress induces ethylene biosynthesis (Jackson and Campbell 1976; Bradford and Dilley 1978) and its accumulation due to entrapment in water (Drew 1992). Ethylene concentration increases under waterlogging conditions due to increasing activity of ACC-synthase and ACC-oxidase in root and shoot, respectively (Olson et al. 1995; English et al. 1995). Under waterlogging conditions, oxygen supply to plant roots is reduced, and root environment becomes anaerobic which further reduces root permeability, water absorption, and mineral uptake by the plant. Consequently, symptoms like the closing of stomata, reduced photosynthesis, inhibition of stem, and root growth occur (Jackson 1985; Grichko and Glick 2001). ACC, synthesized in plant roots under anaerobic condition, is transported to shoot, where it is oxidized to produce ethylene and causes abnormal growth like leaf epinasty (Jackson 1997). It has been observed that under waterlogging conditions acetaldehyde and ethanol intermediates are accumulated in plant roots. Oxidative phosphorylation of mitochondria is blocked due to reduced oxygen, and cells undergo anaerobic fermentation, which damages plant tissues (Liao and Lin 1994). In an excessively moist soil, bacteria such as *Enterobacter cloacae* and *P. putida* predominate over fungi and actinomycetes (Grichko and Glick 2001). ACC deaminase-synthesizing strains of *E. cloacae* CAL2 and *P. putida* UW4 were studied for their role in plant growth improvement under flood conditions which significantly improved leaf chlorophyll content and shoot growth of tomato plants (Grichko et al. 2005). Mycorrhizal fungi are also reported to have a major role in mitigating flood stress (Grover et al. 2011). Recently, the role of PGPR in protecting plants from flood stress has been investigated by Barnawal et al. (2012) and Li et al. (2013). Glick (2014) have also discussed potential of PGPR in combating flood stress.

4.11 Future Challenges and Conclusion

To enhance the agriculture productivity without harming the agroecosystems is the key challenge for agronomists. Farmers have always tried to improve the chemical and physical conditions of their soils, to make it nutrient rich, to retain moisture, and to ease the growth of the plant, but roles played by soil microbes are generally ignored (East 2013). In this context, the use of PGPM for biofertilization, prevention from deadly diseases, alleviating abiotic and biotic stresses, and remediation of contaminated sites can be very useful. However, for this to achieve a better understanding of plant–microbe interactions at biochemical and molecular level is necessary.

Despite their wide applications in agroecosystems, a fresh perspective focused on their extensive applicability to mitigate environmental problems has been ignored. Unfamiliarity of the role of PGPM in remediation of pollutants, management of degraded lands, and alleviation of stresses is due to lack of deeper understanding of their functioning related to interaction with microbes and plants. In recent years, substantial progress has been made in affirming the role of PGPM in combating abiotic and biotic stresses. "Omics" approaches which include genomics and proteomics also provide insight on structural and functional aspects of genes and proteins, whereas metabolomics is helping in the identification and quantification of cellular metabolites (Swarupa et al. 2016). These approaches can be useful in deducing the pathways and designing tailor made bioformulations with multiple applications. A number of PGPM strains are now known or being used to combat abiotic stresses which can in the future also be used to mitigate impacts of climate change.

Recently, efforts to study the rhizospheric microbiome are on priority (Turner et al. 2013; Spence et al. 2014). Rhizosphere microbiome provides a holistic perspective to understand the plant–microbe interactions. It comprises the greatest diversity of microorganisms directly interacting with a given plant; therefore, a tremendous capacity to influence plant fitness and adaptation (Coats and Rumpho 2014). The detailed study or complete analysis of rhizospheric microbiome (also considered as the second genome of the plant) is not very easy (Berendsen et al. 2012). However, this could provide ample amount of information regarding the positive or negative influence on plant growth and fitness such as how the beneficial mutualistic microbes or pathogens take part in decomposition, nutrient solubilization, nutrient cycling, secretion of plant growth hormones, antagonism toward pathogens, and induction of plant immune system (Lakshmanan et al. 2014). There are several studies, often referred to as plant–soil feedback experiments, showing the effect of the rhizospheric microbiome on plant community composition (Bever et al. 2012). Schlaeppi and Bulgarelli (2015) proposed that local microbiome information could be applied in the development of future microbial inoculants. With an agricultural viewpoint, Bulgarelli et al. (2013) also stated that thousands of strains of PGPR have been isolated during the past few decades but the exact mode of a potentially beneficial microorganism is still very much a black box. Also the bioformulations used at present lack in replicability

and quality. This situation can be improved by utilizing latest technology and designing future bioformulations with PGM along with their metabolites or other additives (Arora and Mishra 2016).

PGPM are tools in sustainable agriculture and environmental management. Their role in the regeneration of fertile soils, degradation of pollutants and wastes, and mitigating the effects of climate change are core areas where future research and explorations are required. In the near future, by the expansion research and bioengineering tools, the role of PGPM in agroecosystems will also expand and their applications may also extend to provide a sustainable solution to various environmental problems. Better understanding of plant–microbe interactions can further provide solution for a wide variety of problems related to sustainable agriculture, soil quality, and remediation of marginal lands by impact of climate change.

References

Abbott LK, Murphy DV (2007) Biological soil fertility: a key to sustainable land use in agriculture. Springer, London, p 254

Achal V, Savant VV, Sudhakara Reddy M (2007) Phosphate solubilization by wide type strain and UV-induced mutants of *Aspergillus tubingensis*. Soil Biol Biochem 39(2):695–699

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58(4):921–929

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saudi Univ Sci 26:1–20

Ahmad M, Zahir ZA, Asghar HN, Asghar M (2011) Inducing salt tolerance in mung bean through coinoculation with rhizobia and plant-growth-promoting rhizobacteria containing 1-aminocyc lopropane-1-carboxylate deaminase. Can J Microbiol 57(7):578–589

Ahmed E, Holmstrom SJ (2014) Siderophores in environmental research: roles and applications. Microb Biotechnol 7:196–208

Akema T, Futai K (2005) Ectomycorrhizal development in a *Pinus thunbergii* stand in relation to location on a slope and effect on tree mortality from pine wilt disease. J For Res 10:93–99

Aktar MW, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. Interdisc Toxicol 2:1–12

Alami Y, Achouak W, Marol C, Heulin T (2000) Rhizosphere soil aggregation and plant growth promotion of sunflowers by exopolysaccharide producing *Rhizobium* sp. strain isolated from sunflower roots. Appl Environ Microbiol 66:3393–3398

Ali SS, Vidhale NN (2013) Bacterial siderophore and their application: a review. Int J Curr Microbiol App Sci 2(12):303–312

Ali SZ, Sandhya V, Grover M, Kishore N, Rao LV, Venkateswarlu B (2009) *Pseudomonas* sp. strain AKM-P6 enhances tolerance of sorghum seedlings to elevated temperatures. Biol Fertil Soils 46(1):45–55

Allen JW, Shachar-Hill Y (2009) Sulfur transfer through an arbuscular mycorrhiza. Plant Physiol 149:549–560

Allison SD, Martiny JBH (2008) Resistance, resilience, and redundancy in microbial communities. Proc Natl Acad Sci U S A 105:11512–11519

Altomare C, Norvell WA, Bjorkman G, Harman G (1999) Solubilization of phosphates and micronutrients by the plant growth-promoting and biocontrol fungus *T. Harzianum* Rifaii 1295–22. Appl Environ Microbiol 65:2926–2933

Anandham R et al (2014) Early plant growth promotion of maize by various sulfur oxidizing bacteria that uses different thiosulfate oxidation pathway. Afr J Microbiol Res 8(1):19–27

Anastasi A, Tigini V, Varese GC (2013) The bioremediation potential of different ecophysiological groups of fungi. In: Goltapeh EM, Danesh YR, Varma A (eds) Fungi as bioremediators, soil biology, vol 32. Springer, Berlin, pp 29–49

Anitha A, Rabeeth E (2010) Degradation of fungal cell walls of phytopathogenic fungi by lytic enzyme of *Streptomyces griseus*. Afr J Plant Sci 4:061–066

Antoun H, Prevost D (2005) Ecology of plant growth promoting rhizobacteria. In: Siddiqui ZA (ed) PGPR: biocontrol and biofertilization. Springer, Dordrecht, pp 1–38

Arkhipova TN, Veselov SU, Melentiev AI, Martynenko EV, Kudoyarova GR (2005) Ability of bacterium Bacillis subtilis to produce cytokinins and to influence the growth and endogenous hormone content of lettuce plants. Plant Soil 272:201–209

Arora NK, Singhal V, Maheshwari DK (2006) Salinity-induced accumulation of poly-b-hydroxybutyrate in rhizobia indicating its role in cell protection. World J Microbiol Biotechnol 22(6):603–606

Arora NK, Khare E, Oh JH, Kang SC, Maheshwari DK (2008) Diverse mechanisms adopted by fluorescent *Pseudomonas* PGC2 during the inhibition of *Rhizoctonia solani* and *Phytophthora capsici*. World J Microbiol Biotechnol 24(4):581–585

Arora NK, Mishra J (2016) Prospecting the roles of metabolites and additives in future bioformulations for sustainable agriculture. Appl Soil Ecol 107:405–407. doi:10.1016/j.apsoil.2016.05.020

Arora NK, Tewari S, Singh S, Lal N, Maheshwari DK (2012) PGPR for protection of plant health under saline conditions. In: Maheshwari DK (ed) Bacteria in agrobiology: stress management. Springer, Berlin, pp 239–258

Arshad M, Saleem M, Hussain S (2007) Perspectives of bacterial ACC deaminase in phytoremediation. Trends Biotechnol 25:356–362

Asada K (2000) The Water–Water cycle as alternative photon and electron sinks. Philos Trans R Soc 355:1419–1431

Ashford AE, Peterson CA, Carpenter JL, Cairney JWG, Allaway WG (1988) Structure and permeability of the fungal sheath in the Pisonia mycorrhiza. Protoplasma 147:149–161

Auge RM (2001) Water relation, drought and VA mycorrhizal symbiosis. Mycorrhiza 11:3–42

Auld B (2002) Fungi as biocontrol agents: progress, problems and potential. Plant Pathol 51(4):518

Autry AR, Fitzgerald JW (1990) Sulfonate S: a major form of forest soil organic sulfur. Biol Fertil Soils 10:50–56

Baars O, Zhang X, Morel FMM, Seyedsayamdost MR (2016) The siderophore metabolome of *Azotobacter Vinelandii*. Appl Environ Microbiol 82(1)

Bano A, Fatima M (2009) Salt tolerance in *Zea mays* (L.) following inoculation with *Rhizobium* and *Pseudomonas*. Biol Fertil Soils 45:405–413

Barassi CA, Ayrault G, Creus CM, Sueldo RJ, Sobrero MT (2006) Seed inoculation with Azospirillum mitigates NaCl effects on lettuce. Sci Hortic 109:8–14

Barnawal D, Bharti N, Maji D, Chanotiya CS, Kalra A (2012) 1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase-containing rhizobacteria protect Ocimum sanctum plants during waterlogging stress via reduced ethylene generation. Plant Physiol Biochem 58:227–235

Barriuso J, Solano BR, Fray RG, Camara M, Hartmann A, Manero FJG (2008) Transgenic tomato plants alter quorum sensing in plant growth-promoting rhizobacteria. Plant Biotechnol J 6:442–452

Belimov AA, Safronova VI, Sergeyeva TA, Egorova TN, Matveyeva VA, Tsyganov VE et al (2001) Characterization of plant growth-prompting rhizobacteria isolated from polluted soils and containing 1-aminocyclopropane-1-carboxylate deaminase. Can J Microbiol 47:642–652

Belimov A, Dodd I, Hontzeas N, Theobald J, Safronova V, Davies W (2009) Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signaling. New Phytol 181:413–423

Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol 35:1044–1051

Benhamou N, Garand C, Goulet A (2002) Ability of nonpathogenic *Fusarium oxysporum* strain Fo47 to induce resistance against *Pythium ultimum* infection in cucumber. Appl Environ Microbiol 68:4044–4060

Bent E (2006) Induced systemic resistance mediated by plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF). In: Tuzun S, Bent E (eds) Multigenic and induced systemic resistance in plants. Springer, Boston, pp 225–258

Berendsen RL, Pieterse CM, Bakker PA (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486

Bever JD, Platt TG, Morton ER (2012) Microbial population and community dyanamics on plant roots and their feedbacks on plant communities. Annu Rev Microbiol 66:265–283

Bhandari G (2014) An overview of agrochemicals and their effects on environment in Nepal. Appl Ecol Environ Sci 2:66–73

Biari A, Gholami A, Rahmani HA (2008) Growth promotion and enhanced nutrient uptake of maize (*Zea mays* L.) by application of plant growth promoting rhizobacteria in arid region of Iran. J Biol Sci 8:1015–1020

Bisht S, Pandey P, Aggarwal H, Sood A, Sharma S, Kumar V, Bisht NS (2014) Utilization of endophytic strain *Bacillus* sp. SBER3 for biodegradation of polyaromatic hydrocarbons (PAH) in soil model system. Eur J Soil Biol 60:67–76

Bisht S, Pandey P, Sood A, Sharma S, Bisht NS (2010) Biodegradation of anthracene and napthlene by chemotactically active rhizobacteria of *Populus deltoids*. Braz J Microbiol 41:922–930

Bohnert HJ (2007) Abiotic stress. eLS. Wiley, Hoboken, NJ

Bossis E, Lemanceau P, Latour X, Gardan L (2000) The taxonomy of *Pseudomonas fluorescens* and *Pseudomonas putida*: current status and need for revision. Agronomie 20:51–63

Bou-Abdallah F (2010) The iron redox and hydrolysis chemistry of the ferritins. Biochim Biophys Acta 1800:719–731

Boukhalfa H, Lack J, Reilly SD, Hersman L, Neu MP (2003) Siderophore production and facilitated uptake of iron and plutonium in *P. putida*. AIP Conf Proc 673:343–344

Boyer LR, Brain P, Xu XM, Jeffries P (2015) Inoculation of drought-stressed strawberry with a mixed inoculum of two arbuscular mycorrhizal fungi: effects on population dynamics of fungal species in roots and consequential plant tolerance to water deficiency. Mycorrhiza 25(3):215–227

Bradford KJ, Dilley DR (1978) Effects of root anaerobiosis on ethylene production, epinasty, and growth of tomato plants. Plant Physiol 61:506–509

Bradford MA (2013) Thermal adaptation of decomposer communities in warming soils. Front Microbiol 4:333

Bradford MA, Davies CA, Frey SD, Maddox TR, Melillo JM, Mohan JE, Reynolds JF, Treseder KK, Wallenstein MD (2008) Thermal adaptation of soil microbial respiration to elevated temperature. Ecol Lett 11:1316–1327

Bradshaw AD, Johnson MS (1992) Revegetation of metalliferous mine waste: the range of practical techniques used in Western Europe. In: Minerals, metals and the environment. Institute of Mining and Mettalurgy, London, p 491

Brazil GM, Kenefick L, Callanan M, Haro A, Lorenzo VD, Dowling DN, O'Gara F (1995) Construction of a Rhizosphere pseudomonad with potential to degrade polychlorinated biphenyls and detection of bph gene expression in the rhizosphere. Appl Environ Microbiol 61:1946–1952

Bresson J, Varoquaux F, Bontpart T, Touraine B, Vile D (2013) The PGPR strain *Phyllobacterium brassicacearum* STM196 induces a reproductive delay and physiological changes that result in improved drought tolerance in Arabidopsis. New Phytol 200:558–569

Bruijn FJD (ed) (2015) Biological nitrogen fixation, 2 volume set. Wiley–Blackwell, Hoboken, NJ, p 2060

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154(2):275–304

Bruser T, Lens P, Truper HG (2000) The biological sulphur cycle. In: Lens P, Pol LH (eds) Environmental technologies to treat sulphur pollution. IWA Publishing, London, pp 47–86

Bucking H, Kafle A (2015) Role of arbuscular mycorrhizal fungi in the nitrogen uptake of plants: current knowledge and research gaps. Agronomy 5:587–612

Budzikiewicz H (2010) Microbial siderophores. In: Kinghorn AD, Falk H, Kobayashi J (eds) Progress in the chemistry of organic natural products, vol 92. Springer, Vienna, pp 1–75

Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P (2013) Structure and functions of the bacterial microbiota of plants. Annu Rev Plant Biol 64:807–838
Burd GI, Dixon DC, Click BR (1998) A plant growth promoting bacterium that decreases nickel toxicity in seedlings. Appl Environ Microbiol 64:3663–3668
Cairney JWG (2000) Evolution of mycorrhiza systems. Naturwissenschaften 87:467–475
Cakmak I (2009) Enrichment of fertilizers with zinc: an excellent investment for humanity and crop production in India. J Trace Elem Med Biol 23(4):281–289
Cakmakci R, Donmez F, Aydn A, Sahin F (2006) Growth promotion of plants by plant growth promoting rhizobacteria under green house and two different field soil conditions. Soil Biol Biochem 38:1482–1487
Cameron KC, Di HJ, Moir JL (2013) Nitrogen losses from the soil/plant system: a review. Ann Appl Biol 162:145–173
Carlile MJ, Watkinson SC (1996) Saprotrophs and ecosystems. In: Carlile MJ, Watkinson SC (eds) The fungi. Academic, New York, p 608
Carnejo P, Meier S, Borie G, Rillig MC, Borie F (2008) Glomalin-related soil protein in a Mediterranean ecosystem affected by a copper smelter and its contribution to Cu and Zn sequestration. Sci Total Environ 406:154–160
Cass A, McKenzie N, Cresswell H (1996) Physical indicators of soil health. In: Walker J, Reuter DJ (eds) Indicators of catchment health: a technical perspective. CSIRO, Melbourne, pp 89–107
Cawoy H, Bettiol W, Fickers P, Ongena M (2011) *Bacillus*-based biological control of plant diseases. In: Stoytcheva M (ed) Pesticides in the modern world: pesticides use and management. InTech, Rijeka, pp 273–302
Chakraborty S, Tiedemann AV, Teng PS (2000) Climate change: potential impact on plant diseases. Environ Pollut 108:317–326
Chattopadhyay A, Bhatnagar NB, Bhatnagar R (2004) Bacterial insecticidal toxins. Crit Rev Microbiol 30:33–54
Chaudhry Q, Blom-Zandstra M, Gupta S, Joner EJ (2005) Utilising the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. Environ Sci Pollut Res 12(1):34–48
Cheeseman JM (1988) Mechanisms of salinity tolerance in plants. Plant Physiol 87:57–550
Chen S, Jin W, Liu A, Zhang S, Liu D, Wang F, Lin X, He C (2013) Arbuscular mycorrhizal fungi (AMF) increase growth and secondary metabolism in cucumber subjected to low temperature stress. Sci Hortic 160:222–229
Cheng Z, Park E, Glick BR (2007) 1-Aminocyclopropane-1-carboxylate deaminase from *Pseudomonas putida* UW4 facilitates the growth of canola in the presence of salt. Can J Microbiol 53:912–918
Coats VC, Rumpho ME (2014) The rhizosphere microbiota of plant invaders: an overview of recent advances in the microbiomics of invasive plants. Front Microbiol 5:368
Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959
Contreras-Cornejo HA, Macias-Rodriguez L, Cortes-Penagos C, Lopez-Bucio J (2009) *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. Plant Physiol 149:1579–1592
Copping LG (2009) The manual of biocontrol agents. BCPC Publications, Hampshire, p 852
Correa A, Cruz C, Ferrol N (2015) Nitrogen and carbon/nitrogen dynamics in arbuscular mycorrhiza: the great unknown. Mycorrhiza 25:499–515
Crowley DA (2006) Microbial siderophores in the plant rhizosphere. In: Barton LL, Abadía J (eds) Iron nutrition in plants and rhizospheric microorganisms. Springer, Dordrecht, pp 169–189
Daane LL, Harjono I, Zylstra GJ, Haggblom MM (2001) Isolation and characterization of polycyclic aromatic hydrocarbon– degrading bacteria associated with the rhizosphere of salt marsh plants. Appl Environ Microbiol 67:2683–2691

Dardanelli MS, Fernández de Córdoba FJ, Rosario Espuny M, Rodríguez Carvajal MA, Soria Díaz ME, Gil Serrano AM, Okon Y, Megías M (2008) Effect of Azospirillum brasilense coinoculated with Rhizobium on Phaseolus vulgaris flavonoids and Nod factor production under salt stress. Soil Biol Biochem 40:2713–2721

Datta B, Chakrabartty PK (2014) Siderophore biosynthesis genes of *Rhizobium* sp. isolated from *Cicer arietinum* L. 3 Biotech 4(4):391–401

de Lima Procópio RE, da Silva IR, Martins MK, de Azevedo JL, de Araújo JM (2012) Antibiotics produced by *Streptomyces*. Braz J Infect Dis 16:466–471

Diacono M, Castrignanò A, Troccoli A, De Benedetto D, Basso B, Rubino P (2012) Spatial and temporal variability of wheat grain yield and quality in a Mediterranean environment: a multivariate geostatistical approach. Field Crop Res 131:49–62

Dimkpa C, Weinand T, Asch F (2009) Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Environ 32:1682–1694

Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2(8):621–631

Drew MC (1992) Soil aeration and plant root growth metabolism. Soil Sci 154:259–268

Drew MC, Hole PS, Picchioni GA (1990) Inhibition by NaCl of net CO2 fixation and yield of cucumber. J Am Soc Hortic Sci 115:472–477

Droux M (2004) Sulfur assimilation and the role of sulfur in plant metabolism: a survey. Photosyn Res 79:331–348

Dzantor EK (2007) Phytoremediation: the state of rhizosphere "engineering" for accelerated rhizodegradation of xenobiotic contaminants. J Chem Technol Biotechnol 82:228–232

East R (2013) Soil science comes to life – plants may be getting a little help with their tolerance of drought and heat. Nature 501:18–19

Egamberdieva D, Lugtenberg B (2014) PGPR to alleviate salinity stress on plant growth. In: Miransari M (ed) Use of microbes for the alleviation of soil stresses. Springer, New York, pp 73–96

Elsharkawy MM, Shimizu M, Takahashi H, Hyakumachi M (2012) The plant growth-promoting fungus *Fusarium equiseti* and the arbuscular mycorrhizal fungus *Glomus mosseae* induce systemic resistance against Cucumber mosaic virus in cucumber plants. Plant Soil 361:397–409

English PJ, Lycett GW, Roberts JA, Jackson MB (1995) Increased 1-aminocyclopropane-1-carboxylic acid oxidase activity in shoots of flooded tomato plants raises ethylene production to physiologically active levels. Plant Physiol 109:1435–1440

Erickson LE (1997) An overview of research on the beneficial effects of vegetation in contaminated soil. Ann N Y Acad Sci 829:30–35

Estevez J, Dardanelli MS, Megias M, Rodriguez-Navarro DN (2009) Symbiotic performance of common bean and soybean co inoculated with rhizobia and *Chryseobacterium balustinum* Aur9 under moderate saline conditions. Symbiosis 49:29–36

Eswaran H, Reich P, Beigroth F (1997) Global distribution of soils with acidity. In: Moniz AC et al (eds) Plant soil plant interactions at low pH. Braz Soil Sci Soc Am, Madison, pp 159–164

Fageria NK, Baligar VC, Wright RJ (1990) Iron nutrition of plants: an overview on the chemistry and physiology of its deficiency and toxicity. Pesq Agropec Bras. Brasilia 25:553–570

Fahad S, Hussain S, Bano A, Saud S, Hassan S, Shan D, Khan FA, Khan F, Chen Y, Wu C, Tabassum MA, Chun MX, Afzal M, Jan A, Jan MT, Huang J (2015) Potential role of phytohormones and plant growth-promoting rhizobacteria in abiotic stresses: consequences for changing environment. Environ Sci Pollut Res Int 22(7):4907–4921

Falkenmark M (2013) Growing water scarcity in agriculture: future challenge to global water security. Philos Trans R Soc A 371:20120410

FAO (2011) World Census of Agriculture: analysis and international comparison of the results (1996–2005). FAO Statistical Development Series No. 13, Rome

FAO (2013) Food and agriculture organization. Food security and gender: linkages, disparities, and priorities for action. In: Background paper presented at the international conference on forests for food security and nutrition, Rome, 13–15 May, 2013

FAO (2015) The state of food insecurity in the world meeting the 2015 international hunger targets: taking stock of uneven progress. Food and Agric Org of the Uni Nat, Rome p 62
Fatima Z, Saleemi M, Zia M, Sultan T, Aslam M, Rehman RU, Chaudhary MF (2009) Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. Afr J Biotechnol 8:219–225
Fattah QA (2005) Plant resources for human development. Third International Botanical Society, Dhaka
Ferguson JA, Korte F (1977) Epoxidation of aldrin to exodieldrin by soil bacteria. Appl Environ Microbiol 34:7–13
Fernández V, Ebert G, Winkelmann G (2005) The use of microbial siderophores for foliar iron application studies. Plant Soil 272(1):245–252
Fernando WGD, Ramarathnam R, Krishnamoorthy AS, Savchuk SC (2005) Identification and use of potential bacterial organic antifungal volatiles in biocontrol. Soil Biol Biochem 37:955–964
Ferro AM, Sims RC, Bugbee B (1994) Hycrest crested wheatgrass accelerates the degradation of pentachlorophenol in soil. J Environ Qual 23:272–279
Figueiredo MVB, Burity HA, Martinez CR, Chanway CP (2008) Alleviation of water stress effects in common bean (Phaseolus vulgaris L.) by co-*inoculation Paenibacillus* x *Rhizobium tropici*. Appl Soil Ecol 40:182–188
Filion M, St-Arnaud M, Fortin JA (1999) Direct interaction between the arbuscular mycorrhizal fungus Glomus intraradices and different rhizosphere microorganisms. New Phytol 141:525–533
Fletcher J, Bender C, Budowle B, Cobb WT, Gold SE, Ishimaru CA, Luster D, Melcher U, Murch R, Scherm H, Seem RC, Sherwood JL, Sobral BW, Tolin SA (2006) Plant pathogen forensics: capabilities, needs, and recommendations. Microbiol Mol Biol Rev 70(2):450–471
Flowers TJ (2004) Improving crop salt tolerance. J Exp Bot 55(396):307–319
Fravel D (2005) Commercialization and implementation of biocontrol. Annu Rev Phytopathol 43:337–359
Freney JR (1967) Sulfur-containing organics. In: McLaren AD, Peterson GH (eds) Soil biochemistry. Marcel Dekker, New York, pp 229–252
Gaddie GM (1993) Interaction of fungi with toxic metals. New Phytol 124:25–60
Gahan J, Schmalenberger A (2014) The role of bacteria and mycorrhiza in plant sulfur supply. Front Plant Sci 5:723
Galinski EA, Truper HG (1994) Microbial behavior in salt-stressed ecosystems. FEMS Microbiol Rev 15:95–108
Ganeshan G, Kumar AM (2005) *Pseudomonas fluorescens,* a potential bacterial antagonist to control plant diseases. J Plant Interact 1(3):123–134
Garon D, Krivobok S, Seigle-Murandi F (2000) Fungal degradation of fluorene. Chemosphere 40:91–97
Glick BR (1995) The enhancement of plant growth by free living bacteria. Can J Microbiol 41:109–114
Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Lett 251:17
Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica, Article ID 2012963401
Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169:30–39
Godsey CB, Schmidt JP, Schlegel AJ, Taylor RK, Thompson CR, Gehl RJ (2003) Correcting iron deficiency in corn with seed row-applied iron sulfate. Agron J 95:160–166
Gonzales-Chavez MC, Carrillo-Gonzales R, Wright SF, Nichols KA (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. Environ Pollut 130:317–323
Gothwal R, Nigam V, Mohan M, Sasmal D, Ghosh P (2009) Screening of nitrogen fixers from rhizospheric bacterial isolates associated with important desert plants. Appl Ecol Environ Res 6:101–109
Grichko VP, Glick BR (2001) Ethylene and flooding stress in plants. Plant Physiol Biochem 39:1–9

Grichko VP, Glick BR, Grishko VI, Pauls KP (2005) Evaluation of tomato plants with constitutive, root-specific and stress-induced ACC deaminase gene expression. Russ J Plant Physiol 52:359–364

Grover M, Ali Sk Z, Sandhya V, Rasul A, Venkateswarlu B (2011) Role of microorganisms in adaptation of agriculture crops to abiotic stress. World J Microbiol Biotechnol 27(5):1231–1240

Guilioni L, Wery J, Tardieu F (1997) Heat stress-induced abortion of buds and flowers in pea: is sensitivity linked to organ age or to relations between reproductive organs? Ann Bot 80:159–168

Gutierrez-Manero FJ, Probanza A, Ramos B, Colon-Flores JJ, Lucas-Garcia JA (2003) Effects of culture filtrates of rhizobacteria isolated from wild lupine on germination, growth, and biological nitrogen fixation of lupine seedlings. J Plant Nutr 26:1101–1115

Hamayun M, Khan SA, Khan AL, Rehman G, Kim YH, Iqbal I, Hussain J, Sohn EY, Lee IJ (2010) Gibberellin production and plant growth promotion from pure cultures of *Cladosporium* sp. MH–6 isolated from cucumber (*Cucumis sativus* L.) Mycologia 102:989–995

Hamdia MA, Shaddad MAK, Doaa MM (2004) Mechanism of salt tolerance and interactive effect of *Azospirillum bransilense* inoculation on maize cultivars grown under salt stress conditions. Plant Growth Regul 44:165–174

Han HS, Supanjani E, Lee KD (2006) Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. Plant Soil Environ 52(3):130–136

Harte J (2007) Human population as a dynamic factor in environmental degradation. Popul Env 28:223–236

Hasanuzzaman M, Nahar K, Alam MM, Chowdhury RR, Fujita M (2013) Physiological, biochemical, and molecular mechanisms of heat stress tolerance in plants. Int J Mol Sci 14(5):9643–9684

Hassan S, St-Arnaud M, Labreque M, Hijri M (2010) Phytoremediation: biotechnological procedures involving plants and arbuscular mycorrhizal fungi. In Devarajan Thangadurai CAB, Hijri M (eds) Mycorrhizal biotechnology. CRC Press New York, p 152

Heffer P, Prud'homme M (2015) Fertilizer outlook 2015–2019. In: 83rd International fertilization association annual conference Istanbul (Turkey), 25–27 May 2015

Herridge DF, Peoples MB, Boddey RM (2008) Global inputs of biological nitrogen fixation in agricultural systems. Plant Soil 311:1–18

Hirel B, Tétu T, Lea PJ, Dubois F (2011) Improving nitrogen use efficiency in crops for sustainable agriculture. Sustainability 3(12):1452–1485

Hoagland RE, Zablotowicz RM, Locke MA (1994) Propanil metabolism by rhizosphere microflora. In: Anderson TA, Coats JR (eds) Bioremediation through rhizosphere technology. Am Chem Soc, Washington, pp 160–183

Hole DG, Perkins AJ, Wilson JD, Alexander IH, Grice PV, Evans AD (2005) Does organic farming benefit biodiversity? Biol Cons 122:113–130

Holford ICR (1997) Soil phosphorus: its measurement, and its uptake by plants. Aust J Soil Res 35:227–239

Horinouchi H, Muslim A, Hyakumachi M (2010) Biocontrol of fusarium wilt of spinach by the plant growth promoting fungus *Fusarium equiseti* GF183. J Plant Pathol 92(1):249–254

Horrigan L, Lawrence RS, Walker P (2002) How sustainable agriculture can address the environmental and human health harms of industrial agriculture. Environ Health Perspect 110:445–456

Hossain MM, Sultana F, Kubota M, Koyama H, Hyakumachi M (2007) The plant growth-promoting fungus *Penicillium simplicissimum* GP17–2 induces resistance in *Arabidopsis thaliana* by activation of multiple defense signals. Plant Cell Physiol 48(12):1724–1736

Hossain MM, Sultana F, Kubota M, Koyama H, Hyakumachi M (2008) Systemic resistance to bacterial leaf speck pathogen in Arabidopsis thaliana induced by the culture filtrate of a plant growth-promoting fungus (PGPF) *Phoma sp*. GS8–1. J Gen Plant Pathol 74:213–221

Hossain MM, Sultana F, Miyazawa M, Hyakumachi M (2014) Plant growth-promoting fungus Penicillium spp. GP15–1 enhances growth and confers protection against damping-off and anthracnose in the cucumber. J Oleo Sci 63:391–400

Ibibijen J, Urquiaga S, Ismaili M, Alves BJ, Boddey RM (1996) Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition and nitrogen fixation of three varieties of common beans (*Phaseolus vulgaris*). New Phytol 134:353–360

Idris R, Trifonova R, Puschenreiter M, Wenzel WW, Sessitsch A (2004) Bacterial communities associated with flowering plants of the Ni hyperaccumulator Thlaspi goesingense. Appl Environ Microbiol 70:2667–2677

IFPRI (2000) International food policy research institute. Global study reveals new warning signals: degraded agricultural lands threaten world's food production capacity. IFPRI News Release

Islam KR, Weil RR (2000) Soil quality indicator properties in mid – Atlantic soils as influenced by conservation management. J Soil Water Conserv 55:69–78

Jackson MB (1985) Ethylene and responses of plants to soil waterlogging and submergence. Annu Rev Plant Physiol 36:145–174

Jackson MB (1997) Hormones from roots as signals for the shoots of stressed plants. Trends Plant Sci 2:22–28

Jackson MB, Campbell DJ (1976) Waterlogging and petiole epinasty in tomato: the role of ethylene and low oxygen. New Phytol 76:21–29

Jacobs H, Boswell GP, Ritz K, Davidson FA, Gadd GM (2002) Solubilization of calcium phosphate as a consequence of carbon translocation by Rhizoctonia solani. FEMS Microbiol Ecol 40:65–71

Jacobsen CS (1997) Plant protection and rhizosphere colonization of barley by seed inoculated herbicide degrading *Burkholderia* (*Pseudomonas*) *cepacia* DBO1 (pRO101) in 2,4-D contaminated soil. Plant Soil 189(1):139–144

Jankong P, Visoottiviseth P, Khokiattiwong S (2007) Enhanced phytoremediation of arsenic contaminated land. Chemosphere 68:1906–1912

Jones DL, Shannon D, Murphy DV, Farrar J (2004a) Role of dissolved organic nitrogen (DON) in soil N cycling in grassland soils. Soil Biol Biochem 36:749–756

Jones DL, Hodge A, Kuzyakov Y (2004b) Plant and mycorrhizal regulation of rhizodeposition. New Phytol 163:459–480

Joseph B, Patra RR, Lawrence R (2007) Characterization of plant growth promoting Rhizobacteria associated with chickpea (*Cicer arietinum* L). Int J Plant Prod 1(2):141–152

Kamprath EJ, Nelson WL, Fitts JW (1956) The effect of pH, sulphate and phosphate concentrations on the adsorption of sulphate by soils. Soil Sci Soc Am Proc 20:463–466

Kang S, Khan AL, Waqas M, You YH, Kim JH, Kim JK, Hamayun M, Lee IJ (2014) Plant growth promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in Cucumis sativus. J Plant Int 9(1):673–682

Kangwankraiphaisan T, Suntornvongsagul K, Sihanonth P, Klysubun W, Gadd GM (2013) Influence of arbuscular mycorrhizal fungi (AMF) on zinc biogeochemistry in the rhizosphere of *Lindenbergia philippensis* growing in zinc-contaminated sediment. Biometals (3):489–505

Kannahi M, Senbagam N (2014) Studies on siderophore production by microbial isolates obtained from rhizosphere soil and its antibacterial activity. J Chem Pharma Res 6(4):1142–1145

Kapulnik Y, Okon Y (2002) Plant growth promotion by rhizosphere bacteria. In: Waisel Y, Eshel A, Kafkafi U (eds) Plant roots: the hidden half. Marcel Dekker, New York, pp 869–885

Kaur T, Sharma D, Kaur A, Manhas RK (2013) Antagonistic and plant growth promoting activities of endophytic and soil actinomycetes. Arch Phytopathol Plant Prot 46:1756–1768

Kaushal M, Wani SP (2016) Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. Ann Microbiol 66(1):35–42

Kelley SL, Aitchison EW, Deshpande M, Schnoor JL, Alvarez PJ (2001) Biodegradation of 1,4-dioxane in planted and unplanted soil: effect of bioaugmentation with *Amycolata* sp. CB1190. Water Res 35:3791–3800

Kertesz MA, Mirleau P (2004) The role of soil microbes in plant sulphur nutrition. J Exp Bot 55(404):1939–1945

Kesavan PC, Swaminathan MS (2008) Strategies and models for agricultural sustainability in developing Asian countries. Philos Trans R Soc B Biol Sci 363:877–891

Khalid S, Hafeez NA, Muhammad JA, Ana A, Zahir AZ (2015) Biofortification of iron in chickpea by plant growth promoting rhizobacteria. Pak J Bot 47(3):1191–1194

Khan AL, Hamayun M, Radhakrishnan R et al (2012) Mutualistic association of *Paecilomyces formosus* LHL10 offers thermotolerance to *Cucumis sativus*. A van Leeuw 101(2):267–279
Khan MR, Khan SM (2002) Effect of root-dip treatment with certain phosphate solubilizing microorganisms. Bioresour Technol 85(2):213–215
Khan MS, Zaidi A, Ahemad M, Oves M, Wani PA (2010) Plant growth promotion by phosphate solubilizing fungi – current perspective. Arch Agron Soil Sci 56:73–98
Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture – a review. Agron Sustain Dev 27:29–43
Khare E, Arora NK (2011a) Physiologically stressed cells of fluorescent *Pseudomonas* EKi as better option for bioformulation development for management of charcoal rot caused by *Macrophomina phaseolina* in field conditions. Curr Microbiol 62(6):1789–1793
Khare E, Arora NK (2011b) Dual activity of pyocyanin from *Pseudomonas aeruginosa*—antibiotic against phytopathogen and signal molecule for biofilm development by rhizobia. Can J Microbial 57(9):708–713
Khatibi R (2011) Using sulfur oxidizing bacteria and P solubilizing for enhancing phosphorous availability to Raphanus sativus. Afr J Plant Sci 5(8):430–435
Kingsley MT, Fredrickson JK, Metting FB, Seidler RJ (1994) Environmental restoration using plant-microbe bioaugmentation. In: Hinchee RE, Leeson A, Semprini L, Ong SK (eds) Bioremediation of chlorinated and polyaromatic hydrocarbon compounds. Lewis Publishers, Boca Raton, pp 287–292
Kloepper JW, Schroth MN (1978) Association of *in vitro* antibiosis with inducibility of increased plant growth by *Pseudomonas spp*. (Abstr). Phytopathol News 12:136
Kobayashi T, Nishizawa NK (2012) Iron uptake, translocation, and regulation in higher plants. Annu Rev Plant Biol 63:131–152
Koide RT, Goff MD, Dickie IA (2000) Component growth efficiencies of mvcorrhizal and nonmvcorrhizal plants. New Phytol 148:163–168
Krewulak KD, Vogel HJ (2008) Structural biology of bacterial iron uptake. Biochim Biophys Acta 1778:1781–1804
Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJ (2004) Rhizoremediation: a beneficial plant-microbe interaction. Mol Plant Microbe Interact 17:6–15
Kumar P, Dubey RC, Maheshwari DK (2012) *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiol Res 8(6):493–499
Lafrance P, Lapointe M (1998) Mobilization and co-transport of pyrene in the presence of Pseudomonas aeruginosa UG2 biosurfactants in sandy soil columns. Ground Water Monit Remediat 18:139–147
Lakshmanan V, Selvaraj G, Bais HP (2014) Functional soil microbiome: belowground solutions to an aboveground problem. Plant Physiol 166(2):689–700
Larkindale J, Huang B (2005) Effects of abscisic acid, salicylic acid, ethylene and hydrogen peroxide in thermotolerance and recovery for creeping bentgrass. Plant Growth Regul 47:17–28
Lee S, Ka JO, Gyu SH (2012) Growth promotion of *Xanthium italicum* by application of rhizobacterial isolates of *Bacillus aryabhattai* in microcosm soil. J Microbiol 50:45–49
Li J, McConkey BJ, Cheng Z, Guo S, Glick BR (2013) Identification of plant growth-promoting bacteria-responsive proteins in cucumber roots under hypoxic stress using a proteomic approach. J Proteomics 84:119–131
Lian B et al (2002) A comprehensive review of the mechanism of potassium releasing by silicate bacteria. Acta Mineral Sin 22(2):179–183
Liang Y, Lin X, Yamada S, Inoue M, Inosako K (2013) Soil degradation and prevention in green house production. Springerplus 2(Suppl 1):S10
Liao CT, Lin CH (1994) Effect of flooding stress on photosynthetic activities of *Momordica charantia*. Plant Physiol Biochem 32:479–485
Lim JH, Kim SD (2013) Induction of drought stress resistance by multi-functional PGPR *Bacillus licheniformis* K11 in pepper. Plant Pathol J 29(2):201–208

Ling W, Sun R, Gao X, Xu R, Li H (2015) Low-molecular-weight organic acids enhance desorption of polycyclic aromatic hydrocarbons from soil. Eur J Soil Sci 66:339–347
Liu D, Lian B, Dong H (2012) Isolation of *Paenibacillus* sp. and assessment of its potential for enhancing mineral weathering. Geomicrobiol J 29(5):413–421
Lobell DB, Burke MB, Tebaldi C, Mastrandrea MD, Falcon WP, Naylor RL (2008) Prioritizing climate change adaptation needs for food security in 2030. Science 319:607–610
Lopes-Assad ML, Avansini SH, Rosa MM, Carvalho JRP, Ceccato-Antonini SR (2010) The solubilization of potassium-bearing rock powder by *Aspergillus niger* in small-scale batch fermentations. Can J Microbiol 56:598–605
Lu C, Barr DB, Pearson MA, Waller LA (2008) Dietary intake and its contribution to longitudinal organophosphorus pesticide exposure in urban/suburban children. Environ Health Perspect 116:537–542
Lynette AK, Murphy DV (2007) What is soil biological fertility? Soil biological fertility. Springer, Dordrecht, pp 1–15
MacGregor JT (2006) Genetic toxicity assessment of microbial pesticide: needs and recommended approaches. A report to the organization for economic cooperation and development, Paris, pp 1–17
Magdoff F, Weil RR (eds) (2004) Soil organic matter in sustainable agriculture, vol 412. CRC Press, Boca Raton, FL, USA
Maherali H, Klironomos J (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316:1746–1748
Maheshwari DK, Dheeman S, Agarwal M (2015) Phytohormone-producing PGPR for sustainable agriculture. In: Maheshwari DK (ed) Bacterial metabolites in sustainable agroecosystem. Springer, Cham, pp 159–182
Maheshwari DK, Dubey RC, Aeron A, Kumar B, Kumar S, Tewari S, Arora NK (2012) Integrated approach for disease management and growth enhancement of *Sesamum indicum* L. utilizing *Azotobacter chroococcum* TRA2 and chemical fertilizer. World J Microbiol Biotechnol 28(10):3015–3024
Maksimov IV, Abizgil'dina RR, Pusenkova LI (2011) Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens. Appl Biochem Microbiol 47:333–345
Marasco R, Rolli E, Ettoumi B et al (2012) A drought resistance-promoting microbiome is selected by root system under desert farming. PLoS One 7(10):e48479
Marschner H (ed) (2012) Marschner's mineral nutrition of higher plants, vol 89. Academic, London, p 651
Marschner H, Dell B (1994) Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159(1):89–102
Masunaka A, Hyakumachi M, Takenaka S (2011) Plant growth-promoting fungus, *Trichoderma koningi* suppresses isoflavonoid phytoalexin vestitol production for colonization on/in the roots of *Lotus japonicus*. Microbes Environ 26:128–134
Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572
McClung CR, Davis SJ (2010) Ambient thermometers in plants: from physiological outputs towards mechanisms of thermal sensing. Curr Biol 20(24):1086–1092
McCoy CW (1990) Entomogenous fungi as microbial pesticides. In: Baker RR, Dunn PE (eds) New directions in biological control: alternatives for suppressing agricultural pests and diseases (UCLA Symposia on Molecular and Cellular Biology). Wiley–Liss, New York, pp 139–159
McLaren RG, Cameron KC (2004) Soil science: sustainable production and environmental protection. Oxford University Press, Victoria, p 314
McNear JDH (2013) The rhizosphere – roots, soil and everything in between. Nat Edu Know 4(3):1
Meena SV, Maurya BR, Verma JP (2014) Does a rhizospheric microorganism enhance K+ availability in agricultural soils? Microbiol Res 169(5):337–347
Meharg AA, Cairney JWG, Maguire N (1997) Mineralization of 2,4-dichlorophenol by ectomycorrhizal fungi in axenic culture and in symbiosis with pine. Chemosphere 34:2495–2504
Mejare M, Bulow L (2001) Metal-binding proteins and peptides in bioremediation and phytoremediation of heavy metals. Trends Biotechnol 19:67–73

Merry RH (1996) Chemical indicators of soil health. In: Walker J, Reuter DJ (eds) Indicators of catchment health: a technical perspective. CSIRO, Melbourne, pp 109–119
Merzaeva OV, Shirokikh IG (2006) Colonization of plant rhizosphere by actinomycetes of different genera. Microbiol 75(2):226–230
Meyers WH, Kalaitzandonakes N (2012) World population growth and food supply. In: Popp J, Matlock M, Kemper N, Jahn M (eds) The role of biotechnology in a sustainable food supply. Cambridge University Press, Cambridge, pp 1–16
Michalzik B, Kalbitz K, Park JH, Solinger S, Matzner E (2001) Fluxes and concentrations of dissolved organic carbon and nitrogen synthesis for temperate forests. Biogeochemistry 52:173–205
Miller KJ, Wood JM (1996) Osmoadaptation by rhizosphere bacteria. Annu Rev Microbiol 50:101–136
Millner PD, Wright SF (2002) Tools for support of ecological research on arbuscular mycorrhizal fungi. Symbiosis 33:101–123
Milosevic NA, Marinkovic JB, Tintor BB (2012) Mitigating abiotic stress in crop plants by microorganisms. Proc Nat Sci 123:17–26
Mishra S, Arora NK (2012a) Evaluation of rhizospheric *Pseudomona*s and *Bacillus* as biocontrol tool for *Xanthomonas campestris* pv campestris. World J Microbiol Biotechnol 28(2):693–702
Mishra S, Arora NK (2012b) Management of black rot in cabbage by rhizospheric *Pseudomonas* species and analysis of 2, 4-diacetylphloroglucinol by qRT-PCR. Biolo Cont 61(1):32–39
Mishra J, Arora NK (2016) Bioformulations for plant growth promotion and combating phytopathogens: a sustainable approach. In: Bioformulations: for sustainable agriculture. Springer, India, pp 3–33
Mishra J, Tewari S, Singh S, Arora NK (2015) Biopesticides where we stand? In: Arora NK (ed) Plant microbe symbiosis: applied facets. Springer, New Delhi, pp 37–75
Mohan SV, Kisa T, Ohkuma T, Kanaly RA, Shimizu Y (2006) Bioremediation technologies for treatment of PAH contaminated soil and strategies to enhance process efficiency. Rev Environ Sci Biotechnol 5:347–374
Morley GF, Gadd GM (1995) Sorption of toxic metals by fungi and clay minerals. Mycol Res 99:1429–1438
Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250
Murali M, Amruthesh KN (2015) Plant growth-promoting fungus *penicillium oxalicum* enhances plant growth and induces resistance in pearl millet against Downy mildew disease. J Phytopathol 163:743–754
Murali M, Amruthesh KN, Sudisha J, Niranjana SR, Shetty HS (2012) Screening for plant growth promoting fungi and their ability for growth promotion and induction of resistance in pearl millet against downy mildew disease. J Phytol 4(5):30–36
Nadeem SM, Zahir ZA, Naveed M, Arshad M (2007) Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Can J Microbiol 53:1141–1149
Naznin HA, Kimura M, Miyazawa M, Hyakumachi M (2013) Analysis of volatile organic compounds emitted by plant growth-promoting fungus *Phoma sp*. GS8–3 for growth promotion effects on tobacco. Microbes Environ 28:42–49
Nelson LM (2004) Plant growth promoting rhizobacteria (PGPR): prospects for new inoculants. Plant Manag Netw doi:10.1094/CM–2004–0301–05–RV
Newman LA, Reynolds CM (2004) Phytodegradation of organic compounds. Curr Opin Biotechnol 15:225–230
Niu S, Li H, Pare PW, Aziz M, Wang S, Shi H, Li J, Han Q, Guo S, Li J, Guo Q, Ma Q, Zhang J (2015) Induced growth promotion and higher salt tolerance in the halophyte grass Puccinellia tenuiflora by beneficial rhizobacteria. Plant Soil:1–14
Oldeman L R (1998) Soil degradation: a threat to food security? Report 98/01, International Soil Reference and Information Centre, wageningen
Olson DC, Oetiker JH, Yang SF (1995) Analysis of LE-ACS3, an ACC synthase gene expressed during flooding in roots of tomato plants. J Biol Chem 270:14056–14061

Olsson PA, Thingstrup I, Jakobsen I, Baath E (1999) Estimation of the biomass of arbuscular mycorrhizal fungi in a linseed field. Soil Biol Biochem 31:1879–1887

Paul EA, Clark FE (1996) Soil microbiology and biochemistry. Academic, San Diego, p 340

Pelosi C, Barot S, Capowiez Y, Hedde M, Vandenbulcke F (2013) Pesticides and earthworms. A review. Agron Sustain Dev 34:199–228

Peoples MB et al (2009) The contributions of nitrogen-fixing crop legumes to the productivity of agricultural systems. Symbiosis 48:1–17

Pieterse CMJ, Ton J, Van Loon LC (2001) Cross-talk between plant defence signalling pathways: boost or burden? Ag Biotech Net 3:1–8. ABN 068

Pinedo-Rivilla C, Aleu J, Collado IG (2009) Pollutants biodegradation by fungi. Curr Org Chem 13:1194–1214

Pinton R, Varanini Z, Nannipieri P (2001) The rhizosphere as a site of biochemical interactions among soil components, plants and microorganisms. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere. biochemistry and organic substances at the soil-plant interface. Marcel Dekker, Newyork, pp 1–17

Prajapati K, Sharma MC, Modi HA (2012) Isolation of two potassium solubilizing fungi from ceramic industry soils. Life Sci Leaflets 5:71–75

Prasad MNV (2011) A State of the art report on bioremediation, its applications to contaminated sites in India. Published by Ministry of Environment & Forests, Government of India, New Delhi

Prasanna R, Nain L, Rana A, Shivay YS (2016) Biofortification with microorganisms: present status and future challenges. In: Singh U, Praharaj SC, Singh SS, Singh PN (eds) Biofortification of food crops. Springer, India, pp 249–262

Prietzel J, Weick C, Korintenberg J, Seybold G, Thumerer T, Treml B (2001) Effects of repeated (NH4)2SO4 application on sulfur pool in soil, soil microbial biomass, and ground vegetation of two watersheads in the Black Forest/Germany. Plant Soil 230:287–305

Radosevich M, Traina SJ, Hao YL, Tuovinen OH (1995) Degradation and mineralization of atrazine by a soil bacterial isolate. Appl Environ Microbiol 61:297–302

Radzki W, Gutierrez Mañero FJ, Algar E, Lucas García JA, García-Villaraco A, Ramos SB (2013) Bacterial siderophores efficiently provide iron to iron-starved tomato plants in hydroponics culture. Anton Van Leeuw 104(3):321–330

Ramette A, Frapolli M, Défago G, Moënne-Loccoz Y (2003) Phylogeny of HCN synthase-encoding hcnBC genes in biocontrol fluorescent *Pseudomonads* and its relation-ship with host plant species and HCN synthesis ability. Mol Plant Microbe Interact 16:525–535

Ramos SB, Barriuso MJ, Pereyra de la Iglesia MT, Domenech J, Gutierrez MFJ (2008) Systemic disease protection elicited by plant growth promoting rhizobacteria strains: relationship between metabolic responses, systemic disease protection, and biotic elicitors. Phytopathology 98(4):451–457

Ramos Solana B, Pereyra de la Iglesia MT, Probanza A, Lucas Garcia JA, Megias M, Gutiérrez-Mañero FJ (2006) Screening of PGPR to improve growth of Cistus ladanifer seedlings for reforestation of degraded Mediterranean ecosystems. Plant Soil 289:59–68

Rana A, Kabi SR, Verma S, Adak A, Pal M, Shivay YS, Prasanna R, Nain L (2015) Prospecting plant growth promoting bacteria and cyanobacteria as options for enrichment of macro– and micronutrients in grains in rice–wheat cropping sequence. Cog Food Agric 1:1037379

Rana A, Saharan B, Nain L, Prasanna R, Shivay YS (2012) Enhancing micronutrient uptake and yield of wheat through bacterial PGPR consortia. Soil Sci Plant Nutr 58:573–582

Rasool S, Ahmad A, Siddiqi TO, Ahmad P (2013) Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. Acta Physiol Plant 35(4):1039–1050

Reddy MS, Rodolfo II, Patricio SF, William DD, William DB, Riyaz S, Sudini H, Vijay KKK, Armanda A, Gopalkrishnan S (eds) (2014) Recent advances in biofertilizers and biofungicides (PGPR) for sustainable agriculture. Cambridge Scholars Publishing, Newcastle upon Tyne, UK, p 540

Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM (2002) Thermotolarance generated by plant/fungal symbiosis. Science 298:1581
Rehm G, Schmitt M (2002) Copper for crop production. http://www.extension.umn.edu
Rillig MC, Mummey DL (2006) Mycorrhizas and soil structure. New Phytol 171:41–53
Rodŕiguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339
Rodziewicz P, Swarcewicz B, Chmielewska K, Wojakowska A, Stobiecki M (2014) Influence of abiotic stresses on plant proteome and metabolome changes. Acta Physiol Plant 36(1):1–19
Rohrbachen F, St-Arnaud M (2016) Root exudation: the ecological driver of hydrocarbon rhizoremediation. Agronomy 6(19):1–27
Ronchel MC, Ramos JL (2001) Dual system to reinforce biological containment of recombinant bacteria designed for rhizoremediation. Appl Environ Microbiol 67:2649–2656
Ryan PR, Delhaize E, Jones DL (2001) Function and mechanism of organic anion exudation from plant roots. Ann Rev Plant Physiol Plant Mol Biol 52:527–560
Saha N, Liu M, Gajan A, Pile LA (2016) Genome-wide studies reveal novel and distinct biological pathways regulated by SIN3 isoforms. BMC Genomics 17(1):111
Saha R, Saha N, Donofrio RS, Bestervelt LL (2013) Microbial siderophores: a mini review. J Basic Microbiol 53:303–317
Salas-Marina MA (2011) The plant growth-promoting fungus *Aspergillus ustus* promotes growth and induces resistance against different lifestyle pathogens in *Arabidopsis thaliana*. J Microbiol Biotechnol 21:686–696
Saldajeno MGB, Hyakumachi M (2011) The plant growth-promoting fungus Fusarium equiseti and the arbuscular mycorrhizal fungus *Glomus mosseae* stimulate plant growth and reduce severity of anthracnose and damping-off diseases in cucumber (*Cucumis sativus*) seedlings. Ann Appl Biol 159:28–40
Saleem M, Arshad M, Hussain S, Bhatti AS (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Indus Microbiol Biotechnol 34:635–648
Salimpour S, Khavazi K, Nadian H, Besharati H, Miransari M (2010) Enhancing phosphorous availability to canola (Brassica napus L.) using P solubilizing and sulfur oxidizing bacteria. Aust J Crop Sci 4(5):330–334
Sandhya V, Ali SZ, Grover M, Reddy G, Venkateswarlu B (2009) Alleviation of drought stress effects in sunflower seedlings by exopolysaccharides producing Pseudomonas putida strain P45. Biol Fertil Soils 46:17–26
Saravanakumar D, Samiyappan R (2007) ACC deaminase from *Pseudomonas fluorescens* mediated saline resistance in groundnut (*Arachis hypogea*) plants. J Appl Microbiol 102(5):1283–1292
Saravanan VS, Kalaiarasan P, Madhaiyan M, Thangaraju M (2007) Solubilization of insoluble zinc compounds by *Gluconacetobacter diazotrophicus* and the detrimental action of zinc ion (Zn^{2+}) and zinc chelates on root knot nematode *Meloidogyne incognita*. Lett Appl Microbiol 44:235–241
Saravanan VS, Subramoniam SR, Raj SA (2004) Assessing *in vitro* solubilization potential of different zinc solubilizing bacterial (zsb) isolates. Braz J Microbiol 35:121–125
Sasha RC, Cleveland CCC, Townsend AR (2011) Functional ecology of free-living nitrogen fixation: a contemporary perspective. Ann Rev Ecol Evol Syst 42:489–512
Sayyed RZ, Chincholkar SB, Reddy MS, Gangurde NS, Patel PR (2013) Siderophore producing PGPR for crop nutrition and phytopathogen suppression. In: Maheshwari KD (ed) Bacteria in agrobiology: disease management. Springer, Berlin, pp 449–471
Schlaeppi K, Bulgarelli D (2015) The plant microbiome at work. Mol Plant Microbe Interact 28:212–217
Schnoor JL, Aitchison EW, Kelley SL, Alvarez PJJ (1998) Phytoremediation and bioaugmentation of 1,4-dioxane. In: Adv. Biol. Syst. Rem. Conf. Pages, pp 87–91
Schreiner RP, Milhara KL, McDaniel H, Bethlenfalvay GJ (1997) Mycorrhizal fungi influence plant and soil functions and interactions. Plant Soil 188:199–209

Segura A, Rodríguez-Conde S, Ramos C, Ramos JL (2009) Bacterial responses and interactions with plants during rhizoremediation. Microb Biotechnol 4:452–464

Selvakumar G, Panneerselvam P, Ganeshamurthy AN (2012) Bacterial mediated alleviation of abiotic stress in crops. In: Maheshwari DK (ed) Bacteria in agrobiology: stress management. Springer, Berlin, pp 205–224

Semple KT, Morriss AWJ, Paton GI (2003) Bioavailability of hydrophobic contaminants in soils: fundamental concepts and techniques for analysis. Eur J Soil Sci 54:809–818

Sequi P (1989) Le funzioni agronomiche della sostanza organica. In: Patron (ed) Chimica del suolo, Bologna, Italy, pp 279–292

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. SpringerPlus 2:1–14

Sheldrick WF, Syers JK, Lingard J (2002) A conceptual model for conducting nutrient audits at national, regional and global scales. Nutr Cycl Agroecosys 62:61–67

Shelton DR, Khader S, Karns JS, Pogell BM (1996) Metabolism of twelve herbicides by Streptomyces. Biodegradation 7:129–136

Sheng XF, He LY (2006) Solubilization of potassium-bearing minerals by a wild type strain of *Bacillus edaphicus* and its mutants and increased potassium uptake by wheat. Can J Microbiol 52:66–72

Sheng XF, Xia JJ (2006) Improvement of rape (Brassica napus) plant growth and cadmium uptake by cadmium-resistant bacteria. Chemosphere 64(6):1036–1042

Siciliano SD, Germida JJ (1999) Enhanced phytoremediation of chlorobenzoates in rhizosphere soil. Soil Biol Biochem 31:299–305

Siddiqui ZA, Pichtel J (2008) Mycorrhizae: an overview. In: Siddiqui ZA, Akhtar MS, Futai K (eds) Mycorrhizae: sustainable agriculture and forestry. Springer, Dordrecht, pp 1–35

Sieh D, Watanabe M, Devers EA, Brueckner F, Hoefgen R, Krajinski F (2013) The arbuscular mycorrhizal symbiosis influences sulfur starvation responses of *Medicago truncatula*. New Phytol 197:606–616

Sikes BA (2010) When do arbuscular mycorrhizal fungi protect plant roots from pathogens? Plant Sign Beha 5(6):763–765

Sillanpaa M (1990) Micronutrients assessment at the country level. An international study FAO Soils Bulletin 63. Food and Agriculture Organization of the United Nations

Singh P, Kumar V, Agrawal S (2014) Evaluation of phytase producing bacteria for their plant growth promoting activities. Int J Microbiol 2014:1–7

Sivasakthi S, Usharani G, Saranraj P (2014) Biocontrol potentiality of plant growth promoting bacteria (PGPR)-*Pseudomonas fluorescens* and *Bacillus subtilis*: a review. Afr J Agric 9(16):1265–1277

Skowronska M, Filipek T (2013) Life cycle assessment of fertilizers: a review. Int Agrophys 28:101–110

Smith EE, Facelli E, Pope S, Smith FA (2010) Plant performance in stressful environments. Interpreting new and established knowledge of the roles of arbuscular mycorrhizas. Plant Soil 326:3–20

Smith SE, Smith FA (2012) Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. Mycologia 104:1–13

Smith SE, Jakobsen I, Gronlund M, Smith FA (2011) Roles of mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant Physiol 156:1050–1057

Solans M, Vobis G, Cassán F, Luna V, Wall LG (2011) Production of phytohormones by root-associated saprophytic actinomycetes isolated from the actinorhizal plant *Ochetophila trinervis*. World J Microbiol Biotechnol 27:2195–2202

Spaepen S, Vanderleyden J, Okon Y (2009) Plant growth-promoting actions of rhizobacteria. Adv Bot Res 51:283–320

Spago FR, Ishii Mauro CS, Oliveira AG, Beranger JPO, Cely MVT, Stanganelli MM et al (2014) *Pseudomonas aeruginosa* produces secondary metabolites that have biological activity against plant pathogenic Xanthomonas species. Crop Prot 62:46–54

Sparks DL (2000) Bioavailability of soil potassium, D-38–D-52. In: Sumner ME (ed) Handbook of soil science. CRC Press, Boca Raton

Sparks DL, Huang PM (1985) Physical chemistry of soil potassium. In: Munson RD (ed) Potassium in agriculture. Am Soc Agro, Madison, pp 201–276

Spence C, Alff E, Johnson C, Ramos C, Donofrio N, Sundaresan V, Bais H (2014) Natural rice rhizospheric microbes suppress rice blast infections. BMC Plant Biol 14:1–17

Speight J G (2017) Removal of organic compounds from the environment. Environmental organic chemistry for engineers. Butterworth-Heinemann, UK, pp 387–432

Sreevidya M, Gopalakrishnan S, Kudapa H, Varshney RK (2016) Exploring plant growth-promotion actinomycetes from vermicompost and rhizosphere soil for yield enhancement in chickpea. Braz J Microbiol 47(1):85–95

Sriprang R, Hayashi M, Yamashita M, Ono H, Saeki K, Murooka Y (2002) A novel bioremediation system for heavy metals using the symbiosis between leguminous plant and genetically engineered rhizobia. J Biotechnol 99:279–293

Srivastava S, Yadav A, Seema K, Mishra S, Choudhary V, Nautiyal CS (2008) Effect of high temperature on Pseudomonas putida NBRI0987 biofilm formation and expression of stress sigma factor RpoS. Curr Microbiol 56:453–457

Stein AJ (2014) Rethinking the measurement of undernutrition in a broader health context. Glob Food Secur 3:193–199

Stephan MP et al (1979) Physiological studies with *Azospirillum spp*. In: Vose PB, Ruschel AP (eds) Associative N_2-fixation, vol 1. CRC Press, Boca Raton, pp 7–14

Stepien P, Klobus G (2005) Antioxidant defence in the leaves of C3 and C4 plants under salinity stress. Physiol Plant 125:31–40

Sultana F, Hossain MM, Kubota M, Hyakumachi M (2008) Elicitation of systemic resistance against the bacterial speck pathogen in *Arabidopsis thaliana* by culture filtrates of plant growth-promoting fungi. Can J Plant Pathol 30:196–205

Sunithakumari K, Padma DSN, Vasandha S (2016) Zinc solubilizing bacterial isolates from the agricultural fields of Coimbatore, Tamil Nadu, India. Curr Sci 1(10):2

Swarupa V, Pavitra K, Shivashankara KS, Ravishankar KV (2016) Omics-driven approaches in plant-microbe interaction. In: Singh PD, Singh BH, Prabha R (eds) Microbial inoculants in sustainable agricultural productivity, vol 1: Research perspectives. Springer, New Delhi, pp 61–84

Sziderics AH, Rasche F, Trognitz F, Sessitsch A, Wilhelm E (2007) Bacterial endophytes contribute to abiotic stress adaptation in pepper plants (*Capsicum annuum* L.) Can J Microbiol 53:1195–1202

Takase H, Nitanai H, Hoshino K, Otani T (2000) Requirement of the *Pseudomonas aeruginosa* tonB gene for high-affinity iron acquisition and infection. Infect Immun 68:4498–4504

Talibart R, Jebbar M, Gouffi K, Pichereau V, Gouesbet G, Blanco C, Bernard T, Pocard JA (1997) Transient accumulation of glycine betaine and dynamics of endogenous osmolytes in salt-stressed cultures of Sinorhizobium meliloti. Appl Environ Microbiol 63:4657–4663

Tewari S, Arora N (2014a) Multifunctional exopolysaccharides from Pseudomonas aeruginosa PF23 involved in plant growth stimulation, biocontrol and stress amelioration in sunflower under saline conditions. Curr Microbiol 4(69):484–494

Tewari S, Arora NK (2014b) Talc based exopolysaccharides formulation enhancing growth and production of Hellianthus annuus under saline conditions. Cellular Mol Biol 60:73–81

Tewari S, Arora NK (2015) Plant growth promoting fluorescent *Pseudomonas* enhancing growth of sunflower crop. Int J Sci Technol Soi 1(1)

Tewari S, Arora N (2016) Soybean production under flooding stress and its mitigation using plant growth-promoting microbes. In: Mohammad M (ed) Environmental Stresses in Soybean Production: Soybean Production 2, Academic press, San Diego pp 23–40

Tigini V, Prigione V, Di Toro S, Fava F, Varese GC (2009) Isolation and characterization of polychlorinated biphenyl (PCB) degrading fungi from a historically contaminated soil. Microb Cell Fact 8:5

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature 418:671–677

Tordoff GM, Baker AJM, Willis AJ (2000) Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere 41:219–228
Trabelsi D, Mhamdi R (2013) Microbial inoculants and their impact on soil microbial communities: a review. Bio Med Res Int 2013:11. doi:10.1155/2013/863240
Turner BL, Paphazy M, Haygarth PM, McKelvie ID (2002) Inositol phosphates in the environment. Philos Trans R Soc Lond B Biol Sci 357:449–469
Turner TR, James EK, Poole PS (2013) The plant microbiome. Genome Biol 14:1–10
UNFCCC (2007) Climate change: impacts, vulnerabilities and adaptation in developing countries. Climate Change Secretariat (UNFmeFCCC), Bonn, p 5
USCB (2015) United State Census Bureau. International database. http://www.census.gov/population/international/data/idb/informationGateway.php
Vadakattu G, Paterson J (2006) Free living bacteria lift soil nitrogen supply. Farming Ahead 169:40
Vance CP (2001) Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiol 127:390–397
Vassilev SV, Vassileva CG (2006) A new approach for the classification of coal fly ashes based on their origin, composition, properties, and behavior. Fuel 86:1490–1512
Verma VC, Singh SK, Prakash S (2011) Bio-control and plant growth promotion potential of siderophore producing endophytic *Streptomyces* from *Azadirachta indica.* A Juss. J Basic Microbiol 51(5):550–556
Vinale F, Sivasithamparam K, Ghisalberti LE, Marra R, Woo LS, Lorito M (2008) *Trichoderma*-plant-pathogen interactions. Soil Biol Biochem 40:1–10
Vitousek PM, Menge DNL, Reed SC, Cleveland CCC (2013) Biological nitrogen fixation: rates, patterns and ecological controls in terrestrial ecosystems. Philos Trans R Soc B Biol Sci 368(1621):20130119
Vollenweider P, Gunthardt-Goerg MS (2005) Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. Environ Pollut 137:455–465
Vurukonda SSKP, Vardharajula S, Shrivastava M, SkZ A (2016) Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol Res 184:13–24
Wagner SC (2012) Biological nitrogen fixation. Nat Edu Know 3(10):15
Wahid A (2007) Physiological implications of metabolites biosynthesis in net assimilation and heat stress tolerance of sugarcane sprouts. J Plant Res 120:219–228
Wander M (2004) Soil organic matter fractions and their relevance to soil function. In: Magdoff F, Weil RR (eds) Soil organic matter in sustainable agriculture. CRC Press, Boca Raton
Wang H, Dong Q, Zhou J, Xiang X (2013) Zinc phosphate dissolution by bacteria isolated from an oligotrophic karst cave in central China. Front Earth Sci 7:375–383
Waqas M, Khan AL, Kamran M, Hamayun M, Kang SM, Kim YH, Lee IJ (2012) Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. Molecules 17:10754–10773
Welch RM, Graham RD (2004) Breeding for micronutrients in staple food crops from a human nutrition perspective. J Exp Bot 55:353–364
Weller DM (2007) *Pseudomonas* biocontrol agents of soil borne pathogens: looking back over 30 years. Phytopathology 97(2):250–256
Wenzel WW (2009) Rhizosphere processes and management in plant-assisted bioremediation (phytoremediation) of soils. Plant Soil 321:385–408
Weyens N, van der Lelie D, Taghavi S, Newman L, Vangronsveld J (2009) Exploiting plant-microbe partnerships to improve biomass production and remediation. Trends Biotechnol 27:591–598
Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511
Wu SC, Cheung KC, Luo YM, Wong MH (2006) Effects of inoculation of plant growth-promoting rhizobacteria on metal uptake by Brassica juncea. Environ Pollut 140(1):124–135
Xu S, Li J, Zhang X, Wei H, Cui L (2006) Effects of heat acclimation pretreatment on changes of membrane lipid peroxidation, antioxidant metabolites, and ultrastructure of chloroplasts in two cool-season turfgrass species under heat stress. Environ Exp Bot 56:274–285
Yadav J, Verma JP, Tiwari KN (2011) Plant growth promoting activities of fungi and their effect on chickpea plant growth. Asian J Biol Sci 4:291–299

Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN (1982) Living with water stress: evolution of osmolyte systems. Science 217:1214–1222

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Yang X, Wu X, Hao H, He Z (2008) Mechanisms and assessment of water eutrophication. J Zhejiang Univ Sci B 9(3):197–209

Yao L, Wu Z, Zheng Y, Kaleem I, Li C (2010) Growth promotion and protection against salt stress by *Pseudomonas putida* Rs–198 on cotton. Eur J Soil Biol 46:49–54

Yee DC, Maynard JA, Wood TK (1998) Rhizoremediation of trichloroethylene by a recombinant, root–colonizing Pseudomonas fluorescens strain expressing toluene ortho– monooxygenase constitutively. Appl Environ Microbiol 64:112–118

Yi Y, Saleeba JA, Guerinot ML (1994) Iron uptake in *Arabidopsis thaliana*. In: Manthey J, Luster Dand D, Crowley E (eds) Biochemistry of metal micronutrients in the rhizosphere. Lewis Publishers, Chelsea, pp 295–307

Yu X, Ai C, Xin L, Zhou G (2011) The siderophore-producing bacterium, *Bacillus subtilis* CAS15, has a biocontrol effect on *Fusarium* wilt and promotes the growth of pepper. Eur J Soil Biol 47(2):138–145

Yuwono T, Handayani D, Soedarsono J (2005) The role of osmotolerant rhizobacteria in rice growth under different drought conditions. Aust J Agric Res 56:715–721

Zahir ZA, Munir A, Asghar HN, Shahroona B, Arshad M (2008) Effectiveness of rhizobacteria containing ACC-deaminase for growth promotion of peas (Pisum sativum) under drought conditions. J Microbiol Biotechnol 18:958–963

Zahir AZ, Ghani U, Naveed M, Nadeem SM, Asghar HN (2007) Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) under salt-stressed conditions. Arch Microbiol 191(5):415–424

Zaidi A, Khan MS, Ahemad M, Oves M, Wani PA (2009) Recent advances in plant growth promotion by phosphate-solubilizing microbes. In: Khan MS et al (eds) Microbial strategies for crop improvement. Springer, Berlin, pp 23–50

Zaidi S, Usmani S, Singh BR, Musarrat J (2006) Significance of Bacillus subtilis strain SJ–101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in Brassica juncea. Chemosphere 64(6):991–997

Zhang W, Tian Z, Pan X, Zhao X, Wang F (2013) Oxidative stress and non-enzymatic antioxidants in leaves of three edible canna cultivars under drought stress. Hortic Environ Biotechnol 54:1–8

Zhuang X, Chen J, Shim H, Bai Z (2007) New advances in plant growth-promoting rhizobacteria for bioremediation. Environ Int 33:406–413

Phosphate Biofertilizers as Renewable and Safe Nutrient Suppliers for Cropping Systems: A Review

5

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Abstract

Sustainable agriculture highly depends on soil microorganisms to supply essential nutrients for plants and circulate the nutrient cycles in cropping systems. These microorganisms which are commercially formulated and briefly named "biofertilizers" can significantly reduce fossil fuel consumption, environmental degradation, and production cost related to agriculture. Phosphate biofertilizer is one of the most important groups of these beneficial microorganisms which plays a notable role in nutrient preparation for crops. Although these biofertilizers are usually known as phosphate suppliers for cropping systems, they can also provide other macro- and micronutrients to crops. Fungi and bacteria form two major groups of phosphate biofertilizers which can live freely or as symbiont organisms in agricultural soils. Mycorrhiza is a symbiont fungus which increases plant uptake of phosphate, nitrogen, and micronutrients and improves soil structure via formation of an extensive and dense mycelial network connected to plant roots. In contrast, phosphate solubilizing microorganisms are usually free living and able to solubilize insoluble phosphate compounds in soil mainly via releasing a wide range of organic acids and chelating metabolites. However, the effectiveness of these microorganisms is significantly influenced by edaphic factors and field management practices. For example, tillage as a usual practice in most of the cropping systems has negative effects on the absence and activity of mycorrhizal fungi. Application of chemical fertilizers which is another routine operation in modern agriculture also notably reduces the survival and effectiveness of phosphate biofertilizers. This review article presents the results on the main phosphate biofertilizers which can potentially be applied in sustainable agriculture, their action mechanisms, and important factors influencing their effectiveness.

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5.1 Introduction

Nowadays, agriculture relies on chemical fertilizers in order to satisfy the demand of crops with a high yield potential and produce economically viable yields. The synthesis of these fertilizers requires high amounts of fossil fuels as an energy source. Fossil fuels are nonrenewable resources, and their oxidized products such as CO_2 pose hazards to the environment and to human health. Moreover, fossil fuel reserves are finite and therefore unsustainable in long-term scale.

Phosphorus (P) is the second important element after nitrogen which is necessary to survival and growth of plants (Ogbo 2010). However, in the soil solution, it usually exists in very low quantities (a micromolar level) as compared with most of the other vital nutrient elements which are present in millimolar levels (Ozanne 1980). To ameliorate P deficiency, high amounts of chemical P fertilizers are used which can lead to the environmental degradation, pollution of natural resources, water eutrophication, and increased crop production cost. Moreover, a notable section of the P added into the soil as chemical fertilizers is rapidly converted to unavailable compounds such as calcium phosphate or other fixed forms. As reported by Gyaneshwar et al. (2002), about 75–90% of the chemical P fertilizers applied in agricultural soils become unavailable quickly due to P combination with other elements such as Fe, Al, Ca, and Mg depending on the soil pH level. Generally, in the alkaline soils, P is fixed by Ca or Mg, whereas in the acidic ones, it forms insoluble compounds via reaction with Fe or Al. Therefore, there are large reserves of P in most agricultural soils resulted from the massive use of the synthetic P fertilizers (Rodriguez and Fraga 1999); as in a global scale, these reserves can sustain crop yields in their maximum levels for about one century (Goldstein et al. 1993). On the other hand, major P chemical fertilizers are originated from rock phosphates as their mother materials which are known to be finite resources, and their reserves may be depleted during the next 100 years (Herring and Fantel 1993). Phosphate biofertilizers can play an important role in agroecosystems as renewable and ecofriendly nutrient suppliers for plants and are proposed as possible alternatives for conventional chemical P fertilizers. According to Raghuwanshi (2012), the use of these biofertilizers can be included as an efficient approach in Integrated Nutrient Management (INM) and Integrated Plant Nutrition System (IPNS). They can biologically transform soil P from unavailable to available forms.

These biofertilizers contain different types of microorganisms which increase the accessibility of plants to soil P reserves which are unavailable in normal conditions. This can be attributed to their ability to dissolve insoluble P compounds and extension of plant root system via establishment of a symbiotic relationship with the roots of different plant species. These microorganisms belong to different taxonomic groups especially fungi and bacteria.

5.2 Mycorrhiza

An important symbiotic relationship between soil fungi and vascular plant roots is called mycorrhizae which through it nutrients and energy are exchanged between two symbionts (Brundrett 2002). Roots of about 95% of plant species can be

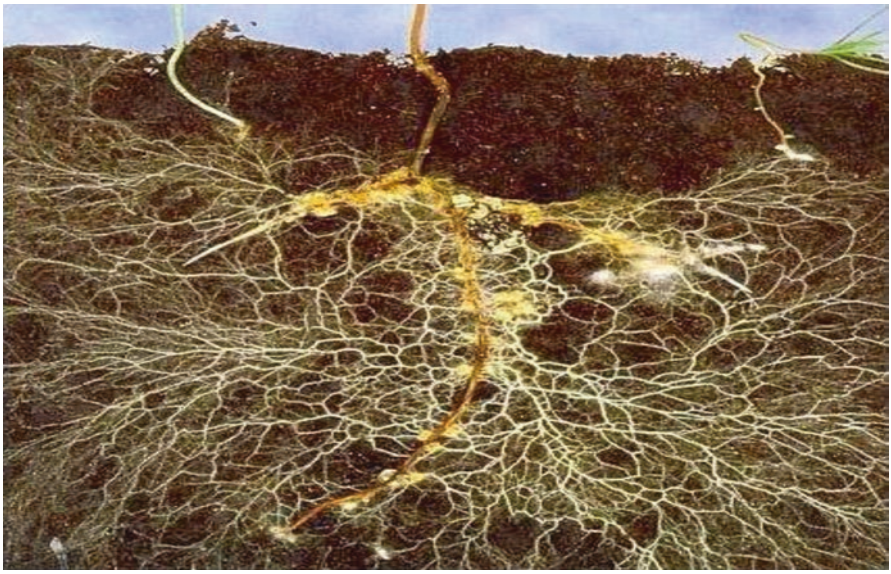

Fig. 5.1 AM fungi form an extensive and dense mycelia network in soil by which cover the depletion zone around plant roots (Source: http://www.sarcozona.org/tag/mycorrhizae)

colonized by soil fungi and establish mutualistic relationships named arbuscular mycorrhizae (AM) (Smith and Read 2008). Terrestrial plants and AM fungi (AMF) have been evolved side by side during their evolutionary history. A symbiotic relationship between AM fungi and land plants has been distinguished in the fossils belonging to Ordovician era, approximately 460 million years before this (Redecker et al. 2000).

Plant roots are colonized by AM, and the fungi transmit nutrient elements such as P into the host plant in exchange for the photoassimilate produced by plant. Arbuscules are highly branched intracellular fungal structures which are formed in the cortex of host plant roots, and at the same time fungi constitute their mycelial network in the soil (Fig. 5.1). P uptake by plants can be enhanced due to symbiotic relationship with AM (Bolan 1991). Moreover, these beneficial microorganisms can increase nitrogen (Barea et al. 1991) and micronutrient (Burkert and Robson 1994) availability to host plants and aggregate soil particles leading to an improved soil structure (Tisdall 1994). However, supply host plant with P which is an extremely nonmobile macronutrient in most soils can be defined as the main benefit caused by AMF (Bucher 2007).

5.2.1 Some Benefits of Mycorrhiza

As mentioned previously, increased phosphorus availability to plants is known as the main advantage resulted from the symbiosis with AMF. Because of low solubility and mobility, P is proposed as one of the most limiting essential soil elements

needed for plant survival and growth. It is estimated that crop inoculation with AMF can reduce the use of P chemical fertilizers by 80% in field conditions (Jakobsen 1995). In a study, plants inoculated with AM showed a sixfold increase in Pi and fourfold increase in the other nutrients as compared with uninoculated fertilized plants. Other workers showed that inoculation with AM fungi increased plant ability to utilize soluble P from rock phosphate (Antunes and Cardoso 1991; Guissou et al. 2001).

Moreover, mycorrhizal roots can acquire nitrogen organic compounds, such as amino acids and small peptides, and transport them to host plants (Bajwa and Read 1985; Bajwa et al. 1985). Ericoid, a group of mycorrhizal fungi, can degrade organic nitrogen and transmit it to mycorrhizal plants in the experiments conducted in controlled environments (Abuzinadah and Read 1986; Read 1991; Read et al. 1989). Michelsen et al. (1996) also suggested that ericoid mycorrhizae enabled the host plants to access soil organic N sources under natural conditions.

AM fungi can also effectively protect soil against erosion. This can be achieved by their extraradical hyphae which are able to connect soil particles (Miller and Jastrow 1992) leading to an improved soil aggregate stability and consequently a lower soil erodibility. AMF can also produce a sticky glycoprotein named glomalin which cements soil particles (Wright and Upadhyaya 1998; Wright et al. 1999; Rillig et al. 2002) and improves the stability of soil aggregates via binding soil particles (Peters 2002).

5.2.2 Mechanisms by Which Mycorrhiza Interacts with Plants and Improves P and N Availability for Them

Before the physical contact between plants and AMF (i.e., at the pre-symbiotic stage), it is known that some molecular signals are exchanged between them. Some studies have been shown that on the one hand AMF modulate root gene expression (Kosuta et al. 2003; Weidmann et al. 2004), intracellular signaling (Navazio et al. 2007; Kosuta et al. 2008), development (Oláh et al. 2005), and metabolism (Gutjahr et al. 2009) via diffusion of some produced compounds. On the other hand, plants release some special biochemicals via their roots which stimulate fungi to establish a symbiotic relationship (Gianinazzi-Pearson et al. 1989; Siqueira et al. 1991; Tsai and Phillips 1991; Giovannetti et al. 1996; Buee et al. 2000). Strigolactones (SLs) have been distinguished as the main secondary metabolites which are produced by host plants and are able to stimulate the symbiont fungi (Akiyama et al. 2005; Besserer et al. 2006). Some important morphological and developmental events in AM fungi including spore germination, hyphal branching, and increasing fungal respiration and mitochondrial activity are usually induced by SLs (Besserer et al. 2006, 2008).

The soil volume exploited by plants can be extended by several times when plant roots are in association with AMF mycelial network (Fig. 5.2). Therefore, P uptake can be achieved more efficiently by a mycorrhizal than a non-mycorrhizal plant root system (Smith and Read 2008). In other words, mycorrhizal plants can access

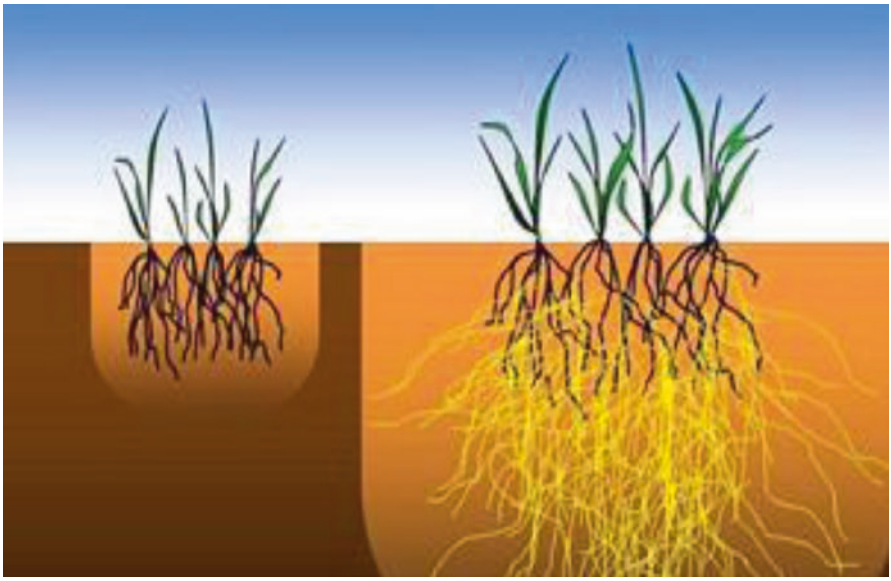

Fig. 5.2 The soil volume which can be explored by a plant can be increased by several times of magnitude via the network of fungal mycelium connected to AM roots (Source: http://www.dirt-goddessseeds.com/category_s/1901.htm)

nutrients such as phosphorus which exist outside the rhizosphere zone where they are not accessible for non-mycorrhizal plants. This is achieved through the fungal mycelial network connected to plant root system (Friese and Allen 1991). For example, one centimeter of colonized roots might produce 50–150 cm of extraradical hyphae (Harley 1989). Moreover, in comparison with plant roots, fungal hyphae are much thinner (Bago et al. 1998), which can enable them to penetrate in the soil microscopic pores which are unavailable to plant roots.

Another mechanism by which AMF increase P availability to plants is related to their ability to produce different organic acids (Lapeyrie 1988) which can transform soil mineral phosphates from insoluble to soluble forms. This inevitably leads to the higher plant access to acid-labile insoluble P compounds such as calcium phosphate. In addition, phosphatase produced by AMF can enable them to release P from organic phosphate forms (Koide and Shreinner 1992).

Although the plant growth-promoting effect of AMF is mainly attributed to their ability to dissolve insoluble P compounds and increase phosphate uptake by plants, there are some evidences on the effectiveness of these fungi to increase nitrogen accessibility to plants (Ames et al. 1984; Azcón-Aquilar et al. 1993). Matsumura et al. (2013) reported that under different amino acid treatments, the nitrogen content of mycorrhizal plants was notably higher than that for non-mycorrhizal plants. In another study, Hobbie and Hobbie (2006) observed that in arctic tundra, 61–86% of the nitrogen acquired by plants was resulted from an ectomycorrhizal symbiotic relationship. Govindarajulu et al. (2005) also found that AM fungi are able to obtain

soil inorganic nitrogen by their extraradical mycelium which then is converted to arginine and translocated to the intra-radical fungal mycelium located in the roots of host plant.

Some studies have demonstrated that the nitrogen present in the soil organic compounds can be accessible to AMF (Hodge et al. 2001; Whiteside et al. 2009; Hodge and Fitter 2010). Hodge and Fitter (2010) showed that decomposing soil organic materials are responsible for 31% of the nitrogen acquired by AMF hyphae system. This can be explained by the AMF ability to produce a diverse range of hydrolytic enzymes including cellulase, pectinase, and xyloglucanase in their external mycelial network (Garcia-Romera et al. 1991; Garcia-Garrido et al. 1992). It clearly is known that these enzymes are responsible to decompose the soil organic matters.

Chitinases are another group of metabolites produced by AMF species which are proposed as one of the factors involved in plant root protection against soil pathogens (Azcón-Aguilar and Barea 1997; Gianinazzi-Pearson 1996). Whiteside et al. (2012) showed that recalcitrant (i.e., a molecule with relatively large and complex structure) organic N compound such as chitosan can be absorbed by AMF in situ.

5.3 Phosphate Solubilizing Microorganisms (PSMs)

P is proposed as one of the most important elements participant in growth, development, and biological processes of different organisms. It is also known as an essential limiting factor for plants due to its insufficient solubility and mobility in soils (Vessey 2003) especially in extraordinary pH conditions. However, insoluble P compounds such as calcium phosphate and apatite can be solubilized by phosphate solubilizing microorganisms (PSMs) mainly bacteria and fungi which are in association with plant roots. *Bacillus* and *Pseudomonas* are known as the most important genera of mineral phosphate solubilizing bacteria (PSB) (Illmer and Schinner 1992), while main genera of fungi involved in P solubilization process are *Aspergillus* and *Penicillium* (Motsara et al. 1995). In soil, bacterial and fungal PSMs form 1–50 and 0.1–0.5% of the total soil phosphate solubilizing microorganisms, respectively. It means that the number of PSB is higher by 2–150 times than that for fungal solubilizing agents (Kucey 1983). Generally, production of organic acids and chelating factors by PSMs can explain their ability to solubilize insoluble phosphate compounds (Deinum et al. 1996; Dong and Pierdominici 1995).

However, there are some evidences which indicate inorganic acids can also be produced by PSMs. For example, the bacteria belonging to the genus *Acidithiobacillus* produce sulfuric acid via reaction with elemental sulfur (Garcia Junior 1992). This biologically produced acid plays an effective role in natural P solubilizing process via reducing soil pH which consequently leads to the improved plant growth (Stamford et al. 2002). However, it has been shown in both liquid and solid media that fungi have a higher ability to produce organic acids and therefore are more efficient to solubilize insoluble P compounds when compared with PSB (Venkateswarlu et al. 1984).

In a soil with P limited resources, PSMs can notably increase plant accessibility to this important element. According to Mohammadi et al. (2015), in a weedy condition along with a reduced sowing uniformity (i.e., when high intra- and interspecific competitions were intensified), phosphate biofertilizers containing fungi and bacteria could significantly improve soybean yield indicating the essential role of these microorganisms to support plants in a P limited condition.

5.4 Mechanisms by Which PSMs Improve P Availability for Plants

The improvement of P availability by PSMs can be achieved through different mechanisms (Fig. 5.3). However, it seems that the production of different organic acids by PSMs is the main reason explaining their solubilizing activity (Alam et al. 2002). Diverse organic acids such as gluconic, ketogluconic, oxalic, citric, succinic, fumaric, tartaric, α-ketobutyric, lactic, itaconic, isovaleric, isobutyric, acetic, malic, glyoxylic, and malonic can be produced by PSMs. The results of some studies show that the most efficient organic acid involved in P solubilization process is gluconic acid which is produced by Gram-negative bacteria (Goldstein et al. 1993; Kim et al. 1998). Khan et al. (2009) also suggested that gluconic and ketogluconic are the main low molecular weight organic acids produced by PSMs which are able to solubilize insoluble phosphate compounds in soil. The glucose oxidative metabolism by glucose dehydrogenase in the presence of a cofactor named pyrroloquinoline quinone (PQQ) is the mechanism by which gluconic acid is produced by PSMs (Fig. 5.4).

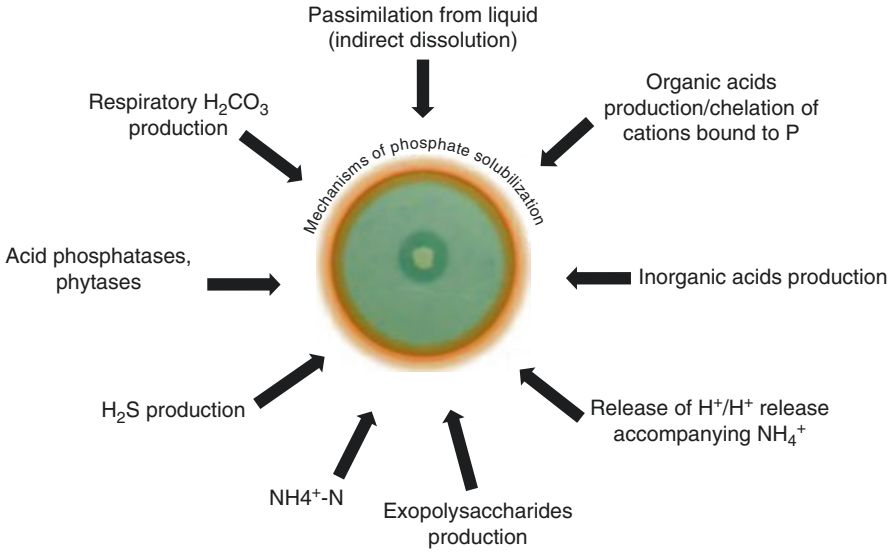

Fig. 5.3 Different mechanisms by which PSMs improve P availability for plants (Adapted from Zaidi et al. 2009)

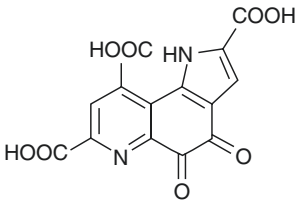

Fig. 5.4 Molecular structure of pyrroloquinoline quinone (PQQ) which acts as a cofactor in gluconic acid production process (Adapted from Matsumura et al. 2014)

In general, the reduced soil pH caused by organic acids produced by PSMs can explain their ability to dissolve insoluble P compounds (Nahas 1996). However, it appears that increasing P solubilization rate cannot be achieved by acidifying reaction alone (Subha Rao 1982). According to Kucey (1988), another major factor influencing solubilization process is the capacity of organic acids to chelate insoluble P compounds; as in a study when 0.05 M EDTA was added to the medium, solubilization rate was the same as inoculation with *Penicillium bilaii*.

PSMs can also produce inorganic acids, synthesize exopolysaccharides, and release H^+ as other important mechanisms contributing to inorganic P solubilization process (Gamalero and Glick 2011). Moreover, phosphatase produced by PSMs can play a key role in solubilization of organic P compounds (Park et al. 2011).

5.5 Factors Influencing the Efficiency of P-Related Microorganisms

The effectiveness of PSMs as biofertilizers can be influenced by diverse factors. Ho and Ko (1985) showed that after artificial introduction of PSMs into the soil, the size or density of their populations was decreased quickly. The success level of PSMs after introducing them into the soils highly depends upon their ability to compete with other soil microorganisms and the presence of a notable PSM saprophytic capacity. According to Kucey et al. (1989), the effectiveness of the inoculated PSMs to improve plant growth and yield can be varied in relation to several factors including:

1. If inoculated PSM can survive and colonize in the plant rhizosphere.
2. Its competitive ability with native microorganisms.
3. Essence and characteristics of the inoculated soils and plant varieties.
4. Inadequate rhizospheric nutritional level which can lead to the sufficient organic acid production by PSMs to dissolve insoluble P compounds.
5. PSM infirmity to dissolve soil P.

It is concluded that extensive studies should be carried out to distinguish the PSM strains with high durability and competitive ability under the environments with high complexity such as a plant rhizosphere in order to access to highly efficient P biofertilizers.

5.5.1 Soil Factors

Edaphic factors including soil composition (Bashan et al. 1995), physiological condition, temperature, pH, water content (Van Elsas et al. 1991), and the existence of recombinant plasmids (Van Veen et al. 1997) can significantly affect the survival of the inoculated PSMs. While competition, predation, and the growth of plant roots which supplies the substrates needed to PSMs form the main biotic factors influencing PSM survival as inoculants. Since the survival of AMF as obligatory endosymbionts only depends on the carbohydrates produced by the root cells of host plants, all edaphic agents determining the metabolism and growth of host will certainly affect AMF efficiency.

The soils with high buffering capacity can notably reduce PSM efficiency to solubilize insoluble P compounds, especially when PSM strains are not able to release acceptable levels of organic acids. Khan et al. (2007) also found that the presence of diverse environmental conditions is an important reason which can explain the variation in PSM efficiency. The low effectiveness of PSMs can be related to an unsuitable soil environment as may be observed in high alkaline soils. As in the soils with high alkalinity level that are commonly found in arid and semi-arid climatic conditions (e.g., many areas of Iran) and usually have high temperatures and salinity levels, PSMs may colonize plant roots poorly resulting in a low P solubilizing activity. Therefore, it seems that searching for PSM strains with high efficiency in unfavorable environmental conditions is necessary.

5.5.2 Agronomic Practices

Sole cropping, conventional tillage, and fertilizer application are some of the common techniques to produce yield in most modern agricultural systems which can negatively affect AMF abundant and diversity in soils (Helgason et al. 1998; Oehl et al. 2005).

5.5.2.1 Tillage Practices

Tillage operations have been shown to reduce the number of AMF spores present in the soil (Kabir et al. 1998) and AM fungi colonization in some agricultural crops (Jasper et al. 1989; Miller et al. 1995; McGonigle and Miller 1996). Annual soil disturbances produced by conventional tillage systems showed reducing effects on AMF colonization when compared with reduced tillage practices (Miller and Jastrow 1992; Miller et al. 1995; Al-Karaki 1998; Miller 2000).

In general, conservation tillage practices have positive effects on AM fungi parameters and other soil factors. Positive consequences caused by no tillage consisted of higher soil carbon, nitrogen, sulfur, and phosphorus quantities and a greater AM fungal propagules remaining in the soil as compared with conventional tillage, as well as a simultaneously increased phosphorus accessibility for subsequent crops.

In a study, the amount of total glomalin produced by AMF enhanced in the soils under reduced tillage and no tillage than conventional tillage and soil carbon content was known as an important factor determining this enhancement (Borie et al. 2006).

5.5.2.2 Fertilization

AMF diversity and abundance have increasingly been declined in response to mineral nutrient application in agroecosystems (Lin et al. 2012; Liu et al. 2012). Among the mineral nutrients, Pi and nitrate have solely shown adverse effects on AMF, while these beneficial fungi were not negatively affected even by high levels of other essential elements including potassium, calcium, magnesium, sulfate, and iron.

Although the adverse effect of Pi on AMF has been recognized for a long time (Abbott et al. 1984; Thomson et al. 1986; Amijee et al. 1989; Breuillin et al. 2010; Balzergue et al. 2011), the increased AMF-plant symbiotic relationship caused by N deficiency can significantly overcome the reducing influence resulted from high P levels on AMF. This indicates that symbiosis can be enhanced by plants as long as there are limiting levels of one of these two important elements in rhizosphere.

Phosphorus

Crop production through the extensive use of chemical P fertilizers can notably decline AMF existence and abundance in soils (Johnson 1993). In a P-enriched environment, plant roots are not usually colonized severely by AMF (Amijee et al. 1989) as it has been indicated that when adequate accessible P is present in the soil, the growth of certain plant species may be reduced due to AMF colonization (Son and Smith 1995).

Pi can systemically suppress AM development which is in relation to the nutritional condition of host plant shoot. Inasmuch as a notable section of the photosynthate produced by host plant is usually used by AMF (Smith et al. 2009; Douds et al. 2000), the inhibiting effect of the elevated Pi levels on AMF development may be attributed to an energy-saving negative feedback mechanism in the environments in which the P needed for plant can adequately be provided in the absence of a symbiotic relationship with fungi. In other words, at a high level of phosphorus, plant preferentially adopts a nonexpensive and direct approach to acquire P (Nagy et al. 2008), and therefore, the plant root colonization by AMF can significantly be declined.

Moreover, long-term previous P applications can also affect AM fungi colonization of subsequent crops (Kahiluoto et al. 2000; Dekkers and van der Werff 2001). Dekkers and van der Werff (2001) reported that after 10 years without P fertilization, AM fungi colonization of winter wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) was greater when previous long-term annual P fertilization ranged from 0 to 17.5 kg ha^{-1} compared to when the rate of P application was 52.5 kg ha^{-1}.

The main metabolites including amino acids and carbohydrates which are secreted by host plant roots and are usable for AMF can be reduced in a P-enriched

soil (Graham et al. 1981; Thomson et al. 1986). The genes involved in carotenoid biosynthesis and those responsible for symbiotic relationship, e.g., PT4, were suppressed in the presence of Pi (Breuillin et al. 2010). In contrast, the roots exposed to a P-deficient condition can exude some essential flavonoid signals which induce the growth and activity of AMF at the pre-symbiotic phase (Nair et al. 1991).

Based on some conducted studies, low quantities of strigolactones (key factor to trigger plant-AMF symbiosis) can usually be produced and exuded by different plant species when the soil phosphorus is high (Yoneyama et al. 2007a, b; López-Ráez et al. 2008), and strigolactones may not be present in the plant root exudates exposed to high P levels, and consequently these plants don't show stimulating effects on AMF.

Therefore, it can be assumed that the suppressive effect of P-enriched soils on AMF symbiosis is related to the decreased plant ability to produce sufficient levels of strigolactones in these conditions (Bouwmeester et al. 2007; Yoneyama et al. 2007b). Balzergue et al. (2011) reported that the exudates extracted from the plant roots developed in a P-enriched soil were not able to induce branching of fungal hyphae.

Other researchers also demonstrated that there is a negative correlation between the levels of strigolactones produced by host plant and soil available phosphorus (Yoneyama et al. 2007a, b; López-Ráez et al. 2008) and these metabolites could not be detected in the root exudates obtained from the plants developed in P-enriched environments. However, the number of the roots colonized by AMF and plant ability to produce strigolactones is mainly determined by shoot Pi content compared to the externally soil available phosphorus or the Pi levels which locally exist in plant roots (Balzergue et al. 2011).

Nitrogen

Previous studies in controlled environments and the field have found that low N levels (20 mM N) increased mycorrhizal infection (Goulart et al. 1995, 1996; Stribley and Read 1976). Whiteside et al. (2012) suggested that increasing nitrogen accessibility can decrease plant tendency to establish a symbiotic relationship with AMF, because the cost-effectiveness of fungal association is significantly reduced under this condition. Consequently, in the soils with high N levels, a decreased AMF frequency can be expected as is usually happened in different ecosystems (Treseder 2004). Cappellazzo et al. (2008) also reported that the ability of AM fungus *G. mosseae* to transport amino acids was notably declined in the presence of high inorganic nitrogen levels. The suppressing effects of N-enriched environments on AM colonization and activity have been demonstrated in several works. For example, Whiteside et al. (2012) observed a lower AMF ability to organically derived nitrogen uptake when accessible nitrogen was increased. In their study, the use of nitrogen fertilizer notably reduced the rate of specific uptake (i.e., per unit biovolume) of labile organic N by AMF.

However, if soil available N is so low that it reduces plant growth, establishment of the mycorrhizal association may be affected. In a study, the limited N supply to the host plants could have resulted in a reduced C supply to support mycorrhizal association, thus leading to a reduced mycorrhizal infection level (Yang et al. 2002).

Other studies showed that serious nitrogen deficiency in plants may contribute to low root carbohydrate content which lowers infection levels in vesicular-arbuscular mycorrhizal associations (Hepper 1983; Same et al. 1983). It can be concluded that the presence of a critical N level to achieve an efficient plant-AM association in soil is necessary.

5.5.2.3 Rotation

Since the development of AM fungi is biotrophic (Morton 1990), the absence of mycorrhizae hosts could cause a decrease in soil residual AM propagules and their vitality for crops seeded afterward in a rotation.

Including non-mycorrhizal crops in rotation might affect the concentration and vitality of indigenous AM species in soil, thereby affecting the growth of AM-dependent crops following in the rotation (Dalpè and Monreal 2004). Gavito and Miller (1998) reported that intra-radical AM colonization of corn (*Zea mays* L.) was delayed in field plots when canola rather than corn was the previous crop.

In general, the crops belonging to *Chenopodiaceae*, *Brassicaceae*, and *Caryophyllaceae* (Barker et al. 1998) families don't form symbiotic associations with AM fungi, and thus including them in rotations can significantly reduce the absence and activity of AM fungi in agroecosystem soils. Moreover, since AM fungi are obligate symbionts and their survival is fully dependent to live hosts, including black fallow in a rotation has negative effects on these beneficial microorganisms.

Conclusion

In general, phosphate biofertilizers can be proposed as suitable alternatives to synthetic chemical fertilizers which are extensively applied in modern agricultural ecosystems. Maintaining and invigorating these beneficial microorganisms via adoption of appropriate agronomic practices and introducing them into the agricultural soils intentionally can notably reduce fossil fuel consumption and environmental hazards caused by chemical inputs used in cropping systems while reclaiming the soil ecosystem. These microorganisms which mainly belong to fungi and bacteria groups can increase crop accessibility to nutrient reserves in soil via different mechanisms such as formation of a dense and extensive mycelial network connected to crop roots and production of a wide range of organic acids and chelating metabolites. However, some conventional operations which are extensively used in crop production systems today have shown negative effects on these beneficial microorganisms which consequently have been led to the increased dependency of these systems to external inputs.

It is concluded that in order to attain the self-sufficient and sustainable agricultural systems, the essential role of phosphate-related microorganisms as efficient nutrient suppliers for crops should seriously be considered. Moreover, the reasonable crop production practices including the use of conservation tillage (no or reduced tillage), organic manures instead of synthetic chemical fertilizers, and suitable and black fallow-free crop rotations as well as the artificial introduction of these microorganisms as biofertilizers into the agricultural soils should be included in cropping system management programs.

References

Abbott LK, Robson AD, De Boer G (1984) The effect of phosphorus on the formation of hyphae in soil by the vesicular arbuscular mycorrhizal fungus, *Glomus fasciculatum*. New Phytol 97:437–446

Abuzinadah RA, Read DJ (1986) The role of proteins in the nitrogen nutrition of ectomycorrhizal fungi. New Phytol 103:481–493

Akiyama K, Matsuzaki K, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827

Alam S, Khalil S, Najma A, Rashid M (2002) In vitro solubilization of inorganic phosphate by phosphate solubilizing microorganisms (PSM) from maize rhizosphere. Int J Agric Biol 4:454–458

Al-Karaki GN (1998) Benefit, cost and water-use efficiency of arbuscular mycorrhizal durum wheat grown under drought stress. Mycorrhiza 8:41–45

Ames RN, St. Porter LK, John TV, Reid CPP (1984) Nitrogen sources and 'A' values for vesicular- arbuscular and non-mycorrhizal sorghum grown at three rates of 15^{N}-ammonium sulphate. New Phytol 97:269–276

Amijee F, Tinker PB, Stribley DP (1989) Effects of phosphorus on the morphology of VA mycorrhizal root-system of leek (*Allium porrum* L). Plant Soil 119:334–336

Antunes V, Cardoso EJBE (1991) Growth and nutrient status of citrus plants as influenced by mycorrhiza and phosphorus application. Plant Soil 131:11–19

Azcón-Aguilar C, Barea JM (1997) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. Mycorrhiza 6:457–464

Azcón-Aquilar C, Alba C, Montilla M, Barea JM (1993) Isotopic (15^{N}) Evidence of the use of less available N forms by VA mycorrhizas. Symbiosis 15:39–48

Bago B, Azcon-Aguilar C, Goulet A, Piche Y (1998) Branched absorbing structures (BAS): a feature of the extraradical mycelium of symbiotic arbuscular mycorrhizal fungi. New Phytol 139:375–388

Bajwa R, Read DJ (1985) The biology of mycorrhiza in the ericaceae. IX. Peptides as nitrogen sources for the ericoid endophyte and for mycorrhizal and non-mycorrhizal plants. New Phytol 101:459–467

Bajwa R, Abuarghub S, Read DJ (1985) The biology of mycorrhiza in the ericaceae X. The utilization of proteins and the production of proteolytic enzymes by the mycorrhizal endophyte and by mycorrhizal plants. New Phytol 101:469–486

Balzergue C, Puech-Pagès V, Bécard G, Rochange SF (2011) The regulation of arbuscular mycorrhizal symbiosis by phosphate in pea involves early and systemic signalling events. J Exp Bot 62:1049–1060

Barea JM, El-Atrach F, Azcon R (1991) The role of VA mycorrhizas in improving plant N acquisition from soil as assessed with 15^{N}. The use of stable isotopes in plant nutrition. In: Fitton C (ed) Soil fertility and environmental studies. Joint AIEA, FAO, Division, Vienna, pp 677–808

Barker SJ, Tagu D, Dalpè G (1998) Regulation of root and fungal morphogenesis in mycorrhizal symbiosis. Plant Physiol 116:1201–1207

Bashan Y, Puente ME, Rodriquea MN, Toledo G, Holguin G, Ferrera-Cerrato R, Pedrin S (1995) Survival of *Azorhizobium brasilense* in the bulk soil and rhizosphere of 23 soil types. Appl Environ Microbiol 61:1938–1945

Besserer A, Puech-Pages V, Kiefer P, Gomez-Roldan V, Jauneau A, Roy S, Portais JC, Roux C, Bécard G, Séjalon-Delmas N (2006) Strigolactones stimulate arbuscular mycorrhizal fungi by activating mitochondria. PLoS Biol 4:e226

Besserer A, Bécard G, Jauneau A, Roux C, Séjalon-Delmas N (2008) GR24, a synthetic analog of strigolactones, stimulates the mitosis and growth of the arbuscular mycorrhizal fungus *Gigaspora rosea* by boosting its energy metabolism. Plant Physiol 148:402–413

Bolan NS (1991) A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plant. Plant Soil 134:189–207

Borie F, Rubio R, Rouanet JL, Morales A, Borie G, Rojas C (2006) Effects of tillage systems on soil characteristics, glomalin and mycorrhizal propagules in a Chilean Ultisol. Soil Tillage Res 88:253–261

Bouwmeester HJ, Roux C, Lopez-Raez JA, Bécard G (2007) Rhizosphere communication of plants, parasitic plants and AM fungi. Trends Plant Sci 12:224–230

Breuillin F, Schramm J, Hajirezaei M, Ahkami A, Favre P et al (2010) Phosphate systemically inhibits development of arbuscular mycorrhiza in *Petunia hybrida* and represses genes involved in mycorrhizal functioning. Plant J 64:1002–1017

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154:275–304

Bucher M (2007) Functional biology of plant phosphate uptake at root and mycorrhizal interfaces. New Phytol 173:11–26

Buee M, Rossignol M, Jauneau A, Ranjeva R, Bécard G (2000) The pre-symbiotic growth of arbuscular mycorrhizal fungi is induced by a branching factor partially purified from root exudates. Mol Plant Microbe Interact 13:693–698

Burkert B, Robson A (1994) Zn uptake in subterranean clover (*Trifolium subterraneum* L.) by three vesicular-arbuscular mycorrhizal fungi in a root free sandy soil. Soil Biol Biochem 26:1117–1124

Cappellazzo G, Lanfranco L, Fitz M, Wipf D, Bonfante P (2008) Characterization of an amino acid permease from the endomycorrhizal fungus *Glomus mosseae*. Plant Physiol 147:429–437

Dalpè Y, Monreal M (2004) Arbuscular mycorrhizae inoculum to support sustainable cropping systems. Crop Manag 3. doi:10.1094/CM-2004-0301-09-RV

Deinum B, Sulastri RD, Zeinab MHJ, Maassen A (1996) Effects of light intensity on growth, anatomy and forage quality of two tropical grasses (*Brachiaria brizantha* and P*anicum maximum* var. trichoglume). Neth J Agric Sci 44:111–124

Dekkers TBM, van der Werff PA (2001) Mutualistic functioning of indigenous arbuscular mycorrhizae in spring barley and winter wheat after cessation of long-term phosphate fertilization. Mycorrhiza 10:195–201

Dong M, Pierdominici MG (1995) Morphology and growth of stolons and rhizomes in three clonal grasses, as affected by different light supply. Vegetatio 116:25–32

Douds DD, Pfeffer PE, Shachar-Hill Y (2000) Carbon partitioning, cost, and metabolism of arbuscular mycorrhizas. In: Kapulnik Y, Douds DD (eds) Arbuscular mycorrhizas: physiology and function. Kluwer Academic Publishers, Dordrecht

Friese CF, Allen MF (1991) The spread of VA mycorrhizal fungal hyphae in the soil–inoculum types and external hyphal architecture. Mycologia 83:409–418

Gamalero E, Glick BR (2011) Mechanisms used by plant growth-promoting bacteria. In: Maheshwari DK (ed) Bacteria in agrobiology: plant nutrient management. Springer, Berlin, pp 17–46

Garcia Junior O (1992) O enxofre e suas transformações microbianas. In: Cardoso E, Saito MT, Neves MCP (eds) Microbiologia do solo Campinas: SBCS, pp 243–255

Garcia-Garrido JM, Garcia-Romera I, Ocampo JA (1992) Cellulase production by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe. New Phytol 121:221–226

Garcia-Romera I, Garcia-Garrido JM, Ocampo JA (1991) Pectolytic enzymes in the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. FEMS Microbiol Lett 78:343–346

Gavito ME, Miller MH (1998) Changes in mycorrhiza development in maize induced by crop management practices. Plant Soil 198:185–192

Gianinazzi-Pearson V (1996) Plant cell responses to arbuscular mycorrhizal fungi: getting to the roots of the symbiosis. Plant Cell 8:1871–1883

Gianinazzi-Pearson V, Branzanti B, Gianinazzi S (1989) In vitro enhancement of spore germination and early hyphal growth of a vesicular-arbuscular mycorrhizal fungus by root exudates and plant flavonoids. Symbiosis 7:243–255

Giovannetti M, Sbrana C, Citernesi AS, Avio L (1996) Analysis of factors involved in fungal recognition responses to host-derived signals by arbuscular mycorrhizal fungi. New Phytol 133:65–71

Goldstein AH, Rogers RD, Mead G (1993) Mining by microbe. Bio Technol 11:1250–1254
Goulart BL, Demchak K, Yang WQ (1995) Organic matter and nitrogen level effects on mycorrhizal infection in 'Bluecrop' highbush blueberry plants. J Small Fruit Viticult 3:151–164
Goulart BL, Demchak K, Yang WQ (1996) Effect of cultural practices on field grown 'Bluecrop' highbush blueberries, with emphasis on mycorrhizal infection levels. Acta Hortic 46:271–278
Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Doud DD, Allen JW, Bücking H, Lammers PJ, Shachar-Hill Y (2005) Nitrogen transfer in the arbuscular mycorrhizal Symbiosis. Nature 435:819–823
Graham JH, Leonard RT, Menge JA (1981) Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. Plant Physiol 68:548–552
Guissou T, Bâ AM, Guinko S, Plenchette C, Duponnois R (2001) Mobilisation des phosphates naturels de kodijari par des jujubiers (*Ziziphus mauritiana* Lam.) mycorhizes dans un sol acidifié avec de la tourbe. Fruits 56:261–269
Gutjahr C, Novero M, Guether M, Montanari O, Udvardi M, Bonfante P (2009) Presymbiotic factors released by the arbuscular mycorrhizal fungus Gigaspora margarita induce starch accumulation in Lotus japonicus roots. New Phytol 183:53–61
Gyaneshwar P, Naresh Kumar G, Parekh LJ, Poole PS (2002) Role of soil microorganisms in improving P nutrition of plants. Plant Soil 245:83–93
Harley JL (1989) The significance of mycorrhiza. Mycol Res 92:129–139
Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998) Ploughing up the wood-wide web. Nature 394:431
Hepper CM (1983) The effect of nitrate and phosphate on the vesicular–arbuscular mycorrhizal infection of lettuce. New Phytol 93:389–399
Herring JR, Fantel RJ (1993) Phosphate rock demand into the next century: impact on world food supply. Nat Resour Search 2:226–246
Ho WC, Ko WH (1985) Effect of environmental edaphic factors. Soil Biol Biochem 17:167–170
Hobbie JE, Hobbie EA (2006) N-15 in symbiotic fungi and plants estimates nitrogen and carbon flux rates in Arctic tundra. Ecology 87:816–822
Hodge A, Fitter AH (2010) Substantial nitrogen acquisition by arbuscular mycorrhizal fungi from organic material has implications for N cycling. Proc Natl Acad Sci U S A 107:13754–13759
Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413:297–299
Illmer P, Schinner F (1992) Solubilization of inorganic phosphates by microorganisms isolated from forest soil. Soil Biol Biochem 24:389–395
Jakobsen I (1995) Transport of phosphorus and carbon in VA mycorrhizas. In: Varma A, Hock B (eds) Mycorrhiza. Springer, Berlin, pp 297–324
Jasper DA, Abbott LK, Robson AD (1989) Soil disturbance reduces the infectivity of external hyphae of vesicular-arbuscular mycorrhizal fungi. New Phytol 112:93–99
Johnson NC (1993) Can fertilization of soil select less mutualistic mycorrhizae? Ecol Appl 3:749–757
Kabir Z, O'Halloran IP, Widden P, Hamel C (1998) Vertical distribution of arbuscular mycorrhizal fungi under corn (*Zea mays* L.) in no-till and conventional tillage systems. Mycorrhiza 8:53–55
Kahiluoto H, Ketoja E, Vestberg M (2000) Promotion of utilization of arbuscular mycorrhiza through reduced P fertilization 1. Bioassays in a growth chamber. Plant Soil 227:191–206
Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture-a review. Agron Sustain Dev 27:29–43
Khan AA, Jilani G, Akhtar MS (2009) Phosphorus solubilising bacteria: occurance, mechanisms and their role in crop production. J Agric Biol Sci 1:48–58
Kim KY, Jordan D, McDonald GA (1998) *Enterobacter agglomerans*, phosphate solubilizing bacteria and microbial activity in soil: effect of carbon sources. Soil Biol Biochem 30:995–1003
Koide TR, Shreinner PR (1992) Regulation of vesicular arbuscular mycorrhizal symbiosis. Annu Rev Plant Physiol Plant Mol Biol 43:557–581

Kosuta S, Chabaud M, Lougnon G, Gough C, Dénarié J, Barker DG, Bécard G (2003) A diffusible factor from arbuscular mycorrhizal fungi induces symbiosis-specific MtENOD11 expression in roots of *Medicago truncatula*. Plant Physiol 131:952–962

Kosuta S, Hazledine S, Sun J, Miwa H, Morris RJ, Downie JA, Oldroyd GE (2008) Differential and chaotic calcium signatures in the symbiosis signaling pathway of legumes. Proc Natl Acad Sci U S A 105:9823–9828

Kucey RMN (1983) Phosphate solubilizing bacteria and fungi in various cultivated and virgin Alberta soils. Can J Soil Sci 63:671–678

Kucey RMN (1988) Effect of *Penicillium bilaji* on the solubility and uptake of P and micronutrients from soil by wheat. Can J Soil Sci 68:261–270

Kucey RMN, Janzen HH, Legget ME (1989) Microbial mediated increases in plant available phosphorus. Adv Agron 42:199–228

Lapeyrie F (1988) Oxalate synthesis from soil bicarbonate by the mycorrhizal fungus *Paxillus involutus*. Plant Soil 110:3–8

Lin XG, Feng YZ, Zhang HY, Chen RR, Wang JH et al (2012) Long-term balanced fertilization decreases arbuscular mycorrhizal fungal diversity in an arable soil in north China revealed by 454 pyrosequencing. Environ Sci Technol 46:5764–5771

Liu YJ, Shi GX, Mao L, Cheng G, Jiang SJ et al (2012) Direct and indirect influences of 8 yr of nitrogen and phosphorus fertilization on *Glomeromycota* in an alpine meadow ecosystem. New Phytol 194:523–535

López-Ráez JA, Charnikhova T, Gómez-Roldán V et al (2008) Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. New Phytol 178:863–874

Matsumura A, Taniguchi S, Yamawaki K, Hattori R, Tarui A, Yano K, Daimon H (2013) Nitrogen uptake from amino acids in maize through arbuscular mycorrhizal symbiosis. Am J Plant Sci 4:2290–2294

Matsumura H, Umezawa K, Takeda K, Sugimoto N, Ishida T, Samejima M, Ohno H, Yoshida M, Higarashi K, Nakamura N (2014) Discovery of a eukaryotic pyrroloquinoline quinone dependent oxidoreductase belonging to new auxiliary activity family in the database of carbohydrate-active enzymes. PLoS One 9:e104851

McGonigle TP, Miller MH (1996) Mycorrhizae, P absorption and yield of maize in response to tillage. Soil Sci Soc Am J 60:1856–1861

Michelsen A, Schmidt IK, Sleep D (1996) Leaf 15^N abundance of subarctic plants provides field evidence that ericoid, ectomycorrhizal and non- and arbuscular mycorrhizal species access different sources of soil nitrogen. Oecologia 105:53–63

Miller MH (2000) Arbuscular mycorrhizae and the phosphorus nutrition of maize: a review of Guelph studies. Can J Plant Sci 80:47–52

Miller RM, Jastrow JD (1992) The role of mycorrhizal fungi in soil conservation. In: Behtlenfalvay GJ, Linderman RG (eds) Mycorrhizae in sustainable agriculture. ASA Special Publication 54. ASA, Madison, pp 29–44

Miller MH, McGonigle TP, Addy HD (1995) Functional ecology of vesicular-arbuscular mycorrhizas as influence by phosphate fertilization and tillage in an agricultural ecosystem. Crit Rev Biotechnol 15:241–255

Mohammadi GR, Chatrnour S, Jalali-honarmand S, Kahrizi D (2015) The effects of planting arrangement and phosphate biofertilizer on soybean under different weed interference periods. Acta Agric Slov 105:313–322

Morton JB (1990) Species and clones of arbuscular mycorrhizal fungi (*Glomales*, *Zygomycetes*): their role in macro and micro evolutionary processes. Mycotaxon 37:493–515

Motsara MR, Bhattacharyya PB, Srivastava B (1995) Biofertilizers their description and characteristics. In: Biofertilizer technology, marketing and usage, a sourcebook- cum-glossary, fertilizer development and consultation organisation 204–204, A Bhanot Corner, 1–2 Pamposh Enclave, New Delhi, 110048, pp 9–18

Nagy R, Drissner D, Amrhein N, Jakobsen I, Bucher M (2008) Mycorrhizal phosphate uptake pathway in tomato is phosphorus-repressible and transcriptionally regulated. New Phytol 181:950–959

Nahas E (1996) Factors determining rock phosphate solubilization by microorganism isolated from soil. World J Microb Biot 12:18–23

Nair MG, Safir GR, Siqueira JO (1991) Isolation and identification of vesicular-arbuscular mycorrhiza-stimulatory compounds from clover (*Trifolium repens*) roots. Appl Environ Microbiol 57:434–439

Navazio L, Moscatiello R, Genre A, Novero M, Baldan B, Bonfante P, Mariani P (2007) A diffusible signal from arbuscular mycorrhizal fungi elicits a transient cytosolic calcium elevation in host plant cells. Plant Physiol 144:673–681

Oehl F, Sieverding E, Ineichen K, Ris EA, Boller T, Wiemken A (2005) Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. New Phytol 165:273–283

Ogbo FC (2010) Conversion of cassava wastes for biofertilizer production using phosphate solubilizing fungi. Bioresour Technol 101:4120–4124

Oláh B, Brière C, Bécard G, Dénarié J, Gough C (2005) Nod factors and a diffusible factor from arbuscular mycorrhizal fungi stimulate lateral root formation in *Medicago truncatula* via the DMI1/DMI2 signalling pathway. Plant J 44:195–207

Ozanne PG (1980) Phosphate nutrition of plants – general treatise. The role of phosphorus in agriculture. In: Khasawneh FE, Sample EC, Kamprath EJ (eds) American Soc Agron Crop Sci Soc America, Soil Sci Soc America, Madison, pp 559–589

Park JH, Bolan N, Megharaj M, Naidu R (2011) Isolation of phosphate solubilizing bacteria and their potential for lead immobilization in soil. J Hazard Mater 185:829–836

Peters S (2002) Mycorrhiza 101. Reforestation Technologies International, Salinas

Raghuwanshi R (2012) Opportunities and challenges to sustainable agriculture in India. NEBIO 3:78–86

Read DJ (1991) Mycorrhizas in ecosystems. Experientia 47:376–390

Read DJ, Leake JR, Langdale AR (1989) The nitrogen nutrition of mycorrhizal fungi and their host plants. In: Boddy L, Marchant R, Read DJ (eds) Nitrogen, phosphorous and sulphur utilization by fungi. Cambridge University Press, New York, pp 181–204

Redecker D, Kodner R, Graham LE (2000) Glomalean fungi from the Ordovician. Science 289:1920–1921

Rillig MC, Wright SF, Eviner VT (2002) The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant Soil 238:325–333

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Same BI, Robson AD, Abbott LK (1983) Phosphorus, soluble carbohydrates and endomycorrhizal infection. Soil Biol Biochem 15:593–597

Siqueira JO, Safir GR, Nair MG (1991) Stimulation of vesicular-arbuscular mycorrhiza formation and growth of white clover by flavonoid compounds. New Phytol 118:87–93

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic/Elsevier, New York

Smith FA, Grace EJ, Smith SE (2009) More than a carbon economy: nutrient trade and ecological sustainability in facultative arbuscular mycorrhizal symbioses. New Phytol 182:347–358

Son CL, Smith SE (1995) Mycorrhizal growth responses: interaction between photon irradiance and phosphorus nutrition. New Phytol 108:305–314

Stamford NP, Silva JA, Freitas ADS, Araujo Filho JT (2002) Effect of sulphur inoculated with *Acidithiobacillus* in a saline soil grown with *Leucena* and *mimosa* tree legumes. Bioresour Technol 81:53–59

Stribley DP, Read DJ (1976) The biology of mycorrhiza in the ericaceae VI. The effects of mycorrhizal infection and concentration of ammonium nitrogen on growth of cranberry (*Vaccinium macrocarpon* Ait.) in sand culture. New Phytol 77:63–72

Subha Rao NS (1982) Advances in agricultural microbiology. In: Subha Rao NS (ed) Oxford and IBH Publ Co, pp 229–305

Thomson BD, Robson AD, Abbott LK (1986) Effects of phosphorus on the formation of mycorrhizas by *Gigaspora calospora* and *Glomus fasciculatum* in relation to root carbohydrates. New Phytol 103:751–765

Tisdall JM (1994) Possible role of soil microorganisms in aggregation in soils. Plant Soil 159:115–121
Treseder KK (2004) A meta-analysis of mycorrhizal responses to nitrogen, phosphorus, and atmospheric CO_2 in field studies. New Phytol 164:347–355
Tsai SM, Phillips DA (1991) Flavonoids released naturally from alfalfa promote development of symbiotic *Glomus* spores in vitro. Appl Environ Microbiol 57:1485–1488
Van Elsas JD, Van Overbeek LS, Fouchier R (1991) A specific marker pat for studying the fate of introduced bacteria and their DNA in soil using a combination of detection techniques. Plant Soil 138:49–60
Van Veen JA, Leonard S, Van Overbeek LS, Van Ellsas JD (1997) Fate and activity of microorganisms introduced into soil. Microbiol Mol Biol R 61:121–135
Venkateswarlu B, Rao AV, Raina P, Ahmad N (1984) Evaluation of phosphorus solubilization by microorganisms isolated from arid soil. J Indian Soc Soil Sci 32:273–277
Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586
Weidmann S, Sanchez L, Descombin J, Chatagnier O, Gianinazzi S, Gianinazzi-Pearson V (2004) Fungal elicitation of signal transduction-related plant genes precedes mycorrhiza establishment and requires the dmi3 gene in *Medicago truncatula*. Mol Plant Microbe Interact 17:1385–1393
Whiteside MD, Treseder KK, Atsatt PR (2009) The brighter side of soils: quantum dots track organic nitrogen through fungi and plants. Ecology 90:100–108
Whiteside MD, Digman MA, Gratton E, Treseder KK (2012) Organic nitrogen uptake by arbuscular mycorrhizal fungi in a boreal forest. Soil Biol Biochem 55:7–13
Wright SF, Upadhyaya A (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil 198:97–107
Wright SF, Starr JL, Paltineau IC (1999) Changes in aggregate stability and concentration of glomalin during tillage management transition. Soil Sci Soc Am J 63:1825–1829
Yang WQ, Goulart BL, Demchak K, Li Y (2002) Interactive effects of mycorrhizal inoculation and organic soil amendments on nitrogen acquisition and growth of highbush blueberry. J Am Soc Hortic Sci 127:742–748
Yoneyama K, Xie X, Kusumoto D, Sekimoto H, Sugimoto Y, Takeuchi Y, Yoneyama K (2007a) Nitrogen deficiency as well as phosphorus deficiency in sorghum promotes the production and exudation of 5-deoxystrigol, the host recognition signal for arbuscular mycorrhizal fungi and root parasites. Planta 227:125–132
Yoneyama K, Yoneyama K, Takeuchi Y, Sekimoto H (2007b) Phosphorus deficiency in red clover promotes exudation of orobanchol, the signal for mycorrhizal symbionts and germination stimulant for root parasites. Planta 225:1031–1038
Zaidi A, Khan MS, Ahemad M, Oves M (2009) Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiol Immunol Hung 56:263–284

Bacterial Probiotics: A Truly Green Revolution

6

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Abstract

Throughout history, the evolution and progress of all human civilizations have been closely linked to the evolution and development of agriculture, since this is the basis of food production to sustain population and ensure social stability.

At the beginning of the twentieth century, due to great advances in medicine, world population increased significantly. This fact was derived to a situation in which the need to significantly increase the ability to produce food was necessary in order to feed all those people. And so, the Green Revolution in the 1960s–1980s was derived in a great increase of crops yields, saving many millions of people from starvation. One of the key factors in the Green Revolution was the application of synthetic fertilizers and pesticides. Despite obvious benefit

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of these products in the amount of food produced, chemical fertilizers and pesticides have many negative impacts in health and environment.

Many bacterial strains have been described as plant probiotics, and, by improving availability of nutrients and plant health, they produce an increase in crops yields in an eco-friendly manner. The growing concern about protecting environment, human health, and the need to produce more food with the limited resources for an exponentially growing population in the Earth is making that many worldwide companies are increasingly producing and commercializing bacterial-based biofertilizers, and the plant probiotics market is growing all around the world – the new Green Revolution is here.

6.1 Introduction: From Hunter-Gatherers to the New Green Revolution

Agriculture is the basis of the society; ancient civilizations owed their existences to it. In other words, at the time when humans were able to obtain food from agriculture and livestock, they began to establish the foundation of civilizations (Fig. 6.1).

The first agricultural practice was the domestication of different plant species (Khush 2001). The archaeological remains are set in Mesopotamia, where wheat and barley were firstly grown. The rest of the world followed soon after; for example, in America the natives began to cultivate and harvest corn, tomato, and potato, among other crops, whereas in Asia people started the cultivation of rice (Carpanetto and Bianchini 2011).

Along with the population migration to large cities, the land for agriculture and ability to make more productive crops had to increase, in order to avoid the endangering of the social stability. In 1750, in Britain, as elsewhere in Europe, grain prices began to rise due to the continuous population growth. Thus, it was the time to renew agriculture, leading to the so-called British Agricultural Revolution during the years 1750–1880. During this time, there was an increase in the amount of land, labor, rotation with leguminous plants, and improvements in drainage systems, resulting in an increase in yield per hectare. Countries like Britain, Denmark, the Netherlands, and Belgium were pioneers in this agricultural revolution (Zanden 1991; Ang et al. 2010).

At the beginning of the twentieth century, new technologies appeared that facilitated an increase in agricultural production. Among those techniques, the use of mechanical equipment was the beginning of several changes in agricultural practices. Due to this, the countries adopting this mechanization experienced a significant increase in agricultural production. In addition, crop rotation, better quality seeds, and the use of fertilizers began to be common practices. The origin of the first fertilizers was diverse; for example, in regions of Belgium, fertilizers came from urban wastes, and, in contrast, Britain imported guano from Latin America (Zanden 1991).

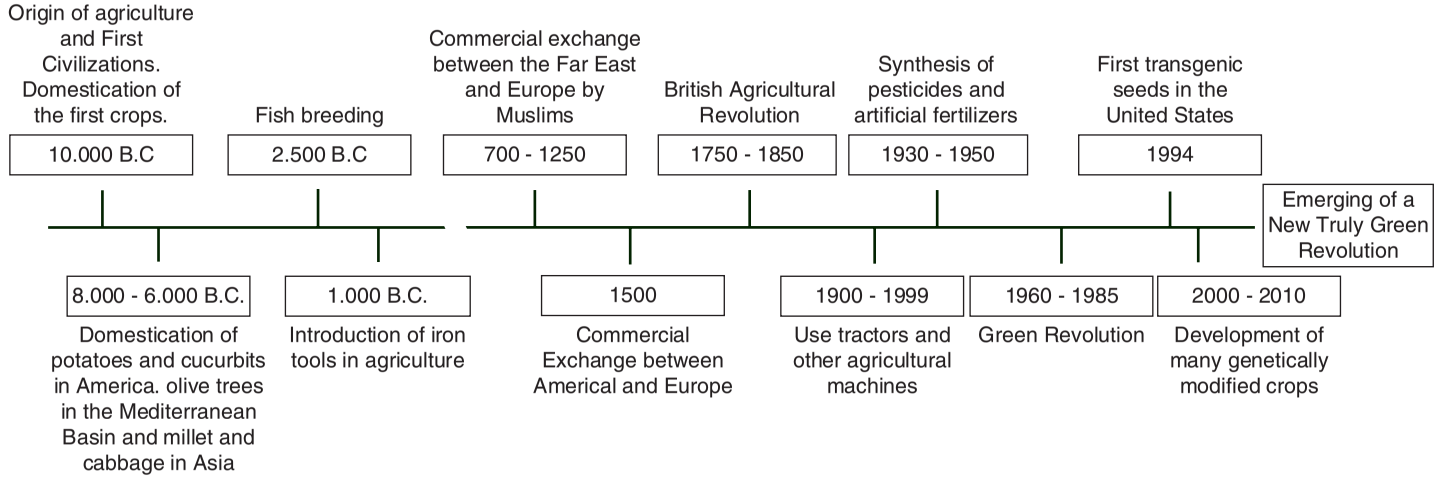

Fig. 6.1 Foundation of civilizations

Since the mid-twentieth century to the twenty-first century, the population of the overpopulated areas increased twofold (Khush 2001). Between 1960 and 1970, famines threatened many areas around the whole planet. To tackle the problem, good agricultural productivity was required, which reduces food insecurity and poverty and also improves human nutrition (Sayer and Cassman 2013). As a result, governments and companies began to make considerable investments in agriculture, which had an impact in infrastructures, market development, and new policy areas, in addition to prioritize agricultural research (Pingali 2012; Gomez et al. 2013). This was the origin of the Green Revolution, which appears between 1966 and 1985 (Pingali 2012).

One of the techniques that made the Green Revolution successful was the genetic improvement of major crops, selecting best genetic traits in order to increase productivity, adaptation to different environments, resistance to harmful abiotic factors, resistance to biotic stress, and reduced harvest periods. The use of these new hybrids increased their yield per hectare (Skorov 1973; Khush 2001). Genetic breeding was not the only factor in the Green Revolution; improvements in the irrigation, cultivation methods, sowing/harvesting timings, the application of chemical fertilizers, and weed/pest control must be also considered (Skorov 1973; Conway and Barbie 1988; Peng et al. 1999; Pingali 2012). Unquestionably, the crucial impact of the Green Revolution was the development and application of chemical fertilizers on crops. Also improvements in agricultural practices employed during the Green Revolution were successful; for example, land employed for cereal cultivation increased only by 30%, whereas cereal production was increased threefold. If we evaluate the most important crops since the beginning of the Green Revolution until the new millennium, all of them increased their yields, for example, 208% in wheat, 109% in rice, 157% in corn (maize), and 78% in potato crops (Pingali 2012). This revolution had important impacts in society, reducing poverty levels in many regions. In addition, food prices decreased, benefiting consumers and disadvantaged people, both in urban and rural areas. The Green Revolution avoided, or at least reduced, the occurrence of famines, population undernourishment, and death from starvation (Khush 2001; Pingali 2012).

Despite the advantages already discussed, it should also be mentioned that the Green Revolution had several disadvantages. The extensive farming of monocultures with similar genotypes increased the problem of pests and diseases, which also degenerated with the use of pesticides. Another problem was the disproportional use of chemical fertilizers. Some fertilizers and pesticides, especially when are improperly used, can affect human health and pollute groundwater, causing consequent effects on aquatic systems and the loss of genetic diversity (Conway and Barbie 1988). Also, the enormous amount of energy and water needed for their synthesis contribute to the depletion of natural resources, as well as to the global warming. Finally, the investment required to increase yields was high; only rich farmers could afford it, producing a disparity between rich and poor farmers. For example, in Africa, the lack of infrastructures, inadequate marketing, and scarce incentives were some of the reasons why this continent did not develop well along the revolution (Pingali 2012).

According to the Population Division of United Nations, the planet adds about 70 million people each year (Soby 2013). The world population is estimated to reach around 9.5 billion people in 2050. This inevitably translates to a need for increasing food production, as we have seen before; "History repeats itself." Nowadays, we have to admit that we are on the limit of the biophysical and environmental barriers to obtain higher yields. Some people think that the first thing necessary to increase yield is deforestation and exploiting water resources, thereby reducing biodiversity. However, the destruction of natural environments is not a suitable option; it is time to start innovating in agriculture in order to increase production without affecting the environment (Sayer and Cassman 2013). By the year 2050, food production should be duplicated, meaning an increase of 2.4% per year; however, at this moment that level is well below (1.3%) (Araus et al. 2014). In other words, the population is growing faster than food is produced. To make matters worse, currently, 15% of the population (868 million people) suffers malnutrition (Gomez et al. 2013).

Ideally, production increase should be achieved with less land, less water, less labor, and fewer chemical products. The goal is to obtain high-yield crops that are more resistant to biotic and abiotic stresses (Khush 2001; Araus et al. 2014). Therefore, the scientific community should develop further research in order to increase crop production and try to completely avoid the previously presented problems.

Some bacterial strains have the capability to promote plant growth (PGP) through several different mechanisms (García-Fraile et al. 2015). Some of them are common rhizosphere inhabitants (PGPR: plant growth-promoting rhizobacteria). Some others live as epiphytes over plant tissues or even inside their plant host, as endophytes, without inducing any disease. In some cases, as in the rhizobia-legume symbiosis, the bacteria live inside nodules – plant organs specifically created for accommodating their microsymbionts. Combinations of one or several of these bacteria are formulated into products and applied to the fields as biofertilizers, increasing crop yields by the availability and uptake of mineral nutrients for plants, without a total dependence on chemical fertilizers and, therefore, protecting the environment. It is time to begin a *New Green Revolution* that will use the advantages that certain microorganisms, the so-called plant probiotics, have on crop development and production.

6.2 Proofs of Plant Probiotic Potential as Crop Enhancers

For many years, scientists from all around the world have developed and published uncountable studies showing the great potential of beneficial microorganisms inoculation for yield increase in many and diverse agricultural crops. In this section, we detail several studies performed in the interaction of probiotic bacteria with different significant crops (Table 6.1).

Table 6.1 Examples of bacterial strains which showed plant growth promotion capabilities in different crops

PGPB	Crop	Reference
Pseudomonas putida P13 *Microbacterium laevaniformans* P7 *Pantoea agglomerans* P5	Potato	Malboobi et al. (2009)
Burkholderia tropica MTo-293	Tomato	Bernabeu et al. (2015)
Pseudomonas fluorescens SS5	Tomato	Ahirwar et al. (2015)
Azotobacter chrococcum *Azospirillum brasilense*	Tomato	Ramakrishnan and Selvakumar (2012)
Rhizobium leguminosarum TPV08 *Rhizobium leguminosarum* PETP01	Tomato	García-Fraile et al. (2012)
Bacillus megaterium MFD-2 *Bacillus subtilis* BA-142 *Pantoea agglomerans* FF *Acinetobacter baumannii* CD-1	Cucumber	Dursun et al. (2010)
Pseudomonas putida P13 *Pantoea agglomerans* P5	Cucumber	Isfahani and Besharati (2012)
Mesorhizobium ciceri TAL-1148 *Ochrobactrum ciceri* Ca-34^T	Chickpea	Imran et al. (2015)
Piriformospora indica PI *Mesorhizobium ciceri* MR *Pseudomonas argentinensis* LPGPR1	Chickpea	Mansotra et al. (2015)
Pseudomonas putida NBRIRA *Bacillus amyloliquefaciens* NBRISN13	Chickpea	Kumar et al. (2016)
Azospirillum brasilense Ab-V5	Maize	Ferreira et al. (2013)
Mycobacterium phlei MbP18	Maize	Egamberdiyeva (2007)
Pseudomonas spp. *Bacillus amyloliquefaciens* *Bacillus subtilis*	Rice	Cong et al. (2009)
Azospirillum sp. B510	Rice	Isawa et al. (2010), Bao et al. (2013)
Rhizobium tropici SEMIA 4080 *Azospirillum brasilense* Ab-V5	Soybean	Hungria et al. (2013)
Rhizobium sp. BARIRGm901	Soybean	Alam et al. (2015)
Bradyrhizobium diazoefficiens USDA110 *B. japonicum* THA6	Soybean	Prakamhang et al. (2015)
Rhizobium leguminosarum bv *phaseoli* LBM1123 *R. leguminosarum* bv *phaseoli* LCS0306	Bean	Mulas et al. (2011)
Rhizobium leguminosarum TPV08 *Rhizobium cellulosilyticum* ALA10B2^T	Bean	Díez-Méndez et al. (2015)

6.2.1 Fruit and Vegetables

Based on the total amount of production per year, potato is the first crop in the world ranking (Zaidi et al. 2015). However, this plant requires a considerably high dose of nutrients. Some studies showed that the application of plant probiotic bacteria in

single or combined formulations increase potato yields. In this sense, *Pseudomonas putida* P13, *Microbacterium laevaniformans* P7, and *Pantoea agglomerans* P5, applied either alone or in co-inoculation, had beneficial effect on this crop, in both greenhouse and field trials. The mixture of *P. agglomerans* P5 or *M. laevaniformans* P7 combined with *Pseudomonas putida* P13 substantially augmented biomass and plant growth in potato. Specifically, the mixture of *P. agglomerans* P5 and *P. putida* P13 enhanced the potato yield between 20 and 25% (Malboobi et al. 2009).

According to Dorais et al. (2008), tomato is the second crop in the world ranking based on its annual worldwide production. Therefore, several approaches for augmenting the number of fruits and their quality in this plant are being tested. Bernabeu et al. (2015) showed that the inoculation of tomato seedlings with *Burkholderia tropica* MTo-293 resulted in an effective colonization and an important improvement of the tomato production in two different seasons. Ahirwar et al. (2015) showed a consistent improvement in fruit production per plant (57%) with the application of a *Pseudomonas fluorescens* SS5 inoculation. Ramakrishan and Selvakumar (Ramakrishnan and Selvakumar 2012) also reported an increase in the amount of fruits per plant, total production per plant, and average fruit weight when tomato crops were inoculated with *Azotobacter chrococcum* and *Azospirillum brasilense*, used both alone and co-inoculated; however, the best results were obtained in the co-inoculation treatment. Also, García-Fraile et al. (2012) described a higher tomato production after *Rhizobium leguminosarum* TPV08 and *Rhizobium leguminosarum* PETP01 inoculations.

Another highly consumed vegetable is the cucumber. In 2007, the worldwide production was 4.46 million tons (FAOstat 2007); plants inoculated with *Acinetobacter baumannii* CD-1, *Bacillus subtilis* BA-142, *Bacillus megaterium* MFD-2, and *Pantoea agglomerans* FF exhibited significant positive effects on total and per plant fruit weight and number of fruits per plant compared to un-inoculated plants. In particular, inoculation with *B. megaterium* MFD-2 induced the highest fruit weight, whereas *P. agglomerans* FF showed the highest fruit number per plant (Dursun et al. 2010). Moreover, Isfahani and Besharati (2012) detected a yield boost in cucumber plants when inoculated with *Pseudomonas putida* P13 and *Pantoea agglomerans* P5 cultivated in soils with or without addition of chemical fertilizers.

Several other studies reported that rhizobial strains also increase the production of other important fruits and vegetables as lettuce, carrot, pepper, and strawberry crops (Flores-Félix et al. 2013; Silva et al. 2014; Flores-Félix et al. 2015).

6.2.2 Cereals

Maize is an important crop in temperate and semiarid climatic regions. Despite the high yields already being obtained for this crop by using genetically modified varieties, new ways of fertilization are being studied to satisfy its increasing demand. Ferreira et al. (2013) showed that grain yields were increased up to 29% when *Azospirillum brasilense* Ab-V5 and nitrogen were applied to the fields, compared to the treatment with just nitrogen fertilization; however, the authors describe how this

response depends on the type of soil. Moreover, Egamberdiyeva (2007) showed that the maize inoculation with *Mycobacterium phlei* MbP18 significantly increased (38%) total dry matter of this crop grown in a calcisol soil, compared to the un-inoculated control.

Rice is another cereal crop which researchers have focused a lot of attention to, trying to further increase its yield, because of the high level of consumption of its grains in the world. Cong et al. (2009) indicated that inoculation with *Bacillus amyloliquefaciens*, *Bacillus subtilis*, *Pseudomonas spp*., and an unidentified soil yeast significantly increased grain in approximately 270 kg per hectare during two successive seasons. In addition, Isawa et al. (2010) reported an improvement in seed production with the application of *Azospirillum sp.* B510 as bio-inoculant, results which were confirmed by Bao et al. (2013).

6.2.3 Legumes

Nowadays, soybean is the main source of proteins for millions of humans and the most economically important legume in the world. Therefore, many studies to improve its production have been performed. Hungria and collaborators (Hungria et al. 2013) obtained an increased soybean yield (19.6%) when the seeds were co-inoculated with *Rhizobium tropici* SEMIA 4080 and *Azospirillum brasilense* Ab-V5, compared to un-inoculated plants. Alam et al. (2015) analyzed soybean root hair colonization by *Rhizobium sp*. BARIRGm901, showing that effective bacterial attachment significantly enhanced the production compared with un-inoculated soybean plants. Moreover, co-inoculation with *Bradyrhizobium diazoefficiens* USDA110 and *B. japonicum* THA6 strains increases the yield per hectare more than 44% (Prakamhang et al. 2015).

Due to its nutritional value, chickpea is one of the most widely cultivated legumes. Imran et al. (2015) evaluated the effects of the inoculation with *Ochrobactrum ciceri* Ca-34^T and *Mesorhizobium ciceri* TAL-1148 in this crop. The co-inoculation produces more biomass (62%) and grain yield (111%) compared to un-inoculated plants. Similarly, Mansotra et al. (2015) have reported that a co-inoculation with *Piriformospora indica* PI, *Pseudomonas argentinensis* LPGPR1, and *Mesorhizobium ciceri* MR significantly improved the yield in comparison with *Mesorhizobium* single application. Besides increasing the growth parameters, Kumar et al. (2016) described that chickpea inoculated with a mixture of the strains *Pseudomonas putida* NBRIRA and *Bacillus amyloliquefaciens* NBRISN13 improved plant development under drought stress.

Bean is the most abundant legume included in human diets. According to Mulas and collaborators (Mulas et al. 2011), Spain, Italy, and Greece are the main common bean producers in the EU. However, the field extension dedicated to this crop has decreased during the last years, due to the important amounts of NPK fertilizers that this crop requires. For this reason, new ways of fertilization are being analyzed. The inoculation with rhizobia, as the strains *Rhizobium leguminosarum* bv *phaseoli* LBM1123 and *R. leguminosarum* bv *phaseoli* LCS0306, resulted in a

significant increase of aerial biomass, yield, and total N in seed compared to uninoculated treatments. Additionally, Díez-Méndez et al. (2015) showed an important enhancement of bean plants cultivated under greenhouse conditions when co-inoculated with the strains *R. leguminosarum* TPV08 and *R. cellulosilyticum* ALA10B2^T.

6.3 Plant Probiotics Enhance Not Just Crop Yields but also Their Quality

During the last decades, food quality and safety have been the principal issues on the European political agenda (Commission of the European Communities 1999). Currently, consumers become increasingly concerned about food safety and quality (Trienekens and Zuurbier 2008). Buyers demand food products with high and consistent quality. Thus, agriculture sustainability has emerged as one of the most significant concerns nowadays (Chauhan et al. 2015) because agriculture progresses can improve both food quality and safety (Conceição et al. 2016). In this sense, plant beneficial bacteria may play an important role in the improvement of both production and nutritional quality of crops (Ahemad and Kibret 2014). Several studies support the inoculation of plant growth-promoting rhizobacteria (PGPR) as enhancers of nutritional value and yield production in diverse crops. Thus, this section will be focused in the improvement of nutritional quality of the crops by different bacterial plant probiotics (Table 6.2).

Table 6.2 Nutritional value improved by PGPR

Microorganisms	Plant	Nutritional value	References
Bacillus megaterium TV-91C	Cabbage *(Brassica oleracea)*	Chlorophyll content	Turan et al. (2014)
Bacillus subtilis TV- 17C			
Pantoea agglomerans RK-92			
Bacillus sp. PSB10	Chickpea (*Cicer arietinum)*	Chlorophyll content	Wani and Khan (2010)
Pseudomonas thivervalensis Serratia marcescens	Corn (*Zea mays)*	Chlorophyll content	Shahzad et al. (2013)
Rhizobium MRP1	Pea (*Pisum sativum*)	Nitrogen content	Ahemad and Khan (2009b, 2010a, 2011a)
Rhizobium phaseoli	*Vigna radiata* L	Nitrogen content	Zahir et al. (2010)
Mesorhizobium MRC4	Chickpea(*Cicer arietinum)*	Nitrogen content	Ahemad and Khan (2009a, 2010b, 2010e)
Rhizobium MRL3	Lentil (*Lens culinaris)*	Nitrogen content	Ahemad and Khan (2010c, d, 2011b)

(continued)

Table 6.2 (continued)

Microorganisms	Plant	Nutritional value	References
Rhizobium leguminosarum TPV08 + *Rhizobium cellulosilyticum* ALA10B2^T	Bean (*Phaseolus vulgaris)*	Nitrogen content	Díez-Méndez et al. (2015)
Rhizobium strains PEPT01 and TPV08	pepper leaves (*Capsicum annuum*)	Plant sterols	Silva et al. (2014)
Rhizobium strains PEPT01 and TPV08	pepper leaves (*Capsicum annuum)*	Volatile compounds	Silva et al. (2014)
Pseudomonas fluorescens, *Bacillus subtilis*, *Azospirillum brasilense*	Peppermint *(Mentha piperita)*	Volatile compounds	Santoro et al. (2011)
Pseudomonas fluorescens WCS417r, *Bacillus subtilis* 09, *Sinorhizobium meliloti* Rm1021, and *Bradyrhizobium* sp. USDA 4438	*Origanum majorana L.*	Lipid oil	Banchio et al. (2008)
Phyllobacterium PEPV15	Strawberry (*Fragaria ananassa*)	Ascorbic acid	Flores-Félix et al. (2015)
Pseudomonas fluorescens N21.4	Blackberry (*Rubus fruticosus)*	Flavonoids	Garcia-Seco et al. (2015)
Chryseobacterium balustinum Aur 9	Bean *Phaseolus vulgaris*	Flavonoids	Dardanelli et al. (2010)

6.3.1 Nitrogen and Protein Content

Diverse studies showed that some rhizobial strains are able to improve nitrogen and protein content in the seeds of different legumes. *Rhizobium phaseoli*, in the presence of tryptophan, significantly increased *Vigna radiata* L. grain nitrogen concentration in comparison with the control (Zahir et al. 2010). Several studies reported that *Rhizobium* strain MRL3 increased seed protein content in *Lens culinaris* (Ahemad and Khan 2010c, d, 2011b). Moreover, *Phaseolus vulgaris*-nodulating bacterium *Rhizobium leguminosarum* TPV08 co-inoculated with the non-nodulating cellulase and cellulose overproducer strain *Rhizobium cellulosilyticum* ALA10B2^T significantly increased nitrogen content in beans, in comparison with the single treatments of each bacterial strain and the un-inoculated treatment (Díez-Méndez et al. 2015). *Rhizobium* sp. MRP1 improved both nitrogen and protein content on *Pisum sativum* under laboratory conditions (Ahemad and Khan 2009b, 2010a, 2011a). Finally, *Mesorhizobium* strain MRC4, improved seed protein content in *Cicer arietinum* (Ahemad and Khan 2009a, 2010b, 2010e).

6.3.2 Chlorophyll Content

Chlorophyll is a green compound commonly found in plant stems and green leaves. This molecule is able to convert the energy of sunlight into chemical energy through the process termed as photosynthesis, in which carbon dioxide and water are

transformed into oxygen and glucose. By taking chlorophyll into our bodies, we elevate the amount of hemoglobin in our blood, which is translated to an improvement in energy and blood circulation and oxygenation. Diverse studies demonstrated that the application of rhizobacterial plant probiotics increased chlorophyll content in several crops. Turan and collaborators (Turan et al. 2014) studied the effect on *Brassica oleracea* (cabbage) after inoculation with different rhizospheric bacteria (*Bacillus megaterium* strain TV-91C, *Bacillus subtilis* strain TV-17C, and *Pantoea agglomerans* strain RK-92) and showed that all strains were able to increase chlorophyll content. Wani and collaborators (2007) published the ability of *Bacillus* sp. PSB10 isolated from *Cicer arietinum* nodules as enhancer of chlorophyll content in chickpea. Moreover, rhizobacterial isolates classified within the species *Serratia marcescens* and *Pseudomonas thivervalensis*, which harbored ACC deaminase activity, presented a substantial augmentation in chlorophyll content in *Zea mays* compared with the non-inoculated treatment (Shahzad et al. 2013).

6.3.3 Phytosterols, Oils, and Volatile Organic Compounds (VOCs)

Phytosterols are cholesterol-like compounds present in most vegetables, appearing in high concentrations especially in vegetable oils. Their capability to decrease blood cholesterol levels, protect against some types of cancer, and modulate the immune system has been described (Piironen et al. 2000). Health experts suggest that complementing the diet with sterols from vegetable origin is an efficient and bio-safe manner to decrease the risk of suffering a coronary disease (Abu Mweis and Jones 2008). This strategy was tested and proved among dyslipidemic patients who needed extra treatments to lower lipids (Gupta et al. 2011). Silva et al. (2014) showed that the analysis by HPLC-DAD of alkaline hydrolysis obtained extracts revealed that some sterols are considerably augmented in pepper leaves after application of a *Rhizobium* strain as inoculant.

Essential oils contained in certain plants are used as chiral substances in synthetic organic chemistry; also, they are employed to produce highly functional and economic value molecules through biotechnological processes (Sangwan et al. 2001). In food and pharmaceutical industries, these oils are used as food and beverage flavoring, perfumes, or fungicides and insecticides (Deans and Svoboda 1990). Banchio et al. (2008) showed the properties of PGPRs in essential oil concentration in the aromatic plant *Origanum majorana L.* Four strains were tested in the study (*Bradyrhizobium* sp. USDA 4438, *Bacillus subtilis* 09, *Sinorhizobium meliloti* Rm1021, and *Pseudomonas fluorescens* WCS417r), showing that the essential oil concentration was increased in inoculated plants. Specifically, plants inoculated *P. fluorescens* WCS417r *had an increase in* essential oil yield of 0.14% (w/v), and the authors propose this strain as an interesting commercial bio-inoculant for *O. majorana* crops.

Volatile organic compounds (VOCs) are molecules liberated from aerial parts of the plant into the atmosphere and from roots into the soil. The primary functions of volatile compounds are to defend plants against herbivores and pathogens; to attract pollinators, seed dispersers, and other beneficial animals and microorganisms; and

to serve as signals in plant-to-plant communication (Dudareva et al. 2006). Plants produce and deliver large amounts of volatile organic compounds, flowing into some characteristic smells. Plant smells have always been recognized for their commercial and aesthetic value, and they are emitted not only from flowers and fruits but also from vegetative tissues (Marin-Loaiza and Cespedes 2007). In this sense, Silva et al. (2014) described how pepper plant leaves were richer than the control plants in these compounds when inoculated with two rhizobial strains; all leaves from inoculated plants contained greater levels of methyl salicylate, the major volatile organic compound. Santoro and collaborators (Santoro et al. 2011) examined the influence of released VOCs from rhizobacteria in the plant growth and essential oil composition of the aromatic plant *Mentha piperita* (peppermint) inoculated with three PGPR strains belonging to the genera *Bacillus*, *Pseudomonas*, and *Azospirillum*; plants exposed to VOCs produced by *Pseudomonas* or *Bacillus* were significantly bigger than control plants, and regarding the production of essential oils, plants treated with *Pseudomonas* VOCs showed a twofold increase in the monoterpenes content as well as a significantly higher content in pulegone and menthone. These results show how VOCs synthesized by rhizobacteria induce the biosynthesis of secondary metabolites as well as promote plant growth and crop yields in some plant species.

6.3.4 Carotenoid, Vitamin, and Flavonoid Content

Lycopene is a bright red carotenoid which is also an intermediate molecule in other carotenoids biosynthesis pathway. This compound is found in important concentrations in several fruits as red grapefruit, tomato, watermelon, or guava (Stahl and Sies 1996). Lycopene and some of its derivatives, such as β-carotene, are effective free-radical removals, and its inclusion in the diet is related with a reduction in cancer occurrence (DiMascio et al. 1989; Giovannucci et al. 1995; Gerster 1997; Rao and Agarwal 1998; Giovannucci 1999). Tomatoes are one of the vegetables with a higher content in lycopene, and their capability to eliminate active oxygen species (AOS) has been described (Rao et al. 1998; Toor and Savage 2005). The reddening of tomatoes is also due to lycopene, so this compound is very essential not only for the nutritional value of the plant but also for its marketable quality (Dumas et al. 2003). Ordookhani et al. (2010) showed the influence of PGPRs and AMF (arbuscular mycorrhizal fungi) in tomato Hybrid GS −15 plants: higher levels in lycopene and antioxidants contents were obtained in plants inoculated with a mixture of *Pseudomonas*, *Azotobacter*, *Azospirillum*, and AMF.

L-Ascorbic acid is a derivate form of the commonly known vitamin C. Humans, primates, and other mammals are unable to synthesize ascorbic acid, due to the lack of functionality of the gene encoding the L-gulono-gamma-lactone oxidase enzyme that catalyzes the last step in its biosynthesis (Chatterjee 1973; Nishikimi et al. 1994). Since this molecule carries out several essential antioxidant and metabolic functions, it is essential to acquire it through the diet. Flores-Félix et al. (2015) inoculated a *Phyllobacterium* strain in strawberry plants showing an increment in

vitamin C content in the strawberries from those plants inoculated with the bacterium.

Folic acid (vitamin B9) and its derivate molecules are communally termed folates (Hanson and Gregory 2011). Deficiencies of folates in human diet produce several diseases and deficiencies as birth defects (i.e., anemia or spina bifida) or higher risks of developing certain types of cancer or vascular diseases (Hanson and Gregory 2011), and animals are incapable of synthesizing folates, depending on what plants ingest for their obtaining. Bona et al. (2015) showed that the inoculation of strawberry plants grown under greenhouse conditions with a mixture of mycorrhizas and two plant growth-promoting bacterial strains, both of them able to synthesize siderophores, solubilizes phosphates, and produce the phytohormone indole-3-acetic acid (AIA), improved the qualitative and nutritional features of strawberry plants and their fruits, which had a higher concentration of folic acid, as well as vitamin C, sugars, and various organic acids.

One of the most important groups of secondary metabolites in plants is flavonoids. They are essential for the plant, playing important functions in the metabolism of vegetables (Tahara 2007), but also for animals incorporating plants into their diet, having being described their potential benefits in the prevention of some chronic diseases (Martin et al. 2013). Garcia-Seco et al. (2015) conducted a flavonoid study in blackberries with the application of PGPR inoculants. Blackberry plants (*Rubus* spp., Rosaceae) produce one of the forest berries with more pharmacologic applications (Hummer 2010), with an interesting potential for improving human health (Cuevas-Rodríguez et al. 2010; Hassimotto and Lajolo 2011). Blackberries contain the genetic and, therefore, enzymatic machinery to synthesize phenylpropanoids, and these genes can be elicited by plant growth-promoting bacteria (Lattanzio 2013). Phenylpropanoids, as flavonoids, are phenolic compounds, but phenylpropanoid and flavonoid synthesis is driven through different metabolic pathways. Nevertheless, Garcia-Seco et al. (2015) showed that application of *Pseudomonas fluorescens* N21.4 into blackberry plants induced overexpression of some of the genes implicated in the flavonoid biosynthetic pathway, with an observation of a higher concentration of these substances in the berries. Moreover, flavonoids are also essential signal molecules for the establishment of the rhizobia-legume symbiosis (Mandal et al. 2010). Dardanelli et al. (2010) examined the flavonoid exudation patterns of *Phaseolus vulgaris*, in the presence or lack of the PGPR *Chryseobacterium balustinum* Aur9, describing that the presence of this bacterium in the *P. vulgaris* rhizosphere determines the exuded flavonoid pattern and providing several possible explanations to this effect: (i) the increase of the plant's root surface as a response of the presence of the bacterium, (ii) the induction of genes implicated in other flavonoid biosynthetic pathways, or (iii) the bacterium involvement in the catabolism of the plant-exuded flavonoids.

Although all very promising results have been obtained with the application of bacterial probiotics in different crops, improving crop yields and food quality in an eco-friendly manner, it is also important to dedicate research efforts to the study of PGP microorganism safety, since the application of pathogenic ones in the fields could have fatal effects on plants, animals, or human health. Moreover, it is

necessary to develop proper biofertilizer application methods, as an appropriate application could improve inoculants efficiency and enhance bacterial adherence, being both factors of utmost importance for a successful inoculation that would increase crop production. In the next section of this chapter, we will deepen in the concepts bacterial inoculants safety and plant probiotic technologies.

6.4 Strain Selection and Technologies for Application of Plant Probiotics

6.4.1 Strain Selection Meets Biosafety Concept

The effectiveness of inoculants and the knowledge of plant growth-promoting mechanisms are very important factors for the production of biofertilizers, but, before field application, it should be necessary to check the biosafety of these strains both environmentally and for animal and human health (Berg 2009; Selvakumar et al. 2014).

A number of studies have highlighted this need for more comprehensive research on the biosafety of microorganisms isolated from plants that could be used as PGPR or plant probiotic bacteria. In the last decades, numerous studies have shown the presence in the rhizosphere of a large number of microorganisms that are taxonomically very closely related to well-known opportunistic microbes that can cause disease in immunocompromised individuals. In fact, some authors consider the rhizosphere as a large reservoir for opportunistic pathogenic bacteria for humans (Berg et al. 2005, 2013).

From the rhizosphere of several diverse plant species, *Burkholderia* strains with PGPR activity have been isolated, which were related to the *B. cepacia* group. Hence, its use has been hampered because of their potential health risk (Chiarini et al. 2006). Recently, new species from the same genus that come from a separate phylogenetic lineage have been isolated from roots of plants and described. Some of these new *Burkholderia* species described have the ability to induce nodule formation and atmospheric nitrogen fixing on legumes (Moulin et al. 2001; Garau et al. 2009; Estrada-de Los Santos et al. 2013). Recent studies show that the mutualistic species associated with plants are clearly different from those described as pathogenic (Angus et al. 2014).

Similarly, within the genus *Ochrobactrum*, some species (*O. lupini* and *O. cytisi*) have been described as able to induce nitrogen-fixing nodules on legume roots and are closely related to the opportunistic human pathogen *O. anthropi* (Trujillo et al. 2005; Zurdo-Piñeiro et al. 2007). Romano et al. (2009) have shown that human pathogenic strains of *Ochrobactrum anthropi* form a subpopulation different from plant-associated strains. Therefore, it is necessary to deepen our understanding at molecular, physiological, and biochemical level when characterizing these strains and establish clear criteria for determining where is the limit for defining a strain as safe depending on their taxonomical proximity to potential human pathogens.

The situation in the genus *Micromonospora* differs greatly from the two examples mentioned before. Just as in previously mentioned genera, several strains of this genus have been described to have strong activity as PGPB not associated with biological nitrogen fixation (Martínez-Hidalgo et al. 2014a, b) and good potential as biocontrol agents (Martínez-Hidalgo et al. 2015). However, within the genus *Micromonospora*, no clinical isolates have been described; hence, we may conclude that this genus can be considered biologically safe.

As mentioned before, adequate characterization of the selected strains is necessary. Furthermore, testing their pathogenicity and ecotoxicity should ensure their safety, in order to avoid using strains that possess even a minimal risk to health or the environment. *Caenorhabditis elegans* can be used as a model to evaluate the pathogenic potential of selected strains and has been used in the case of *Burkholderia* isolates (Angus et al. 2014). It is also required to know if the strains tested produce antibiotics or other toxic or harmful compounds at ecological level (Zachow et al. 2009), considering that in the ecosystem in which the bacterium is released, there is wide range of micro- and macroscopic organisms that could be affected: microbiota, nematofauna, annelids, insects and other invertebrates, small mammals, and higher organisms (Stephens and Rask 2000; Köhler and Triebskorn 2013).

Vílchez et al. (2016) have proposed an evaluation system to evaluate the biosafety of the bacterial strains used in bio-inoculants: This modular system of evaluation is based on a panel of assays on model organisms for all trophic levels to assess the environmental impact of bio-inoculants. This system is named Environmental and Human Safety Index (EHSI) (Vílchez et al. 2016). Besides the standardization that EHSI would provide, it also prevents the high economic cost of testing the environmental impact of bio-inoculants that are now used and avoids assays on vertebrate animals as is currently established by the legal framework of the European Union. Monetary cost is an important advantage of this index, due to the current controversy among companies that produce inoculants and European authorities on defining the tests required in order to allow the use of bacterial inoculants safely. Currently, large multinational companies in the inoculant sector want to establish a series of tests with a high economic cost that the small industry would not be able to assume.

The use of microbial-based fertilizers is determined by their inclusion in formulation schemes and must have an extended shelf life until its field application (Malusá and Vassilev 2014). There are important steps to follow related with manufacture and formulation of microbial inocula (Vassilev et al. 2015). Until now, studies on inoculants have been focused in obtaining good PGP strains, neglecting the production process. More attention is needed for the optimization of the production schemes and formulation procedures, to ensure the efficacy and correct application of biofertilizers.

A high-quality biofertilizer must have a high density and viability of the bacterial inocula and a long shelf life; in other words, biofertilizers should ensure the success of the growth promotion after their application (Herridge and Peoples 1990; Somasegaran and Hoben 1994).

6.4.2 Proper Technologies for the Application of Biofertilizers

Apart from the selection of proper PGP bacterial strains, one of the main concerns in the formulation of an effective biofertilizer and the success in its commercialization is the adequate selection of a carrier. Carrier materials are considered as the preferred delivery systems of microbial species to field conditions (Bashan 1998). Many substances of different origins are susceptible to be used as carriers. Basically, carriers can be classified into four groups: (i) soils (i.e., peat, zeolite, clay, or talc), (ii) waste materials (i.e., biochar, vermicompost, bagasses, or farm manures), (iii) inorganic/inert materials (i.e., vermiculite, perlite, rock phosphate, or alginate beads), and (iv) liquids and lyophilized microorganisms (Herrmann and Lesueur 2013). Selection of carriers varies depending on several factors, being among the most important ones in the maintenance of the bacterial shelf life, the application system, the sterilization easiness, and the production costs (García-Fraile et al. 2015). The most commonly used carrier is peat, especially when bacterial strains are included in the formulation. However, its use must become conscious due to the negative impact of its extraction on several worldwide ecosystems. Alternative materials are starting to be more used; nevertheless, there is a lack of innovative procedures for obtaining in soil biofertilization (Bashan et al. 2014; Vassilev et al. 2015).

Despite of this fact, several studies reported some recent advances in carriers' improvements, fulfilling one or some of the problematic issues previously mentioned. In this sense, there are some candidates to be taken into account for biofertilization formulation. Bio-encapsulation of PGPR strains onto polymers, such as alginate beads, is reported as a good strategy to maintain almost unlimited shelf life of the microorganisms used and release them progressively in the soil through their biodegradation process (Bashan and González 1999; García-Fraile et al. 2015). Moreover, polymers as alginate offer protection to microbial cells against biotic and abiotic stresses. Bashan et al. (2002) reported a method using alginate microbeads for the inoculation of *Azospirillum brasilense* in wheat and tomato, enhancing plant development. The benefits of bio-encapsulation of beneficial microorganisms are clear, becoming a highly favorable strategy in biofertilizer formulation development (Schoebitz et al. 2013; Vassilev et al. 2015). Additionally, recent works mentioned other polymers, such as hydrogel or bacterial exopolysaccharides, which have some advantages, providing interesting results to be explored in depth (Rodrigues et al. 2015; Suman et al. 2016).

Several authors described the benefits of vermicompost in the survival of beneficial PGPR species (*Azotobacter chroococcum*, *Rhizobium leguminosarum*, *Bacillus megaterium*, and *B. mucilaginous*) and strengthen their synergistic effects when they are in combination, improving crop quality and yield and enhancing soil properties (Song et al. 2015; Bharti et al. 2016). However, these effects are subjected to the dose of vermicompost and the crop type, converting them in a disadvantage.

Biochar is also considered as a good carrier for amending soils, serving as a delivery direct mechanism to bacterial strains to plant roots. Recent studies showed that pyrolyzed pinewood biochar does not appear to affect plant growth-promoting traits from some species of *Enterobacter* and *Pseudomonas*, included in

biofertilizer formulation, which should be applied directly to the agrarian soils. This kind of biochar performed as well as peat maintains higher population densities in comparison with vermiculite (Hale et al. 2014, 2015; Sun et al. 2016).

Other solid carriers were also tested and compared among them in several studies, such as zeolite, vermiculite, talc, or coriander husk in the framework of bacterial survival, plant growth enhancement, or low cost production (Joshi et al. 2007; Arora et al. 2014; Maheshwari et al. 2015; Tripathi et al. 2015). Moreover, liquid carriers (oils, culture broths, etc.) are also reported as suitable as solid carriers for some crops (Vander Gheynst et al. 2006; Albareda et al. 2008).

Once PGPR(s) and carrier(s) are selected under in vitro or greenhouse conditions in different crops and the prospects of the formulation are analyzed, the engineering of the industrialization process is required. Moreover, industrial production must be strictly calculated, since the economic costs of the production of the biofertilizer are the key for ensuring its commercialization. In this sense, the whole process must be controlled by quality standards. Crucial steps in the manufacture procedure requiring quality control are fermentation, carrier preparation (involves sterilization), carrier inoculation, packaging, and term storage (Herrmann and Lesueur 2013; García-Fraile et al. 2015).

In conclusion, a complete understanding of the complex relationships between soil and crop types, microbial strains and carrier material is fully required to commercialize in a successful manner a biofertilizer formulation with high potential. Moreover, this economic success is subjected to the collaboration between researchers, companies of the private sector, and farmers, who are the latest recipients of the final product. For this reason, field trials and demonstrations prior to commercialization are as important as the rest of the cited key steps.

6.4.3 Product Registration and Marketing

When a company wants to register a new product based on microorganisms, it has to select to which group it will belong. If the company decides that its product has an effect as a biocontrol agent, then the product must be registered as "plant protection product (PPP)." The microorganisms in these inoculants are used to control pests and diseases. In the EU, this kind of products are habitually denoted as biopesticides and are controlled according to Regulation 1107/2009, regarding the release of PPPs in the market. Because of that, they are still handled in a comparable way to chemicals, because there is no legal provision yet for their specific registering. Although, for the registration of a product, a PPP dossier with information of all the active microorganisms included in the formulation must be provided, including the physical, biological, and chemical properties, to guarantee the human, animal, and environmental safety, as well as its efficacy, must be provided (Kamilova and de Bruyne 2013).

On the other hand, companies may decide to register a product as an inoculant/biofertilizer (Kamilova et al. 2015). There are not many specific guidelines in the European Union, which set quality limitations or regulatory and environmental standards for biofertilizers and their use. This is a problem to create homogeneous rules between the different countries and its different regions.

6.4.4 European Countries

Spain, for example, allows autonomic administrations to regulate their own biofertilizer standards. The best example is found in Andalucía, the Spanish region with the maximum percentage of organic farming, which allows the use of products designed by "a group of organisms that are applied to soil or seeds to improve plant nutrition," as mycorrhiza, rhizobia, or *Azotobacter*, among others (Malusá and Vassilev 2014). Chapter IV of Royal Decree 506/2013 mentions that products containing raw organic, animal, or vegetable origin may not exceed certain values of detrimental microorganisms like *Salmonella* spp. and *Escherichia coli*. There is no mention about beneficial microorganisms. In this scenario, there is a double way to approve microbial inoculants in Spain. The first category includes biofertilizers, which are regulated by the Fertilizer Law included in the Royal Decree 506/2013. Plant strengtheners are included in the second category, regulated by the so-called OMDF-list, which means "Other Meanings of Defense and Fortifiers." For both categories, a submission of technical information, safety information, and efficacy tests is required (Kamilova and de Bruyne 2013).

In the "Legislative Decree 29 April 2010" from Italy, mycorrhizal fungi inocula and some microbial inoculants are included in the groups "Products with action on the soil and plants" and "Products with specific action." In the label of these products, the organic matrix used and the name of the microbial species included must be indicated. If a company seeks authorization for a new microbial inoculant, the Italian Ministry of Public Health would prove if this or these strain(s) act as a biofungicide or not. Furthermore, the use of genetically modified organisms or pathogens, such as *Escherichia coli*, *Salmonella* spp., and other aerobic mesophilic microbes and nematodes, is prohibited (Kamilova and de Bruyne 2013; Malusá and Vassilev 2014).

In Germany, the new plant defense law speaks about plant fortifiers, which are allowed in the market if they do not comprise microbe included in the definition stated in the Pesticide Regulation 1107/2009/EC. Several scientific institutions evaluate if these products are safe for human and animal health and environment, making a monthly list with the different substances and microorganisms that increase plant resistance.

In France, French authorities evaluate biofertilizers, in order to confirm the quality and human, animal, and environmental safety before being placed on the market. Furthermore, all companies must provide a product dossier of each biofertilizer. The authorities can authorize microbial inoculants, even if they do not state effects in nutrition.

The United Kingdom and Ireland lack specific laws for microbial inoculants; their products do no bear plant defense claims. Even so, companies check with the Chemical Regulation Directorate and to the Ministry of Agriculture, in the United Kingdom and Ireland, respectively, to verify the quality of their products.

Nowadays in the Netherlands, the EU pesticide Regulation 1107/2003/EC and fertilizer Regulation 2003/2003/EC are followed in a very severe way. However, for decades, numerous products including PGPRs were allowed in the market

regardless of the law. Fortunately, the legal environment is now changing, and marketed products must be approved by Regulation 1107/2009/EC (Kamilova and de Bruyne 2013).

6.4.5 China and India

In China, legal quality of biofertilizers is based on eight different parameters, which ranges from content of carbon and water or pH until the granules dimension in case of solid products. But, by the Chinese standard, the most important of these eight parameters is the amount of living cells, which determine the quality of the final product (Suh et al. 2006).

In India, another overpopulated country that depends mostly on agricultural production to feed people, the Indian Ministry of Agriculture sets the standards for quality parameters in biofertilizers, such as the minimum counts of viable cells, the pH, or the efficiency character. To each product, a detailed procedure is specified, in order to ensure and maintain their quality control procedures (Malusá and Vassilev 2014).

6.4.6 American Countries

In the American continent, the different nations are responsible for controlling the production and use of biofertilizers, through collaborations among them. The United States is one of the countries where the quality of these biofertilizers is set at the discretion of manufacturers. However, other American countries have strict controls and legislations, such as Brazil, Uruguay, Canada, Argentina, and Cuba (Moreno-Gomez et al. 2012).

Argentina has a relaxed normative, allowing the commercialization of biofertilizers based on rhizobia; however, it lacks regulations for other types of microorganisms such as *Pseudomonas*, *Bacillus*, or fungi, as well as for controlling contaminations and strain origin (Corvalan et al. 2007; Moreno-Gomez et al. 2012). Here, biological fertilizers are registered in the National Health and Agroalimentary Quality Service (SENASA), which, along with other divisions of the government, determines biofertilizer adoption, restriction, prohibition, or marketing. The regulations in this country establish minimal concentrations of bacteria in each biofertilizer product at the time of production and expiration. It is intended to extend the legislation to molecular, physiological, and morphological characterizations, genetic stability, ability of the inoculants to resist abiotic stress, and the confirmation of the absence of pathogens, among other parameters (Corvalan et al. 2007).

In the case of Brazil, there are certification processes and control, making that the 91% of their products within the established normative (Hungria and Campo 2007; Moreno-Gomez et al. 2012.).

Despite the extensive normative in those American countries, there is still a lack of specific or effective regulations in other countries of this continent, such as Bolivia,

Venezuela, Peru, Mexico, Colombia, and others (Abela and Valenzuela, 2007; Moreno-Gomez et al. 2012; Moreno-Sarmiento et al. 2007; Zúñiga, 2007).

Independently from the regulations of each Latin American country, the common interest in biofertilizers originated the creation, together with Spain and Portugal, of the Ibero-American Network for Biological Fertilizers for Agriculture and Environment (BIOFAG) in 2003. This network aims to integrate knowledge and technologies to increase the safe use of biofertilizers. BIOFAG consists of 60 research groups and companies in 12 countries of Latin America and the Iberian Peninsula (Moreno-Gomez et al. 2012).

Given the disparity between different inoculant legislations in different countries, a global initiative for the regulation of biofertilizers is necessary and should be agreed, in order to develop further biofertilizer market and make it more equitable in every country.

6.5 Worldwide Commercialization of Plant Probiotics

In the last years, there is a worldwide increasing worrying about environmental issues, and many countries' governments are developing regulations protective with the environment. In addition, the demand for eco-products is expanding, especially in developed countries. All this is causing a worldwide increasing of the demand for biofertilizers, and, therefore, the microbial biofertilizer market is growing every year, being expected an increase of 14% by 2020 (García-Fraile et al. 2015). A review of some of the currently commercialized microbial based biofertilizers is given in Table 6.3.

Seizing upon the opportunity of the current and future expansion of the biofertilizers market, several multinational companies are already distributing and selling biofertilizers all around the world. For instance, the company Novozymes produces different bacterial biofertilizers capable of nitrogen fixation and phosphate solubilization which are sold in the United States, Brazil, Canada, and the Asiatic, European, and Australian continents. Between their most popular products, Nitragin Gold® or Cell-Tech® is formulated with strains of rhizobia capable of nodulating legumes, and TagTeam® is based on a combination of rhizobia with the fungus *Penicillium bilaii*. These solutions are presented in liquid or granules and also mixed with peat. Besides, Novozymes and Monsanto have recently built a business alliance termed the BioAg Alliance. The combination of Monsanto and Novozymes competences resulted in one of the most advanced industrial microbial platform. The association aims to develop novel biofertilizers on the basis of tests with hundreds of bacterial strains in thousands of field conditions to (i) choose those bacteria offering a reliable advantage for crop yields, (ii) detect possible strain interactions, and (iii) exclude redundancies (Bjørndal F., Novozymes press officer, personal communication). Also Rizobacter, a significant company involved in the experimentation of bacterial biofertilizers and funded in Argentina in 1977, sells biofertilizers based on rhizobacterial formulations for legume seed, not just in its own country, but also in other Latin American countries, the United States, Africa, and Europe.

Table 6.3 Bacterial-based commercial biofertilizers

Biofertilizer	Brand	Composition
Nitragin Gold®	Novozymes	Rhizobia
Cell-Tech®	Novozymes	Rhizobia
TagTeam®	Novozymes	Rhizobia + *Penicillium bilaii*
Accomplish®	Loveland Products, Inc	PGPR + enzymes + organic acids + chelators
Nodulator®	BASF Canada Inc.	*Bradyrhizobium japonicum*
Nodulator® N/T	BASF Canada Inc.	*Bacillus subtilis MBI 600* + *Bradyrhizobium japonicum*
Nodulator® PRO	BASF Canada Inc.	*Bacillus subtilis* + *Bradyrhizobium japonicum*
Nodulator® XL	BASF Canada Inc.	*Rhizobium leguminosarum* biovar *viciae 1435*
Bioboots®	Brett-Young Seeds	*Delftia acidovorans*
Bioboots®	Brett-Young Seeds	*Delftia acidovorans* + *Bradyrhizobium* sp.
EVL coating®	EVL Inc.	PGPR consortia
Nitrofix®	Labiofam	*Azospirillum* sp.
Bioativo®	Instituto de Fosfato Biológico	PGPR consortia
VitaSoil®	Symborg	PGPR consortia
Azotobacterin®	JSC “Industrial Innovations”	*Azospirillum brasilense* B-4485
Mamezo®	Tokachi Federation of Agric. Coop. (TFAC)	Rhizobia in peat
R-Processing Seeds®	Tokachi Federation of Agric. Coop. (TFAC)	Legume seed coated with rhizobia
Hyper Coating Seeds®	Tokachi Federation of Agric. Coop. (TFAC)	Grass legume seeds coated with rhizobia
Life®	Biomax	PGPR consortia
BioMix®	Biomax	PGPR consortia
Biozink®	Biomax	PGPR consortia
Biodine®	Biomax	PGPR consortia
Rhizosum N	Biosym Technologies	*Azotobacter vinelandii*
Rhizosum P	Biosym Technologies	*Bacillus megaterium*
Rhizosum K	Biosym Technologies	*Frateuria aurantia*
Rhizosum Micros	Biosym Technologies	*Azospirillum*
Rhizosum Aqua	Biosym Technologies	PGPR consortia

In 1993, the Canadian government started to set the bases for the regulation of biotechnological products. Nowadays, the Canadian Food Inspection Agency, organization with the power to regulate fertilizer substances of chemical and biological origin, regulates the efficiency requirements for biofertilizers, their environmental safety constraints, and the prerequisites for their registration. All registered biofertilizers can be accessed at their website (www. inspection.gc.ca);

the list contains more than 200 microbe-based supplements for farm plants with an average of 10^6 to 10^9 living cells per milliliter or gram of product. Most of them contain rhizobial strains and are designed for legume crops. Still, some companies manufacture bacterial biofertilizers containing other than rhizobial strains. For instance, BASF Canada Inc. produces formulas with a mixture of *Bradyrhizobium japonicum* and *Bacillus subtilis* which are globally distributed in countries all around the world. Brett-Young Seeds manufactures the inoculant Bioboots®, based on the bacterium *Delftia acidovorans*, which is provided in three different formulations, two of them are designed for canola, and the difference between them is the formulation – one uses peat as carrier while the other is provided in liquid – the third inoculant is intended for soybean and combines the bacterium *D. acidovorans* with a strain of *Bradyrhizobium*. EVL Inc. formulates a biofertilizer based on the PGP *Lactobacillus helveticus* and the bio-stimulant EVL coating®, a mixture of several synergic microorganisms which was designed to be applied in combination with chemical fertilizers. The company sells directly the product, but also its license to be distributed by other companies or included in combination with their own products (Macouzet-Garcia, M., Scientific Director of EVL, personal communication). To end, Novozymes BioAg Limited commercializes several products in Canada which include *Bacillus amyloliquefaciens* combined with the fungus *Trichoderma virens*.

In the United States, many companies commercialize microbial biofertilizers, and their acceptance between farmers is increasing. One example is Custom Biologicals, Inc., a company dedicated to the manufacturing and distribution of microbial products for a variety of environmental and agricultural applications. Between others, they commercialize the following products:

Biota Max™, which is a soil probiotic that includes both beneficial fungal and bacterial soil strains with the innovative formulation of an effervescent tablet

Custom B5, another soil probiotic that includes five different species of plant growth-promoting bacteria belonging to the genus *Bacillus*, which is provided either as a liquid concentrate or on a tablet formulation

Custom N2, which contains the nitrogen-fixing bacterium *Paenibacillus polymyxa*, and it is sold as biofertilizer as well as a soil amendment

The firm Loveland Products developed Accomplish®, a biochemical fertilizer formulated with microbial strains in combination with organic acids, enzymes, and chelators and registered as an organic product by the WSDA (Washington State Department of Agriculture). The company indicates that this biofertilizer increases the accessibility of nutrients naturally present in the soil or provided with chemical fertilizers and improves root length and volume, so plants can better uptake nutrients and water. Experimental trials performed in 2010 by Loveland Products in cooperation with the University of Minnesota show higher yields in soybean and corn crops amended with this organic fertilizer. Launched as a new, retail division of reputable Reforestation Technologies International (RTI), Xtreme Gardening sells beneficial biological inoculants. Xtreme Gardening commercializes AZOS,

which contains 1.2×10^9 CFUs of nitrogen-fixing bacterium (*Azospirillum brasilense*) that also synthesizes the phytohormone IAA (indole-3-acetic-acid) – natural plant growth hormone that produces larger crop yields. Also, the American company, Plant Success, produces and distributes inoculants which combine mycorrhiza and bacteria, being remarkable because of the great number of microbial strains included in their formulations Great White®, containing 16 mycorrhizal species, 14 bacterial species, and 2 *Trichoderma* species, and Plant Success Soluble®, which combines 19 mycorrhizal species, bacterial species, and two *Trichoderma* species.

In Mexico, Biofabrica SXXI, an enterprise dedicated to the research, development, and production of biofertilizers, commercializes Azofer® and Rhizofer®. Rhizofer® contains 0.5×10^4 cells of *Rhizobium etli* per gram of product, and it is sold as biofertilizer for legume crops. Azofer® is elaborated with the bacterium *Azospirillum brasilense*, which fixes atmospheric nitrogen and produces phytohormones, stimulating the plant growth.

Cuba is principally an agrarian nation that continuously implements policies aiming more sustainable practices. Since 1991, scientific institutes develop microbial fertilizers. Presently, the corporation Labiofam S. A. manufactures Nitrofix®, a formulation based on *Azospirillum* able to fix nitrogen and produce phytohormones, which promotes sugar cane and other tropical crops growth.

As reported by the International Plant Nutrition Institute (IPNI), a global and nonprofit organization dedicated to the responsible management of plant nutrition, approximately 60,000 or 70,000 tons of biological fertilizers are sold yearly in Brazil and applied to maize, beans, sugarcane, rice, soybean, carrots, tomatoes, citrus, cotton, forage, and eucalyptus crops. Numerous big firms commercialize biofertilizers in the country. Some examples are the Instituto de Fosfato Biológico (IFB) Ltda., Embrafós Ltda., Liderfós Ltda. IBF, and Biofosfatos do Brasil Ltda. IBF manufactures a product named Bioativo®, which contains a mix of beneficial microorganisms that fix atmospheric nitrogen and solubilize phosphates, organic matter, and macro- and micro-essential nutrients and which patent was registered in the Brazilian Institute for Intellectual Property with the patent number PI-9401724-7.

Reports from the IPNI made public that every year countries in the South American Cone – Paraguay, Argentina, Uruguay, and Bolivia – seed more than 30 million hectares of soybean, being more than 70 percent of those inoculated with *Bradyrhizobium*-based inoculants. Additionally, maize and wheat plantations are occasionally inoculated with *Azospirillum* and *Pseudomonas* in South American countries (Abela & Valenzuela, 2007; Moreno-Sarmiento et al. 2007; Zúñiga, 2007).

In Europe, the biofertilizer sector is one of the most advanced in the planet, and the majority of governments in the EU have developed policies to reinforce its extension. Economic studies predict a value of the biological fertilizers market in Europe of more than four and a half thousand billion dollars by 2017. Symborg, a biofertilizer company under expansion, sells VitaSoil®, a mixture of microorganisms with 2.3×10^6 living cells per milliliter of product, specific for cereal

crops, horticultural and floral plants, citrus and other fruit trees, vineyards, and tobacco plantations. In Spain, the firm Biosym Technology S.L. manufactures and commercializes several microbial-based fertilizing products, which are also distributed by the company Agrogenia. Some of the bacterial biofertilizers they are selling are:

Rhizosum N, based on the nitrogen-fixing bacterium *Azotobacter vinelandii*
Rhizosum Aqua, based on a nitrogen-fixing strain of *Azospirillu*m
Rhizosum P, which contains a *Bacillus megaterium* phosphate solubilizer strain
Rhizosum K, with the potassium solubilizer *Frateuria aurantia*
Rhizosum Micros, a microbial mix solubilizing several micronutrients

In a personal communication, Emilio Marín, Managing Director of Biosym Technology S.L., explained how the company isolates microbial strains from many ecosystems and screens them for bioefficacy, selecting satisfactory ones. The selected candidate microbes have to be GRAS (generally considered as safe) and nonpathogenic to animals, plants, and human beings. The product is tested for presence of common human pathogens before packing for end use. In case of bacterial consortia based biofertilizers, each bacterium is produced separately, and spores/cells are blended with a medium to make the consortium. The final products are encapsulated in natural origin biopolymers.

In Russia, the firm JSC "Industrial Innovations" produces biofertilizers based on bacterial strains. This company produces Azotobacterin® is one of the most commonly applied bio-inoculants. This product contains the nitrogen-fixing bacterium *Azospirillum brasilense* B-4485, and studies performed by the enterprise report up to 20% increase in the yield of several crops as wheat, barley, maize, carrot, and cabbage, with the application of this inoculum.

Many Asiatic governments are making great efforts to promote a more sustainable agriculture, which is promoting the biofertilizer market. This market also relies on the expansion of the ecologic foodstuff industry. The economic prosperity in several countries of this continent has led to an escalation of the demand of ecological products. Despite this, the majority of farmers are restrained about making innovations in their traditional fertilization means.

In 2006, the FNCA Biofertilizer Project Group in Asia edited and published the Biofertilizer Manual (Group FBP 2006). In this publication, authors state that at that moment, only the Tokachi Federation of Agricultural Cooperatives (TFAC) was manufacturing and selling microbial-based fertilizers in Japan. In the TFAC's factory, three products were manufactured: Mamezo®, a peat substrate containing rhizobia; R-Processing Seeds®, consisting in legume seeds containing rhizobia; and Hyper Coating Seeds®, comprising legume grass seeds in a calcium carbonate pill enclosing rhizobial strains.

Annually, India invests roughly 1.5 billion dollars in biofertilizers and biopesticides. Organic agricultural crops occupy more than hundred thousand hectares in the country, and this area is in expansion every year; biofertilizers play a critical role in ecological farming, and therefore their application is likely going to increase, being already perceived a reduction in the use of chemical products. The

National Project on Development and use of Biofertilizers (NPDB) and six regional branches of the National Biofertilizer Development Centre were created by the Indian Administration in order to produce and distribute biofertilizers, develop quality standards and control products, train producers and farmers, and promote these eco-friendly fertilizers in the Indian agriculture. Biomax, one of the main suppliers of biological fertilizers in the world, is centered in India and sells various bio-inoculants containing microorganism, designed for a wide-ranging diversity of crops and with different plant-promoting capabilities such as nitrogen fixation or phosphate, iron, magnesium, and zinc solubilization (i.e., Life®, BioMix®, Biozink®, Biodine®). Other examples of large Indian biofertilizer manufacturing companies are Ajay Biotech Ltd., National Fertilizers Ltd., Madras Fertilizers Ltd., Gujarat State Fertilizers and Chemicals Ltd., T. Stanes and Company Ltd., Camson Bio Technologies Ltd., and Rashtriya Chemicals and Fertilizers Ltd.

In 1996, the Ministry of Agriculture in China launched regulations for the management and registration of biological fertilizers. Ten years after, in 2006, 511 products had been already registered. At the moment, few big companies produce and sell biofertilizers in the Chinese market. Possibly, the most important company of bacterial-based fertilizers is China Bio-Fertilizer AG. The company produces several inoculants based on phosphate- and potassium-solubilizing bacterial strains. The enterprise states that, according to their own research, their products allow the reduction of chemical fertilizers by 30% meanwhile the production increases up to 30%, varying with the different crops.

6.6 Conclusions and Future Perspectives

According to Cirera and Masset (2010), production of agricultural lands should be incremented in about a hundred percent during the present century to sustain the increasing human population in the planet. Half a century ago, the humanity was facing a similar problem, and the Green Revolution allowed a worldwide increasing of the crops yields, saving millions of people from starvation and malnutrition. Nevertheless, several studies have highlighted the enormous amount of problems associated with chemical fertilizers:

A huge demand of energy consumption for their synthesis – related with the depletion of natural resources and the contribution to the greenhouse effect and the climatic change

Atmosphere, soil, and water pollution – due to fertilizer decomposition and lixiviation processes

Several human health problems – as a result of chemical fertilizer rests in food

Therefore, one of the main challenges that humanity is facing nowadays is the development of a new *truly* Green Revolution capable to get us a productive and sustainable cultivation which guarantees food provision for the increasing human population in the world with the limited surface of agricultural land and protecting the planet and human health. Application of bacterial plant probiotics in crops might

be a possible solution to achieve those goals; nevertheless, much work is needed in order for this to become a reality, as the selection and adjustment of bacterial biofertilizers to efficiently promote the different agricultural crops in all the agronomic systems as well as the development of new technologies for the lifetime improvement of biofertilizers and political efforts to promote bacterial biofertilizer registration, production, distribution, and application in the fields.

References

Abela J and Valenzuela R (2007) Situación de los cultivos leguminosos y los Biofertilizantes en Bolivia. In: Izaguirre-Mayoral M, Labandera C, Sanjuan J (eds) Biofertilizantes en Iberoamérica: una visión técnica, científica y empresarial. CYTED, BIOFAG, Uruguay. pp 61–67

Abu Mweis SS, Jones PJ (2008) Cholesterol-lowering effect of plant sterols. Curr Artheroscl Rep 10(6):467–472

Ahemad M, Khan MS (2009a) Effect of insecticide-tolerant and plant growth-promoting *Mesorhizobium* on the performance of chickpea grown in insecticide stressed alluvial soils. J Crop Sci Biotechnol 12(4):217–226

Ahemad M, Khan MS (2009b) Toxicity assessment of herbicides quizalafop-p-ethyl and clodinafop towards *Rhizobium* pea symbiosis. Bull Environ Contam Toxicol 82(6):761–766

Ahemad M, Khan MS (2010a) Comparative toxicity of selected insecticides to pea plants and growth promotion in response to insecticide-tolerant and plant growth promoting *Rhizobium leguminosarum*. Crop Prot 29(4):325–329

Ahemad M, Khan MS (2010b) Ameliorative effects of *Mesorhizobium* sp. MRC4 on chickpea yield and yield components under different doses of herbicide stress. Pesticide Biochem Physiol 98(2):183–190

Ahemad M, Khan MS (2010c) Insecticide-tolerant and plant-growth-promoting Rhizobium improves the growth of lentil (*Lens esculentus*) in insecticide-stressed soils. Pest Manag Sci 67(4):423–429

Ahemad M, Khan MS (2010d) Growth promotion and protection of lentil (*Lens esculenta*) against herbicide stress by *Rhizobium* species. Ann Microbiol 60(4):735–745

Ahemad M, Khan MS (2010e) Improvement in the growth and symbiotic attributes of fungicide-stressed chickpea plants following plant growth promoting fungicide-tolerant *Mesorhizobium* inoculation. Afr J Basic Appl Sci 2(3–4):111–116

Ahemad M, Khan MS (2011a) Effect of tebuconazole-tolerant and plant growth promoting *Rhizobium* isolate MRP1 on pea–Rhizobium symbiosis. Sci Hortic 129(2):266–272

Ahemad M, Khan MS (2011b) Plant-Growth-Promoting fungicide-tolerant rhizobium improves growth and symbiotic characteristics of lentil (*Lens esculentus*) in fungicide-applied soil. J Plant Growth Regul 30(3):334–342

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26(1):1–20

Ahirwar NK, Gupta G, Singh V, Rawlley RK, Ramana S (2015) Influence on growth and fruit yield of tomato (*Lycopersicon esculentum* Mill.) plants by inoculation with *Pseudomonas fluorescence* (SS5): possible role of plant growth promotion. Int J Curr Microbiol App Sci 4:720–730

Alam F, Bhuiyan MA, Alan SS, Waghmode TR, Kim PJ, Lee YB (2015) Effect of *Rhizobium sp*. BARIRGm901 inoculation on nodulation, nitrogen fixation and yield of soybean (*Glycine max*) genotypes in gray terrace soil. Biosci Biotechnol Biochem 79:1660–1668

Albareda M, Rodríguez-Navarro DN, Camacho M, Temprano FJ (2008) Alternatives to peat as a carrier for rhizobia inoculants: solid and liquid formulations. Soil Biol Biochem 40(11):2771–2779

Ang J, Banerjee R and Madsen J (2010) Innovation, technological change and the British Agricultural Revolution. Centre for Applied Macroeconomic Analysis. The Australian National University, CAMA Working Paper 11/2010

Angus AA, Agapakis CM, Fong S, Yerrapragada S, Estrada-de los Santos P, Yang P, Song N, Kano S, Caballero-Mellado J, de Faria SM, Dakora FD, Weinstock G, Hirsch AM (2014) Plant-Associated symbiotic Burkholderia species lack hallmark strategies required in mammalian pathogenesis. PLoS ONE 9(1): e83779. doi:10.1371/journal.pone.0083779

Araus J, Li J, Parry M, Wang J (2014) Phenotyping and other breeding approaches for a New Green Revolution. J Integr Plant Biol 56:422–424

Arora NK, Tiwari S, Singh R (2014) Comparative study of different carriers inoculated with nodule forming and free living plant growth promoting bacteria suitable for sustainable agriculture. J Plant Pathol Microbiol 5:229

Banchio E, Bogino PC, Zygadlo J, Giordano W (2008) Plant growth promoting rhizobacteria improve growth and essential oil yield in *Origanum majorana* L. Biochem Syst Ecol 36(10):766–771

Bao Z, Sasaki K, Okubo T, Anda M, Hanzawa E, Kakizaki K, Sato T, Mitsui H, Minamisawa K (2013) Impact of *Azospirillum sp.* B510 inoculation on rice-associated bacterial communities in a paddy field. Microbes Environ 28:487–490

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16(4):729–770

Bashan Y, González LE (1999) Long-term survival of the plant-growth-promoting bacteria *Azospirillum brasilense* and *Pseudomonas fluorescens* in dry alginate inoculant. Appl Microbiol Biotechnol 51(2):262–266

Bashan Y, Hernandez JP, Leyva LA, Bacilio M (2002) Alginate microbeads as inoculant carriers for plant growth-promoting bacteria. Biol Fertil Soils 35(5):359–368

Bashan Y, de-Bashan LE, Prabhu SR, Hernandez JP (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant and Soil 378(1–2):1–33

Berg G, Eberl L, Hartmann A (2005) The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. Environ Microbiol. 7(11):1673–1685

Berg G (2009) Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Berg G, Alavi M, Schmid M, Hartmann A (2013) The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. Molecular Microbial Ecology of the Rhizosphere, De Bruijn, FJ (Ed.). Wiley, New York, USA., ISBN-13, 1477764743, 1209–1216

Bernabeu PR, Pistorio M, Torres-Tejerizo G, Estrada-De los Santos P, Galar ML, Boiardi JL, Luna MF (2015) Colonization and plant growth-promotion of tomato by *Burkholderia tropica*. Sci Hortic 191:113–120

Bharti N, Barnawal D, Wasnik K, Tewari SK, Kalra A (2016) Co-inoculation of *Dietzia natronolimnaea* and *Glomus intraradices* with vermicompost positively influences *Ocimum basilicum* growth and resident microbial community structure in salt affected low fertility soils. Appl Soil Ecol 100:211–225

Bona E, Lingua G, Manassero P, Cantamessa S, Marsano F, Todeschini V, Copetta A, D'Agostino G, Massa N, Avidano L, Gamalero E, Berta G (2015) AM fungi and PGP pseudomonads increase flowering, fruit production, and vitamin content in strawberry grown at low nitrogen and phosphorus levels. Mycorrhiza 25(3):181–193

Carpanetto D, Bianchini P (2011). Atlas histórico del mundo. Tikal-Susaeta Eds. Madrid, Spain. ISBN-13: 978-8499281247

Chatterjee IB (1973) Evolution and the biosynthesis of ascorbic acid. Science 182(4118):1271–1272

Chauhan H, Bagyaraj DJ, Selvakumar G, Sundaram SP (2015) Novel plant growth promoting rhizobacteria—prospects and potential. Appl Soil Ecol 95:38–53

Chiarini L, Bevivino A, Dalmastri C, Tabacchioni S, Visca P (2006) Burkholderia cepacia complex species: health hazards and biotechnological potential. Trends Microbiol, 14(6):277–286

Cirera X, Masset E. Income distribution trends and future food demand. (2010) Philosophical Transactions of the Royal Society B: Biological Sciences 365(1554):2821–34.
Conceição P, Levine S, Lipton M, Warren-Rodríguez A (2016) Toward a food secure future: ensuring food security for sustainable human development in Sub-Saharan Africa. Food Policy 60:1–9
Cong PT, Dunga TD, Hiena TM, Hien NT, Choudhury AT, Kecskés ML, Kennedy IR (2009) Inoculant plant growth-promoting microorganisms enhance utilisation of urea-N and grain yield of paddy rice in southern Vietnam. Eur J Soil Biol 45:52–61
Conway G, Barbie E (1988) After the Green Revolution: sustainable and equitable agricultural development. Futures 20:651–670
Corvalan D, Dubois M, Medana M, Perticari A, Racca R and Ruiz O (2007) Situación actual y perspectivas del mercado de semillas y biofertilizantes en la Argentina. In: Izaguirre-Mayoral M, Labandera C, Sanjuan J (eds) Biofertilizantes en Iberoamérica: una visión técnica, científica y empresarial. CYTED, BIOFAG, Uruguay. pp 61–67
Cuevas-Rodríguez EO, Yousef GG, García-Saucedo PA, López-Medina J, Paredes-López O, Lila MA (2010) Characterization of anthocyanins and proanthocyanidins in wild and domesticated Mexican blackberries (*Rubus* spp.) J Agric Food Chem 58(12):7458–7464
Dardanelli MS, Manyani H, González-Barroso S, Rodríguez-Carvajal MA, Gil-Serrano AM, Espuny MR, López-Baena FJ, Begollín RA, Megías M, Ollero FJ, Ollero FJ (2010) Effect of the presence of the plant growth promoting rhizobacterium (PGPR) *Chryseobacterium balustinum* Aur9 and salt stress in the pattern of flavonoids exuded by soybean roots. Plant and Soil 328(1–2):483–493
Deans SG, Svoboda KP (1990) The antimicrobial properties of marjoram (*Origanum majorana* L.) volatile oil. Flavour Fragr J 5(3):187–190
Díez-Méndez A, Menéndez E, García-Fraile P, Celador-Lera L, Rivas R, Mateos PF (2015) *Rhizobium cellulosilyticum* as a co-inoculant enhances *Phaseolus vulgaris* grain yield under greenhouse conditions. Symbiosis 67:1351–1357
DiMascio P, Aaiser S, Sies H (1989) Lycopene as the most effective biological carotenoid singlet oxygen quencher. Arch Biochem Biophys 274:532–538
Dorais M, Ehret DL, Papadopoulos AP (2008) Tomato (*Solanum lycopersicum*) health components: from the seed to the consumer. Phytochem Rev 7:231–250
Dudareva N, Negre F, Nagegowda DA, Orlova I (2006) Plant volatiles: recent advances and future perspectives. Crc Cr Rev Plant Sci 25(5):417–440
Dumas Y, Dadomo M, Di Lucca G, Grolier P (2003) Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. J Agric Food Chem 83(5):369–382
Dursun A, Ekinci M, Dönmez MF (2010) Effects of foliar application of plant growth promoting bacterium on chemical contents, yield and growth of tomato (*Lycopersicon esculentum* L.) and cucumber (*Cucumis sativus* L.) Pak J Bot 42:3349–3356
Egamberdiyeva D (2007) The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. Appl Soil Ecol 36:184–189
Estrada-de Los Santos P, Vinuesa P, Martínez-Aguilar L, Hirsch AM, Caballero-Mellado J (2013) Phylogenetic analysis of Burkholderia species by multilocus sequence analysis. Curr Microbiol. 67:51–60
Estrada-de los Santos P, Rojas-Rojas FU, Tapia-García EY, Vásquez-Murrieta MS & Hirsch AM (2016) To split or not to split: an opinion on dividing the genus Burkholderia. Ann Microbiol. 66:1303–1314
FAOSTAT (2007) United Nations Food and Agriculture Organization, Rome. FAOSTAT. http://faostat.fao.org/default.aspx
Ferreira AS, Pires RR, Rabelo PG, Oliveira RC, Luz JMQ, Brito CH (2013) Implications of *Azospirillum brasilense* inoculation and nutrient addition on maize in soils of the Brazilian Cerrado under greenhouse and field conditions. App Soil Ecol 72:103–108
Flores-Félix JD, Ménendez E, Rivera LP, Marcos-García M, Martínez-Molina P, Mateos PF, Velázquez E, García-Fraile P, Rivas R (2013) Use of Rhizobium leguminosarum as a potential biofertilizer for *Lactuca sativa* and *Daucus carota* crops. J Plant Nutr Soil Sci 000:1–7
Flores-Félix JD, Silva LR, Rivera LP, Marcos-García M, García-Fraile P, Martínez-Molina E, Mateos PF, Velázquez E, Andrade P, Rivas R (2015) Plants probiotics as a tool to produce

highly functional fruits: the case of *Phyllobacterium* and vitamin C in strawberries. PLoS One 10(4):e0122281
Garau G, Yates RJ, Deiana P, Howieson JG (2009) Novel strains of nodulating Burkholderia have a role in nitrogen fixation with papilionoid herbaceous legumes adapted to acid, infertile soils. Soil Biol Biochem, 41(1):125–134
García-Fraile P, Robledo M, Ramírez-Bahena M-H, Flores-Félix JD, Fernández MT, Mateos PF, Rivas R, Igual JM, Martínez-Molina E, Peix Á, Velázquez E (2012) Rhizobium promotes non-legumes growth and quality in several production steps: towards a biofertilization of edible raw vegetables healthy for humans. PLoS One 7(5):e38122
García-Fraile P, Menéndez E, Rivas R (2015) Role of bacterial biofertilizers in agriculture and forestry. AIMS Bioeng 2(3):183–205
Garcia-Seco D, Zhang Y, Gutierrez-Mañero FJ, Martin C, Ramos-Solano B (2015) Application of *Pseudomonas fluorescens* to blackberry under field conditions improves fruit quality by modifying flavonoid metabolism. PLoS One 10(11):e0142639
Gerster H (1997) The potential role of lycopene for human health. J Am Coll Nutr 16(2):109–126
Giovannucci E (1999) Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. J Natl Cancer I 91(4):317–331
Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA, Willett WC (1995) Intake of carotenoids and retino in relation to risk of prostate cancer. J Natl Cancer I 87(23):1767–1776
Gomez M, Barrett C, Raney T et al (2013) Post-green revolution food systems and the triple burden of malnutrition. Food Policy 42:129–138
Group FBP, editor (2006) Biofertilizer Manual. Tokyo, Japan: Japan Atomic Industrial Forum.
Gupta AK, Savopoulos CG, Ahuja J, Hatzitolios AI (2011) Role of phytosterols in lipid-lowering: current perspectives. QJM 104(4):301–308
Hale L, Luth M, Kenney R, Crowley D (2014) Evaluation of pinewood biochar as a carrier of bacterial strain *Enterobacter cloacae* UW5 for soil inoculation. Appl Soil Ecol 84:192–199
Hale L, Luth M, Crowley D (2015) Biochar characteristics relate to its utility as an alternative soil inoculum carrier to peat and vermiculite. Soil Biol Biochem 81:228–235
Hanson AD, Gregory IIIJF (2011) Folate biosynthesis, turnover, and transport in plants. Annu Rev Plant Biol 62:105–125
Hassimotto N, Lajolo FM (2011) Antioxidant status in rats after long-term intake of anthocyanins and ellagitannins from blackberries. J Agric Food Chem 91(3):523–531
Herridge DF, Peoples MB (1990) Ureide assay for measuring nitrogen fixation by nodulated soybean calibrated by 15N methods. Plant Physiol 93:495–503
Herrmann L, Lesueur D (2013) Challenges of formulation and quality of biofertilizers for successful inoculation. Appl Microbiol Biotechnol 97(20):8859–8873
Hummer KE (2010) Rubus pharmacology: antiquity to the present. Hort Sci 45(11):1587–1591
Hungria M and Campo R (2007) Inoculantes Microbianos: Situação no Brasil. In: Izaguirre-Mayoral M, Labandera C, Sanjuan J (eds) Biofertilizantes en Iberoamérica: una visión técnica, científica y empresarial. Una Denad Internacional, S.A. Montevideo, Uruguay
Hungria M, Nogueira MA, Araujo RS (2013) Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. Biol Fertil Soils 49:791–801
Imran A, Mirza MS, Shah TM, Malik KA, Hafeez FY (2015) Differential response of kabuli and desi chickpea genotypes toward inoculation with PGPR in different soils. Front Microbiol 6:895
Isawa T, Yasuda M, Awazaki H, Minamisawa K, Shinozaki S, Nakashita H (2010) *Azospirillum* sp. strain B510 enhances rice growth and yield. Microbes Environ 25:58–61
Isfahani FM, Besharati H (2012) Effect of biofertilizers on yield and yield components of cucumber. J Biol Earth Sci 2:83–92
Joshi P, Rayalu S, Bansiwal A, Juwarkar AA (2007) Surface modified zeolite, a novel carrier material for *Azotobacter chroococcum*. Plant and Soil 296(1–2):151–158
Kamilova F, de Bruyne R (2013) Plant growth promoting microorganisms: the road from an academically promising result to a commercial product. In: de Bruijn FJ (ed) Molecular microbial ecology of the rhizosphere, vol 1-2. Wiley, Hoboken, NJ, pp 677–686

Kamilova F, Okon Y, de Weert S, Hora K (2015) Commercialization of microbes: manufacturing, inoculation, best practice for objective field testing and registration. In Principles of plant-microbe interactions, Springer I. P. 319-327

Khush G (2001) Green revolution: the way forward. Nat Rev Genet 2:815–822

Köhler HR, Triebskorn R (2013) Wildlife ecotoxicology of pesticides: can we track effects to the population level and beyond?. Science, 341(6147):759–765

Kumar M, Mishra S, Dixit V, Agarwal L, Chauhan PS, Nautiyal CS (2016) Synergistic effect of Pseudomonas putida and Bacillus amyloliquefaciens ameliorates drought stress in chickpea (*Cicer arietinum* L.) Plant Sign Behave 11(1)

Lattanzio V (2013) Phenolic compounds: introduction. In: Natural Products. Springer, Berlin Heidelberg, pp 1543–1580

Maheshwari DK, Dubey RC, Agarwal M, Dheeman S, Aeron A, Bajpai VK (2015) Carrier based formulations of biocoenotic consortia of disease suppressive *Pseudomonas aeruginosa* KRP1 and *Bacillus licheniformis* KRB1. Ecol Eng 81:272–277

Malboobi MA, Behbahani M, Madani H, Owlia P, Deljou A, Yakhchali B, Moradi M, Hassanabadi H (2009) Performance evaluation of potent phosphate solubilizing bacteria in potato rhizosphere. World J Microbiol Biotechnol 25:1479–1484

Malusá E, Vassilev N (2014) A contribution to set a legal framework for biofertilisers. Appl Microbiol Biotechnol 98:6599–6607

Mandal SM, Chakraborty D, Dey S (2010) Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signal Behav 5(4):359–368

Mansotra P, Sharma P, Sharma S (2015) Bioaugmentation of Mesorhizobium cicer, Pseudomonas spp. and Piriformospora indica for Sustainable Chickpea Production. Physiology and Molecular Biology of Plants 21(3):385–393

Marin-Loaiza J C and Cespedes C L (2007) Volatile compounds from plants. Origin, emission, effects, analysis and agro applications

Martin C, Zhang Y, Tonelli C, Petroni K (2013) Plants, diet, and health. Annu Rev Plant Biol 64:19–46

Martínez-Hidalgo P, Galindo-Villardón P, Trujillo ME, Igual JM, Martínez-Molina E (2014a) Micromonospora from nitrogen-fixing nodules of alfalfa (Medicago sativa L.). A new promising plant probiotic bacteria. Sci Rep. 4:6389. DOI:10.1038/srep06389

Martínez-Hidalgo P, Olivares J, Delgado A, Bedmar E, Martínez-Molina E (2014b) Endophytic Micromonospora from Medicago sativa are apparently not able to fix atmospheric nitrogen. Soil Biol Biochem. 74:201–203

Martínez-Hidalgo P, García JM, Pozo MJ (2015) Induced systemic resistance against Botrytis cinerea by Micromonospora strains isolated from root nodules. Front Microbiol. 6:922. doi:10.3389/fmicb.2015.00922

Moreno-Gomez B, Rascon-Cruz Q and Aguado-Santacruz G (2012) Manejo y Calidad de los Biofertilizantes. In Aguado-Santacruz G (ed). Introducción al Uso y Manejo de los Biofertilizantes en la Agricultura. INIFAP/SAGARPA, México. pp 115–150

Moreno-Sarmiento N, Moreno-Rodríguez L and Uribe-Vélez D (2007) Biofertilizantes para la Agricultura en Colombia. In: Izaguirre-Mayoral M, Labandera C, Sanjuan J (eds) Biofertilizantes en Iberoamérica: una visión técnica, científica y empresarial. CYTED, BIOFAG, Uruguay. pp 38–45

Moulin L, Munive A, Dreyfus B, Boivin-Masson C (2001) Nodulation of legumes by members of the ß-subclass of Proteobacteria. Lett Nature. 411:948–950

Mulas D, García-Fraile P, Carro L, Ramírez-Bahena M-H, Casquero P, Velázquez E, Gonzalez-Andrés F (2011) Distribution and efficiency of Rhizobium leguminosarum strains nodulating Phaseolus vulgaris in Northern Spanish soils: Selection of native strains that replace conventional N fertilization. Soil Biol Biochem 43:2283–2293

Nishikimi M, Fukuyama R, Minoshima S, Shimizu N, Yagi K (1994) Cloning and chromosomal mapping of the human nonfunctional gene for L-gulono-gamma-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man. J Biol Chem 269(18):13685–13688

Ordookhani K, Khavazi K, Moezzi A, Rejali F (2010) Influence of PGPR and AMF on antioxidant activity, lycopene and potassium contents in tomato. Afr J Agric Res 5(10):1108–1116
Peng J, Richards D, Hartley N, Murphy G et al (1999) 'Green revolution' genes encode mutant gibberellin response modulators. Nature 400:256–261
Piironen V, Lindsay DG, Miettinen TA, Toivo J, Lampi AM (2000) Plant sterols: biosynthesis, biological function and their importance to human nutrition. J Agric Food Chem 80(7):939–966
Pingali P (2012) Green revolution: impacts, limits, and the path ahead. Proc Natl Acad Sci U S A 109:12302–12308
Prakamhang J, Tittabutr P, Boonkerd N, Teamtisong K, Uchiumi T, Abe M, Teaumroong N (2015) Proposed some interactions at molecular level of PGPR co-inoculated with *Bradyrhizobium diazoefficiens* USDA110 and *B. japonicum* THA6 on soybean symbiosis and its potential of field application. Appl Soil Ecol 85:38–49
Ramakrishnan K, Selvakumar G (2012) Effect of biofertilizers on enhancement of growth and yield on tomato (*Lycopersicum esculentum* Mill.) Int J Res Bot 2:20–23
Rao AV, Agarwal S (1998) Bioavailability and in vivo antioxidant properties of lycopene from tomato products and their possible role in the prevention of cancer. Nutr Cancer 31(3):199–203
Rao AV, Waseem Z, Agarwal S (1998) Lycopene content of tomatoes and tomato products and their contribution to dietary lycopene. Food Res Int 31(10):737–741
Rodrigues AC, Vendruscolo CT, da Silveira Moreira A, Santana MVS, de Paula Oliveira JE, Bonifacio A, Figueiredo MADVB (2015) *Rhizobium tropici* exopolysaccharides as carriers improve the symbiosis of cowpea-*Bradyrhizobium-Paenibacillus*. Afr J Microbiol Res 9(37):2037–2050
Romano S, Aujoulat F, Jumas-Bilak E, Masnou A, Jeannot JL, Falsen E, Teyssier C (2009) Multilocus sequence typing supports the hypothesis that Ochrobactrum anthropi displays a human-associated subpopulation. BMC Microbiol, 9(1):1
Sangwan NS, Farooqi AHA, Shabih F, Sangwan RS (2001) Regulation of essential oil production in plants. Plant Growth Regul 34(1):3–21
Santoro MV, Zygadlo J, Giordano W, Banchio E (2011) Volatile organic compounds from rhizobacteria increase biosynthesis of essential oils and growth parameters in peppermint (*Mentha piperita*). Plant Physiol Biochem 49(10):1177–1182
Sayer J, Cassman K (2013) Agricultural innovation to protect the environment. Proc Natl Acad Sci U S A 110:8345–8348
Schoebitz M, López MD, Roldán A (2013) Bioencapsulation of microbial inoculants for better soil–plant fertilization. A review. Agron Sustain Dev 33(4):751–765
Selvakumar G, Panneerselvam P, Ganeshamurthy AN (2014) Biosafety of novel bioinoculants. J Biofertil Biopestici. 5(2):1000154. DOI: 10.4172/2155-6202.1000145
Shahzad SM, Arif MS, Riaz M, Iqbal Z, Ashraf M (2013) PGPR with varied ACC-deaminase activity induced different growth and yield response in maize (*Zea mays* L.) under fertilized conditions. Eur J Soil Biol 57:27–34
Silva LR, Azevedo J, Pereira MJ, Carro L, Velazquez E, Peix A, Valentao P, Andrade PB (2014) Inoculation of the nonlegume *Capsicum annuum* L. with *Rhizobium* Strains. II. Changes in sterols, triterpenes, fatty acids, and volatile compounds. J Agric Food Chem 62(3):565–573
Skorov G (1973) The green revolution and social progress. World Dev 1:13–21
Soby S (2013) The end of the green revolution. J Agr Environ Ethic 26:537–546
Somasegaran P, Hoben HJ (1994) Handbook for Rhizobia, Methods in legume-Rhizobium technology. Springer, New York
Song X, Liu M, Wu D, Griffiths BS, Jiao J, Li H, Hu F (2015) Interaction matters: synergy between vermicompost and PGPR agents improves soil quality, crop quality and crop yield in the field. Appl Soil Ecol 89:25–34
Stahl W, Sies H (1996) Lycopene: a biologically important carotenoid for humans? Arch Biochem Biophys 336(1):1–9
Stephens JHG, Rask HM (2000) Inoculant production and formulation. Field Crops Res, 65(2): 249–258

Suh JS, Jiarong P, Toal PV (2006) Quality control of biofertilizers. Biofert Manual:112–115
Suman A, Verma P, Yadav AN, Srinivasamurthy R, Singh A, Prasanna R (2016) Development of hydrogel based bio-inoculant formulations and their impact on plant biometric parameters of wheat (*Triticum aestivum* L.) Int J Curr Microbiol App Sci 5(3):890–901
Sun D, Hale L, Crowley D (2016) Nutrient supplementation of pinewood biochar for use as a bacterial inoculum carrier. Biol Fertil Soils 52(4):515–522
Tahara S (2007) A journey of twenty-five years through the ecological biochemistry of flavonoids. Biosci Biotech Bioch 71(6):1387–1404
Toor RK, Savage GP (2005) Antioxidant activity in different fractions of tomatoes. Food Res Int 38(5):487–494
Trienekens J, Zuurbier P (2008) Quality and safety standards in the food industry, developments and challenges. Int J Prod Econ 113(1):107–122
Tripathi S, Das A, Chandra A, Varma A (2015) Development of carrier-based formulation of root endophyte *Piriformospora* indica and its evaluation on *Phaseolus vulgaris* L. World J Microbiol Biotechnol 31(2):337–344
Trujillo ME, Willems A, Abril A, Planchuelo AM, Rivas R, Ludena D, Mateos PF, Martínez-Molina E, Velazquez E (2005) Nodulation of Lupinus albus by strains of Ochrobactrum lupini sp. nov. Appl Environ Microbiol. 71:1318–1327
Turan M, Ekinci M, Yildirim E, Günes A, Karagöz K, Kotan R, Dursun A (2014) Plant growth-promoting rhizobacteria improved growth, nutrient, and hormone content of cabbage (*Brassica oleracea*) seedlings. Turkish J Agric Forestry 38(3):327–333
Vander Gheynst JS, Scher H, Guo HY (2006) Design of formulations for improved biological control agent viability and sequestration during storage. Ind Biotech 2(3):213–219
Vassilev NM, Vassileva M, Lopez A, Martos V, Reyes A, Maksimovic I, Eichler-Löbermann B, Malusà E (2015) Unexploited potential of some biotechnological techniques for biofertilizer production and formulation. Appl Microbiol Biotechnol 99:4983–4996
Vílchez JI, Navas A, González-López J, Arcos SC, Manzanera M (2016) Biosafety Test for Plant Growth-Promoting Bacteria: Proposed Environmental and Human Safety Index (EHSI) Protocol. Front Microbiol 6:1514. DOI: 10.3389/fmicb.2015.01514
Wani P, Khan M, Zaidi A (2007) Co-inoculation of nitrogen-fixing and phosphate-solubilizing bacteria to promote growth, yield and nutrient uptake in chickpea. Acta Agronomica Hungarica 55(3):315–323
Wani PA, Khan MS. Bacillus species enhance growth parameters of chickpea (Cicer arietinum L.) in chromium stressed soils. (2010). Food Chem Toxicol 48:3262–3267
Zachow C, Berg C, Müller H, Meincke R, Komon-Zelazowska M, Druzhinina IS, Berg G (2009) Fungal diversity in the rhizosphere of endemic plant species of Tenerife (Canary Islands): relationship to vegetation zones and environmental factors. ISME J, 3(1):79–92
Zahir ZA, Shah MK, Naveed M, Akhter MJ (2010) Substrate-dependent auxin production by *Rhizobium phaseoli* improves the growth and yield of *Vigna radiata* L. under salt stress conditions. J Microbiol Biotechnol 20(9):1288–1294
Zaidi A, Ahmad E, Khan MS, Saif S, Rizvi A (2015) Role of plant growth promoting rhizobacteria in sustainable production of vegetales: current perspective. Sci Hortic 193:231–239
Zanden J (1991) The first green revolution: the growth of production and productivity in European agriculture, 1870-1914. Econ Hist Rev 44:215–239
Zúñiga D (2007) Leguminosas y producción de biofertilizantes en el Perú. In: Izaguirre-Mayoral M, Labandera C, Sanjuan J (eds) Biofertilizantes en Iberoamérica: una visión técnica, científica y empresarial. CYTED, BIOFAG, Uruguay. pp 61–67
Zurdo-Piñeiro JL, Rivas R, Trujillo ME, Vizcaíno N, Carrasco JA, Chamber M, Palomares A, Mateos PF, Martínez-Molina E, Velázquez E (2007) Ochrobactrum cytisi sp. nov., isolated from nodules of Cytisus scoparius in Spain. Int J Syst Evol Microbiol. 57:784–788

Plant-Microbe Interactions in Adaptation of Agricultural Crops to Abiotic Stress Conditions

7

Hassan Etesami and Gwyn A. Beattie

Abstract

Abiotic stresses are an increasing challenge to crop production all over the world. These stresses include high and low temperatures, salinity, flooding, drought, nutrient limitation, and toxic metals and organic pollutants. The costs associated with abiotic stresses are potentially enormous, indicating a need for sound, affordable, environmentally friendly approaches to decrease the adverse effects of these stresses on plants. Unlike animals, plants cannot use avoidance and escape as mechanisms of stress tolerance; consequently, their evolution is marked by the development of highly beneficial interactions with their more mobile companions, microbes. Some of these interactions involve highly sophisticated symbioses that confer stress tolerance, such as with mycorrhizae and rhizobia that help ameliorate nutritional and water deficiencies, while others are more transitory. The agricultural application of beneficial microorganisms is increasingly of widespread interest, with many research programs evaluating microbial strains for their ability to provide protection against a single stress, such as phosphate limitation and cross-protection against multiple stresses. Knowledge of the underlying physiological mechanisms by which diverse microbes mediate stress tolerance, including cross-protection, is critical to the effective use of these microbes to assure sustained agricultural production in changing environmental conditions. Here we provide an overview of current knowledge on the physiological impacts and modes of action of microbial mitigation of abiotic stress symptoms in plants. We indicate further research avenues to enable better use of the protection capabilities of root-colonizing beneficial microbes in agricultural

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production systems affected by a changing climate. As a complement to previous reviews summarizing the mechanisms of resistance to biotic stresses, this review will focus on the mechanisms underlying microbially mediated abiotic stress tolerance, especially tolerance conferred by plant growth-promoting rhizobacteria (PGPRs).

7.1 Introduction

Food security is a fundamental need for society and one that is being threatened by an exponentially increasing global population, unsustainable agricultural practices, and a changing global climate. Developing countries in Africa and Asia account for approximately 80% of the projected population growth, and, with an estimated 800 million people in these countries already undernourished, the Food and Agriculture Organization (FAO) of the United Nations predicts that a 60% increase in world food production over the next two decades is required to sustain these populations (Jewell et al. 2010). This will require a significant increase in grain yields of major crop plants, including rice, wheat, and maize (Godfray et al. 2010). Climate change has exacerbated the frequency and severity of droughts, floods, and high temperatures, causing yield reductions in our major cereal crops (Carmen and Roberto 2011; David and Christopher 2007), and decreased predictability of rainfall in many parts of the world (Jewell et al. 2010).

The pressure to increase agricultural output has increased cultivation on marginal lands and accelerated the rate of land degradation. For example, irrigation has led to salinity across large tracts of agricultural land (Shahbaz and Ashraf 2013), as illustrated by the threat of dry land salinity in almost 32 million hectares in Iran. Similarly, the widespread and intense application of fertilizers has incurred environmental costs in the form of nitrate contamination of groundwater, greenhouse gas (GHG) production associated with industrial nitrogen fixation, GHG production due to microbial-mediated denitrification, and depletion of global phosphorus reserves. The need for fertilizers to achieve higher yields has also contributed to rising costs for farmers worldwide. Our challenge for achieving global food security in the upcoming decades is to increase yields in a sustainable manner; that is, growers need access to a portfolio of production practices that collectively enable sufficiently high yields and profits to meet global food demand, but with sufficiently low environmental costs as to ensure sustainability (Glick 2012, 2014).

Abiotic stress conditions are among the most important constraints to global agricultural production (Jewell et al. 2010; Shrivastava and Kumar 2015), with losses due to abiotic stress estimated at 70% worldwide (Acquaah 2007). Plant-associated microorganisms are increasingly recognized for their potential contributions to enhancing crop growth, crop nutrition, and crop tolerance to abiotic stress (Fig. 7.1).

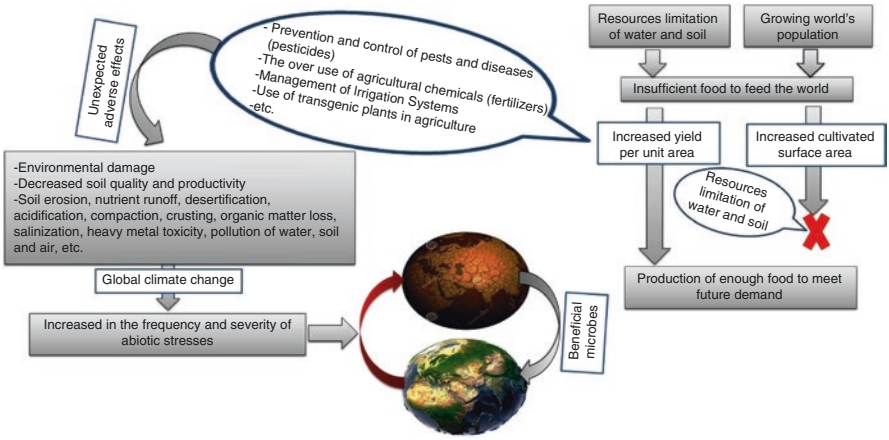


Fig. 7.1 Role of beneficial microbes in sustainable agriculture and environmental health (converting drought earth to green one)

A growing body of research is expanding our understanding of how plant growth-promoting microorganisms (PGPMs) enable agricultural plants to maintain productivity under stressed conditions (Dimkpa et al. 2009; Grover et al. 2010; Porcel et al. 2011; Shrivastava and Kumar 2015). Knowledge of these mechanisms has increased dramatically in the past 15–20 years (Glick 2012) and may serve as the basis for new strategies for selecting or engineering crop plants for an increased ability to cope with climate change-induced stresses (Grover et al. 2010). This chapter will focus on the role of PGPMs in the adaptation of agricultural crops to abiotic stress conditions and will outline the major yield-limiting abiotic stresses faced by crop plants: drought, salinity, nutrient deficiency, temperature, and metal toxicity. For each stress, we will highlight mechanisms by which PGPMs enhance plant tolerance to the stress, if known, and will focus especially on stresses associated with climate change.

7.2 Abiotic Stresses

Stress may be defined as any condition negatively affecting a living organism. Abiotic stresses originate from the surrounding environment, such as in the form of a physical or chemical condition that hinders plant growth, whereas biotic stresses are caused by living organisms, such as those that cause disease or damage. Abiotic stresses encountered by agricultural plants can broadly include low water availability in the form of salinity or drought, flooding, high and low temperatures, high light or ozone, anoxic conditions, high or low pH, nutrient deficiency, and exposure to detrimental chemicals including some agricultural inputs, toxic metals, and organic pollutants.

7.2.1 Effect of Abiotic Stresses on Plants

Abiotic stresses can cause deleterious effects in almost all phenological plant stages. They can cause changes in biochemistry, morphology, and physiology that adversely affect plant growth and productivity (Paul 2012; Wang et al. 2001). These are illustrated by the ability of various stresses to disrupt metabolism, promote membrane disorganization, generate reactive oxygen species, inhibit photosynthesis, reduce the potential for nutrient uptake, and alter hormone levels (Hasegawa et al. 2000). The following section focuses on some of the most serious abiotic stresses limiting the productivity of agricultural crops.

7.2.1.1 Salinity Stress

Soil salinity is one of the most devastating environmental factors limiting the productivity and quality of crop plants in the semiarid areas of the world (Allakhverdiev et al. 2000; Dodd and Perez-Alfocea 2012; Jamil et al. 2011; Ondrasek et al. 2009; Paul 2012; Paul and Lade 2014; Ramadoss et al. 2013a; Shahbaz and Ashraf 2013; Shanker and Venkateswarlu 2011; Yamaguchi and Blumwald 2005). It is responsible for major reductions in cultivated land area, particularly in areas where rising sea levels are promoting encroachment into agricultural lands, and is affecting extensive areas of land in both developed and developing countries. Soils are considered to have high salinity if their electrical conductivity (EC) at saturation is above 4 dS m^{-1} (America 2001), that is, above about 40 mM NaCl (Munns and Tester 2008). This salinity can result from the combined processes of irrigation, fertilization, and desertification (Munns and Tester 2008). The FAO reported that more than 1 billion hectares of land throughout the world have been affected by salinity (Ahmad 2013; FAO 2008). Because of global climate change, this area is increasing (Shrivastava and Kumar 2015) and is estimated to exceed 50% of the arable land by the year 2050 (Jamil et al. 2011).

Salinity has a broad range of negative effects on plant growth. It can reduce germination, plant vigor, and crop yield for many crops, from cereals to horticultural species (Munns and Tester 2008). It has a particularly negative effect on biological nitrogen fixation, as it can reduce nodulation, nitrogen fixation, and the total nitrogen content in legumes (van Hoorn et al. 2001; Mensah and Ihenyen 2009; Rabie and Almadini 2005; Egamberdieva et al. 2013; Paul and Lade 2014). This is consistent with the finding that symbiotic nitrogen fixation is among the most sensitive plant processes to water deficits, with sensitivity occurring during both the formation of symbiotic nodules and the subsequent period of nitrogen fixation and plant nitrogen uptake (Sinclair et al. 2010). Salinity also reduces the photosynthetic capacity of plants, due at least in part to the partial closure of stomata (Meloni et al. 2003) and can reduce protein synthesis and lipid metabolism (Parida and Das 2005).

Almost all micro- and macronutrients decrease in plant roots and shoots with increasing salinity of the growth medium (Paul and Lade 2014). The high Na^+ and Cl^- content on the roots affects the activity of the uptake systems and alters competitive interactions among ions for binding and transport into root cells (Tester and Davenport 2003); this can affect the uptake of nutrients as well as water (Paul and

Lade 2014). The accumulation of Na^+ and Cl^- ions may cause metabolic disturbances, with buildup in the intercellular spaces leading to cell dehydration and death. The dehydration that accompanies high salinity is associated with oxidative stress (Hichem et al. 2010; Johnson et al. 2003) and thus can involve damage to membranes and other macromolecules by ROS (Carmen and Roberto 2011; Pitzschke et al. 2006; Porcel et al. 2011). ROS-induced cellular damage is associated with drought and heat stress as well as salinity (Zhu 2001a), with all potentially promoting protein denaturation (Smirnoff 1998) and activating similar cell signaling pathways (Knight 2000; Shinozaki and Yamaguchi-Shinozaki 2000; Zhu 2001b; Zhu 2002) and responses, including the accumulation of stress proteins, antioxidants, and compatible solutes (Cushman and Bohnert 2000; Pitzschke et al. 2006; Wang et al. 2003).

In general, saline conditions inhibit plant growth through two phases. During the first phase, inhibition is mainly achieved by the decreased water availability due to higher solute concentration of the soil solution. Salt stress increases external osmotic pressure, which decreases leaf water potential and turgor, and ultimately causes stomatal closure. If the salt stress is prolonged, the second, salt-specific phase sets in, and ion toxicity is the main factor that constrains plant metabolism and survival (Chen et al. 2007; Munns 2002; Munns 2005; Munns and Tester 2008; Pandolfi et al. 2012; Sanchez et al. 2008; Tester and Davenport 2003).

7.2.1.2 Drought Stress

By some estimates, drought is the most significant environmental stress impacting global agricultural production (Cattivelli et al. 2008; Kijne 2006; Tuberosa and Salvi 2006). Approximately, 60% of all crops produced in developing countries are grown without irrigation (FAO 2009b), indicating that the majority of crops are vulnerable to drought. Agriculture accounts for approximately 70% of global water use, and irrigation account for up to 90% of total water withdrawals in arid nations (Council 2008; FAO 2009a). The water withdrawal requirement for irrigation is expected to increase by another 14% in developing countries by 2030, with an increase of 10% for every 1 °C increase in temperature in arid and semiarid regions (Grover et al. 2010); these statistics illustrate how strategies to decrease water demands for agriculture will be critical.

Similar to salinity stress, soil water deficits can reduce the photosynthetic capacity, increase photorespiration, and cause increased ROS production (Miller et al. 2010). On a whole-plant level, water-stressed plants wilt and are unable to sequester assimilates into the appropriate plant organs. Severe drought conditions result in reduced yield and plant death (Jewell et al. 2010).

7.2.1.3 Salinity-Induced Nutrient Deficiencies

Crop performance may be adversely affected by salinity-induced nutrient deficiencies. Nitrogen (N) accounts for about 80% of the total mineral nutrients absorbed by plants, and inadequate N is often a growth-limiting nutritional stress (Marschner 1995). Salinity reduces N uptake and accumulation (Feigin 1985). Salinity also reduces phosphorus (P) uptake and accumulation (Paul and Lade 2014); this effect

is due to a reduction in P availability due to the formation of calcium phosphate precipitates (Navarro et al. 2001; Parida and Das 2005; Rogers et al. 2003). The maintenance of adequate levels of potassium (K) is also jeopardized by salinity, with sodium-induced K deficiency implicated in various crops (Botella et al. 1997).

7.2.1.4 Temperature Stress

Global climate change is predicted to result in increases in the air temperature on Earth's surface by 3–5 °C in the coming 50–100 years (Polle and Luo 2014), with a consequent increase in frequency and intensity of drought and heat waves (Hansen et al. 2012). High soil temperatures in tropical and subtropical areas are already a major problem for crop production. Higher temperatures influence photosynthetic rate, plant water relations, flowering, and fruit set. In some regions, low temperatures limit the productivity and areas of cultivation of agricultural crops (Grover et al. 2010).

7.2.1.5 Pollutant Stress

Heavy metals are natural constituents of the environment, but due to indiscriminate use, heavy metal contamination has become a serious problem worldwide, including in some agricultural regions (Luo et al. 2011). All heavy metals are toxic to plants when present in high soil concentrations (Riesen and Feller 2005). High concentrations of heavy metals can decrease nutrient uptake, inhibit various enzymatic activities, induce oxidative stress, inhibit root and shoot growth, and lower yields (Athar and Ahmad 2002; Sandalio et al. 2001). Heavy metals can also be accumulated by agriculturally important crops; their entry into the food chain can negatively impact animal and human health (Sanità di Toppi and Gabbrielli 1999). In addition to heavy metals, many organic contaminants can be present in soil (Chen et al. 2015) and impact plant health.

7.3 Response of Plants to Stresses

Plants can generally tolerate moderate and transient exposure to environmental stresses, with severe stress sometimes causing extreme responses, including inhibiting flowering and seed formation and inducing senescence or plant death. Among the general responses of plants to stress (Fig. 7.2), a primary response is typically modification of gene expression to promote adaptation to a specific stress (Bhatnagar-Mathur et al. 2008).

An example of this is the induction of genes that promote the synthesis and accumulation of compatible solutes in response to soil water deficits. Other examples are the activation of ROS scavenging systems, transporter systems, and proteins involved in plant hormone regulation (Des Marais and Juenger 2010; Hiz et al. 2014; Parida and Das 2005; Santner et al. 2009). Plant hormone signaling pathways may be involved in biotic and abiotic stress responses (Egamberdieva et al. 2015; Khan and Khan 2013; Li et al. 2012; Shabani et al. 2009). Among the most extensively studied hormonal pathways, including the methyl jasmonate, salicylic acid,

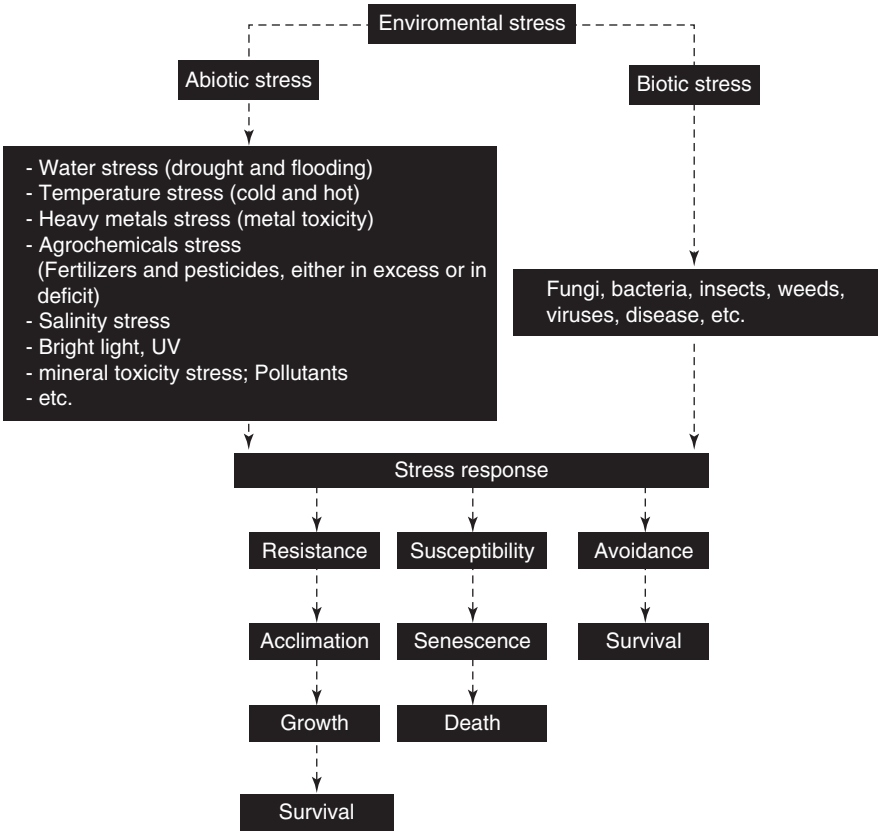


Fig. 7.2 The effect of environmental stresses on plant survival (Hopkins and Huner 2009)

and ethylene pathways (Egamberdieva et al. 2015; Khan and Khan 2013; Li et al. 2012; Shabani et al. 2009), only ethylene will be discussed here due to its key role in plant responses to a variety of abiotic stresses.

7.3.1 Ethylene Production

The phytohormone ethylene (C_2H_4) is found only in gaseous form and is produced endogenously by almost all plants (Babalola 2010); it mediates a wide range of developmental processes as well as responses to stress. Ethylene is an efficient plant growth regulator (Arshad and Frankenberger 2002), but can inhibit growth when its concentrations are high. Stress conditions such as flooding, wounding, drought, chilling, pathogen infection, and salinity may induce the accumulation of ethylene to high concentrations (Babalola 2010; Gnanamanickam 2006). The ethylene biosynthetic pathway is of interest because of the presence of microbial enzymes that interfere with this pathway, described below. Ethylene is synthesized from the

precursor 1-aminocyclopropane-1-carboxylate (ACC) by the enzyme ACC oxidase, and this ACC is derived from S-adenosyl methionine (SAM) by the enzyme ACC synthase (ACS). This synthesis occurs in two phases in response to stress. First, low levels of ethylene induce the expression of stress-related genes; and second, high levels of ethylene cause plant growth inhibition and detrimental effects such as chlorosis and abscission (Glick et al. 2007b), as well as inhibition of developmental processes including root elongation, lateral root growth, root hair formation, and nodulation (Alikhani et al. 2010; Belimov et al. 2009; Botella et al. 2000; Guinel and Sloetjes 2000; Ligero et al. 1999; Mayak et al. 2004; Pierik et al. 2006; Saleem et al. 2007; Yuhashi et al. 2000).

7.4 Improving Plants to Increase Abiotic Stress Tolerance

Plant breeding and plant engineering are both tools that have been used to improve the tolerance of crops to abiotic stress (Jewell et al. 2010; Araus et al. 2008; Ashraf and Akram 2009; Dwivedi et al. 2010; Mittler and Blumwald 2010; Sreenivasulu et al. 2007; Valliyodan and Nguyen 2006; Witcombe et al. 2008). The major constraint to improving the abiotic stress tolerance of crops are the gaps in our understanding of the complex physiological, biochemical, developmental, and genetic mechanisms involved in stress tolerance and the difficulty in combining favorable alleles to create improved high-yielding genotypes. Transgenic plants are primarily tested under controlled greenhouse conditions, with only a few reports of evaluating performance under field conditions. Challenges to enhancing the stress tolerance of crops include the polygenic nature of stress resistance, the time requirements for introducing multiple genes into a plant, and insufficient knowledge of the dynamics of the expression of the introduced genes following a stress stimulus (Bhatnagar-Mathur et al. 2008; Dodd and Perez-Alfocea 2012; Manchanda and Garg 2008; Schubert et al. 2009). Unfortunately, transgenic approaches and molecular breeding programs for improving crop tolerance to stress have generally not brought promising results in farmers' fields (Bhatnagar-Mathur et al. 2008; Dodd and Perez-Alfocea 2012; James et al. 2008; Munns and Tester 2008; Ramadoss et al. 2013a; Schubert et al. 2009; Wang et al. 2003) with some notable exceptions (Munns et al. 2006). In general, the development of stress-tolerant crop varieties through genetic engineering and plant breeding is an essential but a long and expensive process.

7.5 Using Microbes to Enhance the Abiotic Stress Tolerance of Plants

Microorganisms have been found to enhance the growth of diverse crops grown under a range of stresses. This finding suggests that microbial inoculants can be an effective practice to alleviate crop stress and one that complements the development of stress-tolerant plant germplasm (Paul and Lade 2014). Microbial inoculants that alleviate plant stress offer a possible cost-effective, environmentally friendly,

agricultural input, which can generally be developed in a shorter time frame than new plant germplasm (Dodd and Perez-Alfocea 2012; Shrivastava and Kumar 2015). Additional advantages of developing microbial products over plant products for improving plant abiotic stress tolerance include the more rapid screening and modification of microbes than plants, the relative ease with which multiple plant growth-promoting (PGP) traits can be identified or engineering in a single microbe, and the potential application of a single inoculant to multiple crops. Evidence supporting the contribution of microbes to abiotic stress tolerance will be elaborated below, but includes the finding that genetic differentiation in plant-associated microbes can drive local adaptation of plants to their environment (Rodriguez and Redman 2008) and the evolutionary history of mutualistic interactions between plants and microbes that helped drive plant adaptation to stressful conditions (Glick 2012).

7.6 The Rhizosphere as a Selective Force for Microbes

The rhizosphere, which is the region of soil influenced by plant roots, offers an environment rich in nutrients for microorganisms (Bais et al. 2006). Plants release as much as 20% of their fixed carbon into the rhizosphere. These rhizodeposits contain an array of organic compounds, including sugars (such as glucose, xylose, fructose, maltose, sucrose, and ribose), organic acids (such as citric, malic, lactic, succinic, oxalic, and pyruvic acids), amino acids, fatty acids, nucleotides, putrescine, and vitamins. Microbes use these compounds for nutrition and as signal molecules to indicate the presence of the plant (Lugtenberg 2015). Microbes also influence the chemical milieu of the rhizosphere by secreting an array of compounds, including enzymes, waste products, secondary metabolites, and even phytohormones, which may influence plant growth and defense (Ortíz-Castro et al. 2009).

Although the microbial communities on roots are diverse, the composition of these communities may be influenced by the plant (Sessitsch et al. 2002), suggesting the possibility that plants select or enrich for microbes that provide a benefit to the plant. Distinct plant species, and even distinct cultivars, have been found to have a detectable influence on the diversity of their rhizosphere communities (Berg and Smalla 2009; Buée et al. 2009; Hartmann et al. 2008; Acosta-Martínez et al. 2008; Marschner et al. 2001; Germida and Siciliano 2001; Manter et al. 2010; Siciliano and Germida 1999; Van Overbeek and Van Elsas 2008). Whereas some microbes are found primarily outside of the roots, those that colonize the intercellular sites within roots, designated endophytes, may have the greatest impact on the plant due to their close proximity to the plant tissues and thus greater opportunities for chemical exchange.

A breadth of rhizosphere microorganisms have been found to promote plant growth in the absence of plant exposure to environmental stress. These are generally referred to as plant growth-promoting rhizobacteria (PGPRs) (Hayat et al. 2010), but can more generically be called plant growth-promoting microorganisms

(PGPMs). About 2–5% of rhizobacteria exert a beneficial effect on plant growth following inoculation onto plants in a soil containing competitive microflora (Paul and Lade 2014). PGPRs include bacteria of diverse genera such as *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Rhizobium*, and *Serratia* (Gray and Smith 2005), as well as *Streptomyces* spp. (Dimkpa et al. 2009; Tokala et al. 2002). Although the arbuscular mycorrhizal fungi (AMF) (Jeffries et al. 2003) and symbiotic nitrogen-fixing bacteria also provide clear benefits to plant growth, they are generally not regarded as PGPMs (Franche et al. 2008). PGPMs may benefit plants via a diversity of mechanisms (Hayat et al. 2010; Lugtenberg and Kamilova 2009; Paul 2012), and these include, but are not limited to, the production of a compounds such as plant growth regulators, siderophores, and enzymes that influence plant hormone accumulation, biological nitrogen fixation, and activities that enhance nutrient solubilization, protection from phytopathogens, and protection from abiotic stresses. The main focus of this review is on the mechanisms by which rhizosphere bacteria, including endophytes, benefit plant health by modulating the effects of abiotic stress.

7.7 Mechanisms by Which Microbes Enhance Abiotic Stress Tolerance in Plants

The importance of environmental stress in reducing crop yields has fostered a large body of research exploring the role of rhizosphere organisms in plant health in stressed environments (Grover et al. 2010; Paul 2013; Paul and Lade 2014; Venkateswarlu et al. 2008; Yang et al. 2009). Bacteria and fungi have been identified that can improve plant yields in these stressed environments (Banik et al. 2006; Barassi et al. 2006; Belimov et al. 2015; Chakraborty et al. 2015; Choudhary 2012, 2015; Dahmardeh et al. 2009; Damodaran et al. 2014; Davies et al. 2011; del Amor Francisco and Cuadra-Crespo 2012; Dimkpa et al. 2009; Egamberdieva et al. 2008; Egamberdieva 2009; Egamberdieva 2011; Etesami and Alikhani 2016a, b; Fu et al. 2010; Gray and Smith 2005; Hamilton et al. 2016; Kaymak et al. 2009; Khan et al. 2012; Mayak et al. 2004; Milošević et al. 2012; Nadeem et al. 2007; Paul 2012; Paul and Nair 2008; Ramadoss et al. 2013a; Rojas-Tapias et al. 2012; Shrivastava and Kumar 2015; Soleimani et al. 2011; Tiwari et al. 2011; Upadhyay et al. 2009; Yang et al. 2009, 2010; Yildirim and Taylor 2005; Vurukonda et al. 2016). The bacterial genera that have been implicated in these benefits include *Rhizobium*, *Bacillus*, *Pseudomonas*, *Pantoea*, *Paenibacillus*, *Burkholderia*, *Achromobacter*, *Azospirillum*, *Microbacterium*, *Methylobacterium*, *Variovorax*, and *Enterobacter* (Barassi et al. 2006; Dodd and Perez-Alfocea 2012; Grover et al. 2010; Nia et al. 2012; Ramadoss et al. 2013a; Selvakumar et al. 2009; Upadhyay et al. 2009; Yang et al. 2009, 2010; Yildirim and Taylor 2005). Some of these organisms are capable of activating systemic changes in plants that confer tolerance to abiotic stress; this phenomenon has been designated *induced systemic tolerance* (Yang et al. 2009). This section explores our understanding, to date, of the mechanisms by which PGPMs alleviate abiotic stress tolerance in plants.

7.7.1 Indole Acetic Acid Production

Plants regulate their resource allocation to roots versus shoots to optimally balance the size of their root systems, which are critical for nutrient and water uptake, and their aboveground tissues, which are required for photosynthesis and reproduction. Some microbes promote greater root growth by interfering with this regulation. Although this interference could conceivably come at the cost of fitness to the plant, many studies have shown that under conditions of environmental stress, microbial inoculants that promote plant root growth provide measurable benefits to plant growth and/or plant health. In particular, inoculation of plants with various PGPRs have been shown to enhance the formation of lateral roots and root hairs (Paul and Lade 2014) and roots with a larger root surface area (Diby et al. 2005; Paul and Sarma 2006); collectively, these morphological changes increase the opportunities for water and nutrient uptake.

A major mechanism by which bacteria can influence plant root system development is via the production of the auxin indole-3-acetic acid (IAA). This phytohormone is a major hormone used by plants to regulate growth. It is involved in a breadth of physiological processes including plant cell division and differentiation, germination, vascular development, and root growth. Of particular interest for the study of PGPR is the influence of IAA on root length and the initiation of lateral roots. Low IAA production levels generally increase root length and lateral root initiation, whereas high levels promote the opposite. The ability of bacteria to produce IAA was recognized, in part, due to the negative impacts of high IAA production on plant development (Costacurta and Vanderleyden 1995; Ludwig-Müller 2004). IAA production has been detected in diverse bacteria, including methylobacteria, streptomycetes, cyanobacteria, and archaea, with the percentage of soil bacteria capable of IAA production estimated to be as high as 80% (Khalid et al. 2004). At present, the majority of known PGPRs are capable of IAA production (Hayat et al. 2010).

The contribution of IAA production by *Azospirillum brasilense* to root growth under nonstressed conditions has been well documented (Spaepen and Vanderleyden 2011; Spaepen et al. 2007). *A. brasilense* strains induce morphological changes in plant roots (Spaepen et al. 2008). For example, cell-free supernatants of *A. brasilense* cultures induced root elongation and increased root surface area, dry weight, and lateral root development in rice (El-Khawas and Adachi 1999) and soybean (Molla et al. 2001) under hydroponic conditions. Moreover, similar responses by bean plants were observed upon the exogenous application of IAA and PGPR strains (Remans et al. 2008). Lastly, the association of a loss of IAA production via mutagenesis with a loss of root enhancement activity provided direct evidence that IAA production was at least one mechanism responsible for plant growth promotion by *A. brasilense* (Kundu et al. 1997).

The contribution of IAA production to root growth under environmentally stressful conditions has been identified with other microbes. Sadeghi et al. (2012) demonstrated that a *Streptomyces* isolate produced IAA and promoted growth of wheat under high salinity conditions. Similarly, Yao et al. (2010) found that an IAA-producing *Pseudomonas putida* strain promoted the growth of cotton seedlings under high salinity conditions. The finding that this *P. putida* strain also inhibited

production of the stress-inducible phytohormone abscisic acid illustrates the complexity of elucidating mechanisms underlying plant growth promotion. The benefits of IAA production by PGPR may be augmented by the production of an enzyme, ACC deaminase (Etesami et al. 2014a, 2015a, b); this enzyme is described below. Bacteria can produce other phytohormones as well, including cytokinins (Arkhipova et al. 2007) and gibberellins (Botinni et al. 2004), which may contribute to plant growth under stressful conditions (Arkhipova et al. 2007), but the research on these phytohormones in PGPR is far less extensive than for IAA.

7.7.2 ACC Deaminase Production

PGPRs that produce the enzyme ACC deaminase can modulate plant growth by modulating the level of ethylene in the roots (Glick 2014; Singh et al. 2011). The enzyme ACC deaminase catalyzes the cleavage of ACC, which is an immediate precursor of ethylene in the ethylene biosynthetic pathway (Glick et al. 2007a). This cleavage reaction releases ammonia and α-ketobutyrate, both of which can be metabolized by bacteria (Glick et al. 2007b), and concurrently limits further production of ethylene by the plant (Fig. 7.3).

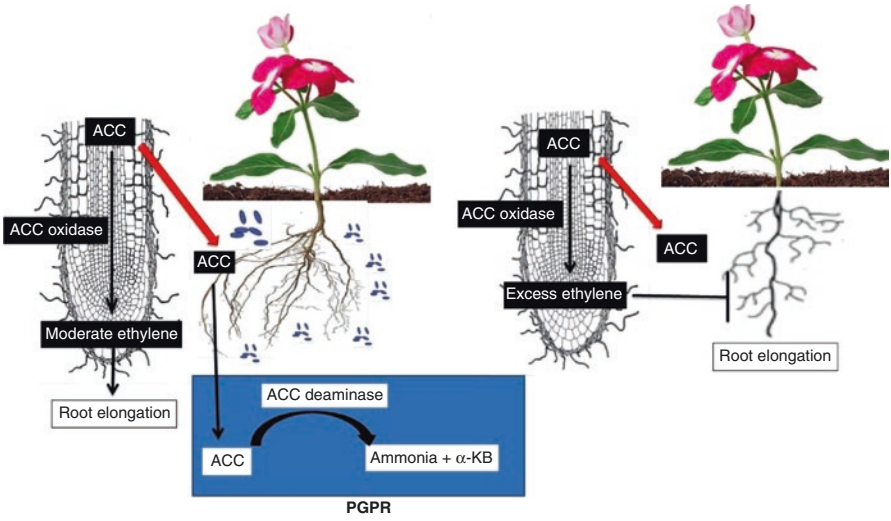


Fig. 7.3 A model to explain how ACC deaminase-producing bacteria lower ethylene in roots. A significant portion of the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) is exuded from plant roots or seeds and is subject to hydrolysis by ACC deaminase-producing bacteria, releasing ammonia and α-ketobutyrate (α-KB). This hydrolysis reduces the ACC levels outside of the plant, which, due to the equilibrium between internal and external ACC levels, results in decreased ACC levels inside the plant. The metabolic benefit of the ACC degradation to the growth of ACC-producing microorganisms increases their growth, and this provides a positive feedback to further decrease the ACC levels, ultimately resulting in a reduction in the endogenous biosynthesis of ethylene (Glick 2012)

As described above, plants respond to a variety of stresses by accumulating ethylene, and this accumulation can inhibit root development. By decreasing ACC levels in plants, ACC deaminase-producing microorganisms decrease plant ethylene levels and alleviate this inhibition (Glick et al. 2007b). Interestingly, the cost of overriding this endogenously induced growth inhibition does not seem to outweigh the benefit for plants grown under stressful conditions, as plants inoculated with ACC deaminase-producing bacteria, or expressing a bacterial ACC deaminase transgene, develop a more extensive root system and exhibit enhanced tolerance to environmental stresses (Arshad et al. 2007; Safronova et al. 2006; Stearns et al. 2005).

ACC deaminase-producing PGPRs have been used successfully to protect a variety of plant species against growth inhibition resulting from stress exposure (Ali et al. 2012, 2014; Barnawal et al. 2012; Etesami et al. 2014b; Glick 2014; Li et al. 2012, 2013; Ramadoss et al. 2013b; Shakir et al. 2012; Siddikee et al. 2011). Although a diversity of bacteria and fungi express ACC deaminase, this activity has been studied most extensively in PGPRs (Glick 2005), including the genera *Achromobacter*, *Acidovorax*, *Alcaligenes*, *Enterobacter*, *Klebsiella*, *Methylobacterium*, *Pseudomonas*, *Rhizobium*, and *Variovorax* (Esquivel-Cote et al. 2010). Bacteria in the symbiotic nitrogen-fixing genus *Bradyrhizobium* can also reduce ethylene accumulation in plants, and this activity enhances nodulation by helping prevent plant suppression of new nodule primordia. Unlike PGPR, however, these symbiotic bacteria do not secrete ACC deaminase, but rather secrete a modified amino acid, rhizobitoxine, that inhibits the ethylene biosynthetic enzymes ACC synthase and β-cystathionase (Sugawara et al. 2006). To date, rhizobitoxine production has been found in some plant pathogens, but not in PGPRs.

7.7.3 Increased Nutrient Mobilization

Many of the soil nutrients required by plants are present in soil but not in an available form because they are in the form of insoluble precipitates or are bound to inorganic and organic soil constituents. Plant nutrient deficiency is stressful but can also exacerbate the adverse effects of other abiotic stresses (Baligar et al. 2001; Grieve and Grattan 1999; Khoshgoftarmanesh et al. 2010). Several studies show that plants exposed to environmental stresses require additional supplies of mineral nutrients to minimize the adverse effects of stress (Endris and Mohammad 2007; Heidari and Jamshid 2010; Kaya et al. 2002). The best-characterized mutualistic interactions in the rhizosphere, namely, the AMF- and symbiotic nitrogen-fixing bacteria-plant interactions, help plants overcome nutrient deficiencies that are incurred in soils with low fertility (Glick 2012). PGPRs can enhance the availability of these nutrients by increasing their solubility or uptake. A diversity of mechanisms by which microorganisms may increase nutrient availability is shown in Fig. 7.4. In this section, we will discuss the ways in which PGPR can be applied to improve crop health and productivity in nutrient-poor environments. The discussion will focus on the most limiting nutrient, N, with some discussion of P and iron, for which the solubility is especially affected by the soil pH (Jewell et al. 2010).

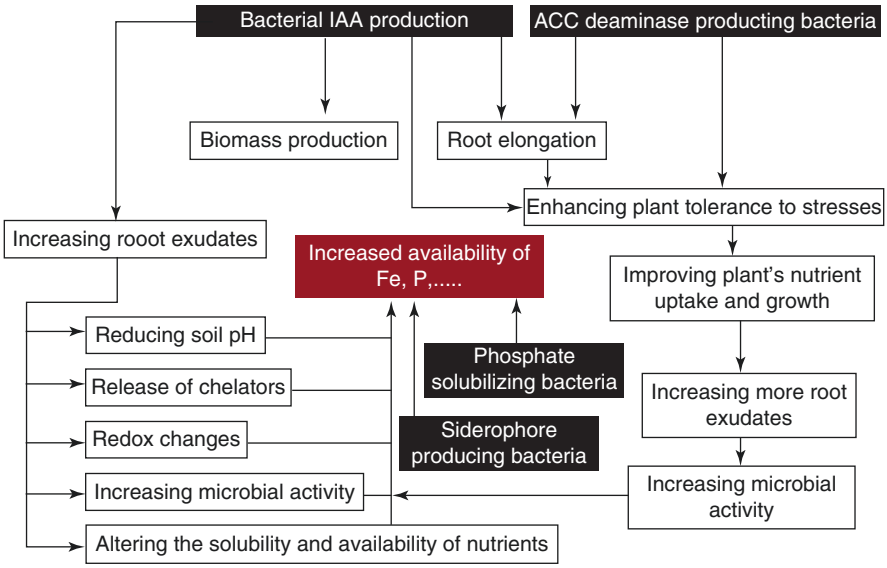


Fig. 7.4 Schematic representation of mechanisms by which PGPR may affect nutrient availability in the rhizosphere (Etesami et al. 2015a)

7.7.3.1 Nitrogen

Environmental stress can reduce the fixed nitrogen that legumes obtain from symbiotic nitrogen fixation. The process of infection and formation of root nodules is strongly repressed by ethylene (Peters and Crist-Estes 1989; Sugawara et al. 2006); therefore, the ethylene that accumulates in response to environmental stresses such as drought and salinity may be responsible, at least in part, for stress-mediated reductions in available nitrogen. This mechanism suggests that PGPR interference in ethylene signaling (Sect. 7.7.2) could help ensure continued fixation under stressful conditions. Other environmental stresses, including iron and phosphorus (P) deficiencies, also depress symbiotic nitrogen fixation, such as by reducing nodule mass, leghemoglobin content, and nitrogenase activity (Garcia et al. 2015; Tang et al. 1990; Pereira and Bliss 1989).

PGPRs may alleviate the effects of these stresses on symbiotic nitrogen fixation by a variety of mechanisms, as shown in Fig. 7.5. These include producing IAA that increases the size of the root system (Parmar and Dadarwal 1999) or number of root hairs (Yahalom et al. 1991), thus increasing the opportunities for nodulation (Glick 2012; Theunis 2005).

They also include enhancing the uptake of iron and P and thus preventing nutrient deficiencies that inhibit nodulation and nodule function. Co-inoculation of PGPR with rhizobia has been explored as a mechanism of ensuring adequate fixed nitrogen under stressful conditions. For example, Egamberdieva et al. (2015) showed that co-inoculation of *Mesorhizobium* spp. symbionts with *Pseudomonas extremorientalis* strain TSAU20 restored growth and nodulation to a legume, *Glycyrrhiza uralensis*, exposed to high salinity. This strain produces IAA

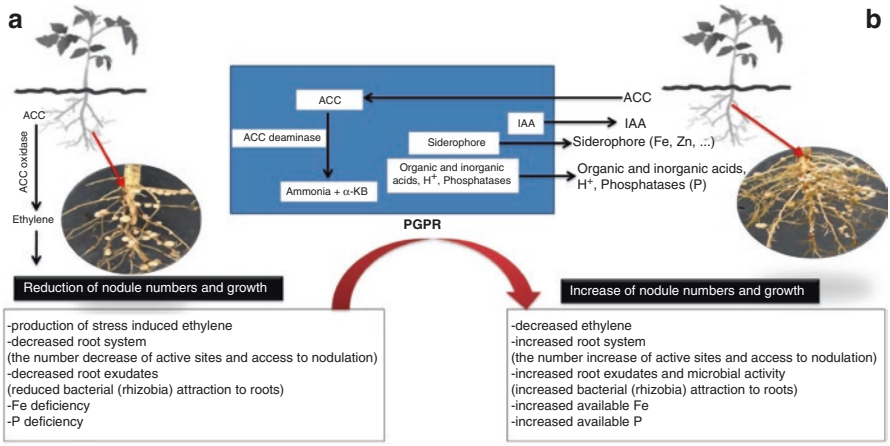


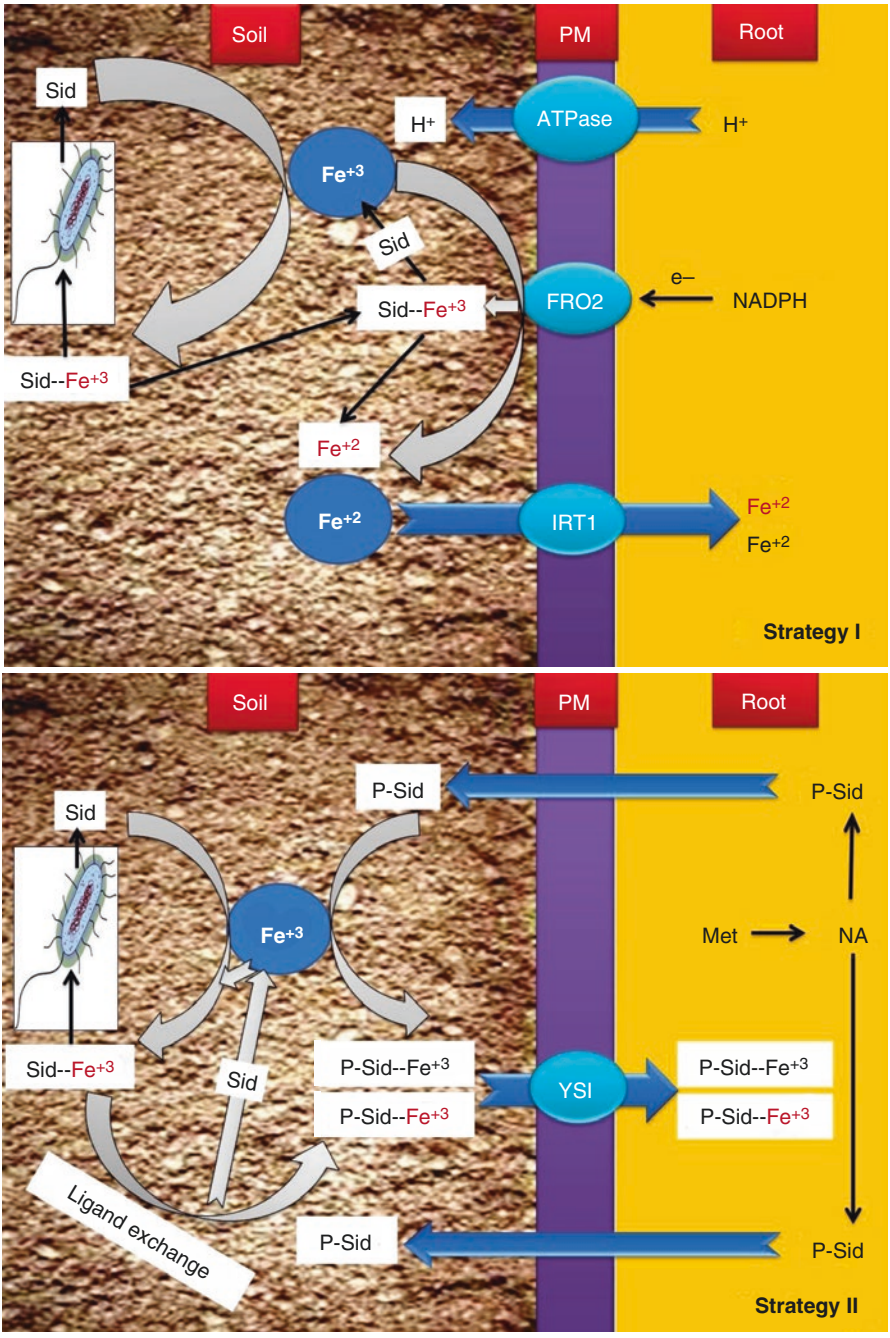
Fig. 7.5 Mechanisms by which PGPRs may alleviate the effects of stress on nodule number and nitrogen fixation in a legume plant. A, Stress-affected legume plant in the absence of inoculation by PGPR; B, stress-affected legume plant inoculated with PGPRs

(Egamberdieva and Kucharova 2009) and was previously shown to alleviate salt stress in the legume *Galega officinalis* when applied alone or with the rhizobial symbiont (Egamberdieva et al. 2013).

Environmental stresses such as salinity and drought may also influence plant N content by reducing root growth and thus reducing the surface area for uptake of nutrients, such as inorganic fertilizers. PGPRs that enhance root growth may therefore restore N nutrition. Although many bacteria enhance plant growth under high salinity conditions (Egamberdieva and Kucharova 2009; Selvakumar et al. 2009; Upadhyay et al. 2009), *Azospirillum* spp. were shown to increase plant N content (Nia et al. 2012) and the uptake of N, as well as P and K, under high salinity and other stressful environmental conditions (Carmen and Roberto 2011). Similarly, a *Streptomyces* sp. isolate increased wheat growth in saline soils as well as increased the concentration of N, P, Fe, and Mn in wheat shoots grown in normal and saline soil (Sadeghi et al. 2012).

7.7.3.2 Iron

Despite the abundance of iron in soils, its availability for plants and microbes is low (Schmidt 2003; Wintergerst et al. 2007). Under aerobic conditions, iron exists predominantly as ferric iron (Fe^{3+}), which reacts to form highly insoluble hydroxides and oxyhydroxides (Desai and Archana 2011; Zuo and Zhang 2010). These insoluble precipitates have a very low bioavailability to plants and microbes and are even less bioavailable in alkaline soils, such as calcareous soils, than in acidic soils. Concentrations of soluble Fe^{3+} are optimal for most plants at 10^{-4} to 10^{-8} M, but are insufficient for growth at 10^{-9} M or lower. Due to the generally low bioavailability of iron, plants and microbes have evolved effective mechanisms for iron sequestration. Plants have two strategies for mobilizing iron (Fig. 7.6).


Soil
PM
Root
Sid
H+
ATPase
H+
Fe+3
Sid
e–
Sid--Fe+3
FRO2
NADPH
Sid--Fe+3
Fe+2
Fe+2
IRT1
Fe+2
Fe+2
Strategy I
Soil
PM
Root
P-Sid
P-Sid
Sid
Met
NA
Fe+3
Sid--Fe+3
Sid
P-Sid--Fe+3
P-Sid--Fe+3
YSI
P-Sid--Fe+3
P-Sid--Fe+3
Ligand exchange
P-Sid
P-Sid
Strategy II

In the first, designated *Strategy I*, the soil is acidified in the rhizosphere through the activity of a root plasma membrane-bound H^+-ATPase, Fe^{3+} is reduced into ferrous iron (Fe^{2+}) by an iron chelate reductase enzyme, and Fe^{2+} is taken up by a membrane-bound Fe^{2+} transporter (Hartmann et al. 2008). All plant species except grasses use this iron acquisition mechanism. In contrast, grasses synthesize, secrete, and take up low-molecular-weight phytosiderophores; we designate this as *Strategy II*. Phytosiderophores are a form of mugineic acids that strongly chelate ferric iron and solubilize it for plant uptake by specialized ferric iron transport proteins, such as the yellow stripe protein in corn (Charlson and Shoemaker 2006; Curie et al. 2001; Guerinot 2010). Strategies I and II are often not sufficient to meet the iron nutritional needs of plants, particularly in calcareous and alkaline soils. One approach to enhance iron mobilization to plants is amending soils with synthetic iron-chelating compounds such as ethylenediaminetetraacetic acid (EDTA) and ethylenediamine-N,N′-bis (2-hydroxyphenylacetic acid) EDDHA could provide a solution, but these are poorly biodegradable and can pose a threat to the environment, the main risk being the accumulation in groundwater (Kaparullina et al. 2011). An alternative approach is to exploit the iron mobilization abilities of microorganisms (Zuo and Zhang 2010).

Iron-chelating compounds called siderophores are produced by diverse plant-associated bacteria. These low-molecular-weight organic compounds are induced under low-iron conditions. Following secretion, they bind ferric iron with a high affinity, increasing its solubilization from iron oxide precipitates in the soil; the subsequent binding of the Fe^{3+}-siderophore complexes to highly specific bacterial siderophore receptors facilitates concurrent uptake and reduction of Fe^{3+} to Fe^{2+}

Fig. 7.6 Schematic representations of iron uptake systems of plant roots and role of siderophore (Sid)-producing bacteria in enhancing iron availability for plant. Plants acquire iron from the soil through the plasma membrane (PM) of their root epidermal cells by two different strategies (Strategies I and II). In non-grass species (Strategy I), acidification of the rhizosphere occurs in part through the activity of a plasma membrane (PM) H^+-ATPase. This H^+ excretion contributes to the solubilization of Fe^{+3}, which is reduced to Fe^{+2} by the FRO2 ferric chelate reductase, transferring electrons (e^-) from NADPH to Fe^{+3}. Fe^{+2} is then transported through the plasma membrane of root epidermal cells by the iron-regulated transporter 1 (IRT1) (Lemanceau et al. 2009). In grasses, Strategy II involves the synthesis of phytosiderophores (P-Sid) from nicotianamine (NA), which is derived from methionine (Met). P-Sid is secreted from the roots by an uncharacterized mechanism into the rhizosphere where it chelates Fe^{+3}. The Fe^{+3}-P-Sid complex is then transported into the epidermal cells of the roots by proteins such as the yellow stripe 1 (YS1) transporter. Bacteria do not take up Fe^{+3}-Sid complexes, but rather obtain iron through a reduction-based mechanism involving Fe-Sid membrane receptors, acquiring Fe^{2+} while releasing Sid for subsequent reuse. Sid increases the Fe^{+3} pools in the rhizosphere, increasing Fe^{+3} available to the root P-Sid. Given the differences in the binding affinity among siderophores, P-Sids that have a higher affinity for Fe^{3+} than Sid may acquire it via ligand exchange

(Boukhalfa and Crumbliss 2002). PGPR inoculants that increased iron uptake into plants with concurrent stimulation of plant growth have been reported (Barzanti et al. 2007; Burd et al. 2000; Carrillo-Castañeda et al. 2002; Lemanceau et al. 2009). Moreover, a contribution of microbial siderophores to the iron nutrition of plants using Strategies I and II has been reported (Jin et al. 2006; Johnson et al. 2002; Rasouli-Sadaghiani et al. 2014; Robin et al. 2008; Vansuyt et al. 2007).

The opportunities for ligand exchange between microbial siderophores and phytosiderophores suggest a role for microbial siderophores in enhancing the iron nutrition of gramineous plants. In particular, when plants use Strategy II for iron acquisition, their phytosiderophores may be able to compete with the bacterial siderophores for iron binding, as supported by studies showing differences among bacterial siderophores in their affinity for iron (Dulla et al. 2010), and the fact that a siderophore or phytosiderophore with a high affinity for Fe^{3+} will be able to steal the Fe^{3+} from one with a lower affinity for Fe^{3+}. This mechanism of uptake, designated *ligand exchange*, was first demonstrated with a fungal siderophore that enhanced iron uptake by barley (Yehuda et al. 1996). However, the extent to which bacterial siderophores are produced in sufficiently high amounts in the rhizosphere to impact the iron nutrition of plants remains an unanswered question (Crowley et al. 1988).

Microbial siderophores may also enhance iron uptake by plants that use Strategy I for iron acquisition. One proposed mechanism is via the transfer of ferric ions from a microbial siderophore to a plant ferric chelate reductase, thus promoting iron reduction and transport into the plant. The extent to which this mechanism facilitates enhanced iron uptake into the plant is not yet known (Crowley et al. 1988). Alternatively, bacteria may alter the signaling pathways that are involved in the physiological responses of the plant to iron deficiency. For example, IAA has a major role in activating plant responses that lead to rhizosphere acidification and iron acquisition by roots (Bacaicoa et al. 2011), with roles for ethylene implicated as well (Lucena et al. 2006). The ability of bacteria to produce IAA (Sect. 7.7.3) and alter plant ethylene signaling (Sect. 7.7.2) suggests mechanisms by which PGPR may influence the iron nutrition of plants. The finding that PGPRs enhance iron acquisition in non-gramineous (Sharma et al. 2003; Johnson et al. 2002; Vansuyt et al. 2007) indicates that one or more of these mechanisms may promote physiologically relevant levels of iron uptake into plants.

7.7.3.3 Phosphorus

Phosphorus (P) is generally the second most limiting nutrient for plant growth after nitrogen. P is soluble in a monobasic ($H_2PO_4^-$) or dibasic (HPO_4^{2-}) form, but even in soils with abundant P, usually only about 1% of the soil P is actually in a soluble form. Over 90% of soil P is generally insoluble in an inorganic form, such as apatite, or in an organic form, such as inositol phosphate (soil phytate), phosphomonoesters, and phosphotriesterase (Khan et al. 2007); these require mineralization before they become plant available. Due to limited P bioavailability in most soils, P limitation is often a limiting factor for plant growth (Khan et al. 2007). Plant strategies to acquire P include the root exudation of organic acids or enzymes to chelate inorganic P or enzymatically release phosphate from organic compounds (Hong et al. 2008; Park et al. 2007; Xiao et al. 2007). In addition, plants adjust their root architecture to

low-P conditions through inhibition of primary root growth, promotion of lateral root growth, enhancement of root hair development, and cluster root formation, which all promote P acquisition by plants (Jain et al. 2007; Ma et al. 2003; Niu et al. 2013; Osmont et al. 2007). Lateral roots, in particular, play an important role in P acquisition by increasing soil exploration (Zhu et al. 2005), the absorptive surface of the root system (Pérez-Torres et al. 2008), and P solubilization (Lynch 2007).

Microorganisms have been widely shown to enhance plant growth by enhancing the bioavailability of P for plants. This is a well-known function of mycorrhizal fungi, which form an extensive network that can extract P from a large volume of soil, but is also a function of many bacterial genera. In fact, enhancing plant P bioavailability is the most common mode of action identified in the PGPRs that have been characterized. The major mechanisms by which PGPR do this are by converting insoluble phosphates such as $Ca_3(PO_4)_2$ (Rodriguez et al. 2004) into soluble forms through the release of organic acids that promote acidification and releasing phosphates from organic phosphates via the secretion of extracellular phosphatases (Glick 2012; Gyaneshwar et al. 2002; Van Der Heijden et al. 2008). The ease of with which P-solubilizing bacteria can be identified using a classic plate assay (Pilovskaya 1948) enabled an extensive number of studies that have screened bacteria for P solubilization activities. These studies have identified diverse bacteria and have often screened and identified strains with resistance to target environmental stresses, such as high salinity (Barassi et al. 2006; Son et al. 2006), high concentrations of heavy metals (Zaidi et al. 2006), and low pH (Son et al. 2006), as well as with an ability to colonize roots and promote plant growth (Taurian et al. 2010). Consequently, P solubilization is a common trait in characterized PGPRs.

7.7.4 Induction of Increased Plant Osmolyte Accumulation

The accumulation of osmolyte compounds can enhance plant tolerance to salinity, drought, and heat (Chen et al. 2007; Dodd and Perez-Alfocea 2012). Osmolytes are low-molecular-weight organic compounds that are used by cells to maintain turgor pressure and cell volume, especially under water-limited conditions. To withstand water limitation, cells of all organisms synthesize metabolites to maintain turgor pressure and full hydration of cytoplasmic constituents, which is critical to the structural integrity of membranes, enzymes, and other cellular components (Majumder et al. 2010). Major types of osmolytes are sugar alcohols (glycerol and methylated inositols), complex sugars (trehalose, raffinose and fructans), quaternary amino acid derivatives (proline, glycine betaine, b-alanine betaine, proline betaine), tertiary amines (ectoine) and sulfonium compounds (dimethyl sulfonium propionate) (Majumder et al. 2010). The accumulation of proline, a widely distributed osmolyte in plants, correlates with tolerance to drought and salinity stress (Szabados and Savouré 2010; Wang et al. 2015). Proline accumulation is a sensitive physiological index of the response of plants to salt and other stresses (Peng et al. 2008) and helps maintain higher leaf water potentials during stress and protect against oxidative stress, potentially by function as an antioxidant (Hayat et al. 2012).

PGPRs can favor osmolyte accumulation in plants exposed to salinity stress. To date, this has been shown for the osmolyte proline. Plants inoculated with PGPR strains showed increased proline accumulation when grown under high salinity conditions (Bano and Fatima 2009; Jha et al. 2011; Kohler et al. 2009; Upadhyay et al. 2012; Zarea et al. 2012), and this occurred in a variety of plant species, including lettuce, wheat, and corn. These results suggest that PGPR can promote an enhanced adaptive response of plants to high external salt concentrations, namely, osmolyte accumulation, indicating that this is one of the many mechanisms by which microbes promote plant tolerance to salinity stress (Bianco and Defez 2009; Munns and Tester 2008; Dodd and Perez-Alfocea 2012).

7.7.5 Exopolymer production

Many soil microbes secrete extracellular polymeric substances (EPSs) into the environment, and these EPS can confer a wide range of benefits to plants. For example, these high-molecular-weight secreted compounds promote soil aggregate stability, which is one of the most important properties controlling plant growth in semiarid environments (Paul and Lade 2014). These EPS, which are comprised primarily of extracellular polysaccharides, proteins, and DNA, are also central to the function and structural integrity of biofilms (Donlan 2002). They serve as a matrix that promotes biofilm adherence to surfaces and as a matrix that binds water, thus contributing greatly to the water-holding capacity of the soil and the biofilms on root surface (Grover et al. 2010). EPS-producing PGPRs have been associated with aggregation of root-adhering soils (Alami et al. 2000; Upadhyay et al. 2011) and improved soil structure (Sandhya et al. 2009), both of which are associated with increased plant resistance to water stress. EPS-producing PGPRs have also been proposed to bind cations including Na^+, thus potentially decreasing the content of Na^+ available for plant uptake and thus helping to ameliorate the negative impact of salinity on plant growth (Ashraf et al. 2004; Geddie and Sutherland 1993; Grover et al. 2010; Upadhyay et al. 2009).

7.7.6 Promotion of Ion Homeostasis in Plants

PGPR may alter ion homeostasis in plants such that the plants are better able to tolerate salinity stress. Salinity stress generally results in the accumulation of Na^+ in leaves due to transport in the transpiration stream and an accompanying deficit in K^+. This physiological response is so consistent that the K^+/Na^+ ratio is used as an indicator of plant salt tolerance (Hamdia et al. 2004). A breadth of reports have documented that PGPR-inoculated plants grown in saline soils have a higher K^+ ion concentration and, in turn, a higher K^+/Na^+ ratio, than uninoculated plants (Jiang et al. 2012; Kasotia et al. 2015; Kohler et al. 2009; Nadeem et al. 2012; Rojas-Tapias et al. 2012; Safronova et al. 2012; Shrivastava and Kumar 2015; Yao et al. 2010; Nadeem et al. 2007; Chang et al. 2014; Egamberdieva et al. 2015; Nadeem et al. 2009; Wang et al. 2016; Mayak et al. 2004). Potassium plays a key role in plant

water stress tolerance and is responsible for stomatal movements in response to changes in leaf water status (Caravaca et al. 2004); thus, PGPR-associated increases in K^+ concentration may help prevent salinity-induced stomatal closure. Although the mechanism by which PGPR alter ion uptake into roots is largely unknown, Zhang et al. (2008) demonstrated that a *Bacillus subtilis* strain can mediate the level of salt tolerance in *Arabidopsis thaliana* by regulating the K^+ transporter HKT1. Interestingly, these changes in HKT1 expression, namely, downregulation in roots and upregulation in shoots, were induced by the mixture of volatile compounds emitted by this *B. subtilis* strain, demonstrating that volatile bacterial signals can affect ion homeostasis and salinity stress tolerance in plants via an effect on a high-affinity K^+ transporter. Some reports also suggest that bacteria influence Ca^{2+} levels, which have an early signaling role in the response to high salinity (Fu et al. 2010).

7.7.7 Induction of Plant Production of Antioxidant Enzymes

Microbes may help prevent or reduce oxidative damage to plants by secreting antioxidant enzymes. The production of oxygen-based radicals is the bane of all aerobic species. These radicals, collectively called reactive oxygen species (ROS), include peroxides, superoxide, hydroxyl radical, and singlet oxygen. Due to their reactive nature, ROS can damage cellular macromolecules and organelles. Various abiotic stresses lead to the production of ROS in plants; these include salinity and drought stress (Hichem et al. 2010; Johnson et al. 2003). To protect against oxidative stress, plant cells produce both antioxidant enzymes and nonenzymatic antioxidants (Hasegawa et al. 2000; Mayak et al. 2004; Miller et al. 2010). Plant tolerance to high salinity has been correlated with high antioxidant enzyme activities, including catalase, ascorbate peroxidase, glutathione reductase, and superoxide dismutase (Apel and Hirt 2004; Miller et al. 2010; Mittova et al. 2002, 2003).

Several studies have reported that rhizobacteria induce plant synthesis of antioxidant enzymes in response to salinity stress (Heidari and Golpayegani 2012; Paul and Lade 2014; Singh et al. 2013; Chakraborty et al. 2013; Nautiyal et al. 2008; Bianco and Defez 2009; Kohler et al. 2009; Jha and Subramanian 2013). These PGPR-induced antioxidant enzymes are believed to contribute to salinity stress tolerance by eliminating hydrogen peroxide from salt-stressed roots (Kim et al. 2005). PGPRs may also alleviate drought stress, as shown following inoculation of maize with five drought-tolerant plant growth-promoting *Pseudomonas* sp. strains (Sandhya et al. 2009).

7.7.8 Reduction of Toxicity of Heavy Metals and Organic Pollutants to Plants

Heavy metals at elevated levels are generally toxic to plants, impairing their metabolism and reducing plant growth. Previous studies have demonstrated that most heavy metal and other xenobiotic contaminants inhibit the root growth of plants

(Arshad et al. 2007). Microbes have a variety of mechanisms for detoxifying heavy metals (Chen et al. 2015; Gadd 2000; Glick 2010; Lim et al. 2003; Lin and Lin 2005; Malik 2004; Soleimani et al. 2011). For example, microbial cell walls have functional groups that can bind heavy metal ions, and this binding and immobilization may have contributed to the reduced uptake of cadmium (Cd) into barley plants following inoculation with PGPR (Luo et al. 2011; Scott and Karanjkar 1992). PGPRs may also promote plant health by reducing the phytotoxic effects of heavy metals, as shown for nickel (Ni) and Cd toxicity following inoculation of *Methylobacterium oryzae* and *Burkholderia* sp. strains on tomato (Madhaiyan et al. 2007).

Organic pollutants may also be harmful to plants. Some endophytes enhance plant tolerance to pollutants and do so by degrading them (Garipova 2014). Organic pollutants may accumulate in plant tissues in the absence of degradative enzymes (Burken 2004), but may be completely degraded in the presence of an endophyte capable of degradation (Lodewyckx et al. 2002a, b; Moore et al. 2006). Although pollutant degradation abilities are found in both endophytes (Barzanti et al. 2007) and free-living bacteria (Mrozik and Piotrowska-Seget 2010), genes encoding the degradative enzymes for specific pollutants occurred more frequently in endophytic bacteria in the presence of those pollutants than in bacteria isolated from soil (Siciliano et al. 2001). The ability of endophytes to degrade pollutants following their extraction from the soil by plants illustrates the contribution of endophytes to both bioremediation of soils and safe cultivation of agricultural products in contaminated soils (Garipova 2014). For example, it was shown experimentally that pea plants treated with the endophyte *Pseudomonas* utilized the 2,4-D herbicide from soil without accumulating it in tissues (Germaine et al. 2006).

Plants synthesize and accumulate ethylene in response to contaminant-induced stresses (Arshad et al. 2007); thus, microbes may influence plant responses to heavy metal contaminants by interfering with ethylene synthesis. Bacteria that produce ACC deaminase (Sect. 7.7.2) have been examined for their ability to mitigate the ill effects of soil contamination caused by xenobiotic chemicals and heavy metals on plants. These bacteria have been shown to promote plant growth in the presence of heavy metals by reducing the stress ethylene synthesized in plants (Arshad et al. 2007, 2008; Belimov et al. 2005; Glick et al. 2007b; Ma et al. 2010; Madhaiyan et al. 2007; Rajkumar et al. 2006; Safronova et al. 2006). In addition to ACC deaminase activity, bacterial siderophores may help plants reduce heavy metal toxicity by increasing the supply of iron to the plant (Burd et al. 2000).

7.7.9 Induction of Resistance to Temperature Stress

Microbes can also increase plant resistance to temperature stress (Su et al. 2015; Selvakumar et al. 2008a, b). For example, a thermotolerant *Pseudomonas* sp. strain induced thermotolerance in sorghum seedlings (Ali et al. 2009), and a *Burkholderia phytofirmans* strain capable of epiphytic and endophytic colonization of grapevines

(Compant et al. 2005) protected plants against heat as well as chilling stress (Ait Barka et al. 2006; Bensalim et al. 1998). The mechanisms by which these benefits are conferred, however, are poorly understood.

7.8 Selection of the Most Stress-Tolerant Microorganisms

The ability to use microbial inoculants to enhance plant tolerance to abiotic stress requires that the introduced microbes tolerate the stressful conditions (Devliegher et al. 1995), as well as compete effectively with the native microflora after inoculation (Rekha et al. 2007). Thus, the selection of most effective root-colonizing bacterial strains is required to fully realize the benefits of inoculation. A general approach to identifying potential PGPR strains to promote plant growth under environmentally stressful conditions has therefore been to isolate organism from a stressful environment, as illustrated by the isolation of halotolerant bacteria from wheat roots grown in saline soil and their subsequent success in stimulating plant growth (Egamberdieva et al. 2008). An alternative approach is to screen bacterial isolates for those that are tolerant to the relevant stress (Lifshitz et al. 1986; Shrivastava and Kumar 2015). In general, microorganisms tolerating extreme environmental conditions have been found suitable for use in various agricultural practices (Egamberdieva and Kucharova 2009). Thus, an effective strategy for the rapid identification of efficacious PGPR strains to use as bioinoculants for stressed crops may be the isolation of indigenous microorganisms from stress-affected soils and from plants grown in such soils, with subsequent screening on the basis of traits contributing to stress tolerance and plant growth promotion and ultimately screening for improved growth or health of plants growth under environmentally stressful conditions (Grover et al. 2010).

7.9 Concluding Remarks and Future Perspectives

Plants developed mutualistic associations with mycorrhizal fungi very early following their transition from aquatic to terrestrial habitats. They embraced these fungi as a means of establishing a network for acquiring enough water and nutrients to meet their needs in their new, water-limited home. This mutualism illustrates the early dependence of plants on microbes in their adaptation to environmental stress. Microbes still play a critical role in conferring the phenotypic plasticity necessary to tolerate a wide range of environmental insults. Although we have identified some of the mechanisms by which bacteria can promote plant tolerance to abiotic stress, ranging from the secretion of bioactive compounds to interference in plant hormonal signaling, many mechanisms have yet to be discovered. Knowledge of these mechanisms, and the full complement of mechanisms expressed by any given microbial inoculant, is important in moving forward in evaluating the growth-promoting potential and plant-protective effects of inoculants in the field. Given the increasing exposure of our agricultural crops to abiotic stresses, and particularly to

drought, salinity, and nutrient limitation, the optimization of inoculants for agricultural use is critical as a complement to the on-going efforts to develop stress-tolerant crop varieties.

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References

Acosta-Martínez V, Dowd S, Sun Y, Allen V (2008) Tag-encoded pyrosequencing analysis of bacterial diversity in a single soil type as affected by management and land use. Soil Biol Biochem 40:2762–2770

Acquaah G (2007) Principles of plant genetics and breeding. Blackwell, Oxford, UK

Ahmad P (2013) Oxidative damage to plants, antioxidant networks and signaling. Academic/Elsevier, San Diego

Ait Barka E, Nowak J, Clement C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PsJN. Appl Environ Microbiol 72:7246–7252

Alami Y, Achouak W, Marol C, Heulin T (2000) Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide-producing *Rhizobium* sp. strain isolated from sunflower roots. Appl Environ Microbiol 66:3393–3398

Ali S, Charles TC, Glick BR (2012) Delay of flower senescence by bacterial endophytes expressing 1-aminocyclopropane-1-carboxylate deaminase. J Appl Microbiol 113:1139–1144

Ali S, Charles TC, Glick BR (2014) Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol Biochem 80:160–167

Ali S, Sandhya V, Grover M, Kishore N, Rao LV, Venkateswarlu B (2009) *Pseudomonas* sp. strain AKM-P6 enhances tolerance of sorghum seedlings to elevated temperatures. Biol Fertil Soils 46:45–55

Alikhani HA, Etesami H, Mohammadi L (2010) Effect of superior indole-3-acetic acid producing Rhizobia and the combination with Ag and L-Tryptophan on wheat growth indices under in vitro conditions. Food Agri Environ 3:949–954

Allakhverdiev SI, Sakamoto A, Nishiyama Y, Inaba M, Murata N (2000) Ionic and Osmotic Effects of NaCl-Induced Inactivation of Photosystems I and II in *Synechococcus* sp. Plant Physiol 123:1047–1056

America SSSO (2001) Glossary of soil science terms. Soil Science Society of America, Madison

Apel K, Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu Rev Plant Biol 55:373–399

Araus JL, Slafer GA, Royo C, Serret MD (2008) Breeding for yield potential and stress adaptation in cereals. Critl Rev Plant Sci 27:377–412

Arkhipova TN, Prinsen E, Veselov SU, Martinenko EV, Melentiev AI, Kudoyarova GR (2007) Cytokinin producing bacteria enhance plant growth in drying soil. Plant Soil 292:305–315

Arshad M, Frankenberger WTJ (2002) Ethylene: agricultural sources and applications. Kluwer Academic, New York, NY, p 342

Arshad M, Saleem M, Hussain S (2007) Perspectives of bacterial ACC deaminase in phytoremediation. Trends Biotechnol 25:356–362

Arshad M, Shaharoona B, Mahmood T (2008) Inoculation with *Pseudomonas* spp. Containing ACC-deaminase partially eliminates the effects of drought stress on growth, yield, and ripening of pea (*Pisum sativum* L.) Project supported by the Higher Education Commission, Islamabad, Pakistan (No. PIN 041 211534 A-031). Pedosphere 18:611–620

Ashraf M, Akram NA (2009) Improving salinity tolerance of plants through conventional breeding and genetic engineering: an analytical comparison. Biotechnol Adv 27:744–752

Ashraf M, Hasnain S, Berge O, Mahmood T (2004) Inoculating wheat seedlings with exopolysaccharide-producing bacteria restricts sodium uptake and stimulates plant growth under salt stress. Biol Fertil Soils 40:157–162

Athar R, Ahmad M (2002) Heavy Metal Toxicity: Effect on plant growth and metal uptake by wheat, and on free living *Azotobacter*. Water Air Soil Pollut 138:165–180

Babalola OO (2010) Beneficial bacteria of agricultural importance. Biotechnol Lett 32:1559–1570

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Baligar VC, Fageria NK, He ZL (2001) Nutrient use efficiency in plants. Commun Soil Sci Plant Anal 32:921–950

Banik P, Midya A, Sarkar BK, Ghose SS (2006) Wheat and chickpea intercropping systems in an additive series experiment: advantages and weed smothering. Eur J Agro 24:325–332

Bano A, Fatima M (2009) Salt tolerance in *Zea mays* (L). following inoculation with Rhizobium and Pseudomonas. Biol Fertil Soils 45:405–413

Barassi CA, Ayrault G, Creus CM, Sueldo RJ, Sobrero MT (2006) Seed inoculation with Azospirillum mitigates NaCl effects on lettuce. Sci Hortic 109:8–14

Barnawal D, Bharti N, Maji D, Chanotiya CS, Kalra A (2012) 1-Aminocyclopropane-1-carboxylic acid (ACC) deaminase-containing rhizobacteria protect *Ocimum sanctum* plants during waterlogging stress via reduced ethylene generation. Plant Physiol Biochem 58:227–235

Barzanti R, Ozino F, Bazzicalupo M, Gabbrielli R, Galardi F, Gonnelli C, Mengoni A (2007) Isolation and characterization of endophytic bacteria from the nickel hyperaccumulator plant Alyssum bertolonii. Microb Ecol 53:306–316

Belimov AA, Dodd IC, Hontzeas N, Theobald JC, Safronova VI, Davies WJ (2009) Rhizosphere bacteria containing 1-aminocyclopropane-1-carboxylate deaminase increase yield of plants grown in drying soil via both local and systemic hormone signalling. New Phytol 181:413–423

Belimov AA, Dodd IC, Safronova VI, Shaposhnikov AI, Azarova TS, Makarova NM, Davies WJ, Tikhonovich IA (2015) Rhizobacteria that produce auxins and contain 1-amino-cyclopropane-1-carboxylic acid deaminase decrease amino acid concentrations in the rhizosphere and improve growth and yield of well-watered and water-limited potato (*Solanum tuberosum*). Ann Appl Biol 167:11–25

Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S, Glick BR (2005) Cadmium-tolerant plant growth-promoting bacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.) Soil Biol Biochem 37:241–250

Bensalim S, Nowak J, Asiedu S (1998) A plant growth promoting rhizobacterium and temperature effects on performance of 18 clones of potato. Am J Pot Res 75:145–152

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1–13

Bhatnagar-Mathur P, Vadez V, Sharma KK (2008) Transgenic approaches for abiotic stress tolerance in plants: Retrospect and prospects. Plant Cell Rep 27:411–424

Bianco C, Defez R (2009) *Medicago truncatula* improves salt tolerance when nodulated by an indole-3-acetic acid-overproducing *Sinorhizobium meliloti* strain. J Exp Bot 60:3097–3107

Botella MÁ, Del Amor F, Amorós A, Serrano M, Martínez V, Cerdá A (2000) Polyamine, ethylene and other physico-chemical parameters in tomato (*Lycopersicon esculentum*) fruits as affected by salinity. Physiol Plantarum 109:428–434

Botella MA, Martinez V, Pardines J, Cerdá A (1997) Salinity induced potassium deficiency in maize plants. J Plant Physiol 150:200–205

Boukhalfa H, Crumbliss AL (2002) Chemical aspects of siderophore mediated iron transport. Biometals 15:325–339

Buée M, Boer W, Martin F, Overbeek L, Jurkevitch E (2009) The rhizosphere zoo: An overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant Soil 321:189–212

Burd GI, Dixon DG, Glick BR (2000) Plant growth-promoting bacteria that decrease heavy metal toxicity in plants. Can J Microbiol 46:237–245

Burken JG (2004) Uptake and metabolism of organic compounds: Green-Liver model. In: Phytoremediation. Wiley, pp 59–84

Caravaca F, Figueroa D, Barea JM, Azcón-Aguilar C, Roldán A (2004) Effect of mycorrhizal inoculation on nutrient acquisition, gas exchange, and nitrate reductase activity of two mediterranean-autochthonous shrub species under drought stress. J Plant Nutr 27:57–74

Carmen B, Roberto D (2011) Soil bacteria support and protect plants against abiotic stresses abiotic stress in plants mechanisms and adaptations. In: Shan A (ed) InTech. pp 143–170

Carrillo-Castañeda G, Juárez Muñoz J, Ramón Peralta-Videa J, Gomez E, Gardea-Torresdey JL (2002) Plant growth-promoting bacteria promote copper and iron translocation from root to shoot in alfalfa seedlings. J Plant Nutr 26:1801–1814

Cattivelli L, Rizza F, Badeck FW, Mazzucotelli E, Mastrangelo AM, Francia E, Marè C, Tondelli A, Stanca AM (2008) Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. Field Crop Res 105:1–14

Chakraborty N, Ghosh R, Ghosh S, Narula K, Tayal R, Datta A, Chakraborty S (2013) Reduction of oxalate levels in tomato fruit and consequent metabolic remodeling following overexpression of a fungal oxalate decarboxylase. Plant Physiol 162:364–378

Chakraborty U, Chakraborty B, Dey P, Chakraborty AP (2015) 15 Role of microorganisms in alleviation of abiotic stresses for sustainable agriculture abiotic stresses in crop plants. 232pp

Chang P, Gerhardt KE, Huang XD, XM Y, Glick BR, Gerwing PD, Greenberg BM (2014) Plant growth-promoting bacteria facilitate the growth of barley and oats in salt-impacted soil: implications for phytoremediation of saline soils. Int J Phytoremediation 16:1133–1147

Charlson DV, Shoemaker RC (2006) Evolution of iron acquisition in higher plants. J Plant Nutr 29:1109–1125

Chen M, Wei H, Cao J, Liu R, Wang Y, Zheng C (2007) Expression of *Bacillus subtilis* proAB genes and reduction of feedback inhibition of proline synthesis increases proline production and confers osmotolerance in transgenic *Arabidopsis*. J Biochem Mole Biol 40:396–403

Chen M, Xu P, Zeng G, Yang C, Huang D, Zhang J (2015) Bioremediation of soils contaminated with polycyclic aromatic hydrocarbons, petroleum, pesticides, chlorophenols and heavy metals by composting: Applications, microbes and future research needs. Biotechnol Adv 33:745–755

Choudhary D (2012) Microbial rescue to plant under habitat-imposed abiotic and biotic stresses. Appl Microbiol Biotechnol 96:1137–1155

Choudhary DK, Kasotia A, Jain S, Vaishnav A, Kumari S, Sharma KP, Varma A (2015) Bacterial-mediated tolerance and resistance to plants under abiotic and biotic stresses. J Plant Growth Regul:1–25

C. Lucena, B. M. Waters, F. J. Romera, M. J. Garcia, M. Morales, E. Alcantara, R. Perez-Vicente, (2006) Ethylene could influence ferric reductase, iron transporter, and H+-ATPase gene expression by affecting FER (or FER-like) gene activity. Journal of Experimental Botany 57(15):4145–4154

Compant S, Reiter B, Sessitsch A, Nowak J, Clement C, Ait Barka E (2005) Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. Appl Environ Microbiol 71:1685–1693

Costacurta A, Vanderleyden J (1995) Synthesis of phytohormones by plant-associated bacteria. Critl Rev Microbiol 21:1–18

Council WW (2008) Water crisis. In: World Water Council (ed) Water at a Glance. World Water Council, Marseilles

Crowley DE, Reid CPP, Szaniszlo PJ (1988) Utilization of microbial siderophores in iron acquisition by oat. Plant Physiol 87:680–685

Curie C, Panaviene Z, Loulergue C, Dellaporta SL, Briat JF, Walker EL (2001) Maize yellow stripe1 encodes a membrane protein directly involved in Fe(III) uptake. Nature 409:346–349

Cushman JC, Bohnert HJ (2000) Genomic approaches to plant stress tolerance. Curr Opin Plant Biol 3:117–124

Dahmardeh M, Ghanbari A, Syasar B, Ramroudi M (2009) Effect of intercropping maize (*Zea mays* L.) with cow pea (*Vigna unguiculata* L.) on green forage yield and quality evaluation. Asian J Plant Sci 8:235

Damodaran T, Rai B, Jha SK, Kannan R, Pandey BK, Vijayalaxmi S, Mishra VK, Sharma DK (2014) Rhizosphere and endophytic bacteria for induction of salt tolerance in gladiolus grown in sodic soils. J Plant Interact 9:577–584

David BL, Christopher BF (2007) Global scale climate–crop yield relationships and the impacts of recent warming. Environ Res Lett 2:014002
Davies WJ, Zhang J, Yang J, Dodd IC (2011) Novel crop science to improve yield and resource use efficiency in water-limited agriculture. J Agri Sci 149:123–131
Des Marais DL, Juenger TE (2010) Pleiotropy, plasticity, and the evolution of plant abiotic stress tolerance. Ann N Y Acad Sci 1206:56–79
Desai A, Archana G (2011) Role of siderophores in crop improvement. In: Maheshwari KD (ed) Bacteria in agrobiology: plant nutrient management. Springer, Berlin, pp 109–139
Devliegher W, Arif M, Verstraete W (1995) Survival and plant growth promotion of detergent-adapted *Pseudomonas fluorescens* ANP15 and *Pseudomonas aeruginosa* 7NSK2. Appl Environ Microbiol 61:3865–3871
Diby P, Sarma YR, Srinivasan V, Anandaraj M (2005) *Pseudomonas fluorescens* mediated vigour in black pepper (*Piper nigrum* L.) under green house cultivation. Ann Microbiol 55:171–174
Dimkpa C, Weinand T, Asch F (2009) Plant-rhizobacteria interactions alleviate abiotic stress conditions. Plant Cell Environ 32:1682–1694
Dodd IC, Perez-Alfocea F (2012) Microbial amelioration of crop salinity stress. J Exp Bot 63:3415–3428
Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis 8:881–890
Dwivedi S, Upadhyaya H, Subudhi P, Gehring C, Bajic V, Ortiz R (2010) Enhancing abiotic stress tolerance in cereals through breeding and transgenic interventions. Plant Breeding Rev 33:31–114
Egamberdieva D (2009) Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. Acta Physiol Plant 31:861–864
Egamberdieva D (2011) Survival of *Pseudomonas extremorientalis* TSAU20 and *P. chlororaphis* TSAU13 in the rhizosphere of common bean (*Phaseolus vulgaris*) under saline conditions. Plant Soil Env 57:122–127
Egamberdieva D, Berg G, Lindström K, Räsänen L (2013) Alleviation of salt stress of symbiotic *Galega officinalis* L. (goat's rue) by co-inoculation of *Rhizobium* with root-colonizing Pseudomonas. Plant Soil 369:453–465
Egamberdieva D, Kamilova F, Validov S, Gafurova L, Kucharova Z, Lugtenberg B (2008) High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. Environ Microbiol 10:1–9
Egamberdieva D, Kucharova Z (2009) Selection for root colonising bacteria stimulating wheat growth in saline soils. Biol Fertil Soils 45:563–571
Egamberdieva D, Li L, Lindström K, Räsänen L (2015) A synergistic interaction between salt-tolerant *Pseudomonas* and *Mesorhizobium* strains improves growth and symbiotic performance of liquorice (*Glycyrrhiza uralensis* Fish.) under salt stress. Appl Microbiol Biotechnol:1–13
El-Khawas H, Adachi K (1999) Identification and quantification of auxins in culture media of *Azospirillum* and *Klebsiella* and their effect on rice roots. Biol Fertil Soils 28:377–381
Endris S, Mohammad MJ (2007) Nutrient acquisition and yield response of Barley exposed to salt stress under different levels of potassium nutrition. Int J Environ Sci Technol 4:323–330
Esquivel-Cote R, Ramírez-Gama RM, Tsuzuki-Reyes G, Orozco-Segovia A, Huante P (2010) *Azospirillum lipoferum* strain AZm5 containing 1-aminocyclopropane-1-carboxylic acid deaminase improves early growth of tomato seedlings under nitrogen deficiency. Plant Soil 337:65–75
Etesami H, Alikhani H, Mirseyed Hosseini H (2015a) Indole-3-Acetic Acid and 1-Aminocyclopropane-1-Carboxylate Deaminase: Bacterial traits required in rhizosphere, rhizoplane and/or endophytic competence by beneficial bacteria. In: Maheshwari DK (ed) Bacterial metabolites in sustainable agroecosystem, Sustainable development and biodiversity, vol 12. Springer International Publishing, New York, pp 183–258
Etesami H, Alikhani HA (2016a) Co-inoculation with endophytic and rhizosphere bacteria allows reduced application rates of N-fertilizer for rice plant. Rhizosphere 2:15. doi:10.1016/j.rhisph.2016.09.003
Etesami H, Alikhani HA (2016b) Rhizosphere and endorhiza of oilseed rape (*Brassica napus* L.) plant harbor bacteria with multifaceted beneficial effects. Biol Con 94:11–24

Etesami H, Alikhani HA, Hosseini HM (2015b) Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. MethodsX 2:72–78

Etesami H, Hosseini H, Alikhani H, Mohammadi L (2014a) Bacterial biosynthesis of 1-aminocyclopropane-1-carboxylate (ACC) deaminase and indole-3-acetic acid (IAA) as endophytic preferential selection traits by rice plant seedlings. J Plant Growth Regul 33:654–670

Etesami H, Mirseyed Hosseini H, Alikhani HA (2014b) Bacterial biosynthesis of 1-aminocyclopropane-1-caboxylate (ACC) deaminase, a useful trait to elongation and endophytic colonization of the roots of rice under constant flooded conditions. Physiol Mole Biol Plant 20:425–434

Eva Bacaicoa, Verónica Mora, Ángel María Zamarreño, Marta Fuentes, Esther Casanova, José María García-Mina, (2011) Auxin: A major player in the shoot-to-root regulation of root Fe-stress physiological responses to Fe deficiency in cucumber plants. Plant Physiology and Biochemistry 49(5):545–556

FAO (2008) Land and plant nutrition management service. http://www.fao.org/ag/agl/agll/spush

FAO (2009a) Aquastat www.faoorg/nr/water/aquastat/data/query/indexhtml

FAO (2009b) Food and Agriculture Organisation of the United Nations. www.faoorg/askfao/topicsListdo?mainAreaId=20263

Feigin A (1985) Fertilization management of crops irrigated with saline water. Plant Soil 89:285–299

Franche C, Lindström K, Elmerich C (2008) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59. doi:10.1007/s11104-008-9833-8

Francisco M. del Amor, Paula Cuadra-Crespo, (2012) Plant growth-promoting bacteria as a tool to improve salinity tolerance in sweet pepper. Functional Plant Biology 39(1):82

Fu Q, Liu C, Ding N, Lin Y, Guo B (2010) Ameliorative effects of inoculation with the plant growth-promoting rhizobacterium *Pseudomonas* sp. DW1 on growth of eggplant (*Solanum melongena* L.) seedlings under salt stress. Agri Water Manage 97:1994–2000

Gadd GM (2000) Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. Curr Opin Biotechnol 11:271–279

Garcia NS, Fu F, Sedwick PN, Hutchins DA (2015) Iron deficiency increases growth and nitrogen-fixation rates of phosphorus-deficient marine cyanobacteria. ISME J 9:238–245

Garipova SR (2014) Perspectives on using endophytic bacteria for the bioremediation of arable soils polluted by residual amounts of pesticides and xenobiotics. Biol Bulletin Rev 4:300–310

Geddie JL, Sutherland IW (1993) Uptake of metals by bacterial polysaccharides. J Appl Bacteriol 74:467–472

Germaine KJ, Liu X, Cabellos GG, Hogan JP, Ryan D, Dowling DN (2006) Bacterial endophyte-enhanced phytoremediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. FEMS Microbiol Ecol 57:302–310

Germida J, Siciliano S (2001) Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars. Biol Fertil Soils 33:410–415

Glick BR (2005) Modulation of plant ethylene levels by the bacterial enzyme ACC deaminase. FEMS Microbiol Llett 251:1–7

Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28:367–374

Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica:1–15

Glick BR (2014) Bacteria with ACC deaminase can promote plant growth and help to feed the world. Microbiol Res 169:30–39

Glick BR, Cheng Z, Czarny J, Duan J (2007a) Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur J Plant Pathol 119:329–339

Glick BR, Todorovic B, Czarny J, Cheng Z, Duan J, McConkey B (2007b) Promotion of plant growth by bacterial ACC Deaminase. Crit Rev Plant Sci 26:227–242

Glenn F. J. Dulla, Ksenia V. Krasileva, Steven E. Lindow, Interference of quorum sensing in Pseudomonas syringae by bacterial epiphytes that limit iron availability. Environmental Microbiology 12(6):1762–1774

Gnanamanickam SS (2006) Plant-associated bacteria, vol 1. Springer, New York

Godfray HCJ, Beddington JR, Crute IR, Haddad L, Lawrence D, Muir JF, Pretty J, Robinson S, Thomas SM, Toulmin C (2010) Food security: The challenge of feeding 9 billion people. Sci 327:812–818

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37:395–412

Grieve CM, Grattan SR (1999) Mineral nutrient acquisition and response by plants grown in saline environments. In: Pessarakli M (ed) Handbook of plant and crop stress, Books in soils, plants, and the environment, vol 2. CRC Press, New York, pp 203–229

Grover M, Ali SZ, Sandhya V, Rasul A, Venkateswarlu B (2010) Role of microorganisms in adaptation of agriculture crops to abiotic stresses. World J Microbiol Biotechnol 27:1231–1240

Guerinot ML (2010) Iron. In: Hell R, Mendel R-R (eds) Cell biology of metals and nutrients. Springer, Berlin, pp 75–94

Guinel FC, Sloetjes LL (2000) Ethylene is involved in the nodulation phenotype of *Pisum sativum* R50 (sym 16), a pleiotropic mutant that nodulates poorly and has pale green leaves. J Exp Bbot 51:885–894

Gyaneshwar P, Kumar GN, Parekh L, Poole P (2002) Role of soil microorganisms in improving P nutrition of plants. In: Food security in nutrient-stressed environments: exploiting plants' genetic capabilities. Springer, pp 133–143

Hamdia MAE-S, Shaddad MAK, Doaa MM (2004) Mechanisms of salt tolerance and interactive effects of *Azospirillum brasilense* inoculation on maize cultivars grown under salt stress conditions. Plant Growth Reg 44:165–174

Hamilton CE, Bever JD, Labbé J, Yang X, Yin H (2016) Mitigating climate change through managing constructed-microbial communities in agriculture. Agri Ecosys Environ 216:304–308

Hansen J, Sato M, Ruedy R (2012) Perception of climate change. Proc Natl Acad Sci U S A 109:E2415–E2423

Hartmann A, Schmid M, Dv T, Berg G (2008) Plant-driven selection of microbes. Plant Soil 321:235–257

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) plant cellular and molecular responses to high salinity. Ann Rev Plant Physiol Plant Mole Biol 51:463–499. doi:10.1146/annurev.arplant.51.1.463

Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598

Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A (2012) Role of proline under changing environments: A review. Plant Signaling Behavior 7:1456–1466

Heidari M, Golpayegani A (2012) Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.) J Saudi Soc Agril Scie 11:57–61

Heidari M, Jamshid P (2010) Interaction between salinity and potassium on grain yield, carbohydrate content and nutrient uptake in pearl millet ARPN. J Agri Biol Sci 5:39–46

Hichem H, Naceur A, Mounir D (2010) Effects of salt stress on photosynthesis, PSII photochemistry and thermal energy dissipation in leaves of two corn (*Zea mays* L.) varieties. Photosynthetica 47:517–526

Hiz MC, Canher B, Niron H, Turet M (2014) Transcriptome Analysis of salt tolerant common bean (*Phaseolus vulgaris* L.) under saline conditions. PLoS One 9:e92598

Hong YF, Liu CY, Cheng KJ, Hour AL, Chan MT, Tseng TH, Chen KY, Shaw JF, SM Y (2008) The sweet potato sporamin promoter confers high-level phytase expression and improves organic phosphorus acquisition and tuber yield of transgenic potato. Plant Mole Bbiol 67:347–361

Hopkins WG, Huner NPA (2009) Introduction to plant physiology. Wiley, Hoboken

Jain A, Poling MD, Karthikeyan AS, Blakeslee JJ, Peer WA, Titapiwatanakun B, Murphy AS, Raghothama KG (2007) Differential effects of sucrose and auxin on localized phosphate deficiency-induced modulation of different traits of root system architecture in *Arabidopsis*. Plant Physiol 144:232–247

James RA, von Caemmerer S, Condon AG, Zwart AB, Munns R (2008) Genetic variation in tolerance to the osmotic stress componentof salinity stress in durum wheat. Functional Plant Biol 35:111–123

Jamil A, Riaz S, Ashraf M, Foolad MR (2011) Gene expression profiling of plants under salt stress. Crit Rev Plant Sci 30:435–458

Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea J-M (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37:1–16

Jewell MC, Campbell BC, Godwin ID (2010) Transgenic plants for abiotic stress resistance. In: Kole C, Michler CH, Abbott AG, Hall TC (eds) Transgenic crop plants. Springer, Berlin, pp 67–132

Jha Y, Subramanian R (2013) Paddy plants inoculated with PGPR show better growth physiology and nutrient content under saline condition. Chilean J Agri Res 73:213–219

Jha Y, Subramanian RB, Patel S (2011) Combination of endophytic and rhizospheric plant growth promoting rhizobacteria in *Oryza sativa* shows higher accumulation of osmoprotectant against saline stress. Acta Physiol Plant 33:797–802

Jiang F, Chen L, Belimov AA, Shaposhnikov AI, Gong F, Meng X, Hartung W, Jeschke DW, Davies WJ, Dodd IC (2012) Multiple impacts of the plant growth-promoting rhizobacterium *Variovorax paradoxus* 5C-2 on nutrient and ABA relations of *Pisum sativum*. J Exp Bot 63:6421–6430

Jin CW, He YF, Tang CX, Wu P, Zheng SJ (2006) Mechanisms of microbially enhanced Fe acquisition in red clover (*Trifolium pratense* L.) Plant Cell Environ 29:888–897

Johnson GV, Lopez A, La Valle Foster N (2002) Reduction and transport of Fe from siderophores. Plant Soil 241:27–33

Johnson HE, Broadhurst D, Goodacre R, Smith AR (2003) Metabolic fingerprinting of salt-stressed tomatoes. Phytochemistry 62:919–928

Kaparullina EN, Doronina NV, Trotsenko YA (2011) Aerobic degradation of ethylenediaminetetraacetate (review). Appl Biochem Microbiol 47:460–473

Kasotia A, Varma A, Choudhary DK (2015) *Pseudomonas*-mediated mitigation of salt stress and growth promotion in *Glycine max*. Agribiol Res 4:31–41

Kaya C, Kirnak H, Higgs D, Saltali K (2002) Supplementary calcium enhances plant growth and fruit yield in strawberry cultivars grown at high (NaCl) salinity. Sci Hortic 93:65–74

Kaymak HC, Guvenc I, Yarali F, Donmez MF (2009) The effects of bio-priming with PGPR on germination of radish (*Raphanus sativus* L.) seeds under saline conditions. Turk J Agric For 33:173–179

Khalid A, Arshad M, Zahir ZA (2004) Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J Appl Mmicrobiol 96:473–480

Khan AL, Hamayun M, Kang S-M, Kim Y-H, Jung H-Y, Lee J-H, Lee I-J (2012) Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of *Paecilomyces formosus* LHL10. BMC Microbiol 12:1

Khan M, Khan NA (2013) Salicylic acid and jasmonates: approaches in abiotic stress tolerance. J Plant Biochem Physiol 1

Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. Agron Sustain Dev 27:29–43

Khoshgoftarmanesh AH, Schulin R, Chaney RL, Daneshbakhsh B, Afyuni M (2010) Micronutrient-efficient genotypes for crop yield and nutritional quality in sustainable agriculture. A review. Agron Sustain Dev 30:83–107

Kijne JW (2006) Abiotic stress and water scarcity: Identifying and resolving conflicts from plant level to global level. Field Crop Res 97:3–18

Kim SY, Lim JH, Park MR, Kim YJ, Park TI, Seo YW, Choi KG, Yun SJ (2005) Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under saline stress. J Biochem Mole Biol 38:218–224

Knight H (2000) Calcium signaling during abiotic stress in plants. Int Rev Cytol 195:269–324

Kohler J, Hernández JA, Caravaca F, Roldán A (2009) Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. Environ Exp Bot 65:245–252

Kundu BS, Sangwan P, Sharma PK, Nandwal AS (1997) Response of pearl millet to phytohormones produced by *Azospirillum brasilense*. Indian J Plant Physiol 2:101–104

Lemanceau P, Expert D, Gaymard F, Bakker PAHM, Briat JF (2009) Chapter 12 Role of iron in plant–microbe interactions. In: Advances in botanical research, vol 51, Academic Press, New York. pp 491–549

Li J, McConkey BJ, Cheng Z, Guo S, Glick BR (2013) Identification of plant growth-promoting bacteria-responsive proteins in cucumber roots under hypoxic stress using a proteomic approach. J Proteome 84:119–131

Li J, Sun J, Yang Y, Guo S, Glick BR (2012) Identification of hypoxic-responsive proteins in cucumber roots using a proteomic approach. Plant Physiol Biochem 51:74–80

Lifshitz R, Kloepper JW, Scher FM, Tipping EM, Laliberté M (1986) Nitrogen-fixing Pseudomonads isolated from roots of plants grown in the Canadian High Arctic. Appl Environ Microbiol 51:251–255

Ligero F, Poveda JL, Gresshoff PM, Caba JM (1999) Nitrate- and inoculation-enhanced ethylene biosynthesis in soybean roots as a possible mediator of nodulation control. J Plant Physiol 154:482–488

Lim PE, Mak KY, Mohamed N, Noor AM (2003) Removal and speciation of heavy metals along the treatment path of wastewater in subsurface-flow constructed wetlands. Water Sci Technol J Int Assoc Water Pollut Res 48:307–313

Lin CC, Lin HL (2005) Remediation of soil contaminated with the heavy metal (Cd^{2+}). J Hazard Mater 122:7–15

Lodewyckx C, Mergeay M, Vangronsveld J, Clijsters H, Van der Lelie D (2002a) Isolation, characterization, and identification of bacteria associated with the zinc hyperaccumulator *Thlaspi caerulescens* subsp. Calaminaria. Int J Phytoremediation 4:101–115

Lodewyckx C, Vangronsveld J, Porteous F, Moore ERB, Taghavi S, Mezgeay M, Lelie Dv d (2002b) Endophytic bacteria and their potential applications. Critl Rev Plant Sci 21:583–606

Ludwig-Müller J (2004) From auxin homeostasis to understanding plant pathogen and plant symbiont interaction: editor's research interests. J Plant Growth Regul 23:1–8

Lugtenberg B (2015) Life of microbes in the rhizosphere. In: Lugtenberg B (ed) Principles of plant-microbe interactions: microbes for sustainable agriculture. Springer, Cham, pp 7–15

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Ann Review Microbiol 63:541–556

Luo S, Wan Y, Xiao X, Guo H, Chen L, Xi Q, Zeng G, Liu C, Chen J (2011) Isolation and characterization of endophytic bacterium LRE07 from cadmium hyperaccumulator *Solanum nigrum* L. and its potential for remediation. Appl Microbiol Biotechnol 89:1637–1644

Lynch JP (2007) Turner review no. 14. Roots of the second green revolution. Aust J Bot 55:493–512

Ma Y, Rajkumar M, Vicente J, Freitas H (2010) Inoculation of Ni-resistant plant growth promoting bacterium *Psychrobacter* sp. strain SRS8 for the improvement of nickel phytoextraction by energy crops. Int J Phytoremediation 13:126–139

Ma Z, Baskin TI, Brown KM, Lynch JP (2003) Regulation of root elongation under phosphorus stress involves changes in ethylene responsiveness. Plant Physiol 131:1381–1390

Madhaiyan M, Poonguzhali S, Sa T (2007) Metal tolerating methylotrophic bacteria reduces nickel and cadmium toxicity and promotes plant growth of tomato (*Lycopersicon esculentum* L). Chemosphere 69:220–228

Majumder AL, Sengupta S, Goswami L (2010) Osmolyte regulation in abiotic stress. In: Pareek A, Sopory SK, Bohnert JH (eds) Abiotic stress adaptation in plants: physiological, molecular and genomic foundation. Springer, Dordrecht, pp 349–370

Malik A (2004) Metal bioremediation through growing cells. Environ Intl 30:261–278

Manchanda G, Garg N (2008) Salinity and its effects on the functional biology of legumes. Acta Physiol Plantarum 30:595–618

Manter DK, Delgado JA, Holm DG, Stong RA (2010) Pyrosequencing reveals a highly diverse and cultivar-specific bacterial endophyte community in potato roots. Microb Ecol 60:157–166
Marschner H (1995) Mineral nutrition of higher plant, 2nd edn. Academic Press, New York
Marschner P, Yang CH, Lieberei R, Crowley DE (2001) Soil and plant specific effects on bacterial community composition in the rhizosphere. Soil Biol Biochem 33:1437–1445
Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166:525–530
Meloni DA, Oliva MA, Martinez CA, Cambraia J (2003) Photosynthesis and activity of superoxide dismutase, peroxidase and glutathione reductase in cotton under salt stress. Environ Exp Bot 49:69–76
Mensah JK, Ihenyen J (2009) Effects of salinity on germination, seedling establishment and yield of three genotypes of mung bean (*Vigna mungo* L. Hepper) in Edo State, Nigeria. Nigerian Ann Nat Sci 8:17–24
Miller GAD, Suzuki N, Ciftci-Yilmaz S, Mittler RON (2010) Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant Cell Environ 33:453–467
Milošević NA, Marinković JB, Tintor BB (2012) Mitigating abiotic stress in crop plants by microorganisms. Zbornik Matice srpske za prirodne nauke:17–26
Mittler R, Blumwald E (2010) Genetic engineering for modern agriculture: challenges and perspectives. Ann Rrev Plant Biol 61:443–462
Mittova V, Tal M, Volokita M, Guy M (2002) Salt stress induces up-regulation of an efficient chloroplast antioxidant system in the salt-tolerant wild tomato species *Lycopersicon pennellii* but not in the cultivated species. Physiol Plantarum 115:393–400
Mittova V, Tal M, Volokita M, Guy M (2003) Up-regulation of the leaf mitochondrial and peroxisomal antioxidative systems in response to salt-induced oxidative stress in the wild salt-tolerant tomato species *Lycopersicon pennellii*. Plant Cell Environt 26:845–856
Molla AH, Shamsuddin ZH, Halimi MS, Morziah M, Puteh AB (2001) Potential for enhancement of root growth and nodulation of soybean co-inoculated with *Azospirillum* and *Bradyrhizobium* in laboratory systems. Soil Biol Biochem 33:457–463
Moore FP, Barac T, Borremans B, Oeyen L, Vangronsveld J, van der Lelie D, Campbell CD, Moore ER (2006) Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: the characterisation of isolates with potential to enhance phytoremediation. Sys Appl Microbiol 29:539–556
Mrozik A, Piotrowska-Seget Z (2010) Bioaugmentation as a strategy for cleaning up of soils contaminated with aromatic compounds. Microbiol Res 165:363–375
Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250
Munns R (2005) Genes and salt tolerance: bringing them together. New Phytol 167:645–663
Munns R, James RA, Lauchli A (2006) Approaches to increasing the salt tolerance of wheat and other cereals. J Exp Bot 57:1025–1043
Munns R, Tester M (2008) Mechanisms of salinity tolerance. Ann Revi Plant Biol 59:651–681
Nicholas Smirnoff, (1998) Plant resistance to environmental stress. Current Opinion in Biotechnology 9(2):214–219
Nadeem SM, Zahir ZA, Naveed M, Arshad M (2007) Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Can J Microbiol 53:1141–1149
Nadeem SM, Zahir ZA, Naveed M, Arshad M (2009) Rhizobacteria containing ACC-deaminase confer salt tolerance in maize grown on salt-affected fields. Can J Microbiol 55:1302–1309
Nadeem SM, Zahir ZA, Naveed M, Nawaz S (2012) Mitigation of salinity-induced negative impact on the growth and yield of wheat by plant growth-promoting rhizobacteria in naturally saline conditions. Ann Microbiol 63:225–232
Nautiyal CS, Govindarajan R, Lavania M, Pushpangadan P (2008) Novel mechanism of modulating natural antioxidants in functional foods: Involvement of Plant Growth Promoting Rhizobacteria NRRL B-30488. J Agri Food Chem 56:4474–4481
Navarro JM, Botella MA, Cerdá A, Martinez V (2001) Phosphorus uptake and translocation in salt-stressed melon plants. J Plant Physiol 158:375–381

Nia SH, Zarea MJ, Rejali F, Varma A (2012) Yield and yield components of wheat as affected by salinity and inoculation with *Azospirillum* strains from saline or non-saline soil. J the Saudi Soc Agri Sci 11:113–121

Niu YF, Chai RS, Jin GL, Wang H, Tang CX, Zhang YS (2013) Responses of root architecture development to low phosphorus availability: a review. Ann Bot 112:391–408

Ondrasek G, Rengel Z, Romic D, Poljak M, Romic M (2009) Accumulation of non/essential elements in radish plants grown in salt-affected and cadmium contaminated environment. Cereal Res Comm 37:9–12

Ortíz-Castro R, Contreras-Cornejo HA, Macías-Rodríguez L, López-Bucio J (2009) The role of microbial signals in plant growth and development. Plant Signaling Behavior 4:701–712

Osmont KS, Sibout R, Hardtke CS (2007) Hidden branches: developments in root system architecture. Annu Rev Plant Biol 58:93–113

Pandolfi C, Mancuso S, Shabala S (2012) Physiology of acclimation to salinity stress in pea (*Pisum sativum*). Environ Exp Bot 84:44–51

Parida AK, Das AB (2005) Salt tolerance and salinity effects on plants: a review. Ecotoxicol Environ Safe 60:324–349

Park MR, Baek S-H, Reyes BG, Yun SJ (2007) Overexpression of a high-affinity phosphate transporter gene from tobacco (NtPT1) enhances phosphate uptake and accumulation in transgenic rice plants. Plant Soil 292:259–269

Parmar N, Dadarwal KR (1999) Stimulation of nitrogen fixation and induction of flavonoid-like compounds by rhizobacteria. J Appl Mmicrobiol 86:36–44

Paul D (2012) Osmotic stress adaptations in rhizobacteria. J Basic Microbiol 52:1–10

Paul D (2013) Osmotic stress adaptations in rhizobacteria. J Basic Microbiol 53:101–110

Paul D, Lade H (2014) Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: a review. Agron Sustain Dev 34:737–752

Paul D, Nair S (2008) Stress adaptations in a plant growth promoting rhizobacterium (PGPR) with increasing salinity in the coastal agricultural soils. J Basic Microbiol 48:378–384

Paul D, Sarma YR (2006) Plant growth promoting rhizhobacteria (PGPR)-mediated root proliferation in black pepper (*Piper nigrum* L.) as evidenced through GS root software. Arch Phytopathol Plant Protect 39:311–314

Peng YL, Gao ZW, Gao Y, Liu GF, Sheng LX, Wang DL (2008) Eco-physiological characteristics of alfalfa seedlings in response to various mixed salt-alkaline stresses. J Integrative Plant biol 50:29–39

Pereira PAA, Bliss FA (1989) Selection of common bean (*Phaseolus vulgaris* L.) for N_2 fixation at different levels of available phosphorus under field and environmentally-controlled conditions. Plant Soil 115:75–82

Pérez-Torres C-A, López-Bucio J, Cruz-Ramírez A, Ibarra-Laclette E, Dharmasiri S, Estelle M, Herrera-Estrella L (2008) Phosphate availability alters lateral root development in *Arabidopsis* by modulating auxin sensitivity via a mechanism involving the TIR1 auxin receptor. Plant Cell 20:3258–3272

Peters NK, Crist-Estes DK (1989) Nodule formation is stimulated by the ethylene inhibitor aminoethoxyvinylglycine. Plant Physiol 91:690–693

Pierik R, Tholen D, Poorter H, Visser EJ, Voesenek LA (2006) The Janus face of ethylene: growth inhibition and stimulation. Trends Plant Sci 11:176–183

Pikovskaya RI (1948) Mobilization of phosphorus in soil in connection with vital activity of some microbial species Mikrobiologiya 17:362–370

Pitzschke A, Forzani C, Hirt H (2006) Reactive oxygen species signaling in plants. Antioxidants Redox Signaling 8:1757–1764

Polle A, Luo Z-B (2014) Biotic and abiotic interactions in plants: Novel ideas for agriculture and forestry in a changing environment. Environ Exp Bot 108:1–3

Porcel R, Aroca R, Ruiz-Lozano JM (2011) Salinity stress alleviation using arbuscular mycorrhizal fungi. A review. Agron Sustain Dev 32:181–200

Rabie G, Almadini AM (2005) Role of bioinoculants in development of salt-tolerance of *Vicia faba* plants under salinity stress. Afr J Biotech 4:210–222

Rubén Bottini, Fabricio Cassán, Patricia Piccoli, (2004) Gibberellin production by bacteria and its involvement in plant growth promotion and yield increase. Applied Microbiology and Biotechnology 65 (5)

Rajkumar M, Nagendran R, Lee KJ, Lee WH, Kim SZ (2006) Influence of plant growth promoting bacteria and Cr^{6+} on the growth of Indian mustard. Chemosphere 62:741–748

Ramadoss D, Lakkineni VK, Bose P, Ali S, Annapurna K (2013a) Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. Springer Plus 2(1):6

Ramadoss D, Lakkineni VK, Bose P, Ali S, Annapurna K (2013b) Mitigation of salt stress in wheat seedlings by halotolerant bacteria isolated from saline habitats. Springer Plus 2:6

Rasouli-Sadaghiani M, Malakouti MJ, Khavazi K, Miransari M (2014) Siderophore efficacy of Fluorescent Pseudomonades affecting labeled iron (59Fe) uptake by wheat (Triticum aestivum L.) genotypes differing in Fe efficiency. In: Miransari M (ed) Use of microbes for the alleviation of soil stresses, Alleviation of soil stress by PGPR and Mycorrhizal Fungi, vol 2. Springer, New York, pp 121–132

Rekha PD, Lai W-A, Arun AB, Young CC (2007) Effect of free and encapsulated *Pseudomonas putida* CC-FR2-4 and Bacillus subtilis CC-pg104 on plant growth under gnotobiotic conditions. Bioresour Technol 98:447–451

Remans R, Beebe S, Blair M, Manrique G, Tovar E, Rao I, Croonenborghs A, Torres-Gutierrez R, El-Howeity M, Michiels J, Vanderleyden (2008) Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (*Phaseolus vulgaris* L.). Plant Soil 302:149–161

Riesen O, Feller U (2005) Redistribution of nickel, cobalt, manganese, zinc, and cadmium via the phloem in young and maturing wheat. J Plant Nutr 28:421–430

Robin A, Vansuyt G, Hinsinger P, Meyer JM, Briat J-F, Lemanceau P (2008) Iron dynamics in the rhizosphere: consequences for plant health and nutrition. Adv Agron 99:183–225

Rodriguez H, Gonzalez T, Goire I, Bashan Y (2004) Gluconic acid production and phosphate solubilization by the plant growth-promoting bacterium *Azospirillum* spp. Naturwissenschaften 91:552–555

Rodriguez R, Redman R (2008) More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. J Exp Bot 59:1109–1114

Rogers ME, Grieve CM, Shannon MC (2003) Plant growth and ion relations in lucerne (*Medicago sativa* L.) in response to the combined effects of NaCl and P. Plant Soil 253:187–194

Rojas-Tapias D, Moreno-Galván A, Pardo-Díaz S, Obando M, Rivera D, Bonilla R (2012) Effect of inoculation with plant growth-promoting bacteria (PGPB) on amelioration of saline stress in maize (*Zea mays*). Appl Soil Ecol 61:264–272

Sadeghi A, Karimi E, Dahaji PA, Javid MG, Dalvand Y, Askari H (2012) Plant growth promoting activity of an auxin and siderophore producing isolate of Streptomyces under saline soil conditions. World J Microbiol Biotechnol 28:1503–1509

Safronova VI, Piluzza G, Zinovkina NY, Kimeklis AK, Belimov AA, Bullitta S (2012) Relationships between pasture legumes, rhizobacteria and nodule bacteria in heavy metal polluted mine waste of SW Sardinia. Symbiosis 58:149–159

Safronova VI, Stepanok VV, Engqvist GL, Alekseyev YV, Belimov AA (2006) Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. Biol Fertil Soils 42:267–272

Saleem M, Arshad M, Hussain S, Bhatti A (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Ind Microbiol Biotechnol 34:635–648

Sanchez DH, Lippold F, Redestig H, Hannah MA, Erban A, Krämer U, Kopka J, Udvardi MK (2008) Integrative functional genomics of salt acclimatization in the model legume Lotus japonicus. The Plant J 53:973–987

Sandalio LM, Dalurzo HC, Gomez M, Romero-Puertas MC, del Rio LA (2001) Cadmium-induced changes in the growth and oxidative metabolism of pea plants. J Exp Bot 52:2115–2126

Sandhya V, Sk ZA, Grover M, Reddy G, Venkateswarlu B (2009) Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. Biol Fertil Soils 46:17–26

Sanità di Toppi L, Gabbrielli R (1999) Response to cadmium in higher plants. Environ Exp Bot 41:105–130

Santner A, Calderon-Villalobos LIA, Estelle M (2009) Plant hormones are versatile chemical regulators of plant growth. Nature Chem Biol 5:301–307

Schmidt W (2003) Iron solutions: acquisition strategies and signaling pathways in plants. Trends Plant Sci 8:188–193

Schubert S, Neubert A, Schierholt A, Sümer A, Zörb C (2009) Development of salt-resistant maize hybrids: The combination of physiological strategies using conventional breeding methods. Plant Sci 177:196–202

Scott JA, Karanjkar AM (1992) Repeated cadmium biosorption by regenerated *Enterobacter aerogenes* biofilm attached to activated carbon. Biotechnol Lett 14:737–740

Selvakumar G, Joshi P, Mishra P, Bisht J, Gupta H (2009) Mountain aspect influences the genetic clustering of psychrotolerant phosphate solubilizing *Pseudomonads* in the Uttarakhand Himalayas. Curr Microbiol 59:432–438

Selvakumar G, Kundu S, Joshi P, Nazim S, Gupta AD, Mishra PK, Gupta HS (2008a) Characterization of a cold-tolerant plant growth-promoting bacterium *Pantoea dispersa* 1A isolated from a sub-alpine soil in the North Western Indian Himalayas. World J Microbiol Biotechnol 24:955–960

Selvakumar G, Mohan M, Kundu S, Gupta AD, Joshi P, Nazim S, Gupta HS (2008b) Cold tolerance and plant growth promotion potential of *Serratia marcescens* strain SRM (MTCC 8708) isolated from flowers of summer squash (*Cucurbita pepo*). Lett Appl Microbiol 46:171–175

Sessitsch A, Reiter B, Pfeifer U, Wilhelm E (2002) Cultivation-independent population analysis of bacterial endophytes in three potato varieties based on eubacterial and Actinomycetes-specific PCR of 16S rRNA genes. FEMS Microbiol Ecol 39:23–32

Shabani L, Ehsanpour AA, Asghari G, Emami J (2009) Glycyrrhizin production by in vitro cultured *Glycyrrhiza glabra* elicited by methyl Jasmonate and salicylic acid. Russ J Plant Physiol 56:621–626

Shahbaz M, Ashraf M (2013) Improving salinity tolerance in cereals. Crit Rev Plant Sci 32:237–249

Shakir MA, Bano A, Arshad M (2012) Rhizosphere bacteria containing ACC-deaminase conferred drought tolerance in wheat grown under semi-arid climate. Soil Environ 31:108–112

Shanker AK, Venkateswarlu B (2011) Abiotic stress in plants—mechanisms and adaptations. InTech, Rijeka

Sharma A, Johri BN, Sharma AK, Glick BR (2003) Plant growth-promoting bacterium Pseudomonas sp. strain GRP 3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). Soil Biol Biochem 35:887–894

Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Curr Opin Plant Biol 3:217–223

Shrivastava P, Kumar R (2015) Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi J Biol Sci 22:123–131

Siciliano SD, Fortin N, Mihoc A, Wisse G, Labelle S, Beaumier D, Ouellette D, Roy R, Whyte LG, Banks MK, Schwab P, Lee K, Greer CW (2001) Selection of specific endophytic bacterial genotypes by plants in response to soil contamination. Appl Environ Microbiol 67:2469–2475

Siciliano SD, Germida JJ (1999) Taxonomic diversity of bacteria associated with the roots of field-grown transgenic *Brassica napus* cv. Quest, compared to the non-transgenic *B. napus* cv. Excel and *B. rapa* cv. Parkland. FEMS Microbiol Ecol 29:263–272

Siddikee MA, Glick BR, Chauhan PS, Wj Y, Sa T (2011) Enhancement of growth and salt tolerance of red pepper seedlings (*Capsicum annuum* L.) by regulating stress ethylene synthesis with halotolerant bacteria containing 1-aminocyclopropane-1-carboxylic acid deaminase activity. Plant Physiol Biochem 49:427–434

Singh A, Sarma BK, Upadhyay RS, Singh HB (2013) Compatible rhizosphere microbes mediated alleviation of biotic stress in chickpea through enhanced antioxidant and phenylpropanoid activities. Microbiol Res 168:33–40

Singh JS, Pandey VC, Singh D (2011) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agri Ecosys Environ 140:339–353

Soleimani M, Akbar S, Hajabbasi MA (2011) Enhancing phytoremediation efficiency in response to environmental pollution stress. Plants Environ 23:10–14

Son HJ, Park GT, Cha MS, Heo MS (2006) Solubilization of insoluble inorganic phosphates by a novel salt- and pH-tolerant *Pantoea agglomerans* R-42 isolated from soybean rhizosphere. Bioresour Technol 97:204–210

Spaepen S, Dobbelaere S, Croonenborghs A, Vanderleyden J (2008) Effects of *Azospirillum* brasilense indole-3-acetic acid production on inoculated wheat plants. Plant Soil 312:15–23

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol 3:a001438

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448

Sreenivasulu N, Sopory SK, Kishor PBK (2007) Deciphering the regulatory mechanisms of abiotic stress tolerance in plants by genomic approaches. Gene 388:1–13

Stearns JC, Shah S, Greenberg BM, Dixon DG, Glick BR (2005) Tolerance of transgenic canola expressing 1-aminocyclopropane-1-carboxylic acid deaminase to growth inhibition by nickel. Plant Physiol Biochem 43:701–708

Steve J. Sinclair, Matthew D. White, Graeme R. Newell, (2010) How Useful Are Species Distribution Models for Managing Biodiversity under Future Climates?. Ecology and Society 15 (1)

Su F, Jacquard C, Villaume S, Michel J, Rabenoelina F, Clément C, Barka EA, Dhondt-Cordelier S, Vaillant-Gaveau N (2015) Burkholderia phytofirmans PsJN reduces impact of freezing temperatures on photosynthesis in *Arabidopsis thaliana*. Frontiers Plant Sci 6

Sugawara M, Okazaki S, Nukui N, Ezura H, Mitsui H, Minamisawa K (2006) Rhizobitoxine modulates plant-microbe interactions by ethylene inhibition. Biotechnol Adv 24:382–388

Szabados L, Savouré A (2010) Proline: a multifunctional amino acid. Trends Plant Sci 15:89–97

Tang C, Robson AD, Dilworth MJ (1990) A split-root experiment shows that iron is required for nodule initiation in *Lupinus angustifolius* L. New Phytol 115:61–67

Taurian T, Soledad Anzuay M, Angelini JG, Tonelli ML, Ludueña L, Pena D, Ibáñez F, Fabra A (2010) Phosphate-solubilizing peanut associated bacteria: screening for plant growth-promoting activities. Plant Soil 329:421–431

Tester M, Davenport R (2003) Na^+ tolerance and Na^+ transport in higher plants. Ann Bot 91:503–527

Theunis M (2005) IAA biosynthesis in rhizobia and its potential role in symbiosis PhD thesis, Universiteit Antwerpen

Tiwari S, Singh P, Tiwari R, Meena KK, Yandigeri M, Singh DP, Arora DK (2011) Salt-tolerant rhizobacteria-mediated induced tolerance in wheat (*Triticum aestivum*) and chemical diversity in rhizosphere enhance plant growth. Biol Fertil Soils 47:907–916

Tokala RK, Strap JL, Jung CM, Crawford DL, Salove MH, Deobald LA, Bailey JF, Morra MJ (2002) Novel plant-microbe rhizosphere interaction involving *Streptomyces* lydicus WYEC108 and the pea plant (*Pisum sativum*). Appl Environ Microbiol 68:2161–2171

Tuberosa R, Salvi S (2006) Genomics-based approaches to improve drought tolerance of crops. Trends Plant Sci 11:405–412

Upadhyay S, Singh D, Saikia R (2009) Genetic diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition. Curr Microbiol 59:489–496

Upadhyay SK, Singh JS, Saxena AK, Singh DP (2012) Impact of PGPR inoculation on growth and antioxidant status of wheat under saline conditions. Plant Biol (Stuttgart, Germany):14, 605–611

Upadhyay SK, Singh JS, Singh DP (2011) Exopolysaccharide-producing plant growth-promoting rhizobacteria under salinity condition. Pedosphere 21:214–222

Valliyodan B, Nguyen HT (2006) Understanding regulatory networks and engineering for enhanced drought tolerance in plants. Curr Opin Plant Biol 9:189–195

Van Der Heijden MG, Bardgett RD, Van Straalen NM (2008) The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecol Lett 11:296–310

van Hoorn JW, Katerji N, Hamdy A, Mastrorilli M (2001) Effect of salinity on yield and nitrogen uptake of four grain legumes and on biological nitrogen contribution from the soil. Agri Water Manage 51:87–98

Van Overbeek L, Van Elsas JD (2008) Effects of plant genotype and growth stage on the structure of bacterial communities associated with potato (*Solanum tuberosum* L.) FEMS Microbiol Ecol 64:283–296

Vansuyt G, Robin A, Briat J-F, Curie C, Lemanceau P (2007) Iron acquisition from Fe-pyoverdine by *Arabidopsis thaliana*. Mole Plant-Microbe Interact 20:441–447

Venkateswarlu B, Desai S, Prasad YG (2008) Agriculturally important microorganisms for stressed ecosystems: challenges in technology development and application. In: Khachatourians GG, Arora DK, Rajendran TP, Srivastava AK (eds) Agriculturally important microorganisms, vol 1. Academic World, Bhopal. pp 225–246

Vurukonda SSKP, Vardharajula S, Shrivastava M, SkZ A (2016) Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol Res 184:13–24

Wang H, Tang X, Wang H, Shao H-B (2015) Proline accumulation and metabolism-related genes expression profiles in *Kosteletzkya virginica* seedlings under salt stress. Frontiers Plant Sci 6:792

Wang Q, Dodd IC, Belimov AA, Jiang F (2016) Rhizosphere bacteria containing 1-aminocyclopropane-1- carboxylate deaminase increase growth and photosynthesis of pea plants under salt stress by limiting Na^+ accumulation. Func Plant Biol 43:161–172

Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1–14

Wang WX, Vinocur B, Shoseyov O, Altman A (2001) Biotechnology of plant osmotic stress tolerance: physiological and molecular considerations. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp 285–292

Wintergerst ES, Maggini S, Hornig DH (2007) Contribution of selected vitamins and trace elements to immune function. Ann Nutr Metabol 51:301–323

Witcombe JR, Hollington PA, Howarth CJ, Reader S, Steele KA (2008) Breeding for abiotic stresses for sustainable agriculture. Philos Trans R Soc Lond B Biol Sci 363:703–716

Xiao B, Huang Y, Tang N, Xiong L (2007) Over-expression of a LEA gene in rice improves drought resistance under the field conditions. Theor Appl Genet 115:35–46

Yahalom E, Dovrat A, Okon Y, Czosnek H (1991) Effect of inoculation with *Azospirillum brasilense strain* Cd and *Rhizobium* on the root morphology of burr medic (*Medicago polymorpha* L.) Israel J Bot 40:155–164

Yamaguchi T, Blumwald E (2005) Developing salt-tolerant crop plants: Challenges and opportunities. Trends Plant Sci 10:615–620

Yang J, Kloepper JW, Ryu C-M (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Yao L, Wu Z, Zheng Y, Kaleem I, Li C (2010) Growth promotion and protection against salt stress by *Pseudomonas putida* Rs-198 on cotton. Eur J Soil Biol 46:49–54

Yehuda Z, Shenker M, Romheld V, Marschner H, Hadar Y, Chen Y (1996) The role of ligand exchange in the uptake of iron from microbial siderophores by gramineous plants. Plant Physiol 112:1273–1280

Yildirim E, Taylor AG (2005) Effect of biological treatments on growth of bean plans under salt stress. Ann Rep Bean Improve Cooperative 48:176–177

Yuhashi K, Ichikawa N, Ezura H, Akao S, Minakawa Y, Nukui N, Yasuta T, Minamisawa K (2000) Rhizobitoxine production by Bradyrhizobium elkanii enhances nodulation and competitiveness on *Macroptilium atropurpureum*. Appl Environm Microbiol 66:2658–2663

Zaidi S, Usmani S, Singh BR, Musarrat J (2006) Significance of *Bacillus subtilis* strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in *Brassica juncea*. Chemosphere 64:991–997

Zarea MJ, Hajinia S, Karimi N, Mohammadi Goltapeh E, Rejali F, Varma A (2012) Effect of *Piriformospora indica* and *Azospirillum* strains from saline or non-saline soil on mitigation of the effects of NaCl. Soil Biol Biochem 45:139–146

Zhang H, Kim MS, Sun Y, Dowd SE, Shi H, Pare PW (2008) Soil bacteria confer plant salt tolerance by tissue-specific regulation of the sodium transporter HKT1. Mole Plant Microbe Interact 21:737–744

Zhu D, Kwon S, Pignatello JJ (2005) Adsorption of single-ring organic compounds to wood charcoals prepared under different thermochemical conditions. Environ Sci Technol 39:3990–3998

Zhu J-K (2001a) Plant salt tolerance. Trends Plant Sci 6:66–71

Zhu JK (2001b) Cell signaling under salt, water and cold stresses. Curr Opin Plant Biol 4:401–406

Zhu JK (2002) Salt and drought stress signal transduction in plants. Ann Rev Plant Biol 53:247–273

Zuo Y, Zhang F (2010) Soil and crop management strategies to prevent iron deficiency in crops. Plant Soil 339:83–95

Diversity and Efficiency of Rhizobia Nodulating *Hedysarum flexuosum* L. in Northwestern of Morocco in Relation to Soil Properties

8

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Abstract

Based on their morphological aspect, 45 strains of rhizobia isolated from root nodules of the wild forage legume *Hedysarum flexuosum* L. sampled from four soil regions of Morocco were tested for their physiological and biochemical characteristics. Their host plants were submitted to analysis of nodule intensity, dry matter yield, and nitrogen content. Moreover, soil samples from the sampling sites of nodulation surveys were collected and analyzed in order to assess the relationship between diversity of *Hedysarum* rhizobia and some soil properties. Even though many of the isolates were from the same plant, they exhibited a wide range of phenotypic diversity in relation to geographical origin. An overall increase in zinc and manganese was the main factor driving compositional differences among rhizobial populations. Their symbiotic efficiency appears to be sensitive to chlorine and aluminum. Although, high chromium in soil may have a positive effect on nodulation and subsequent nitrogen fixation.

8.1 Introduction

Given the great diversity revealed among nitrogen-fixing rhizobia isolated from different legumes, there is an increasing concern in getting knowledge on environmental factors influencing diversity and structure of soil microbial communities. This diversity has been reported to be linked to the large number of leguminous species

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and their wide geographical distribution (Wei et al. 2002). Many researches reputed that legumes can be responsible for variation in the soil bacterial community composition (Bakhoum et al. 2012; Rahi et al. 2012; Lorenzo et al. 2010; Silva et al. 2005), including changes in the communities of symbiotic nitrogen fixers (Rodríguez-Echeverría 2010). However, diversity of rhizobial strains toward their geographical origin remains scattered.

One of the most important forage legumes in the Mediterranean Basin is *Hedysarum* sp. It is a perennial plant, known for its good agronomical traits both in terms of high-quality forage and for soil nitrogen supply. Despite that the genus *Hedysarum* counts more than 100 species, however, only a few species of *Hedysarum* are recorded as being nodulated (Allen and Allen 1981; Sprent 2001). Among this species, *Hedysarum flexuosum* L., often known under the name of sulla, is an important forage legume in the northern parts of Morocco. It is reputed to be tolerant to the stress factors of drought, salinity, and alkaline soil which renders sulla well adapted to marginal areas. The ability of *H. flexuosum* L. to establish a strictly host-specific symbiosis with nitrogen-fixing rhizobia (Glatzle et al. 1986) makes them excellent candidates for use in sustainable agricultural systems.

Considering the potential value of *H. flexuosum* L., we decided to collect and characterize the rhizobia nodulating *H. flexuosum* L. in Northwestern Morocco from different environmental locations with the intent to study some soil properties which drive the phenotypic and efficiency diversity of the rhizobia associated. In the first place, plant samples were collected from each location and analyzed for nitrogen and dry matter content. On the other hand, the bacteria were evaluated in terms of their response of various physiological characters such as salinity stresses, extreme pH, high temperature, heavy metals, and antibiotics tolerance, with attention to select potentially useful strains of rhizobia strains which have to be highly effective in nitrogen fixation, highly competitive, and well adapted to the adverse conditions prevailing in these soils.

8.2 Materials and Methods

8.2.1 Root Nodule and Soil Sampling

The collection of spontaneous nodulated plants of *H. flexuosum* L. was conducted across Northwest part of Morocco during the spring of year 2014. Root nodules were collected from young and green plants at vegetative stage from four sites located in four different regions. From each site, ten plants were randomly collected. Healthy, unbroken, and pink nodules were randomly chosen from each plant. All the nodules were placed on cotton in screw cap plastic tubes containing silica gel as desiccant (Vincent 1970) and stored in 4 °C until isolation. Systematically, rhizosphere soil samples were randomly collected from a depth of 30 cm from the surface of three spots of each sampling site in which *H. flexuosum* L. has been grown naturally. They were mixed, air dried at room temperature, and screened through a 2 mm mesh for physical analysis at National

Table 8.1 Physical soil analysis of the different sites

Sites	Clay (%)	Fine silt (%)	Coarse silt (%)	Fine sand (%)	Coarse sand (%)
Khandak Lihoudi	47.12	26.18	12.87	2.20	1.88
Melloussa	52.63	15.79	10.76	1.84	1.53
Boukhalef	63.83	13.30	0.09	1.49	1.97
Ashakkar	20.41	5.10	0.91	7.60	40.87

Institute of Agronomic Research-Morocco-Rabat and for chemical analysis at the National Center of Scientific and Technical Research (CNRST) in Rabat, Morocco. Soil characteristics are presented in Tables 8.1 and 8.2. Moreover, plants samples were collected from each location and weighed instantly in the field for fresh weight determination. After transportation to the laboratory all samples were oven-dried at 70 °C until reaching a constant weight to determine dry matter.

8.2.2 Rhizobia Isolation

The method of isolating root-nodulating bacteria from nodules was as described by Vincent (1970). After incubation for 3 days at 28 °C, single colonies were picked and checked for purity by repeated streaking on to YEM plate containing Congo red (25 mg/ml) and Gram stain reaction. The pure isolates were stored in 25% (v/v) glycerol at −20 °C.

8.2.3 Cultural Characteristics

Isolates were subjected to different cultural and biochemical tests for identification, namely, Congo red test, growth on peptone glucose agar medium (Vincent 1970), and acid or alkali production in YEM medium containing bromothymol blue (0.025%). All plates were incubated at 28 °C for 6–7 days. Presence of growth was observed after 48 h according to Vincent (1970).

8.2.4 Response to Environmental Stress Factors

The isolates were examined for growth under different stress conditions of high temperature, high salinity, and extreme pH. In the case of temperature tolerance, isolates were kept at 28 (as a control), 35 or 40 °C on YEM plates for 4–5 days. The ability of the isolates to grow in different concentrations of salt was tested by streaking isolates on YEM medium containing 0.5%, 1%, and 2% (w/v) NaCl. Similarly, growth of rhizobial strains was compared at different pH (4.0, 5.0, 8.0, and 9.0) in YEM medium.

Table 8.2 Chemical soil analysis of the different sites

Sites	Water content (%)	pH (H_2O)	OM (%)	$CaCO_3$ (%)	P_2O_5 (ppm)	K_2O (ppm)	N (%)	Ca (%)	Mg (%)	Na (%)	Cl (%)	S (%)	Fe (%)	Mn (%)	Zn (%)	Al (%)	Si (%)	Nb (%)	Cr (%)
Khandak Lihoudi	5.00	7.9	0.9	9.31	17.0	51.20	0.097	3.01	1.04	0.402	–	0.046	3.73	0.0541	0.008	9.61	25.5	–	–
Melloussa	5.66	7.8	1.4	16.58	8.10	138.5	0.146	0.246	0.44	0.224	–	0.060	1.78	–	–	8.19	34.1	–	0.0342
Boukhalef	5.20	7.9	1.4	18.2	13.2	228.8	0.145	0.642	0.62	0.277	0.052	0.080	3.27	0.0431	–	10.7	29.1	0.0016	–
Ashakkar	1.40	8.5	0.7	24.6	3.50	102.3	0.061	0.431	0.77	0.279	–	0.052	3.97	0.0554	0.010	7.92	32.1	–	–

N nitrogen, *Ca* calcium, *Mg* magnesium, *Na* sodium, *Cl* chlorine, *S* sulfur, *Fe* iron, *I* iodine, *Mn* manganese, *Cu* copper, *Zn* zinc, *Al* aluminum, *O* oxygen, *C* carbon, *Sr* strontium, *Br* bromine, *Rb* rubidium, *Si* silicon, *Ti* titanium, *Zr* zirconium, *Ba* barium, *Nb* niobium, *Cr* chromium

8.2.5 Utilization of Carbon and Nitrogen Sources

Isolates were tested for their ability to utilize some carbohydrate as a sole carbon source. For analysis of carbohydrate utilization, a modified YEM agar where yeast extract was reduced to 0.05 g/L (Somasegaran and Hoben 1994) and 0.01% NH_4NO_3 as a source of nitrogen was used. Mannitol was replaced by one of the following carbohydrates to a final concentration of (1%, w/v). Two control media were used for comparison; YEM containing mannitol was used as a positive control and the medium without any carbon source as a negative one. A modified mannitol medium, at which yeast extract was replaced by (0.1%, w/v) of the tested amino acid and mineral salts, was used to investigate the utilization of nitrogen compounds. N-free modified mannitol medium (devoid of any nitrogen source) was used as a control. All the plates were incubated at 28 °C for 2–7 days.

8.2.6 Antibiotic Sensitivity and Heavy Metal Tolerance

All isolates were tested for their sensitivity to eight heavy metals salts, namely, $HgCl_2$, $CuCl_2$, $CdCI_2$, $ZnCl_2$, $MnCl_2.4H_2O$, $CoCl_2.6H_2O$, $AlCl_3$, and $PbCl_2$, and to three antibiotics including kanamycin, erythromycin, and streptomycin. Sensitivity pattern was studied on YEM agar plate containing graded concentration of antibiotics or heavy metals. The stock solution of both antibiotics was prepared in distilled water, and solution was added to YEM medium after filtration through Millipore membrane (0.2 μm porosity). In all experiments growth was recorded after 3 days of incubation at 28 °C in triplicate.

8.2.7 Nodulation Assessment and Effectiveness Evaluation

Productivity and symbiotic efficiency was estimated at the vegetative stage on ten healthy plants collected from each field. A nodule scoring chart was applied to evaluate the infectivity of strains using the chart proposed by (Howieson and Dilworth 2016). Effectiveness of strains in nitrogen fixation was evaluated by scoring total dry matter, plant high and total nitrogen with the Kjeldahl method.

8.2.8 Numerical Analysis

The unweighted pair group method with arithmetic averages (UPGMA) was used for cluster analysis of phenotypic features. The similarity coefficient was computed, and the results are shown as a dendrogram using XLSTAT software (2014). Data obtained from will subject to statistical analysis using SAS software (2002) and followed by mean comparison by Duncan's test. Values are means of three replicates.

8.3 Results and Discussion

8.3.1 Phenotypic Evaluation

A total of 45 bacteria were recovered from root nodules of *H. flexuosum* L. collected from different sites in the region of Tanger. The natural pastures of these plants are found primarily in calcareous clay soils except those in Ashakkar which grow in predominantly sandy soils (Table 8.1). The low level of calcareous found in Khandak Lihoudi soil could be related to shovel structure observed on their root system which acts as bioaccumulator of calcium salts resulting in a localized depletion of $CaCO_3$ from the soil as already proved for *Hedysarum coronarium* L. (Tola et al. 2009). The pH of soils did not vary so much across the study sites confirming the adaptation of this crop to alkaline soils (Moore et al. 2006). Nominal values of soil nitrogen (N), phosphorus (P), and potassium (K) among the three sites varied considerably (Table 8.1), which ultimately affect the plant growth and nitrogen fixation as will be seen below.

The different rhizobial isolates were characterized by studying their presumptive morphological and the physiological characteristics. Generally, most rhizobia are developing a mature colony after days of incubation at 28 °C on YEMA plates. The colonies were characterized by a circular shape, white color, viscous, and differ slightly in their absorption of Congo red dye similarly to other bacteria hosted in the root nodules of the three Mediterranean wild legume species *Hedysarum* (Benhizia et al. 2004). Other interesting and useful characteristics of rhizobia are other growth reactions in the standard YM medium containing bromothymol blue (BTB) as the pH indicator. In our study, all colonies produce an acid reaction YMA-BTB plates and change to yellow after 3 days of incubation at 28 °C. These rhizobia can be qualified as fast-growing rhizobia according to Somasegaran and Hoben (1994). Unlike earlier belief that rhizobia have no ability to grow on glucose peptone agar medium (Somasegaran and Hoben 1994; Vincent 1970), in this study, some isolates grew on this medium and turn the medium to yellow. Finally, all retrieved strains were Gram negative. According to Vincent (1970) and Somasegaran and Hoben (1994), these characteristics are the first clues to the identification of rhizobia.

8.3.1.1 The Numbers Are the Number of Isolates Giving Positive Reaction

Regarding physiological properties of isolated strains (Table 8.3), they showed a large diversity among rhizobia and form heterogeneous group, based on phenotypic characteristics, such as tolerance to pH, salt, temperature and antibiotics, heavy metal, and carbon and nitrogen substrate assimilation tests depending on their geographic origin (Table 8.3). This geographic diversity in rhizobial species composition has been shown to be related to local environmental conditions (Yang et al. 2013; Li et al. 2012). The obtained UPGMA phenogram exhibited a few isolates clustering independently from their geographical origins (Fig. 8.1). All rhizobial strains were included in three distinctive clusters formed at 34% similarity level. Of the three clusters formed, one (C) was composed of rhizobia isolated from two

Table 8.3 Physiological characteristics of root nodule isolates

	Khandak Lihoudi (n = 15)	Melloussa (n = 8)	Boukhalef (n = 12)	Ashakkar (n = 10)
Growth at temperature				
35 °C	4[a]	7	+	9
40 °C	4	4	+	1
Growth at pH				
4	1	+	+	6
5	13	+	+	+
8	+	+	+	+
9	+	4	+	+
NaCl tolerance				
0.5%	14	+	+	+
1%	13	+	+	9
2%	3	3	+	–
Carbohydrate assimilation				
Saccharose	+	+	+	+
Arabinose	+	+	+	+
Glucose	+	+	–	–
Raffinose	+	+	+	5
Utilization of nitrogen sources				
Histidine	10	4	+	5
Asparagine	–	–	–	–
KNO_3	8	+	+	5
NH_4CL	+	+	–	9
Susceptibility to antibiotics (µg/ml)				
Streptomycin				
100	+	3	–	+
250	+	1	–	+
500	+	1	–	+
Erythromycin				
10	14	+	+	+
20	14	+	+	7
50	7	+	+	3
Kanamycin				
100	+	3	–	+
200	+	3	–	8
300	+	2	–	4
Resistance to heavy metals (µg/ml)				
Cadmium				
5	9	+	9	+
10	4	6	9	9
20	4	6	9	9

(continued)

Table 8.3 (continued)

	Khandak Lihoudi (n = 15)	Melloussa (n = 8)	Boukhalef (n = 12)	Ashakkar (n = 10)
Cobalt				
25	+	+	11	+
50	13	7	9	+
100	6	1	9	6
Mercury				
5	+	+	11	+
10	+	7	11	4
20	12	4	4	2
Zinc				
50	2	+	+	8
100	–	+	+	5
200	–	+	+	1
Manganese				
200	10	+	+	5
300	10	+	+	5
400	10	+	+	5
Copper				
200	1	–	–	–
500	–	–	–	–
1000	–	–	–	–
Aluminum				
100	+	+	+	+
200	+	+	+	+
400	–	–	–	–
Lead				
400	13	+	+	8
600	12	+	+	3
1000	–	–	–	–

[a]The numbers are the number of isolates giving positive reaction

different soil origins, namely, Boukhalef and Melloussa; their apparent consistent phenotype profile found among isolates could suggest some degree of genomic relatedness. Instead, the two other clusters (A and B) were composed of isolates originating only from one geographical site (Fig. 8.1). The presence of phenotypic clusters containing only isolates of one soil might indicate an evolution in the rhizobial population with the mutation and/or selection and proliferation of and particular subpopulations in relation to their soil characteristics in which they grow. This probability could explain the repetitive phenotypic profile for some isolates of Boukhalef site (HFB2, HFB4, HFB8, HFB9, HFB12, and HFB15) (Fig. 8.1), suggesting a lack of genetic diversity among isolates of this site. Notably, soils from Boukhalef and Melloussa were characterized by low heavy metal specially zinc and manganese (Table 8.2). This study suggests that metal-contaminated soils may

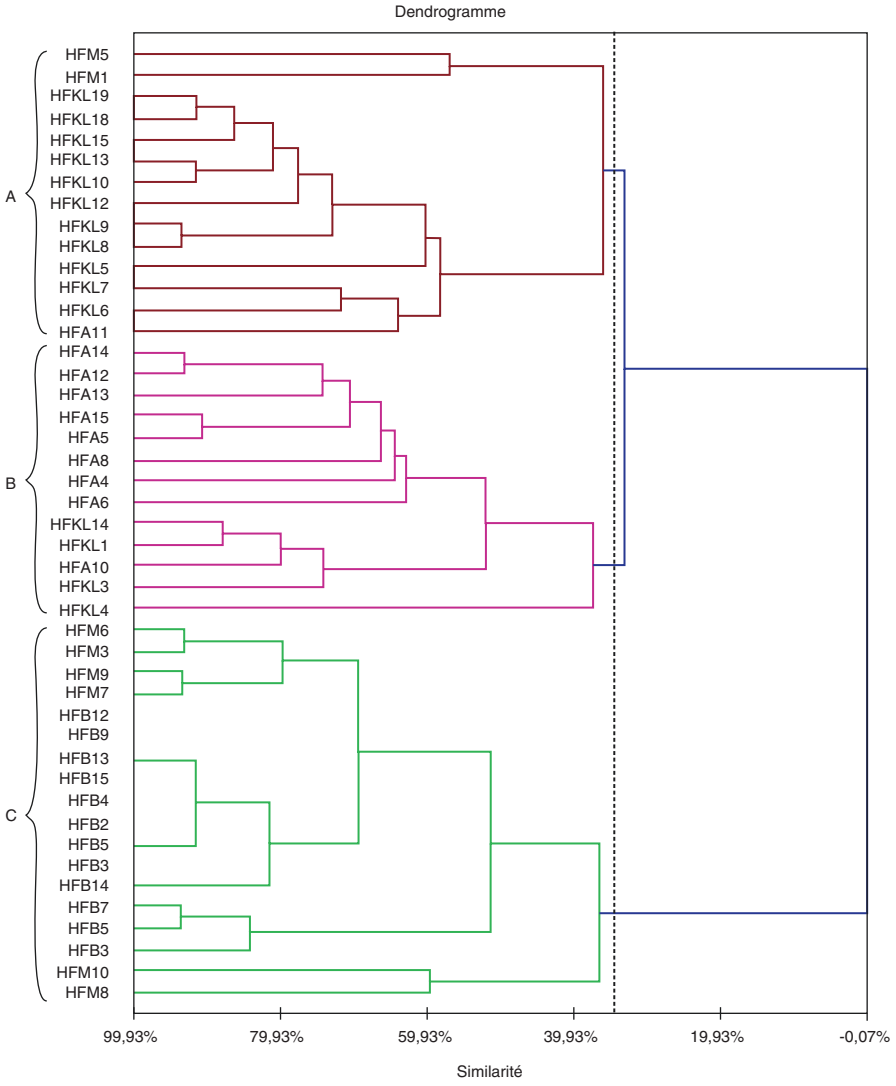

Fig. 8.1 Phenogram showing phenotypic relatedness among 45 isolates from *H. flexuosum* L. nodules growing in different sites of Morocco based on average-linkage cluster analysis of 50 characteristics

preserve a higher diversity of rhizobia as the case of those isolated from Khandak Lihoudi and Ashakkar sites.

By the same token, isolates from different soil showed different resistance to the selected heavy metal (Table 8.3). Metal phenotypes varied within and between each group of isolates. Cluster (C) richer in isolates of Boukhalef and Melloussa showed higher metal tolerance especially to Mn and Zn, suggesting that both metal tolerances may be controlled by same mechanisms of tolerance.

Furthermore, this tolerance was not correlated with their soil origin (Table 8.2). Indeed, in spite of the presence of Mn or Zn in soil of Khandak Lihoudi and Ashakkar sites, their correspondent isolate shows a low tolerance, suggesting no such adaptation to this tows metal. In fact, metal tolerance of rhizobia was demonstrated to be linked to either slow or progressive increase of metal concentration in the soil. Slow metal increase favored the adaptation of more rhizobia to strive with the metal toxicity, contrary to rapid metal charge and long-term effects, and contributed to strong selection of rhizobia strains with high metal tolerance (Giller et al. 1998). This evidence could be ecologically important to investigate the degree of stress imposed by such metal.

8.3.2 Effectiveness Assessment

As well as the phenotypic results, the dry matter yield and nitrogen content of sulla varied from site to site and seemed to be related to the abundance of nodulation (Table 8.4). Thus, all plants assessed in field are either abundant or adequate in nodule. This could be explained by the relative size of the effective native rhizobial populations present in soil in relation to the plant cultivation history and the persistence of their root nodule bacteria in soil (Thami Alami and El Mzouri 2000). The abundance of nodulation found in Melloussa site could be due to abandoned sulla in the last years. Interestingly, the plants from Ashakkar site were only one with the least nodule abundance probably due to the low physical protection of native rhizobia in relation to the low proportion of clay in soil (Table 8.1). Consequently, Ashakkar soil samples did not promote nodule formation. This suggests that the potential of nodulation was not fully displayed in field due to unfavorable environmental conditions such as water availability and levels of nitrogen and phosphorus in soil (Zahran 1999). Accordingly, the low efficiency in terms of nitrogen content recorded in Ashakkar could be related to low level of phosphorus (3.50%) found in soil. Several studies found that nodulation and nitrogen fixation are directly linked to the phosphorus (P) supply. Although, strains of rhizobia differ markedly in tolerance to phosphorus deficiency (Beck and Munns 1985). Not only phosphorus but also mineral

Table 8.4 Nodulation and efficiency of *H. flexuosum* L. evaluated at different sites of Morocco

Sites	Infectivity[1]	Plant height (cm)	Total dry matter (%)	Nitrogen content (%)
Khandak Lihoudi	Abundant	$48.12^{a} \pm 2.65$	$18.00^{a} \pm 0.01$	$3.10^{b} \pm 0.17$
Melloussa	Extremely abundant	$44.50^{a} \pm 0.71$	$14.08^{b} \pm 0.24$	$3.75^{a} \pm 0.08$
Ashakkar	Adequate	$30.50^{b} \pm 2.12$	$10.54^{d} \pm 0.46$	$2.98^{c} \pm 0.24$
Boukhalef	Abundant	$42.50^{a} \pm 3.53$	$12.43^{c} \pm 0.39$	$2.52^{d} \pm 0.21$
S.E.M[2]		2.58	0.67	0.83
Sig.		**	***	***

Values in column followed by letter a, b, c and d differ significantly according to Fisher-protected LSD test ($P < 0.05$)

[1]Infectivity of strains was scored using the chart proposed by Howieson and Dilworth (2016)

[2]*SEM* standard error of the means

nitrogen levels in soil (0.061%) could have a negative effect on symbiotic efficiency. It is widely accepted that the nitrogen-fixing capacity of legumes is influenced by the presence of mineral nitrogen in the soil in which it is grown. Nevertheless, a low dose of nitrogen in the soil can stimulate plant growth until the starts of symbiotic nitrogen fixation (Muller and Pereira 1995). In other hand, adequate potassium (K) fertility proved not only to have positive effect on nodulation and subsequent nitrogen fixation but also alleviate the effects of water shortage (1.40% in Ashakkar site) on symbiotic nitrogen fixation (Sangakkara et al. 1996). However, the absorption by plants of this macronutrients (N, P, K) in addition to others micronutrients such as zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn) could be limited by the presence or absence of native arbuscular mycorrhizal fungi (AMF) even on a calcareous soil (Labidi et al. 2012, 2015; Smith and Read 2008; Azaizeh et al. 1995; Li et al. 1991).

Outstandingly, the nodulation in Boukhalef site was relatively high, but nitrogen content remained limited, probably indicating the low efficiency of the nodulating rhizobia or could be related to high level of chlorine in soil (Table 8.2). In fact, several environmental factors such as physicochemical composition of the soil including heavy metals and water scarcity can affect the infection process and symbiotic nitrogen fixation by *Rhizobium* (Zahran 1999; Ahmad et al. 2012; Arora et al. 2010; Kinkema et al. 2006; Collavino et al. 2005). Soils from Boukhalef were characterized by high aluminum (10.7%) compared to the other sites (Table 8.2). Consequently, rhizobia populations seem to be sensitive to this metal. Studies reported that aluminum is extremely toxic to growth and enzyme activity of both fast- and slow-growing rhizobial species (Arora et al. 2010; Paudyal et al. 2007). Comparatively, the plasmid profiles of ineffective isolates surviving at high concentrations of heavy metals were all very similar (Giller et al. 1989), confirming the observations made above.

In this study the highest nitrogen content (3.75%) were found in Melloussa site (Table 8.4) conjointly with abundant pink nodules, typical of healthy and effective nodules. This result is relatively high comparatively with those obtained by Fitouri et al. (2012a)) for *H. coronarium* L. (max 2.94% in Tunis site). In fact inoculation of *H. coronarium* L. by different rhizobial strains significantly improved air-dry biomass production and the crude protein content. However, this improvement depends all times of the strain used (Fitouri et al. 2012b; Ben Taâmallah 1998). Therefore, testing the ability of the single isolate to induce root nodules on their host plant is primary. As a matter of fact, the high symbiotic efficiency recorded in this site may be as a result of the high level of chromium (Cr) in soil (Table 8.2) as already been demonstrated (Casella et al. 1988).

Conclusion

As has been noted, symbiotic effectiveness of nitrogen-fixing rhizobia varies according to their soil properties in which the plant grown naturally. In the field these factors could be operated interdependently and/or synergistically, affecting ultimately plant growth and symbioses. As a result, identifying the most prevailing factors affecting legume-*Rhizobium* symbiosis remains imperative in order to achieve optimum level of efficiency by culturing sulla in suitable environment conditions.

References

Ahmad E, Zaidi A, Khan MS, Oves M (2012) Heavy metal toxicity to symbiotic nitrogen-fixing microorganism and host legumes. In: Zaidi A et al (eds) Toxicity of heavy metals to legumes and bioremediation. Springer, Wien, pp 29–44

Allen ON, Allen EK (1981) The Leguminosae. Univerdity of Wisconsin Press, Madison. 812 p

Arora NK, Khare E, Singh S, Maheshwari DK (2010) Effect of Al and heavy metals on enzymes of nitrogen metabolism of fast and slow growing rhizobia under explant conditions. World J Microbiol Biotechnol 26:811–816. doi:10.1007/s11274-009-0237-6

Azaizeh HA, Marschner H, Romheld V, Wittenmayer L (1995) Effects of a vesicular-arbuscular mycorrhizal fungus and other soil microorganisms on growth, mineral nutrient acquisition and root exudation of soil-grown maize plants. Mycorrhiza 5:321–327

Bakhoum N, Ndoye F, Kane A, Assigbetse K, Fall D, Sylla SN (2012) Impact of rhizobial inoculation on *Acacia senegal* (L.) Willd. growth in greenhouse and soil functioning in relation to seed provenance and soil origin. World J Microbiol Biotechnol 28:2567–2579

Beck DP, Munns DN (1985) Effect of calcium on the phosphorus nutrition of Rhizobium meliloti. Soil Sci Soc Am J 49:334–337

Ben Taâmallah S (1998) Effets de la disponibilité en eau et de différentes souches de Rhizobium sur la productivité en herbe du sulla (*Hedysarum coronarium*) en Tunisie. Fourrages 154:191–196

Benhizia Y, Benhizia H, Benguedouar A, Muresu R, Giacomini A, Squartini A (2004) Gamma proteobacteria can nodulate legumes of the genus Hedysarum. Syst Appl Microbiol 27:462–468

Casella S, Frassinetti S, Lupi F, Squartini A (1988) Effect of cadmium, chromium and copper on symbiotic and free-living Rhizobium leguminosarum biovar trifolii. FEMS Microbiol Lett 49:343–347

Collavino M, Riccillo PM, Grasso DH, Crespi M, Aguilar OM (2005) GuaB activity is required in Rhizobium tropici during the early stages of nodulation of determinate nodules but is dispensable for the Sinorhizobium meliloti-alfalfa symbiotic interaction. Mol Plant Microbe Interact 18:742–750

Fitouri SD, Trabelsi D, Saïdi S, Zribi K, Ben Jeddi F, Mhamdi R (2012a) Diversity of rhizobia nodulating sulla (*Hedysarum coronarium* L.) and selection of inoculant strains for semi-arid Tunisia. Ann Microbiol 62:77–84. doi:10.1007/s13213-011-0229-2

Fitouri SD, Faysal BJ, Kais Z, Salah R, Ridha M (2012b) Effet de l'inoculation par une souche osmotolerante de Rhizobium sullae sur la croissance et la production en proteine du sulla (Sulla coronarium L.) sous déficit hydrique. J Appl Biosci 51:3642–3651

Giller KE, McGrath SP, Hirsch PR (1989) Absence of nitrogen fixation in clover grown on soil subject to long-term contamination with heavy metals is due to survival of only ineffective rhizobium. Soil Biol Biochem 21:841–848

Giller KE, Witter E, McGrath SP (1998) Toxicity of heavy metals to microorganisms and microbial processes in agricultural soils: a review. Soil Biol Biochem 30:1389–1414

Glatzle A, Schulte-Batenbrock T, Brockwell J (1986) Symbiotic incompatibility between two forage species of Hedysarum, grown in Morocco, and their homologous rhizobia. FEMS Microbiol Lett 37:39–43

Howieson JG, Dilworth MJ (2016) Australian Centre for International Agricultural Research, 2016. Working with rhizobia

Kinkema M, Scott PT, Gresshoff PM (2006) Legume nodulation: successful symbiosis through short and long-distance signalling. Funct Plant Biol 33:1–15. doi:10.1071/FP06056.

Labidi S, Ben Jeddi F, Tisserant B, Debiane D, Rezgui S, Grandmougin-Ferjani A, Lounès-Hadj Sahraoui A (2012) Role of arbuscular mycorrhizal symbiosis in root mineral uptake under $CaCO_3$ stress. Mycorrhiza 22:337–345

Labidi S, Jeddi FB, Tisserant B, Yousfi M, Sanaa M, Dalpé Y, Sahraoui ALH (2015) Field application of mycorrhizal bio-inoculants affects the mineral uptake of a forage legume (*Hedysarum coronarium* L.) on a highly calcareous soil. Mycorrhiza 25:297–309. doi:10.1007/s00572-014-0609-0

Li XL, Marschner H, George E (1991) Acquisition of phosphorus and copper by VA- mycorrhizal hyphae and root-to-shoot transport in white clover. Plant Soil 136:49–57

Li M, Li Y, Chen WF, Sui XH, Li Y, Li Y, Wang ET, Chen WX (2012) Genetic diversity, community structure and distribution of rhizobia in the root nodules of Caragana spp. from arid and semi-arid alkaline deserts, in the north of China. Syst Appl Microbiol 35:239–245

Lorenzo P, Rodríguez-Echeverría S, González L, Freitas H (2010) Effect of invasive *Acacia dealbata* Link on soil microorganisms as determined by PCR-DGGE. Appl Soil Ecol 44:245

Moore GA, Sanford P, Wiley T (2006) Perennial pastures for Western Australia

Muller SH, Pereira PAA (1995) Nitrogen fixation of common bean (*Phaseolus vulgaris* L.) as affected by mineral nitrogen supply at different growth stages. Plant Soil 177:55–61

Paudyal SP, Aryal RR, Chauhan SVS, Maheshwari DK (2007) Effect of heavy metals on growth of Rhizobium strains and symbiotic efficiency of two species of tropical legumes. Sci World 5:27–32

Rahi P, Kapoor R, Young JPW, Gulati A (2012) A genetic discontinuity in root-610 nodulating bacteria of cultivated pea in the Indian trans-Himalayas. Mol Ecol 611(21):145–159

Rodríguez-Echeverría S (2010) Rhizobial hitchhikers from down under: invasional meltdown in a plant-bacteria mutualism? J Biogeogr 37:1611–1622

Sangakkara UR, Hartwig UA, Noesberger J (1996) Soil moisture and potassium affect the performance of symbiotic nitrogen fixation in faba bean and common bean. Plant Soil 184:123–130

Silva C, Vinuesa P, Eguiarte LE, Souza V, Martínez-Romero E (2005) Evolutionary 633 genetics and biogeographic structure of Rhizobium gallicum sensu lato, a widely 634 distributed bacterial symbiont of diverse legumes. Mol Ecol 14:4033–4050

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Academic, London

Somasegaran P, Hoben HJ (1994) Handbook for Rhizobia: methods in Legume-Rhizobium Technology. Springer, New York. 450 pp

Sprent JI (2001) Nodulation in legumes. Royal Botanic Gardens, Kew

Thami Alami I, El Mzouri E (2000) Etude de l'efficacité et de la persistance des souches de Rhizobium de sulla. In: Sulas L (ed) Legumes for Mediterranean forage crops, pastures and alternative uses. CIHEAM, Zaragoza, pp 321–325

Tola E, Henriquez-Sabà JL, Polone E, Dazzo FB, Concheri G, Casella S, Squartini A (2009) Shovel roots: a unique stress-avoiding developmental strategy of the legume plant *Hedysarum coronarium* L. Plant Soil 322:25–37. doi:10.1007/s11104-008-9861-4

Vincent JM (1970) A manual for the practical study of root nodule bacteria. In: IBP Handbook, No 15. Blackwell Scientific Publications Ltd., Oxford

Wei GH, Wang ET, Tan ZY, Zhu ME, Chen WX (2002) Rhizobium indigoferae sp. nov. and Sinorhizobium kummerowiae sp. nov. respectively isolated from Indigofera spp. and *Kummerowia stipulacea*. Int J Syst Evol Microbiol 52:2231–2239

Yang W, Kong Z, Chen W, Wei G (2013) Genetic diversity and symbiotic evolution of rhizobia from root nodules of *Coronilla varia*. Syst Appl Microbiol 36:49–55

Zahran HH (1999) Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev 63:968–989

Increased Soil-Microbial-Eco-Physiological Interactions and Microbial Food Safety in Tomato Under Organic Strategies

9

Nitika Thakur

Abstract

Farmers' profit has been continuously squeezed as the costs have been rising faster than the realization, ushering the law of diminishing returns. The environmental and health impacts have been equally alarming as the toxic residues have entered the whole food chain which increases the incidence of chronic and dreaded diseases like cancer, arthritis, atherosclerosis, corroded membranes, weakened DNA walls, and damaged livers. The situation is really horrendous and calls for an immediate remedial answer, which is none else than reverting back to the organic farming system. Organic agriculture is an eco-friendly management system which upgrades agrological ecosystem health, biodiversity, and soil biological, physical, and chemical properties. Organic cultivation, quality of food, and human health complement the strong environmental arguments for going organic. Organic agriculture initiates self-sustenance, rural development, and nature conservation; the thread that weaves together this ambitious goal is the sustainable use of biodiversity.

9.1 Introduction

Tomato (*Lycopersicon esculentum*; family *Solanaceae*) is known for its popularity, due to the fact that it has been widely grown as an important vegetable throughout the world. It traces its origin from central and south parts of America (Vavilov 1951). It is credited as the second world's largest vegetable crop after potato, but maintains its top position in the list of processed vegetables. Tomato represents a major source of nutrients as a fresh commodity as well as processed product. Tomatoes' unique flavor

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accent as well as taste accounts for its popularity and wide usage. A large amount of tomatoes are being used to prepare products like ketchup, paste, puree, juice, powder, soup, etc. The high dry matter and soluble solids are desirable properties for the canned tomatoes industry since they improve the quality of tomato products (De Pascale et al. 2001). On the other hand, pH values are very important for tomatoes during processing since values which are higher than 4.4 result in mean sensitivity of the pulp to pathogens (thermophilic) (Paulson and Stevens 1974).

The total area under tomato cultivation in the world is 4.81Mha (production, 163.02 million tons; with productivity of 33.9 tons per ha) covering about 2 million producers and 170 countries with certified organic agriculture (Willer and Julia 2015). India holds about an area of 876,410 hectares (production, 17,848,160 MT), whereas the Himachal Pradesh accounts for cultivation area of 17,848 hectares (production, 400,000 MT) (Anonymous 2011) and about 3965.38 ha area under organic certification (Fig. 9.1). Solan district is known for the production of tomato covering 9555 ha area with a production of 3.4 lakh tonnes.

Tomato serves as an important commodity for upgrading the hill farmers in form of crop produced during off-season in Himachal Pradesh (mid-hill), fetching very attractive prices to the farmers. A constant nutrient and water supply is needed for the luxuriant growth of tomato. The rising global energy crises have led to a hike in the cost of chemical fertilizers and pesticides, which would reach beyond the reach of farmers at marginal side. Tomatoes are essential because of the high nutritional and medicinal values contributed to humans as most important role points toward reduction in cardiovascular diseases and certain types of lethal diseases like cancer (Canene et al. 2005). The benefits of tomatoes have been credited to the presence of lycopene which constitutes about 80–90% of the total carotenoid content. The vitamin C content in tomato fruits attributes toward the antioxidant properties of this fruit which cures and prevents diseases. Its value as a vegetable crop has been increased due to the presence of pigment anthocyanin. The adoption of organic strategies is necessary to upgrade the parameters of quality and nutrition. To boost up yields and reduce pest and insect incidence, the agricultural practices have been continuously relying on the use of mineral fertilizers. But the heavy uses of these fertilizers have led to an extensive damage, resulting in the deterioration of beneficial microbes, environmental hazards, and soil fertility.

The researches have shown that the increasing groundwater contamination and surface runoff (nitrate leaching) are the harmful outcomes of the excessive application of chemicals which have been continuously draining the water quality. The rising concerns about the harmful results of using chemical fertilizers have led to a strong urge in alternative strategies to ensure quality with competitive yields thus protecting the crops. Injudicious application of chemicals could cause diverse changes in the biological balances leading to an increase in cancer incidence through the residues (toxic) present in the edible produce. The organic tomatoes are more preferred in comparison to the conventional ones because of better quality, taste, flavor, aroma, texture, storage, and shelf life.

To achieve these properties, usually the farmers often apply large amounts of chemical fertilizers, which exert ill effects on soil and environment and ultimately reduce quality of

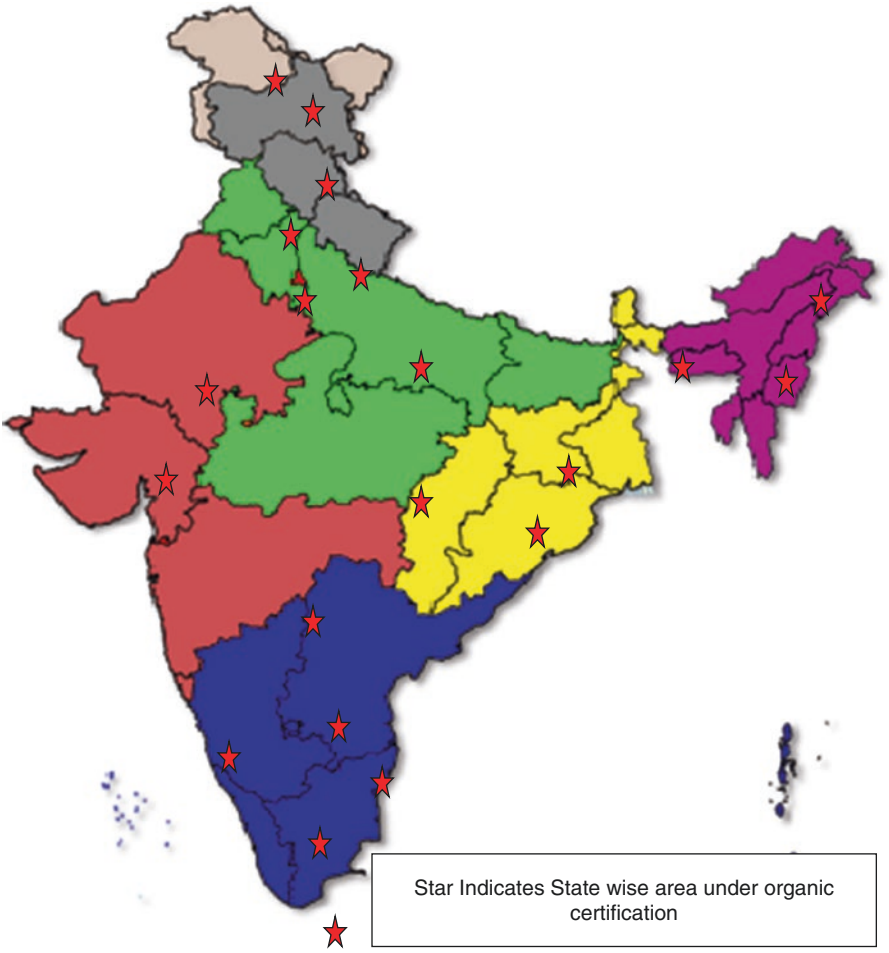

Fig. 9.1 Map indicating the state-wise area under organic certification (Source: Data from APEDA Accredited Certified Agencies in Tracenet)

crop, though increasing the yields. The new approaches in farming system have introduced modern and eco-friendly practices with long-term sustainability. The pattern of organic agricultural land has been increased tremendously from an era of 1999 to the present (Fig. 9.2). The judicial employment of beneficial microbial inoculants (biofertilizers) along with organic manures is considered as an alternative requirement of the crop. The new farming strategies implementing the use of organic supplements have been proven effective in improving soil structure, soil fertility, and crop yields.

Organic matter is an important source of nutrients which is easily supplied to the plants, and their incorporation to the soil would maintain and increase the microbial populations and their activities, which in turn would increase biomass content, respiration rates, and biomass carbon/total carbon ratio. Thus, crop yields have been increased

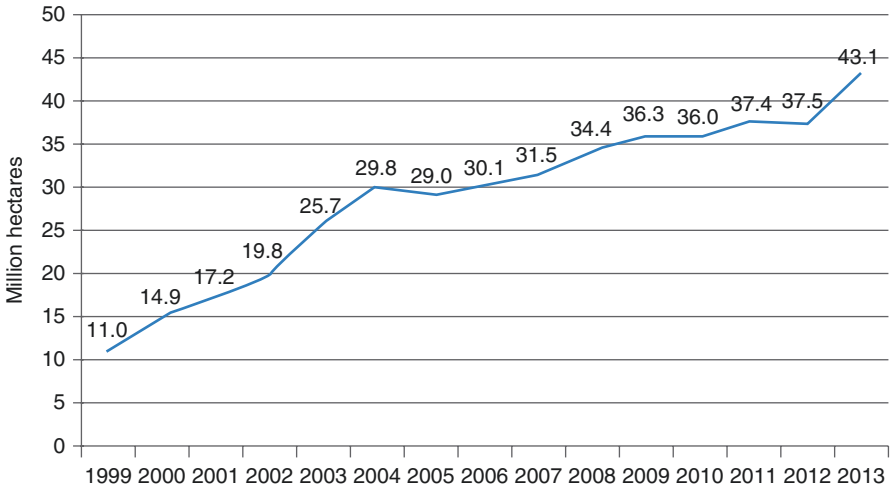

Fig. 9.2 Growth of organic agricultural land (1999–2013): Source-ICCOA (International Competence Center for Organic Agriculture)

with the improvements in soil quality and nutritional profile through the additions of organic supplements (Tonfack et al. 2009). The domestication or scaling up process of a species is the major step in the seedling process where every step needs to be properly executed. The full monitoring phase should start from the handling and managing process of the nursery, finally rating the performance of their survival percentage. The way a seedling is brought up results in assurance of healthy future. Improving quality of a seedling directly affects the survival, growth, and productivity rates of the future product. A good and healthy stock of nursery is essential to raise a good planting material.

The major reasons of seedling mortality on-farm include:

1. The poor health of the seedlings at the time of planting.
2. Unhealthy and poor seedlings are likely to have growth at slower range, thus are less able to compete with weeds or adverse conditions and become more susceptible to insects and pests.
3. Further, in a poor nursery, the wastage of money and time is seen as fewer seedlings will be brought up from a given quantity of seed.
4. Thus sound nursery practice is the foundation of any successful planting program scheme.
5. Soilless culture is a technique for crop production without soil. Crops are grown in the essential nutrient solution or on a proper medium; therefore, soilless culture involves no work such as tools or machines.

Increased disease incidence, lack of healthy soils, and the desire for standardizing optimal conditions for plant growth are leading to the worldwide focus of growing plant in soilless media instead of soil (Winsor and Schwarz 1990).

In addition, treating seeds with beneficial microorganisms provides long-lasting conservation against yield-reducing fungal/bacterial diseases by creating a cover of

protection around the seed root system, which helps in the development of healthier and firm root system, thereby enhancing crop productivity with better yields. The species of *Trichoderma* (*Trichoderma harzianum* and *Trichoderma viride*) are the most important species and have been formulated for about 87 different crops against the soilborne (70) and foliar pathogens (17), respectively (Sharma et al. 2014).

The initiation of agriculture with organic supplements has led to enhance ecosystem health (biodiversity) and biological activity of the soil. The urge to go organic is coupled with the correct combination of organic practices in combination with quality of food and health of human beings. It emphasizes the use of practices generated on farm in preference to use of off-farm inputs, taking into account the specific microclimatic situation which is generally adapted locally.

This is mainly done by implementing a triad of practices including agronomic, biological, and mechanical methods in contrast to synthetic materials (FAO/WHO 1997). The main scenario focuses on maintaining soil fertility for generations, to produce poison-free food for consumers, to secure productivity, and to meet competition from likely cheaper imports, high water percolation, recharging groundwater, development of nitrogen and phosphate-fixing bacteria and microorganisms involved in transferring atmospheric moisture, soil enrichment by transfer of biomass of agro-waste, emergence of mixed farming system, new marketing channels, premium prices, and higher product demand going worldwide (Figs. 9.3 and 9.4). Global markets for organic products are increasing on a wide globe, hence satisfying criteria of food safety (less incidence of diseases like mad cow disease, cancer, etc.), health aspects (over 20% more vitamins and minerals), price premiums (market-led growth, USA), environmental concerns, and sustainability.

Safety and Quality of Food Stands as the Primary Issue to Each Individual The quality can be defined as a complex feature of food that determines perception and acceptability of a consumer. The increasing awareness of a consumer about food, health, and environment has led to an increased interest to go organic.

The data shows that the fruits/vegetables produced organically possess pesticide residues and nitrate levels at much lower stage (below the minimal residual limits) than the conventional fruits and vegetables.

In some reports it has been seen that the organic foods possess higher levels of plant (secondary) metabolites which are beneficial as they link to essential antioxidants such as polyphenolic compounds but also consist and underline some potential concern of health, when one talks about the naturally occurring toxins. As the synthetic chemicals are not preferred (prohibited) in organic cultivation, more biochemical energy can be restored and used effectively for synthesizing the secondary plant metabolites (Jadhav et al. 1981).

Tomato is one of the essential vegetable crops of Solan (HP) grown for both economic and biological reason with million tons of annual production. The present situation figured out presents a clear data of the conventional tomato production in both open-field and greenhouse conditions in Solan (HP) (Fig. 9.5). Due to the suitable climate, there is a great scope for the upgradation and promotion of organic farming.

To support the organic farmers, the various statutory bodies and government have formulated supportive policies in 2010, covering about 30,110 farmers with

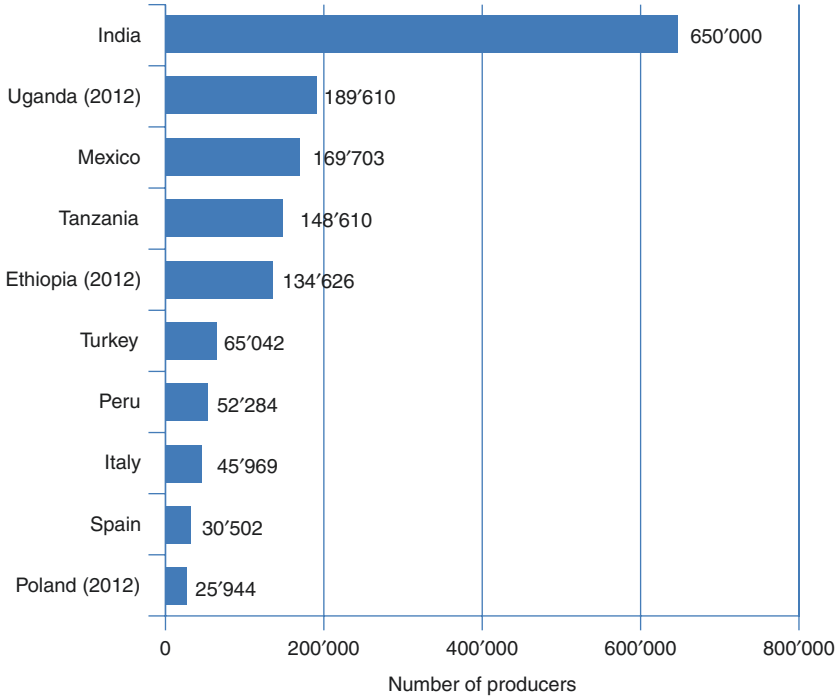

Fig. 9.3 Ten countries with the largest number of organic producers (2013) (Source: ICCOA)

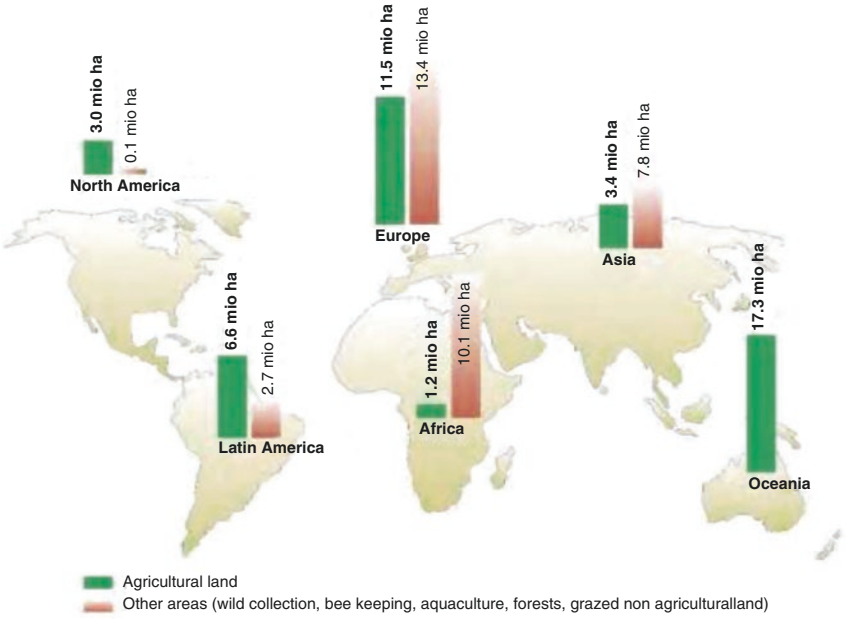

Fig. 9.4 Organic agriculture worldwide: statistics (Source: ICCOA)

the near vision of converting villages (200) to complete bio-villages and 50% assistance to set up individual vermicompost units (20,000). However, government has already started the organic cultivation (registration and certification) process to implement organic fertilizers in tomato production, but the farmers still lack awareness about the incorporation of organic formulation.

Keeping in view the above facts, the present studies were carried out with an open-pollinated and indeterminate tomato variety (cv. Solan Lalima), which has been recently released by University of Horticulture and Forestry (UHF-Nauni) for commercial cultivation of tomato. It shows superiority over the present tomato hybrids available in the markets in terms of fruit quality and productivity. Being open-pollinated variety, it's a suitable option for organic cultivation.

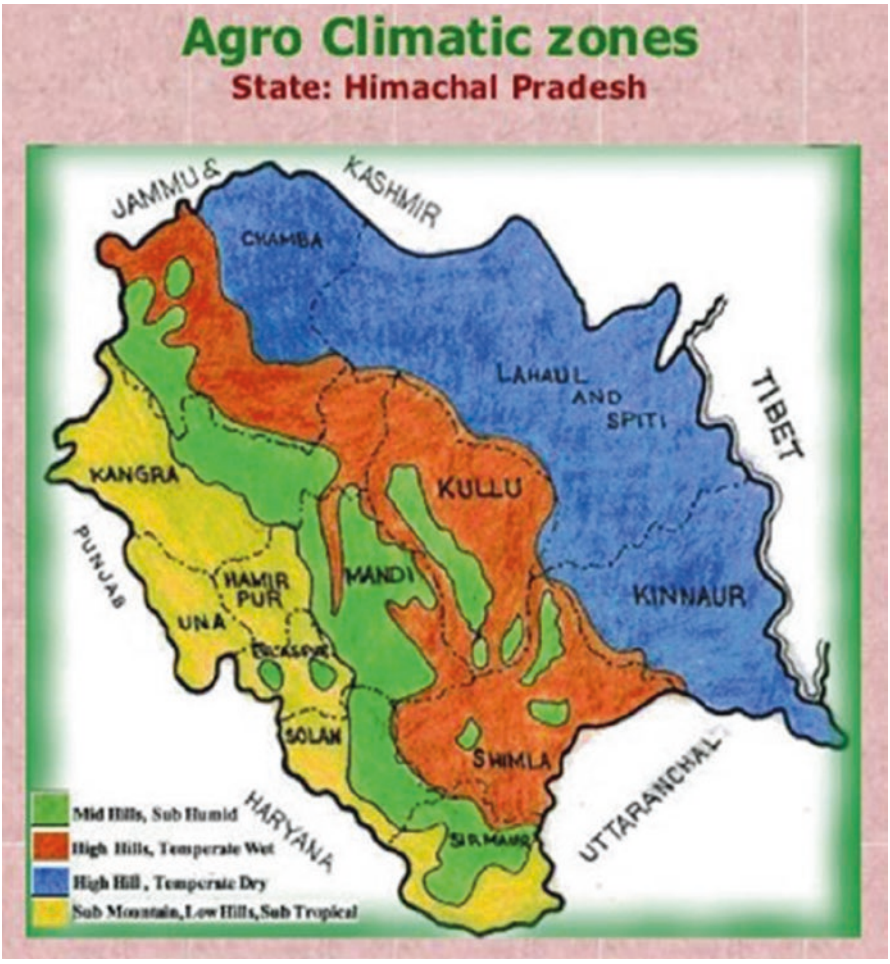

Fig. 9.5 Agroecological zonation of Himachal Pradesh (Source: Centre for Geo-informatics Research and Training, CSK Himachal Pradesh Agricultural University, Palampur, Himachal Pradesh, India)

Therefore, the farmer can produce the seeds at their own farm. The studies were, hence, conducted to see the influence of different organic and inorganic nutrient sources on the soil fertility status, beneficial microbial population, crop quality, yield, economics, and food safety in tomato.

9.2 Materials and Methodology

The present study entitled *The effect of manures and bio-fertilizers on the interaction of microbes with soil and effect on food safety in tomato* was carried out during a tenure of 2 years (2013–2014). The details of the methodology used have been described as under:

9.2.1 Location of the Experimental Field

The experimental field was set up at village Basal (farmer's field), under block Solan, Himachal Pradesh at an (elevation of 1270 m) above mean sea level (30–52′ north and latitude 77–11′ east).

9.2.2 Weather of the Experimental Site

The weather for mid-hill conditions is marked by sub-temperate and subhumid agroclimatic zone (Himachal Pradesh). The rainfall on an average ranges from100 to 300 cm, in the month of August and June.

9.2.3 Experimental Layout

The experiment trial consisted of a primary nursery stage and a secondary field trial. The treatments and procedures followed are separately discussed.

9.2.4 Organic Amendments and Inputs

9.2.4.1 Organic Manures and Fertilizers Used

Organic Manures Used

- FYM (farmyard manure)
- VC (vermicompost)
- Procured from the farmer's field having on-farm inputs

Organic Fertilizers and Agents Used in Biocontrol

- AZO (*Azotobacter*)
- PSB (phosphate solubilizing bacteria)
- Neem cakes, *Trichoderma viride*
- *Pseudomonas fluorescens*
- Asafetida (Poabs Green Pvt. Limited, Kerala)

9.2.5 Organic Package of Practices Adopted for Growing Healthy Tomato Nursery

The tomato seeds (cv. Solan Lalima) were sowed in plastic trays with dimensions measuring 13 × 9 = 117 seeds. The nursery was set up with six organic treatments replicated thrice. The control was laid according to farmer's practice in the field in a seed bed (1 m × 3 m). The different combinations of media were used which contained both soilless and soil growth media mixed with various organic manures and biofertilizers. The detailed description of various treatments is given in Table 9.1.

9.2.6 Seed Source, Seed Variety, and Seed Rate Used

9.2.6.1 Seed Source: From Department of Vegetable Crops – Dr. Y.S. Parmar, UHF, Nauni, Solan)

Table 9.1 The detailed description of various treatments followed during tomato nursery raising

S.no.	Treatments	Combinations of various growth media combined with organic practices
1	**T_1**	FYM + Soil (1:1)
2	**T_2**	FYM+ VC+ Soil(1:1:1)
3	**T_3**	FYM + coco peat + VC + Vermiculite + *Azotobacter* (1:1:1:1:1)
4	**T_4**	FYM + coco peat + Vermiculite + *Azotobacter* (1:1:1:1)
5	**T_5**	FYM + soil + *Azotobacter* (1:1:1)
6	**T_6**	FYM + *Azotobacter* (1:1)
7	**T_7 Control (farmer's practice)**	FYM + soil + no seed treatment + Drenching with Dithane and bevisteen (2.5 g/L and 0.5 g/ L of H_2O)

FYM Farmyard manure, *VC* vermicompost

9.2.6.2 Seed Rate: 400 g/ha (40 gm/biga)

9.2.6.3 Seed Treatment

The seeds were treated with Beejamrut (6 g/40 g seed) and *Trichoderma viride* (0.32 g). The seeds were dried in the shade and again treated the seeds with a mixture of *Azotobacter* and PSB (0.8 g each). Finally dry the seeds in shade and sow within 8 hrs of treatment.

9.2.6.4 Tomato Variety Used

Solan Lalima (open-pollinated and indeterminate variety) variety of tomato shows a superior quality and productivity over the tomato hybrids in the markets which are commonly used by the farmers. Solan Lalima offers an advantage in terms of quality as well as yield increments for the farmers.

9.2.6.5 Treatment of Trays Used for Raising Nursery

The trays were treated with 1:7 formalin.

9.2.6.6 Seedling Treatment

- Neem spray (7 g/L) was given once for 15-day-old seedlings, to protect seedlings from sucking pests like whitefly and thrips.
- The process of drenching (*Pseudomonas fluorescens*, 10 g/L) is done before transplanting to prevent foliar diseases.
- Dipping of root portion of seedling in asafetida suspension (100 g in 5 L of water for 20 min) was done to prevent soilborne pathogens causing wilt diseases, before transplanting. Twenty-day-old tomato seedlings were transplanted to the main experimental field.

9.2.7 Observation and Calculation

Observation was recorded for the following aspects:

- Seedling germination
- Length of root (cm)
- Length of shoot (cm)
- Number of roots
- Seedling vigor
- Incidence of emergence (pre and post) damping-off was calculated.

9.2.8 Field Parameters

9.2.8.1 Experimental Setup of the Field

RBD (randomized block design) was adopted as a field design with eight treatments (replicated five times), consisting of 40 plots measuring 1 m × 3 m where the seedlings were planted at a distance of 90 cm × 30 cm consisting 24 plants per plot. The

T_1–T_6 (six treatments) organic treatments were grown in different blocks, which were laid separately at a 7 m distance from the farmer's (T_7) and chemical treatment (T_8). The doses have been calculated by analyzing the soil and applied manures and biofertilizers and the dozes recommended in organic package of tomato crop.

T_1 312q/ha of farmyard manure + 4 kg/ha *Trichoderma viride*

T_2 78q/ha of vermicompost +*Trichoderma viride* at 4 kg/ha

T_3 312q/ha of VC + *Azotobacter* + phosphate solublizing bacteria + *Trichoderma viride* (4 kg/ha each)

T_4 Farmyard manure at 78q/ha + *Azotobacter* + PSB + *Trichoderma viride* (4 kg/ha)

T_5 *Trichoderma viride* (4 kg/ha) + PSB (4 kg/ha)

T_6 *Trichoderma viride* (4 kg/ha) +*Azotobacter* (4 kg/ha)

T_7 Chemical fertilizers (farmer's practice)+ *Azotobacter*

T_8 **Chemical treatment** (fertilizers + pesticides + weedicides) (Directorate of extension education Dr. YS Parmar, UHF Solan)

9.2.9 Soil Analysis

Before commencement of the experiment, the soil of the experimental area and manures used were analyzed for physiochemical properties. To combat with low and high percentage of NPK and organic carbon, 25% high and low application of manures and biofertilizers were used in accordance with the recommended package.

9.2.9.1 Field Operation Protocol Followed

9.2.9.2 Random Selection from the Field Experiment

A random selection of five plants was considered from each bed. On a whole 200 plants were considered under field parameter analysis.

9.3 Results and Discussion

The studies focused on the use of organic manures and biofertilizers for the two successive years (2013–2014) at village Basal, 5 km away from Solan town, Himachal Pradesh**.** The highlights of the present studies are being discussed under the following headings.

9.3.1 Raising of Healthy Nursery

The focus on choosing a seed which is healthy as well as free from disease is the most essential requirement to have the satisfied product performance. The main stress should be laid on lowering the biotic and abiotic strains which offer limited yield constrains and reduction in incidence of insect pest disease (IPD) which

hinders the economic security of a farmer. The yields have been seen to reduce specially in hills due to the reemergence of pre and post insect pest-like {damping-off (*Pythium aphanidermatum*), bacterial wilt (*Ralstonia solanacearum*), and fusarial wilt (*Fusarium oxysporum*)} and diseases at regular intervals, right from the raising of a nursery to the period of harvesting, where the incidence of most destructive disease, etc. can be witnessed which ruin the crop diversity and quality to the most worst level.

The problem becomes more severe when the crop is attacked by the cascade of diseases one after the other. The use of conventional chemical pesticides is considered the most preferred practices to manage the outbreaks of these diseases, but the indiscriminate chemical approach to deal with these hazards has contributed to adverse effects like soil acidity, impairing soil physical conditions, reducing beneficial microbial population, and continuously degrading organic matter, increasing plant susceptibility to insect pest diseases, and decreasing soil lives. Thus, these eco-friendly agents are highly effective with excellent shelf life, and delivery method is also suitable (Bhagat et al. 2013).

It was observed from the results that all the organic treatments were found effective in increasing the parameters like seedling emergence and vigor index of tomato under both nursery and field conditions, but the maximum increase in vegetative parameters under nursery trials was recorded in treatment T_3 consisting of FYM + VC+ vermiculite+ coco peat+ *Azotobacter* over the control (T_7). The results were excellent for the organic fertilizers incorporated with coco peat, vermiculite, and *Azotobacter* which marked superiority over untreated control, where no seed bio-priming was done and the seedlings were raised in an open-field condition, which was recorded with lower germination percentage and decreased growth parameters in contrast to an organically cultivated nursery with soilless culture.

9.3.1.1 Effect of Seed Treatments on Nursery Growth Parameters

The present results revealed that the application of various combinations of organic treatments for raising tomato seedlings (cv. Solan Lalima) produced significant differences in nursery growth attributes over the conventional treatment (control). The studies revealed the superiority of organic seed treatments (bio-priming with antifungal and antibacterial agents) over the untreated check (control).

The bio-priming and seed treatment carried out in the present studies increased the vegetative attributes related to nursery seedling. The significant effect of seed treatment and bio-priming was in conscience with the studies conducted by Garg et al. (2007), where seed treatment with *T. viride* had a number of effects on aonla seed germination and seedling growth. This in turn made the root system strong and deep which provided with number of benefits like enhanced nitrogen fertilizer use efficiency, increased tolerance to drought, and probably also other abiotic stresses.

This fungus has been reported to keep the conductive tissues healthy by the secretion of some growth hormones since this fungus multiplies on its own; it is different from other seed dressing fungicides. This increase in seedling germination and growth may be attributed to the efficiency of *T. viride* at colonizing seedling roots and enhancing root growth in terms of root length, root hair development, and

depth. Similar studies conducted by Harman et al. (2004) reported another form of *Trichoderma* isolate viz. *T. harzianum* for seed bio-priming in maize, which resulted in increased levels of proteins and exo- and endochitinase in both root and shoot. The increased capability of *Trichoderma* isolated in increasing seedling growth parameters is due to the increased solubilization of some insoluble and soluble minerals under in vitro conditions by the mechanisms, namely, medium acidification, chelating metabolite activity production.

The present observations were strongly supported with similar findings reported on testing organic amendments and agents action toward controlling disease, seedling vigor, and percent emergence in cauliflower (Sharma and Sain 2005), in capsicum (Kabdal et al. 2010), and in tomato (Pietr et al. 2002). The results were at par with Bhagat et al. (2013) where the incorporation of isolates isolated from *Trichoderma* strain and bacterial antagonists used for seed bio-priming of tomato revealed better improvement in emergence of seedlings (%), vigor (%), and biomass. The mechanism related to the hormone secretion and nutrient uptake from the organic matter present in the soil has been highlighted as an important process indulged in promotion of plant growth (Windham et al. 1986; Kleifeld and Chet 1992).

9.3.1.2 Reduction in Damping-Off

The plant diseases (nearly 10–20%) have affected the world food production. However, the heavy use of the chemicals during the past years has given birth to the number of problems related to the environmental concerns, thereby limiting the yield; thus, an eco-friendly approach is gaining popularity which solves the problems related to environmental hazards.

It has been seen that the biological agents seem to have more potential in controlling the postemergence rots where the incidence of disease was reduced to a higher level (59%) as compared to preemergence rot (45.6%). The results of the studies agree with the research conducted by Hooda et al. (2010), where disease reduction in postemergence rot incidence was recorded maximum compared to pre-rot incidence. This was due to the time required for the bioagent inoculum multiplication in rhizosphere and collar region of seedling. Similarly the control of damping-off has been seen through the *Trichoderma viride* and *Pseudomonas fluorescens* application.

The study resulted in enhanced control strategies for damping-off as likewise observed by Kabdal et al. (2010). The present results are at par with the similar findings (Bhagat et al. 2013) where the application of antagonist fungus (*Trichoderma*), as treatment agent for both soil and seed, had a remarkable effect on lowering the incidence of disease and increasing percent yield over control. This can be supported by the studies that stress on the simultaneous application of *Trichoderma* as seed priming and soil incorporation agent as it results in providing a protective cover in the seed coat by the rapid multiplication of bioagents and upgrading a greater strength to compete the pathogens.

The reduction reported in the incidence of damping-off in the present study may be attributed to the mechanisms involved by the biocontrol agents which include a

cascade of antagonistic reaction (antibiosis, volatile toxic metabolite secretion, mycolytic enzymes, parasitism, and competition for space and nutrients), which are considered effective against a series of plant pathogens present in the soil (Khandelwal et al. 2012; Babu and Pallavi 2013).

The present results get strong evidences from the studies conducted on colonization of pea seed by *T. viride* resulting in efficient production of antibiotic production (viridian) in the seed controlling *Pythium* spp. This hydrolytic enzyme combination with the antibiotics may have resulted in an effective level of antagonism (Howell and Stipanovic 1995). The mechanisms of biocontrol process is also supported by the studies conducted by who observed that soil inoculation with *Trichoderma* spores helps in controlling a serious disease called damping-off related to citrus seedling.

Various studies also highlighted that the biosynthesis of siderophores in *P. fluorescens* plays a role in the suppression of pathogens (Costa and Loper 1994) indicating the biocontrol potential against pytopathogenic fungi in both the in vivo and in vitro conditions, respectively (Saraf et al. 2008).

9.3.1.3 Effect of Growing Medium on Nursery Growth Parameters

In recent years, nursery production has transitioned from the use of mineral soil-based potting media to soilless culture. Soilless culture includes hydroponic systems and solid media systems called soilless media: they are made of simple or complex mixtures of materials (Johnson 1985). The combination of these materials is what makes them attractive for use in greenhouse settings, where the environment can be manipulated. Most commonly, soilless media are composite mixes composed of shredded *Sphagnum* peat, shredded coir, composted bark, or sawdust-based materials with the addition of sand, vermiculite, and/or perlite (Ingram et al. 1991). Ideally, these manufactured soilless mixes provide a pathogen-free physical support system necessary for plant growth and thus avoid some of the major problems that are associated with mineral soils. The available nutrients, percent organic matter, pH, and water holding capacities (pore size) of soilless media vary greatly from each other and from mineral or composite soils.

Growing of crops on soil is the conventional practice in crop production; the search for an alternative means of media for cropping came as a result of increasing knowledge in plant nutrition as well as other serious difficulties observed in the use of soil in crop production. Soil possesses numerous limitations for plant growth due to the presence of disease-causing organisms (flora and fauna), poor drainage, and aeration resulting from soil compaction and degradation due to soil erosion and leaching (Mbata and Orji 2008; Ekwu and Mbah 2001). The soilless culture is the new cultivation system of plants that use nutrient solution for raising the plants. The most intensive culture system emphasizes on yield maximizing of crops and the most intense form of agricultural enterprises for commercial production of greenhouse vegetables (Dorais et al., 2001; Grillas et al., 2001; Jensen 1997). The soilless culture in the greenhouse stands as an alternative strategy to the field production carried out for quality vegetable (Pardossi et al. 2002). Therefore, quality of the horticultural crops grown through soilless culture is comparatively superior to the soil cultures conventionally preferred (Massantini et al. 1998).

The present study revealed a superiority of T_3 treatment in terms of soilless media composition, increasing seedling germination percentage, root and shoot length, and vigor percentage over the control. The present findings are in line with the work reported, where the similar effect was observed significant with the combinatorial use of growing media consisting of peat, composted tree bark combined with composted tea wastes, and rice husks. The similar findings gained a strong support through the studies reported by Sahin et al. (2005) who observed the nursery practices followed by the farmers in Nigeria which did not ensure sustainability criteria as the field soils were found generally unsatisfactory for the nursery production as compared to soilless nursery raising media, indicating the seedlings raised in the media with soil were poorer in most vegetative parameters measured in contrast to the soilless medium.

These results are further in agreement with those reported that the composted organic compounds in growing media increased the parameters shoot and root biomass production, in comparison to the field soils which are unsatisfactory for the production of plants (Sahin et al. 2005). The use of FYM as a basal application results in providing additional nutrient to the plant as well as improving soil properties (Reddy and Swamy 2000) and results in proper decomposition and mineralization with solubilizing effect on soil nutrients. Vermicompost is considered the best medium which provides increased levels of oxygen and water to the roots; storage of water and nutrients for the plant; physical, chemical, and biological balance; and requirement for good plant growth (Atefe et al. 2012). Vermicompost as a nursery mixture stands as an excellent growth rejuvenator, as it supplies efficient nutrients to the plant. On the other hand, coco peat improves retention of moisture and thus increases the available nutrient content, porosity, and hydraulic conductivity of the soil (Savithri and Khan 1993). *Azotobacter*, in addition, enhances the process of nitrogen fixation in plants and maintains a direct link for the continuous supply of biological active compounds. The addition of *Azotobacter* may have resulted in the process of nitrogen fixation and production of phytohormones and growth stimulants which aid in controlling many insects and pathogens (Kloepper and Schroth 1980).

9.3.2 Field Trials

The production and consumption areas have been seen to pass through a spectacular breakthrough in India from the past four decades. The farmers have been continuously facing the burning energy cost and inflammations related to high prices due to the use of fertilizers and pesticides. Also, the continuous use of chemical fertilizers is leading to yield reduction and adverse effects on the soil as well as human health. The essential nutrients are required for essential functions and must be provided to the plant at the right time and quantity (Shukla and Naik 1993). With the increase in the process of intensification in cropping, the effect of heavy doses of chemical fertilizers has been analyzed, and the importance of organic materials is being felt for supporting the soil health and productivity.

The growing awareness and interest of both the producer as well as the consumer toward the organic varieties have led to the use of organic cultivation techniques for future use. In addition, higher price of food produced organically than conventionally produced (Oberholtzer et al. 2005) is encouraging producers to go fully organic. The consumer demand has also been seen to divert toward organically produced food which is considered safer and more nutritious to eat (Lester 2006).

9.3.2.1 Vegetative and Quality Attributes

The maximum increase in all the vegetative and crop quality attributes was observed in organic treatment T_3, followed by T_4. The maximum increase was prominent by combined application of manures with biofertilizers and biocontrol agents, followed by the single incorporation of organic manures (T_2 and T_1) and biofertilizers (T_5 and T_6) with biocontrol agents as compared to the control and chemical treatment. Increase in vegetative growth and quality attributes in the present studies may be attributed to T_3 (vermicompost+ PSB+ *Azotobacter* + *T. viride*) with the additional supplementation of vermicompost by *Azotobacter*, phosphate solubilizing bacteria (PSB), and *T. viride*, followed by T_4 (farmyard manure + PSB + *Azotobacter* + *T. viride*).

9.4 Summary and Conclusion

The studies carried out from 2013 to 2014 highlights the importance of soil health and implementing techniques for soil management in agricultural practices. The major work includes protecting soil fertility through improved system of drainage which ultimately sorts the problems related to environmental hazards. The organic methods of cultivation are adopted for disease management by on-farm generated inputs and conservation tillage.

Organic farming depends on an effective biological activity in the soil and contributes to the diversity and increment of beneficial soil microorganisms. The important benefits of this includes: increased mineral uptake, the nutrient supply enhancement, crop vigor improvement, nutrient leaching reduction, soil structure improvement, and resistance to pest and diseases.

It can be concluded that the organic cultivation provides security and safety ensuring the environmental protection and attractive returns to the farmers.

References

Anonymous (2011) Indian horticultural database. National Horticultural Board, Ministry of Agriculture, Government of India

Atefe A, Tehranifar A, Shoor M, Hossein DG (2012) Study of the effect of vermicompost as one of the substrate constituents on yield indexes of Strawberry. J Hortic Sci Ornamen Plants 4(3):241–246

Babu KN, Pallavi PN (2013) Isolation, identification and mass multiplication of *Trichoderma* an important bio-control agent. Int J Pharma Life Sci 4(1):2320–2323

Bhagat S, Bambawale OM, Tripathi AK, Ahmad I, Srivastava RC (2013) Biological management of *Fusarium wilt* of tomato by *Trichoderma* spp. in Andamans. Indian J Hortic 70(3):397–403

Canene AK, Campbell JK, Zaripheh S, Jeffery EH, Erdman JW (2005) The tomato as a functional food. J Nutr 135:1226–1230

Costa JM, Loper JE (1994) Characterization of siderophore production by the biological control agent *Enterobacter cloacae*. Mol Plant Microbe Interact 7:440–448

De Pascale S, Maggio A, Fogliano V, Ambrosino P, Retieni A (2001) Irrigation with saline water improves carotenoids content and antioxidant activity of tomato. J Hortic Sci Biotechnol 76:447–453

Dorais M, Papadopoulos A, Gosselin A (2001) Greenhouse tomato fruit quality. Hortic Rev 26:239–319

Ekwu LG, Mbah BN (2001) Effects of varying levels of nitrogen fertilizer and some potting media on the growth and flowering response of marigold (*Targetes erecta* L.) Niger J Hortic Sci 5:104–109

FAO/WHO (1997) Codex alimentarius food hygiene basic texts. Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Pub. # M-83

Garg N, Singh VK, Prakash O (2007) *Trichoderma viride* treatment for improving seed germination and seedling growth in aonla. Indian J Hortic:213–215

Grillas S, Lucas M, Bardopoulou E, Sarafopoulos S, Voulgari M (2001) Perlite based soilless culture systems: current commercial applications and prospects. Acta Hortic (548):105–114

Harman GE, Petzoldt R, Comis CJ (2004) Interactions between *Trichoderma harzianum* strain T22 and maize inbred line Mo 17 and effects of these interactions on diseases caused by *Pythium ultimum and Colletotrichum graminicola*. Phytopathal 94:147–153

Hooda KS, Joshi D, Dhar S, Bhatt JC (2010) Management of damping off of tomato with botanicals and bio-products in north western Himalayas. Indian J Hortic 68(2):219–223

Howell CR, Stipanovic RD (1995) Mechanism in the biocontrol of *Rhizoctonia solani* induced cotton seedling disease by *Gliocladium virens*: antibiosis. Phytopathology 85:462–472

Ingram DL, Henley RW, Yeager TH (1991) Growth media for container grown ornamental plants. University of Florida IFAS Extension 241, Gainesville

Jadhav SJ, Sharma RP, Salunkhe DK (1981) CRC critical. Rev Toxicol 9:21–104

Jensen M (1997) Food production in greenhouses. In: Plant production in closed ecosystems: the international symposium on plant production in closed ecosystems, Kluwer, Dordrecht, p 1–14

Johnson IR (1985) A model of portioning of growth between the shoots and roots of vegetative plants. Ann Bot 55:421–431

Kabdal P, Hooda KS, Joshi D, Hedau NK, Pandey KN (2010) Biocontrol agents in the health management of capsicum nursery. Indian J Hortic 67(1):70–72

Khandelwal M, Datta S, Mehta J, Naruka R, Makhijani K, Sharma G, Kumar R, Chandra S (2012) Isolation, characterization and biomass production of *Trichoderma viride* using various agro products - a biocontrol agent. Adv Appl Sci Res 3(6):3950–3955

Kleifeld O, Chet I (1992) *Trichoderma harzianum* interaction with plants and effect on growth response. Plant Soil 144:267–272

Kloepper JW, Schroth MN (1980) *Pseudomonas* siderophores: a mechanism explaining diseases-suppressive soils. Curr Microbiol 4:317–320

Lester GE (2006) Organic versus conventionally grown produce: quality differences and guidelines for comparison studies. Hortic Sci 41:296–300

Massantini F, Favilli R, Magnani G, Oggiano N (1998) Soilless culture-biotechnology for high quality vegetables. Soilless Cult 4(2):27–40

Mbata T, Orji MU (2008) Process optimization in the production and preservation of Ugba, a Nigerian fermented food. Int J Microbiol 4:2–62

Oberholtzer L, Dimitri C, Greene C (2005) Price premiums hold on as U.S. organic produce market expands. USDA Economic Research Services, VGS 308-401

Pardossi A, Malorgio F, Campiotti CA, Tognoni F (2002) A comparison between two methods to control nutrient delivery to greenhouse melons grown in recirculating nutrient solution culture. Sci Hortic 92:82–95

Paulson KN, Stevens MA (1974) Relationships among titratable acidity, pH and buffer composition of tomato fruits. J Food Sci 39:254–357

Pietr SJ, Wojtkowiak E, Slusarski C, Stankiewicz M, Lewicka T, Biesiada A (2002) The possible systemic induction of resistance in some vegetables by fungicide resistant *Trichoderma* isolates. In: Proceedings of the 7th working group meeting, "Influence of abiotic and biotic factors on biocontrol agents at Pine Bay, Kusadasi, Turkey", vol. 25. 22-25 May 2002, Bulletin-OILSROP, p 10, 331

Reddy BP, Swamy SN (2000) Effect of FYM, PSB and phosphorus on yield and economics of black gram (*Phaseolus mungo*). Indian J Agric Sci 70:694–696

Sahin U, Ors S, Ercisil S, Anapali O, Esitken A (2005) Effect of pumice amendments on physical soil properties and Strawberry plant growth. J Cent Eur Agric 6(3):361–366

Saraf M, Thakkar A, Patel BV (2008) Biocontrol activity of different species of *Pseudomonas* against phytopathogenic fungi in vivo and in vitro conditions. Int J Biotechnol Biochem 4:217–226

Savithri P, Khan HH (1993) Characteristics of coconut coir peat and its utilization in agriculture. J Plant Crop 22:1–18

Sharma P, Sain SK (2005) Use of biotic agents and abiotic compounds against damping off of cauliflower caused by *Pythium aphanidermatum*. Indian J Phytopathol 58:395–401

Sharma P, Sharma M, Raja M, Shanmugam V (2014) Status of *Trichoderma* research in India: a review. Indian Phytopathol 67(1):1–19

Shukla V, Naik LB (1993) Agro-technique for solanaceous vegetables. Adv Hortic 5

Tonfack LB, Bernadac A, Youmbi E, Mbouapouognigni VP, Ngueguim M, Akoa A (2009) Impact of organic and inorganic fertilizers on tomato vigor, yield and fruit composition under tropical and soil conditions. Fruits 64:167–177

Vavilov N (1951) The origin, variation, immunity and breeding of cultivated plants. Chronol Bot 13:364

Willer, H, Julia L (Eds.) (2015) The World of organic agriculture. Statistics and emerging trends 2015. FIBL-IFOAM report. Bonn, Research Institute of Organic Agriculture (FIBL), Frick and IFOAM-Organics International.

Windham MT, Elad Y, Baker R (1986) A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathology 76:518–521

Winsor GW, Schwarz M (1990) Soilless culture for horticulture crop production. FAO Plant Production and Protection Paper 101.FAO, Rome, Italy, p 188

Emerging Significance of Rhizospheric Probiotics and Its Impact on Plant Health: Current Perspective Towards Sustainable Agriculture

10

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Abstract

Plants act as a shelter for vast numbers of microorganisms known as plant microbiome which is the key to plant health. Microbial population residing in plants interacts with plants through a series of complex mechanism. The plant microbe interactions can be beneficial, neutral or detrimental depending upon the nature of microbiome in the plant. Plant roots and rhizosphere are the most populated regions of plant where microbial activity is highest due to the secretion of bioactive compounds from roots. The beneficial soil microorganisms are also known as plant probiotics and have the potential to improve plant health and fitness both in natural

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and adverse environmental conditions. The microorganism which acts as potential probiotics utilized for the manufacturing of biofertilizers because they serve in promoting plant growth and it is now possible to formulate any type of probiotics, because of their common physiological characters. In the present chapter, the main focus is given to the rhizospheric microbiome which functions as plant probiotics and the importance of rhizospheric probiotics in plant growth promotion during stressed conditions. The chapter also includes the details for the delivery of successful biofertilizers by combining various probiotics and guidelines for their registration for providing a safe and efficient biofertilizer in the market.

10.1 Introduction

Plants act as a shelter for vast numbers of microorganisms known as plant microbiome where cell counts as well as gene densities can outnumber the host itself. Number of work has cited the advantages of microorganisms for growth, nutrition and productivity of plants. So, plants are superorganism which partially depends on their microbiome for the determined task and attributes. As a reward, plants sediment the carbon to their vicinity which comprises as rhizosphere for the growth and development of their microbiome (Raaijmakers et al. 2009; Vorholt 2012) . As of now, the crosstalk linked in plants and their associated microbiome has been considered in detail for numerous leaf invaders, valuable rhizobia and mycorrhizal fungi. Even then, a great number of plant-related microbiome are ready to be explored for their effect on plant growth and nutrition and hence require major attention. The rhizosphere serves as a pool for enormous microorganisms which include archaea, fungi, viruses, oomycetes, bacteria, algae, nematodes, protozoa and arthropods and is the most convoluted environment present on Earth (Bonkowski et al. 2009; Buee et al. 2009). Majority of the rhizospheric microbial communities are associated to a multifarious food nexus which rely on beneficial nutrients secreted by plant roots termed as root exudates comprises of organic acids, amino acids etc. and plays an important role in enhancing microbial diversity and activity. It has been proved that these rhizospheric microbiomes are selectively regulated by plants for their benefits in terms of growth, nutrition and health (Cook et al. 1995) and are well characterized as protozoa, nitrogen-fixing bacteria, biocontrol microorganisms, plant growth-promoting rhizobacteria (PGPR), mycorrhizal fungi and mycoparasitic fungi.

10.2 Rhizosphere Microbiome

Earlier, very little is known about the genetic diversity of soil and rhizosphere microorganisms. Microbial identification using next-generation sequencing technologies has reported that culture methodology is possible for approximately 5% of bacteria that have been cultured, while other colonies have no isolation

techniques yet. For example, in a study of the rarefaction curve obtained through metagenomic analysis of soil, it can be concluded that it was difficult to achieve saturation (Tringe et al. 2005). With the advancement in computational techniques, it has been reported that 1 g of soil harbour well-defined bacterial genomes not less than 1 million surpassing our earlier estimate (Gans et al. 2005). Recently, researchers identified 1,39,819 bacterial and 9,340 crenarchaeotal from four separate soils and enumerated to a total of 52,000 operational taxonomic units (OTUs) (Roesch et al. 2007). *Betaproteobacteria*, *Bacteroidetes* and *Alphaproteobacteria* were the major bacterial communities colonizing the four soils under study (Roesch et al. 2007). The bacterial taxa are the major study of exploration in terms of rhizosphere microbiome compared to other rhizosphere inhabitants and has been found to range between 100 to more than 55,000 OTUs depending on the techniques. In one meta-analysis study, 1200 distinguishable bacterial taxa were obtained from 35 discrete taxonomic orders from 14 distinct types of rhizosphere, out of which 19 clone libraries were obtained from species Proteobacteria which is found to be the most dominant species (Hawkes et al. 2007). Another study employed 454 pyrosequencing methods in the rhizospheric soils of oak with *Acidobacteria* and *Proteobacteria* being prominent (Uroz et al. 2010). In spite of deleterious abiotic environment in soils collected from Antarctic, 732 OTUs were reported associated with two different vascular plants (Teixeira et al. 2010). DeAngelis et al. (2009) have implemented a PhyloChip to identify OTUs in the oat rhizospheric soils. PhyloChip analysis has already detected the rhizospheric bacterial phyla of potato and sugar beet. Moreover, OTUs from 444 to 2015 have been reported in three different varieties of potato rhizosphere grown at two separate fields (Weinert et al. 2011) with *Proteobacteria* (46%), *Actinobacteria* (11%), *Firmicutes* (18%), *Acidobacteria* (3%) and *Bacteroidetes* (7%) being more prevalent. Advancement of PhyloChip generation (G3) offers a reading of nearly 33,000 OTUs in sugar beet seedlings rhizosphere cultivated in the fields of the Netherlands (Mendes et al. 2011). Indistinguishable effect was explained by Roesch et al. (2007) in which the *Proteobacteria* being outnumbered before *Firmicutes*, *Actinobacteria* and *Bacteroidetes*, while unidentified group of bacteria corresponds to group of 16% with respect to OTUs obtained for sugar beet rhizosphere (Mendes et al. 2011). A recent work has explained the contiguous dissemination of rhizospheric bacterial colonies in 600 varieties of *Arabidopsis* to evaluate the mixture of the elemental microbiome through pyrosequencing of 16S rRNA gene segments of bacteria from different plant parts and demonstrated the role of soil pattern on bacterial strains in respective plant parts (Lundberg et al. 2012; Bulgarelli et al. 2012).

Studies on Archaea and other microbial agents in soil have tremendously increased after a breakthrough finding of its ammonia-oxidizing properties (Leininger et al. 2006). Chelius and Triplett (2001) discovered six specific archaeal sequences in the rhizosphere of maize roots. Also, 70 archaeal OTUs constituting about 0.21% of the overall archaeal and bacterial species are remarkably shown through PhyloChip assay residing in the sugar beet rhizosphere grown on agricultural land (Mendes et al. 2013). Though, the importance of Archaea for

defence of plants in response to soil-inhabitant microoganisms is yet to be considered.

10.2.1 Importance of Rhizosphere Microbiome

Though the rhizospheric evolutionary relationship is crucial for consideration, it is desirable to regulate selective activities of microorganisms at individual developmental stages of plant/root proliferation and their role in different time and space. A vast category of molecular methods have been defined for the expression of rhizospheric gene by Barret et al. (2011). A graceful promoter trapping procedure, known as *in vivo* expression technology (IVET), was endorsed to *Pseudomonas fluorescens* genes which records an upregulated expression of genes for nutrient acquisition, stress response and secretion in rhizosphere vicinity (Rainey 1999). Similar work exploiting IVET technology showcases the proteins for *Rhizobium leguminosarum* A34 during rhizosphere colonization that governs environmental sensing, gene expression regulation, metabolic reactions and membrane transport (Barr et al. 2008). Moreover, enormous work dealing with team of rhizosphere reporter genes for functions related to responses of bacteria to carbon, nitrogen, phosphorus availability (Kragelund et al. 1997; Jensen and Nybroe 1999; Ramos et al. 2000; Koch et al. 2001; DeAngelis et al. 2005), temperature and water potential (Ullrich et al. 2000; Herron et al. 2010) is reported. These bioreporters are already utilized to understand bacterial information interchange in the rhizosphere (Loh et al. 2002; Steindler and Venturi 2007; Ferluga and Venturi 2009) along with in situ microcidal compound production (Kulakova et al. 2009; Rochat et al. 2010). The transcriptomics profiling study was also adopted to assess the consequences of root exudates from two sugar beet cultivars on gene expression in *Pseudomonas aeruginosa* (Mark et al. 2005) and concluded that the expression of 104 genes was remarkably modified in response to both root exudates. Microarray is another trending technique to annotate function and activities of numerous microbial colonies. One such gene array covering about 10,000 genes is GeoChip and annotated 150 functions including nitrogen, carbon, sulphur and phosphorus cycling, metal reduction and resistance and organic contaminant degradation (He et al. 2007). GeoChip 3.0 version unlocked the role of pathogen *Candidatus*, *Liberibacter asiaticus* in citrus trees, and concluded an alteration in the make-up and concomitant qualities of rhizosphere microbiome (Trivedi et al. 2011). Recently, 'omics' method offers a tremendous platform to analyse gene transcripts, proteins or metabolites in the plant system and their associated microbial guest. Proteomics approaches have conferred a multiplex synergy in plants and rhizosphere microorganisms in varied agriculture systems (Wang et al. 2011). Tandem MS graph depicts 189 proteins from rice, and its rhizospheric microbiota out of which one-third are difficult to determine (Wang et al. 2011). Bacterial proteins constitute about 22.75% with again the *Proteobacteria* and *Actinobacteria* being the abundant one. A similar work has been carried out

for the rhizosphere of *Rehmannia glutinosa* and rice (Wu et al. 2011; Knief et al. 2011). In case of rice, a total of about 4600 proteins have been reported in which methanogenesis is the most prevalent in rhizosphere as well as phyllosphere (Knief et al. 2011).

Microorganism resides in the vicinity of plant rhizosphere poses several mechanisms involving secretion of phytohormones, solubilizing minerals etc.which subsequently enhances growth of plants (Lugtenberg and Kamilova 2009). The mechanisms involve biofertilization, biocontrol and biostimulation for enhancing plant growth as well as rhizoremediation and resistance to abiotic stress. Several rhizobacteria, from the group of *Proteobacteria* and *Firmicutes* such as *Pseudomonas* spp. and *Bacillus* spp., were reported as having the aforementioned plant growth promoting mechanism. In addition to numerous fungi of Deuteromycetes group, *Trichoderma* spp. and *Gliocladium* spp. were also recognized as having the above mechanisms (Kogel et al. 2006; Qiang et al. 2012). In the past few years, additional knowledge has been acquired on soil inhabitants other than bacteria and fungi of distinct microbial genera, for example, *Planctomycetes* (Hol et al. 2010; Jogler et al. 2012).

10.3 Plant Nutrients Uptake

The microorganism associated with the plant rhizosphere can extensively influence the plant nutrient status. For example, the mycorrhizal fungi, nitrogen-fixing bacteria and other PGPR facilitate the uptake of phosphorus, nitrogen and iron (Richardson et al. 2009; Miransari 2011).

The symbiotic nitrogen-fixing microbes such as *Rhizobium* and mycorrhizal fungi stimulate plant growth and development by exporting nutrients and minerals to plant from the soil (Gianinazzi et al. 2010), maintaining and stabilizing soil structure (Miller and Jastrow 2000), and also shows biocontrol activity (Whipps 2001; Pozo and Azcon-Aguilar 2007). These functions were previously studied and reported (van der Heijden and Sanders 2002; Salvioli and Bonfante 2013). Several nitrogen-fixing bacterial genera other than *Rhizobium* and *Bradyrhizobium* that are inhabitants of rhizosphere were also identified, for example, a study of cowpea rhizosphere discovered that elevated genetic diversity of symbiotic bacterial species in western Amazon (Gaby and Buckley 2011; Guimarães et al. 2012). Pot studies under glasshouse have been carried out by using *Rhizobium*, *Bradyrhizobium* and *Burkholderia* species in cowpea, and it was found that these species were able to form nodules and participate in symbiotic nitrogen fixation (Guimarães et al. 2012). In spite of the enormous research on the N_2 fixing potential of rhizobia species, no studies have been reported on the gene transfer specific for symbiosis in legumes to the non-leguminous plant. Geurts et al. (2012) reported that for transferring specific gene responsible for legume symbiosis to other plants, it is essential to study the difference in the cellular responses elicited by *Rhizobium* and mycorrhizal fungi.

The rhizospheric microbial species are also involved in the stimulation of iron uptake. Iron is abundantly present in soil but mainly exists in insoluble form (i.e. ferric oxide) under neutral or alkaline conditions of soil and is unavailable to microbes. To overcome the scarcity of iron, several microbial species utilize a distinguish mechanism to conquer iron concentrations inside the cells through the production of siderophores (Buckling et al. 2007; Hider and Kong 2010). Other than microbes, plants also reacts towards the iron depletion via enhancing the solubilization of inorganic iron in its rhizosphere, and in some cases it also secretes phytosiderophores which are further moved back into the root tissue (Walker and Connolly 2008). The iron-chelating mechanism is adapted by rice plants to overcome the iron deficiency (Walker and Connolly 2008). It is proved by the previous studies that many bacterial species such as fluorescent pseudomonades elevate the iron uptake with the help of siderophores for graminaceous as well as dicotyledonous plant species (Shirley et al. 2011). Many fungal species are also reported to produce siderophore such as rhizoferrin, a fungal siderophore secreted by *Rhizopus arrhizus,* and recognized as a competent transporter of iron into plants in comparison with the other synthetic chelates (Yehuda et al. 2000). In many cases, PGPRs also initiate the plant's iron uptake mechanism reported in *Bacillus subtilis* GB03 (Zhang et al. 2009). A detailed review on the iron acquisition strategies adapted by rhizospheric microorganisms and plants was studied by Marschner et al. (2011).

A significant proportion of rhizobacterial species are organotrophs, species are characterized as organotrophs, which utilized organic compounds for their growth and development. In majority of the soil the availability of organic compounds is limited and the most common limiting component is carbon which is required for the growth of soil microorganism (Demoling et al. 2007; Rousk and Baath 2007; Vishwakarma et al. 2016). Microbial communities which reside in soils play a major role in releasing the nutritive cations from soil minerals which are further utilized by bacteria itself for their growth as well as nutrition. Bacteria capable of mineral weathering were also isolated from different environmental conditions basically from rhizosphere and also from ectomycorrhizosphere and also facilitate plant growth in contaminated soils (Collignon et al. 2011; Mapelli et al. 2012).

10.4 Role in Growth Promotion During Various Stresses

10.4.1 Biotic Stress

The rhizosphere serve as a barrier against countless bugs present in soil (Cook et al. 1995) at the time of infection in root tissue and prevents its spreading by a process known as antibiosis (Raaijmakers and Mazzola 2012), which can be achieved through secretion of metabolites and acts as growth inhibitor of invading microorganisms because of the reason that rhizospheric microorganisms have an upper hand on antibiotic production (Hoffmeister and Keller 2007; Brakhage and Schroeckh 2011). Some of the biocontrol strains utilize multiple antibiotic compounds with a varied level of antimicrobial activity. Among all, the most important

metabolite is the volatile organic compounds (VOCs). Mostly, they are superior in harmonizing plant growth and mediate communication between microorganisms and plants (Bailly and Weisskopf 2012; Effmert et al. 2012). By definition, VOCs are small molecules (< 300 Da) having high vapour pressures which assist in migrating through the pores in soil filled with water and gases (Insam and Seewald 2010). A diverse strain of bacteria has been identified such as *Stenotrophomonas maltophilia*, *Pseudomonas trivialis*, *P. fluorescens* and *B. subtilis* which can significantly reduce mycelial fungal growth on plant through VOCs secretions (Cavagnaro et al. 2005; Vespermann et al. 2007; Zou et al. 2007; Jamalizadeh et al. 2010). On the other hand, VOCs from bacteria support the growth of ectomycorrhizal fungi and primarily govern the formation of mycorrhizal network (Bonfante and Anca 2009). Finally, same volatiles have also shown to inhibit quorum sensing in bacteria which are evolutionary dissimilar by deregulating the synthesis of the N-acyl-homoserine lactone synthase genes (Chernin et al. 2011).

Moreover, rhizospheric biota can also enhance the plant immune system (De Vleesschauwer and Hofte 2009; Pineda et al. 2010) and are supposed to be enhanced through some phytohormones released from microorganisms like ethylene and jasmonic acid (Zamioudis and Pieterse 2012). In addition, molecules involved in quorum sensing of rhizobacteria can also provide immunity to plant, via the regulation of genes involved in resistant mechanisms like *Pdf1.2*, *MPK3*, *WRKY22*, *MPK6* and *WRKY29* (Mendes et al. 2013). Profound studies have unknotted the effect of rhizobacteria on transcription and metabolites level alterations in plant immune system. Some strains are able to provide resistance through JA/ET transcription pathways (Cartieaux et al. 2008), while some provides immunity through SA transcription pathway in *Arabidopsis* (van de Mortel et al. 2012). Such work supported the statement that rhizobacteria control a varied and intense consequence for immunity and sustainability of plant as well as strengthen the fabrication of secondary metabolites (van de Mortel et al. 2012).

10.4.2 Abiotic Stress

The contribution of rhizospheric microbial communities is essential for some of the species of plants for their survival under severe circumstances (Jorquera et al. 2012). Abiotic stress is considered to be one of the severe stresses of environment that reduces the growth and yield of any crop even on irrigated land throughout the world (Vishwakarma et al. 2017). For instance, *Achromobacter piechaudii* ARV8 isolated from arid and saline soil and is able to enhance the tomato and pepper seedling's biomass when subjected to drought stress (Mayak et al. 2004a, b). Also, plant growth was supported by the rhizobacteria when exposed to flooding (Grichko and Glick 2001). It was also seen that among diverse production systems, growth and yield of plant were intensely influenced by saline nature of soil that can be attributed to drought stress. Bacteria able to tolerate a high concentration of salt can persist with salt stressed ambience, and they, in conjugation with plant, have the ability to express the characteristics that

support growth of plant. For instance, Upadhyay et al. (2009) showed that out of 130 rhizospheric bacterial isolates obtained from wheat plants grown under saline conditions, 24 isolates were found tolerant to high doses of NaCl (8%). All the 24 isolates that tolerated salt stress conditions were found to produce indole acetic acid, 10 were shown to solubilize phosphorous, 8 were found to produce siderophores, 6 were thought to form gibberellin and 2 were found to contain *nifH* gene indicating the ability to fix nitrogen. *Bacillus* was the dominant bacteria found under such conditions (Upadhyay et al. 2009). Most of the halotolerant bacterial strains increased the growth of plant under salt stress conditions, the mechanism of which can be devoted to the decline in ET production through ACC deaminase activity (Siddikee et al. 2010). Another novel halotolerant diazotrophic bacterium capable of phosphate solubilization, producing phytohormones and having ACC deaminase activity, was obtained from the roots of *Salicornia brachiata* (Jha et al. 2012). In reviews by Dodd and Perez-Alfocea (2012), a number of mechanisms are elaborated through which microbes may change plant physiological response under salt stress. Microbial inoculants are of huge interest in agricultural and horticultural prospects to increase the plant growth under cold situations. For instance, *Burkholderia phytofirmans* PsJN is capable of enhancing the growth of grapevine root and its physiological activity at lower temperatures such as 4 °C (Barka et al. 2006). When *Serratia proteamaculans* is inoculated simultaneously with *Bradyrhizobium japonicum*, the growth of soybean was accelerated at 15 °C, since mostly soybean nodule infection and nitrogen fixation are oppressed at mentioned temperature (Zhang et al. 1995, 1996). A number of abiotic parameters can have adverse effect on the plant growth, and these factors may include pH and high levels of toxic components. The major challenge in many agricultural systems all over the world is the contamination of soils with toxic compounds or low pH of soils. In case of pH stressed condition, significant reduction of foliar lesions on corn grown under low pH soil was observed when the plants were treated with *P. fluorescens* strain that produces 2,4-diacetylphloroglucinol (DAPG). Hence it was quite evident from this observation that DAPG producers can tackle with abiotic stress parameters along with their ability to control pathogens (Raudales et al. 2009). Soil contaminants influenced the search for an advanced eco-friendly remediation approach other than physical approaches. Rhizoremediation, a combined approach of plants and microbes (Kuiper et al. 2004), has emerged as a promising technique to remediate the polluted environment. During rhizoremediation, rhizospheric bacteria were stimulated by the exudates of plants in order to facilitate degradation of pollutants. A study of split-root model was conducted using a combined techniques includes T-RFLP, DGGE and 16S rRNA gene pyrosequencing, and it was observed that *Trifolium* and other legumes respond to polycyclic aromatic hydrocarbon contamination systematically (Kawasaki et al. 2012). It was observed that *Verrucomicrobia* and *Actinobacteria* were predominant in the polluted rhizospheres, and the betaproteobacterium *Denitratisoma* was mainly amplified in the presence of the pollutant, which gives an indication that this genus may be essential in the rhizoremediation procedure (Kawasaki et al. 2012).

Also, one of the crucial competitors in rhizoremediation of hydrocarbons was fungi as evident by inoculation of the endophytic fungus *Lewia* sp. in the rhizosphere of *Festuca arundinacea* (Cruz-Hernandez et al. 2012).

Hence, it can be inferred that members of the rhizospheric microbiota can be thought to manage biotic and abiotic stresses on growth of plants providing an environment friendly and sound as substitute for genetic engineering and plant breeding. Nevertheless, the successful utilization of microbial inoculants in the remediation process is not applied globally, and the main reason underlying this is the varying environmental conditions and different plants species, limited shelf life and different registration processes in different countries. For resolution of these restraints, there is a need to have much more rudimentary apprehension on how beneficial rhizospheric microbes commune with the host plant, which molecular and metabolic modifications are made in plants, and the way helpful microorganisms dominate the population dynamics and virulence of phytopathogens.

10.5 Probiotics as a Potential Biofertilizer Candidate

Various classes of soil rhizospheric microbiome which has been classified under specific kingdoms known as bacteria, fungi, protozoa, nematodes, etc. act as a potential probiotics to manufacture biofertilizers because they serve in promoting plant growth and characterized as plant growth-promoting microorganism (PGPM) (Lucy et al. 2004; Smith and Read 2008). Their primary role is to facilitate plant nutrition by providing nutrients ranging from single element like nitrogen by nitrogen-fixing bacteria to a multielements supply. For example, arbuscular mycorrhizal fungi (AMF) association (Bardi and Malusà 2012) leads to an astonishing outcome of plant's output mainly because of enhanced nutrient absorption. Rhizobia a renowned nitrogen-fixing bacteria concomitantly increase the nitrogen uptake in legume plants to 90% of the (Franche et al. 2009) and moreover act as plant growth-promoting rhizobacteria (PGPR) win nonlegumes plants (Hayat et al. 2010). Other symbiotic bacteria such as *Cyanobacteria* upregulate the fixing of nitrogen in various leguminous plants to enhance availability of nitrogen (Wagner 1997). Arbuscular mycorrhizal fungi are categorized as broad spectrum of probiotics which play a major role in plant phosphorus intake (Smith and Read 2008), enhancing phosphorus solubility (Tawaraya et al. 2006). Numerous PGPR are found to stimulate phosphorus solubility in the soil which is present in the form of tricalcium phosphate, hydroxylapatite and rock phosphate (Rodríguez and Fraga 1999; Owen et al. 2015). A divers group of bacterial species are identified as potential potassium solubilizer which can successfully enrich potassium from minerals such as mica, biotite, orthoclases, illite and muscovite (Bennett et al. 2001; Liu et al. 2012), which in turn expand its bioavailability to 15% (Supanjani Han et al. 2006). The quest for new and effective probiotics which offers an advantage to plant nutrition and growth has nurtured interest on species that were least explored. Following, we present a list of probiotics (Table 10.1) which has proved to be an effective biofertilizers for various agricultural plants and crops.

Table 10.1 The table enlists the agricultural probiotics which has shown to enhance the nutrients supply and growth of respective plants and can be employed as biofertilizers

S. no.	Probiotics	Host plant	References
1.	*Azolla*	Rice	Gupta (2004)
2.	*A. lipoferum*	Wheat	El-Komy (2005)
3.	*B. megaterium*	Wheat	El-Komy (2005)
4.	*Pseudomonas*	Chickpea	Mohammadi et al. (2010)
5.	*Azospirillum* and *P. striata*	Maize	Prabakaran and Ravi (1991)
6.	*Azospirillum* and *P. striata*	Cotton	Radhakrishnan (1996)
7.	*A. brasilense*	Maize	Zaddy et al. (1993)
8.	*R. leguminosarum* and *P. putida*	Beans	De Freitas et al. (1993)
9.	*A. mysorens*	Barley	Belimov et al. (1995)
10.	*T. harzianum*	Chickpea	Mohammadi et al. (2011)

10.5.1 Bioformulation Using Probiotics

Biofertilizers are the composition of live or quiescent microorganisms or probiotics, whose designing permits easy marketing with prolonged storage and also without disturbing its efficacy. It offers a suitable method for trading of potent probiotics to the farmer. Bioformulation is a term used for the formulation in which potent or attenuated microorganisms/probiotics are isolated from plant or soil and is known as bioinoculants that are being transported to agricultural field. These bioformulations regulate the possible prosperity of inoculum (Fages 1992). With the increase in technology and its application, it is now possible to formulate any type of probiotics because of their common physiological characters. Hence, the technology developed for a particular probiotics can be employed to other with little standardization (Bashan 1998). Different varieties of probiotics can be employed for manufacturing formulations of probiotics which facilitate enhanced nitrogen and phosphorous fixation, biocontrol agents, PGPR and many more. The bioformulation step includes the isolation of active probiotics (particularly, rhizosphere) which are then mixed with carrier system and nutrients, preservatives and protectant agents. Figure 10.1 shows the detailed procedure for the preparation of probiotics as biofertilizer. A formulation technique may vary depending on probiotic nature, soil properties, plant species, mode of application and the accessible resources. Earlier work has stated that the concentration of inoculum viable cells modulate the inoculum quality (Hitbold et al. 1980; Lupwayi et al. 2000), and so, the formulation plays an active role for biofertilizer making.

The major drawback that hinders for bioformulation is the unsupportive climate conditions, especially in semiarid regions and in India since it upregulates the discrepancy of performance (Sahu and Brahmaprakash 2016). Semiarid areas are also marked with bitter environmental state, high droughts, inadequate irrigation and progressive soil erosion and salinity which results in the wash away of beneficial bacteria (Bashan 1998). These hindrances provide an occasion to develop a platform which offers a cost-effective and resistant bioformulation technique against the above-mentioned conditions.

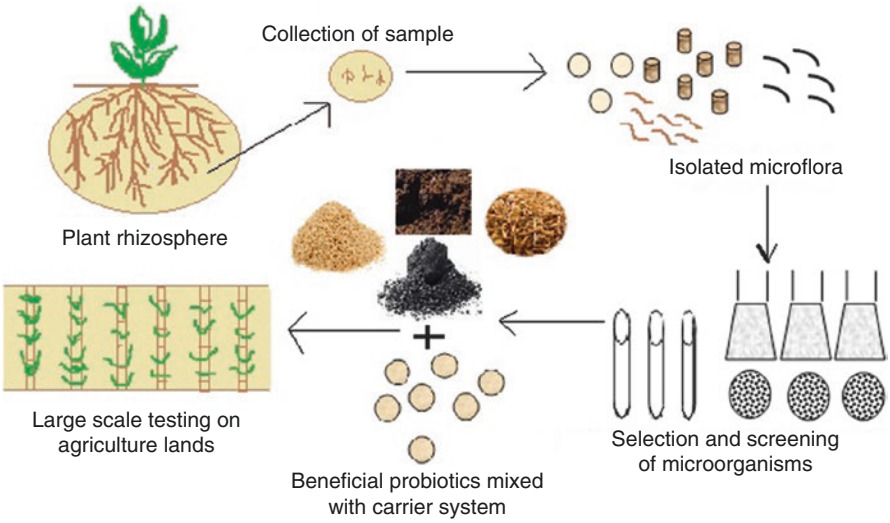

Fig. 10.1. Production of biofertilizers is a five-step process: (1) sample stock preparation, (2) exploration of microbiome, (3) identification of active probiotics, (4) bioformulation of biofertilizers compatible with the selected plant species and (5) evaluation of biofertilizers on farm lands

10.6 Guidelines for Bioproduct Registration

Due to safety concern with biofertilizers and biopesticides on human health, environment and other organisms, Indian government has made it mandatory to register such products. Many countries have made strict rules for registering biopesticides (OECD 1996, 2002; FAO 1988; Leahy et al. 2014). To promote bio-based products, countries have rules and regulation as minimum as possible in comparison with conventional fertilizers and pesticides because bio-based products are already less toxic. A different rules and regulation system has been developed for substances which are mixture of chemical-microbial pesticides aimed to control pests (Desai 2016). Some countries like the USA have incorporated genetically modified plants for pest control mechanism (Smyth and McHughen 2012). Because of the need of good organic foods among people, there is a necessity to regulate the registration of bio-based products at high standard level. However, registration is a continuous challenge in developing countries, particularly for small and medium enterprises (SMEs). SMEs are major contributors of bio-based products production in developing countries, and because of their limited investment, there is no full scale spending in regulatory and dossier procedures. Another problem arises in analysing bioproduct data is the availability of highly experienced and qualified area experts. For handling registration of biopesticides, there is lack of deep scientific understanding between experts which are handling pesticide data. So good laboratory practice (GLP) is helping in universally acceptance of product registration worldwide. Still, basic requirement for registration process varies from country to country. In addition, there are strict rules and regulation implemented for bioproduct registration, but authorities in countries like India and other are treating them as

conventional chemical products. In continuation of this, an act was passed in the USA (Environmental Protection Agency (EPA)) and some other European countries which brought genetically modified plants under biopesticides category (Smyth and McHughen 2012).

10.6.1 Requirements and Regulatory Mechanisms for Bioproducts Registration

The regulatory guidelines for promoting bioproducts are different in different nations, and the guidelines are posted on the respective websites. Biofertilizer registration also needs technical information about the ingredients and bioformulation procedures (Desai 2016). Worldwide registration need data like notional description of biological properties, organism/ingredients, bioefficacies in the laboratory and field, safety/ecotoxicity studies, toxicology, packaging, etc. are required (Desai 2016). Readers and registering applicants can check the related information for any dossier necessity on the respective websites.

All countries that promote bioproducts constitute many regulatory authority boards in order to check the regulation of such products. Based on the world public needs, the authorities update the dossier requirements for registration from time to time, and also according to the needs with respect to the advancement in scientific knowledge, the dossier requirements change (Desai 2016). The various registration processes in regulatory offices from different countries make it complex for registration. More significantly, many of the countries don't ask for information generated through laboratories which have adopted universal good laboratory practices (GLPs) (Desai 2016). So, there is absence of a good laboratory standard and a uniform protocol requirement, and it is making difficult to register biofertilizers for small and medium enterprises (SMEs).

10.7 Conclusion and Future Prospects

Despite the beneficiary role of rhizosphere microbiome for improved agroecosystem, the extensively acknowledged and traditional method restricted their ability to showcase their purpose. Incorporating such methods with latest next-generation sequencing methods to determine rhizomicrobiome will draw fresh perception to rhizosphere biota. Recognition of signals and biomolecules secreted by rhizosphere will help in identifying markers that will unravel the symbiotic relation of plants and beneficial probiotics. These studies will help to optimize crop protection and also expose abundant yet unfamiliar soil probiotics, task and genes for various applications.

Advancement in crop production quality and a transparent lawful structure that assures both manufacturers and farmers are required to maintain the latent profitable growth. Biofertilizers play an important function to increase nutrients supply system, nourishing crop health with little environmental issue. Additional thrust for a broad and productive application of biofertilizers can be deduced from our current

understanding on microbes and scientific growth. Biofertilizers can also function in development of seedlings, growth promotion under the exposure of numerous biotic and abiotic stresses by their improved production of secondary metabolites. The guarantee of usefulness for a biofertilizer in a specified soil against a diversity of crop is, thus, (i) a multifaceted job which has to be monitored while manipulating and relating it to a particular biofertilizer and (ii) a future prospect to enhance the crop improvement and production with such goods into a general procedure for advanced agriculture.

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References

Adeleke RA, Cloete TE, Bertrand A, Khasa DP (2012) Iron ore weathering potentials of ectomycorrhizal plants. Mycorrhiza 22:535–544

Bailly A, Weisskopf L (2012) The modulating effect of bacterial volatiles on plant growth. Plant Signal Behav 7:1–7

Bardi L, Malusà E (2012) Drought and nutritional stresses in plant: alleviating role of rhizospheric microorganisms. In: Haryana N, Punj S (eds) Abiotic stress: new research. Nova Science Publishers Inc, Hauppauge, pp 1–57

Barka EA, Nowak J, Clement C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium, *Burkholderia phytofirmans* strain PsJN. Appl Environ Microbiol 72:7246–7252

Barr M, East AK, Leonard M, Mauchline TH, Poole PS (2008) In vivo expression technology (IVET) selection of genes of Rhizobium leguminosarum biovar viciae A34 expressed in the rhizosphere. FEMS Microbiol Lett 282:219–227

Barret M, Morrissey JP, O'Gara F (2011) Functional genomics analysis of plant growth-promoting rhizobacterial traits involved in rhizosphere competence. Biol Fertil Soils 47:729–743

Bashan Y (1998) Inoculants for plant growth promoting bacteria for use in agriculture. Adv Biotechnol 16:729–770

Belimov AA, Kojemiakov AP, Chuvarliyeva CV (1995) Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. Plant Soil 173:29–37

Bennett PC, Rogers JR, Choi WJ (2001) Silicates, silicate weathering, and microbial ecology. Geomicrobiol J 18:3–19

Berendsen RL, Pieterse CMJ, Bakker P (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17:478–486

Bonfante P, Anca I-A (2009) Plants, mycorrhizal fungi, and bacteria: a network of interactions. Annu Rev Microbiol 63:363–383

Bonkowski M, Villenave C, Griffiths B (2009) Rhizosphere fauna: the functional and structural diversity of intimate interactions of soil fauna with plant roots. Plant Soil 321:213–233

Brakhage AA, Schroeckh V (2011) Fungal secondary metabolites – strategies to activate silent gene clusters. Fungal Genet Biol 48:15–22

Buckling A, Harrison F, Vos M, Brockhurst MA, Gardner A, West SA, Griffin A (2007) Siderophore-mediated cooperation and virulence in Pseudomonas Aeruginosa. FEMS Microbiol Ecol 62:135–141

Buee M, De Boer W, Martin F, van Overbeek L, Jurkevitch E (2009) The rhizosphere zoo: an overview of plant-associated communities of microorganisms, including phages, bacteria, archaea, and fungi, and of some of their structuring factors. Plant Soil 321:189–212

Bulgarelli D, Rott M, Schlaeppi K et al (2012) Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. Nature 488:91–95

Calvaruso C, Turpault MP, Leclerc E, Frey-Klett P (2007) Impact of ectomycorrhizosphere on the functional diversity of soil bacterial and fungal communities from a forest stand in relation to nutrient mobilization processes. Microbial Ecol 54:567–577

Cartieaux F, Contesto C, Gallou A et al (2008) Simultaneous interaction of *Arabidopsis thaliana* with Bradyrhizobium sp. strain ORS278 and Pseudomonas syringae pv. Tomato DC3000 leads to complex transcriptome changes. Mol Plant-Microbe Interact 21:244–259

Cavagnaro TR, Smith FA, Smith SE, Jakobsen I (2005) Functional diversity in arbuscular mycorrhizas: exploitation of soil patches with different phosphate enrichment differs among fungal species. Plant Cell Environ 28:642–650

Chelius MK, Triplett EW (2001) The diversity of archaea and bacteria in association with the roots of *Zea mays* L. Microb Ecol 41:252–263

Chernin L, Toklikishvili N, Ovadis M, Kim S, Ben-Ari J, Khmel I, Vainstein A (2011) Quorum-sensing quenching by rhizobacterial volatiles. Environ Microbiol Rep 3:698–704

Collignon C, Uroz S, Turpault MP, Frey-Klett P (2011) Seasons differently impact the structure of mineral weathering bacterial communities in beech and spruce stands. Soil Biol Biochem 43:2012–2022

Cook RJ, Thomashow LS, Weller DM, Fujimoto D, Mazzola M, Bangera G, Kim DS (1995) Molecular mechanisms of defense by rhizobacteria against root disease. Proc Natl Acad Sci U S A 92:4197

Cruz-Hernandez A, Tomasini-Campocosio A, Perez-Flores L, Fernandez-Perrino F, Gutierrez-Rojas M (2012) Inoculation of seed-borne fungus in the rhizosphere of *Festuca arundinacea* promotes hydrocarbon removal and pyrene accumulation in roots. Plant Soil 363:261–270

De Freitas JR, Gupta VVS, Germida JJ (1993) Influence of *Pseudomonas syringae* R 25 and *P. putida* R 105 on the growth and N2 fixation (ARA) of pea (*Pisum sativum* L.) and field bean (*Phaseolus vulgaris* L.) Biol Fertil Soils 16:215–220

De Vleesschauwer D, Hofte M (2009) Rhizobacteria-induced systemic resistance. Adv Bot Res 51:223–281

DeAngelis KM, Ji PS, Firestone MK, Lindow SE (2005) Two novel bacterial biosensors for detection of nitrate availability in the rhizosphere. Appl Environ Microbiol 71:8537–8547

DeAngelis KM, Brodie EL, DeSantis TZ, Andersen GL, Lindow SE, Firestone MK (2009) Selective progressive response of soil microbial community to wild oat roots. ISME J 3:168–178

Demoling F, Figueroa D, Baath E (2007) Comparison of factors limiting bacterial growth in different soils. Soil Biol Biochem 39:2485–2495

Desai S (2016) Challenges in regulation and registration of biopesticides: an overview. In: Microbial inoculants in sustainable agricultural productivity. Springer, India, pp 301–308

Dodd IC, Perez-Alfocea F (2012) Microbial amelioration of crop salinity stress. J Exp Bot 63:3415–3428

Effmert U, Kalderas J, Warnke R, Piechulla B (2012) Volatile mediated interactions between bacteria and fungi in the soil. J Chem Ecol 38:665–703

El-Komy HMA (2005) Co-immobilization of *A. lipoferum* and *B. megaterium* for plant nutrition. Food Technol Biotechnol 43(1):19–27

Fages J (1992) An industrial view of *Azospirillum* inoculants: formulation and application technology. Symbiosis 13:15–26

FAO (1988) Guidelines on the registration of biological pest control agent. Food and Agriculture Organization of the United Nations, Rome

Ferluga S, Venturi V (2009) *OryR* is a *LuxR*-family protein involved in inter kingdom signaling between pathogenic Xanthomonas oryzae pv. Oryzae and rice. J Bacteriol 191:890–897

Franche C, Lindström K, Elmerich C (2009) Nitrogen fixing bacteria associated with leguminous and non leguminous pants. Plant Soil 321:35–59

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36

Gaby JC, Buckley DH (2011) A global census of nitrogenase diversity. Environ Microbiol 13:1790–1799
Gans J, Wolinsky M, Dunbar J (2005) Computational improvements reveal great bacterial diversity and high metal toxicity in soil. Science 309:1387–1390
Geurts R, Lillo A, Bisseling T (2012) Exploiting an ancient signalling machinery to enjoy a nitrogen fixing symbiosis. Curr Opin Plant Biol 15:438–443
Gianinazzi S, Gollotte A, Binet MN, van Tuinen D, Redecker D, Wipf D (2010) Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20:519–530
Grichko VP, Glick BR (2001) Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiol Biochem 39:11–17
Guimarães AA, Jaramillo PMD, Nóbrega RSA, Florentino LA, Silva KB, de Souza Moreira FM (2012) Genetic and symbiotic diversity of nitrogen-fixing bacteria isolated from agricultural soils in the western Amazon by using cowpea as the trap plant. Appl Environ Microbiol 78(18):6726–6733
Gupta AK (2004) The complete technology book on biofertilizers and organic farming. National Institute of Industrial Research Press, India
Hawkes CV, DeAngelis KM, Firestone MK (2007) Root interactions with soil microbial communities and processes. In: Cardon Z, Whitbeck J (eds) The Rhizosphere. Elsevier, New York, pp 1–3
Hayat R, Ali S, Amara U, Khalid R, Ahmed I (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598
He Z, Gentry TJ, Schadt CW et al (2007) GeoChip: a comprehensive microarray for investigating biogeochemical, ecological and environmental processes. ISME J 1:67–77
Herron PM, Gage DJ, Cardon ZG (2010) Micro-scale water potential gradients visualized in soil around plant root tips using microbiosensors. Plant Cell Environ 33:199–210
Hider RC, Kong X (2010) Chemistry and biology of siderophores. Nat Prod Rep 27:637–657
Hinsinger P, Marschner P (2006) Rhizosphere– perspectives and challenges – a tribute to Lorenz Hiltner. Plant Soil 283:vii–viii
Hinsinger P, Bengough AG, Vetterlein D, Young IM (2009) Rhizosphere: biophysics, biogeochemistry and ecological relevance. Plant Soil 321:117–152
Hitbold AE, Thurlow N, Skipper HD (1980) Evaluation of commercial soybean inoculants by various techniques. Agron J 72:675–681
Hoffmeister D, Keller NP (2007) Natural products of filamentous fungi: enzymes, genes, and their regulation. Nat Prod Rep 24:393–416
Hol WHG, de Boer W, Termorshuizen AJ et al (2010) Reduction of rare soil microbes modifies plant-herbivore interactions. Ecol Lett 13:292–301
Insam H, Seewald MSA (2010) Volatile organic compounds (VOCs) in soils. Biol Fertil Soils 46:199–213
Jamalizadeh M, Etebarian HR, Aminian H et al (2010) Biological control of Botrytis mali on apple fruit by use of Bacillus bacteria, isolated from the rhizosphere of wheat. Arch Phytopathol Plant Protect 43:1836–1845
Jensen LE, Nybroe O (1999) Nitrogen availability to *Pseudomonas fluorescens* DF57 is limited during decomposition of barley straw in bulk soil and in the barley rhizosphere. Appl Environ Microbiol 65:4320–4328
Jha B, Gontia I, Hartmann A (2012) The roots of the halophyte Salicornia Brachiata are a source of new halotolerant diazotrophic bacteria with plant growth-promoting potential. Plant Soil 356:265–277
Jogler C, Waldmann J, Huang XL, Jogler M, Glockner FO, Mascher T, Kolter R (2012) Identification of proteins likely to be involved in morphogenesis, cell division, and signal transduction in Planctomycetes by comparative genomics. J Bacteriol 194:6419–6430
Johnson NC, Graham JH (2013) The continuum concept remains a useful framework for studying mycorrhizal functioning. Plant Soil 363:411–419
Jones D, Hinsinger P (2008) The rhizosphere: complex by design. Plant Soil 312:1–6

Jorquera MA, Shaharoona B, Nadeem SM, de la Luz Mora M, Crowley DE (2012) Plant growth-promoting rhizobacteria associated with ancient clones of creosote bush (*Larrea tridentata*). Microb Ecol 64:1008–1017

Kawasaki A, Watson ER, Kertesz MA (2012) Indirect effects of polycyclic aromatic hydrocarbon contamination on microbial communities in legume and grass rhizospheres. Plant Soil 358:169–182

Knief C, Delmotte N, Chaffron S et al (2011) Metaproteogenomic analysis of microbial communities in the phyllosphere and rhizosphere of rice. ISME J 6:1378–1390

Koch B, Worm J, Jensen LE, Hojberg O, Nybroe O (2001) Carbon limitation induces sigma(S)-dependent gene expression in *Pseudomonas fluorescens in* soil. Appl Environ Microbiol 67:3363–3370

Kogel KH, Franken P, Hückelhoven R (2006) Endophyte or parasite – what decides? Curr Opin Plant Biol 9:358–363

Kragelund L, Hosbond C, Nybroe O (1997) Distribution of metabolic activity and phosphate starvation response of lux-tagged *Pseudomonas fluorescens* reporter bacteria in the barley rhizosphere. Appl Environ Microbiol 63:4920–4928

Kuiper I, Lagendijk EL, Bloemberg GV, Lugtenberg BJJ (2004) Rhizoremediation: a beneficial plant–microbe interaction. Mol Plant-Microbe Interact 17:6–15

Kulakova AN, Kulakov LA, McGrath JW, Quinn JP (2009) The construction of a whole-cell biosensor for phosphonoacetate, based on the LysR-like transcriptional regulator *PhnR* from *Pseudomonas fluorescens* 23F. Microb Biotechnol 2:234–240

Leahy J, Mendelsohn M, Kough J, Jones R, Berckes N (2014) Biopesticide oversight and registration at the U.S. Environmental Protection Agency. In: Coats JR et al (eds) Biopesticides: state of the art and future opportunities. ACS Symposium Series, American Chemical Society, Washington

Leininger S, Urich T, Schloter M et al (2006) Archaea predominate among ammonia-oxidizing prokaryotes in soils. Nature 442:806–809

Lemanceau P, Expert D, Gaymard F, Bakker P, Briat JF (2009) Role of iron in plant–microbe interactions. Adv Bot Res 51:491–549

Leveau JHJ, Uroz S, de Boer W (2010) The bacterial genus Collimonas: mycophagy, weathering and other adaptive solutions to life in oligotrophic soil environments. Environ Microbiol 12:281–292

Liu D, Lian B, Dong H (2012) Isolation of *Paenibacillus* sp. and assessment of its potential for enhancing mineral weathering. Geomicrobiol J 29:413–421

Loh J, Pierson EA, Pierson LS, Stacey G, Chatterjee A (2002) Quorum sensing in plant-associated bacteria. Curr Opin Plant Biol 5:285–290

Lucy M, Reed E, Glick BR (2004) Application of free living plant growth-promoting rhizobacteria. Antonie Van Leeuwenhoek 86:1–25

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556

Lundberg DS, Lebeis SL, Paredes SH et al (2012) Defining the core *Arabidopsis thaliana* root microbiome. Nature 488:86

Lupwayi NZ, Olsen PE, Sonde ES et al (2000) Inoculant quality and its evaluation. Field Crop Res 65:259–270

Lynch JM (1990) The Rhizosphere. John Wiley, Sons, New York

Mapelli F, Marasco R, Balloi A, Rolli E, Cappitelli F, Daffonchio D, Borin S (2012) Mineral-microbe interactions: biotechnological potential of bioweathering. J Biotechnol 157:473–481

Mark GL, Dow JM, Kiely PD et al (2005) Transcriptome profiling of bacterial responses to root exudates identifies genes involved in microbe–plant interactions. Proc Natl Acad Sci U S A 102:17454–17459

Marschner P, Crowley D, Rengel Z (2011) Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis – model and research methods. Soil Biol Biochem 43:883–894

Mayak S, Tirosh T, Glick BR (2004a) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166:525–530
Mayak S, Tirosh T, Glick BR (2004b) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572
Meeting FB (1992) Soil microbial ecology: applications in agricultural and environmental management. Marcel Dekker, New York
Mendes R, Kruijt M, de Bruijn I et al (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097–1100
Mendes R, Paolina G, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37(5):634–663
Miller RM, Jastrow JD (2000) Mycorrhizal fungi influence soil structure. In: Kapulnik Y, Douds DD Jr (eds) Arbuskular mycorrhizas: physiology and function. Kluwer Academic Publishers, London, pp 3–18
Miransari M (2011) Arbuscular mycorrhizal fungi and nitrogen uptake. Arch Microbiol 193:77–81
Mohammadi K, Ghalavand A, Aghaalikhani M, Sohrabi Y, Heidari GR (2010) Impressibility of chickpea seed quality from different systems of increasing soil fertility. Electron J Crop Prod 3(1):103–119
Mohammadi K, Ghalavand A, Aghaalikhani M, Heidari GR, Sohrabi Y (2011) Introducing the sustainable soil fertility system for chickpea (*Cicer arietinum* L). Afr J Biotechnol 10(32):6011–6020
Nelson EB (2004) Microbial dynamics and interactions in the spermosphere. Annu Rev Phytopathol 42:271–309
OECD (1996) Date requirements for registration of biopesticides in OECD member countries: survey results, environment monograph no. 106. Organization for Economic Cooperation and Development, Paris
OECD (2002) Consensus document on information used in assessment of environmental applications involving baculoviruses. Series on harmonisation of regulatory oversight in biotechnology no. 20. ENV/JM/MONO (2002)1 OECD. Organization for Economic Cooperation and Development, Paris
Owen D, Williams AP, Griffi th GW, Withers PJA (2015) Use of commercial bio-inoculants to increase agricultural production through improved phosphorus acquisition. Appl Soil Ecol 86:41–54
Pineda A, Zheng S-J, van Loon JJA, Pieterse CMJ, Dicke M (2010) Helping plants to deal with insects: the role of beneficial soil-borne microbes. Trends Plant Sci 15:507–514
Pozo MJ, Azcon-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393–398
Prabakaran J, Ravi KB (1991) Interaction effect of *A. Brasilense* and *Pseudomonas* sp. A phosphate solubilizer on the growth of *Zea mays*. In: Microbiology Abstracts, XXXI Annual Conference of the Association of Microbiologists of India, TNAU, Coimbatore, January 23–26, p 109
Qiang X, Weiss M, Kogel KH, Schafer P (2012) Piriformospora indica a mutualistic basidiomycete with an exceptionally large plant host range. Mol Plant Pathol 13:508–518
Raaijmakers J, Mazzola M (2012) Diversity and natural functions of antibiotics produced by beneficial and pathogenic soil bacteria. Annu Rev Phytopathol 50:403–424
Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. Plant Soil 321:341–361
Radhakrishnan KC (1996) Role of biofertilizers in cotton productivity. In: National seminar biofertilizer production problem and constraints, TNAU, Coimbatore, January 24–25, p 17
Rainey PB (1999) Adaptation of *Pseudomonas fluorescens* to the plant rhizosphere. Environ Microbiol 1:243–257
Ramos C, Molbak L, Molin S (2000) Bacterial activity in the rhizosphere analyzed at the single-cell level by monitoring ribosome contents and synthesis rates. Appl Environ Microbiol 66:801–809

Raudales RE, Stone E, McSpadden Gardener BB (2009) Seed treatment with 2,4-diacetylphlorog lucinol-producing pseudomonads improves crop health in low-pH soils by altering patterns of nutrient uptake. Phytopathology 99:506–511

Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321:305–339

Rochat L, Pechy-Tarr M, Baehler E, Maurhofer M, Keel C (2010) Combination of fluorescent reporters for simultaneous monitoring of root colonization and antifungal gene expression by a biocontrol pseudomonad on cereals with flow cytometry. Mol Plant-Microbe Interact 23:949–961

Rodríguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Roesch LFW, Fulthorpe RR, Riva A et al (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. ISME J 1:283–290

Rousk J, Baath E (2007) Fungal and bacterial growth in soil with plant materials of different C/N ratios. FEMS Microbiol Ecol 62:258–267

Sahu PK, Brahmaprakash GP (2016) Formulations of biofertilizers–approaches and advances. In: Microbial inoculants in sustainable agricultural productivity. Springer, India, pp 179–198

Salvioli A, Bonfante P (2013) Systems biology and "omics" tools: a cooperation for next-generation mycorrhizal studies. Plant Sci 203:107–114

Shirley M, Avoscan L, Bernaud E, Vansuyt G, Lemanceau P (2011) Comparison of iron acquisition from Fe-pyoverdine by strategy I and strategy II plants. Botany 89:731–735

Siddikee M, Chauhan P, Anandham R, Han GH, Sa T (2010) Isolation, characterization, and use for plant growth promotion under salt stress, of ACC deaminase-producing halotolerant bacteria derived from coastal soil. J Microbiol Biotechnol 20:1577–1584

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Elsevier/Academic, New York/London/Burlington/San Diego, p 605

Smyth SJ, McHughen A (2012) Regulation of genetically modified crops in USA and Canada: Canadian overview. In: Woznaik CA, McHughen A (eds) Regulation of agricultural biotechnology: the United States and Canada. Springer, Dordrecht, pp 15–34

Steindler L, Venturi V (2007) Detection of quorum-sensing N-acyl homoserine lactone signal molecules by bacterial biosensors. FEMS Microbiol Lett 266:1–9

Supanjani Han HS, Jung SJ, Lee KD (2006) Rock phosphate potassium and rock solubilizing bacteria as alternative sustainable fertilizers. Agron Sustain Dev 26:233–240

Tawaraya K, Naito M, Wagatsuma T (2006) Solubilization of insoluble inorganic phosphate by hyphal exudates of arbuscular mycorrhizal fungi. J Plant Nutr 29:657–665

Teixeira LCRS, Peixoto RS, Cury JC, Sul WJ, Pellizari VH, Tiedje J, Rosado AS (2010) Bacterial diversity in rhizosphere soil from Antarctic vascular plants of Admiralty Bay, maritime Antarctica. ISME J 4:989–1001

Tringe SGC, von Mering A, Kobayashi AA et al (2005) Comparative metagenomics of microbial communities. Science 308:554–557

Trivedi P, He Z, Van Nostrand JD, Albrigo G, Zhou J, Wang N (2011) Huanglongbing alters the structure and functional diversity of microbial communities associated with citrus rhizosphere. ISME J 6:363–383

Ullrich MS, Schergaut M, Boch J, Ullrich B (2000) Temperature-responsive genetic loci in the plant pathogen Pseudomonas syringae pv. Glycinea. Microbiology 146:2457–2468

Upadhyay SK, Singh DP, Saikia R (2009) Genetic diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition. Curr Microbiol 59:489–496

Uroz S, Buee M, Murat C, Frey-Klett P, Martin F (2010) Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. Environ Microbiol Rep 2:281–288

van de Mortel JE, de Vos RCH, Dekkers E et al (2012) Metabolic and transcriptomic changes induced in Arabidopsis by the rhizobacterium *Pseudomonas fluorescens* SS101. Plant Physiol 160:2173–2188

van der Heijden MGA, Sanders IR (2002) Mycorrhizal ecology. Springer, New York
Vansuyt G, Robin A, Briat JF, Curie C, Lemanceau P (2007) Iron acquisition from Fe-pyoverdine by *Arabidopsis thaliana*. Mol Plant-Microbe Interact 20:441–447
Vespermann A, Kai M, Piechulla B (2007) Rhizobacterial volatiles affect the growth of fungi and *Arabidopsis thaliana*. Appl Environ Microbiol 73:5639–5641
Vishwakarma K, Sharma S, Kumar N, Upadhyay N, Devi S, Tiwari A. (2016) Contribution of Microbial Inoculants to Soil Carbon Sequestration and Sustainable Agriculture. In: Microbial Inoculants in Sustainable Agricultural Productivity. pp. Springer India. 101–113
Vishwakarma K, Upadhyay N, Kumar N, Yadav G, Singh J, Mishra R.K., Kumar V, Verma R, Upadhyay R.G., Pandey M, Sharma S, (2017) Abscisic Acid Signaling and Abiotic Stress Tolerance in Plants: A Review on Current Knowledge and Future Prospects. Frontiers in Plant Science 08
Vorholt JA (2012) Microbial life in the phyllosphere. Nat Rev Microbiol 10:828–840
Wagner GM (1997) Azolla. A review of its biology and utilisation. Bot Rev 63:1–26
Walker EL, Connolly EL (2008) Time to pump iron: iron-deficiency-signaling mechanisms of higher plants. Curr Opin Plant Biol 11:530–535
Wang HB, Zhang ZX, Li H et al (2011) Characterization of metaproteomics in crop rhizospheric soil. J Proteome Res 10:932–940
Weinert N, Piceno Y, Ding GC et al (2011) PhyloChip hybridization uncovered an enormous bacterial diversity in the rhizosphere of different potato cultivars: many common and few cultivar-dependent taxa. FEMS Microbiol Ecol 75:497–506
Whipps JM (2001) Ecological and biotechnological considerations in enhancing disease biocontrol. In: Vurro M, Gressel J, Butt T, Harman GE, Pilgeram A, St. Leger RJ, Nuss DL (eds) Enhancing biocontrol agents and handling risks, vol 339. IOP Press, Amsterdam, pp 43–51
Wu L, Wang H, Zhang Z, Lin R, Lin W (2011) Comparative metaproteomic analysis on consecutively Rehmannia glutinosa-monocultured rhizosphere soil. PLoS One 6:e20611
Yehuda Z, Shenker M, Hadar Y, Chen YN (2000) Remedy of chlorosis induced by iron deficiency in plants with the fungal siderophore rhizoferrin. J Plant Nutr 23:1991–2006
Zaddy E, Perevolosky A, Okon Y (1993) Promotion of plant growth by inoculation with aggregated and single cell suspension by *Azospirillum brasilense*. Soil Biol Biochem 25:819–823
Zamioudis C, Pieterse CMJ (2012) Modulation of host immunity by beneficial microbes. Mol Plant-Microbe Interact 25:139–150
Zhang F, Lynch DH, Smith DL (1995) Impact of low root temperatures in soybean [*Glycine max* (L.) Merr.] on nodulation and nitrogen fixation. Environ Exp Bot 35:279–285
Zhang F, Dashti N, Hynes R, Smith DL (1996) Plant growth promoting rhizobacteria and soybean [*Glycine max* (L.) Merr.] nodulation and nitrogen fixation at suboptimal root zone temperatures. Ann Bot 77:453–460
Zhang H, Sun Y, Xie X, Kim MS, Dowd SE, Par_e PW (2009) A soil bacterium regulates plant acquisition of iron via deficiency-inducible mechanisms. Plant J 58:568–577
Zou C-S, Mo M-H, Y-Q G, Zhou J-P, Zhang K-Q (2007) Possible contributions of volatile-producing bacteria to soil fungistasis. Soil Biol Biochem 39:2371–2379

The Good, the Bad, and the Ugly of Rhizosphere Microbiome

11

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Abstract

Rhizosphere is the portion of soil that is exposed to the root activity. It is hot spot for microbial activities which support the plant growth and development in different ways. Microbial communities in the rhizosphere referred as rhizosphere microbiome are one of the most diverse regions of the ecosystem existing on Earth. Rhizosphere microbiome is biologically the most diverse part of the ecosystem which contains a large number of microbial communities which interact with the plants differently like the good, the bad, and the ugly microbes of rhizosphere. The good ones are beneficial microbes of the rhizosphere which are involved in plant growth promotion through nutrient uptake in plants, antagonism to plant pathogens, and plant tolerance against abiotic stresses. However, the bad ones are plant parasitic fungi and nematodes which cause diseases of economic importance in important crop plants and result in serious issues of reduction in productivity and food security. Similarly, some rhizosphere microbes avail the opportunity to invade the human body through different courses and cause infectious diseases. These opportunistic microbes are "the ugly" ones as they are the most deleterious in nature. In this chapter, we have discussed in detail the good, the bad, and the ugly members of rhizosphere microbiome. Moreover, we have given a comprehensive account of bolts and nuts of rhizosphere and engineering of rhizosphere for agriculturally sustainability.

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11.1 Introduction

The terrestrial plants are colonized by a huge number of microorganisms in the form of endosphere and their vicinity of rhizosphere. The rhizosphere is a small ecological area in the immediate proximity of the plant roots. Rhizosphere contains huge diversity of microbes which make the zone the biologically most diverse part of the ecosystem on the Earth planet (Berendsen et al. 2012). This zone acts as the interface of microbial interactions with the plant roots. The total set of microbial communities dwelling in locality of root zone is known as rhizosphere microbiome. Most of the diversity of the rhizosphere microbiome is conditioned by bacterial communities which are generally beneficial for the plants (Chaparro et al. 2012; Mendes et al. 2013). Except some fungi which are advantageous for the plants (e.g., mycorrhiza), a large range of fungi present in the rhizosphere is parasitic to the plants and causes serious diseases which may lead to the death of the plants. Similarly, the plant parasitic nematodes are the other category of harmful microbes which also lead to the plant diseases and low productivity in crop plants causing serious economic losses (Abad et al. 2008; Mendes et al. 2013). The most dangerous part of rhizosphere microbiome is opportunistic parasites, mainly bacteria that invade the human body and cause several infectious diseases like cystic fibrosis (caused by the bacterium *Stenotrophomonas maltophilia*) which are difficult to treat (Waters et al. 2007). The majority of microbial communities in the rhizosphere play a vital role to enhance the composition and productivity of natural plant species by ensuring survival and tolerance against different biotic and abiotic stress conditions. They do so by a range of mechanisms which include biofertilization, stimulation of root growth, control of abiotic stress, rhizoremediation, and disease control. These mechanisms are well studied for rhizobacteria belonging to Proteobacteria and Firmicutes, that is, *Pseudomonas* spp. and *Bacillus* spp., and for fungi from Deuteromycetes that is *Trichoderma* spp. Similarly, plant parasitic microbes have developed several strategies to parasitize the plants, and opportunistic human pathogens from the rhizosphere have developed a sophisticated route to reach to their ultimate host. In this chapter, we discuss about the bolts and nuts of rhizosphere microbiome including the factors affecting the microbial communities present in the rhizosphere. Furthermore, the detail of beneficial, plant parasitic, and opportunistic human pathogenic microbes is provided.

11.2 The Rhizosphere Microbiome

Rhizosphere is the portion of soil that is exposed to the root activity. It is hot spot for microbial activities which help the plant growth and development in different ways. The soil connected to the root and often extending a few millimeters from the root system is included in the rhizosphere that serves as a remarkable ecological zone to study plant and microbe interactions (Lynch 1990; Gray and Smith 2005). The term "rhizosphere microbiome" refers to the collective microbial communities associated with the plant rhizosphere. Biologically, the rhizosphere microbiome is the most diverse reservoir of various microbial communities existing on the planet so far (Curtis et al. 2002; Torsvik et al. 2002; Gams 2007; Buée et al. 2009). It has

been reported that soil from the rhizosphere contains up to 1011 microbial cells per gram of the root system (Egamberdieva et al. 2008) and over 30,000 prokaryotic species (Mendes et al. 2011). Plants have developed complex interactions with the microbiota present in the rhizosphere. Most of the rhizosphere populating microbial communities act as synergists who promote plant growth and development, improve nutrient acquisition by the plants, and alleviate abiotic stress by increasing tolerance and induction of defense mechanisms of the plants. These are considered "the good" of rhizosphere microbiomes. However, some microbes parasitize the plants and cause plant diseases, thus rendering detrimental effects on economically important crop plants. These are "the bad" ones of the rhizosphere microbiome. Soil from the rhizosphere contains several microbes, comparatively low in numbers, which can become pathogenic to human beings. The microbes avail this opportunity and cause infectious diseases in humans. These opportunistic microbes are "the ugly" ones as they are the most deleterious in nature by directly infecting the human beings. A schematic diagram of the good, the bad, and the ugly ones of the rhizosphere microbiome is presented in Fig. 11.1 along with examples and their effects on the ecosystem. In rhizosphere region, the microbes and microbe-mediated processes are

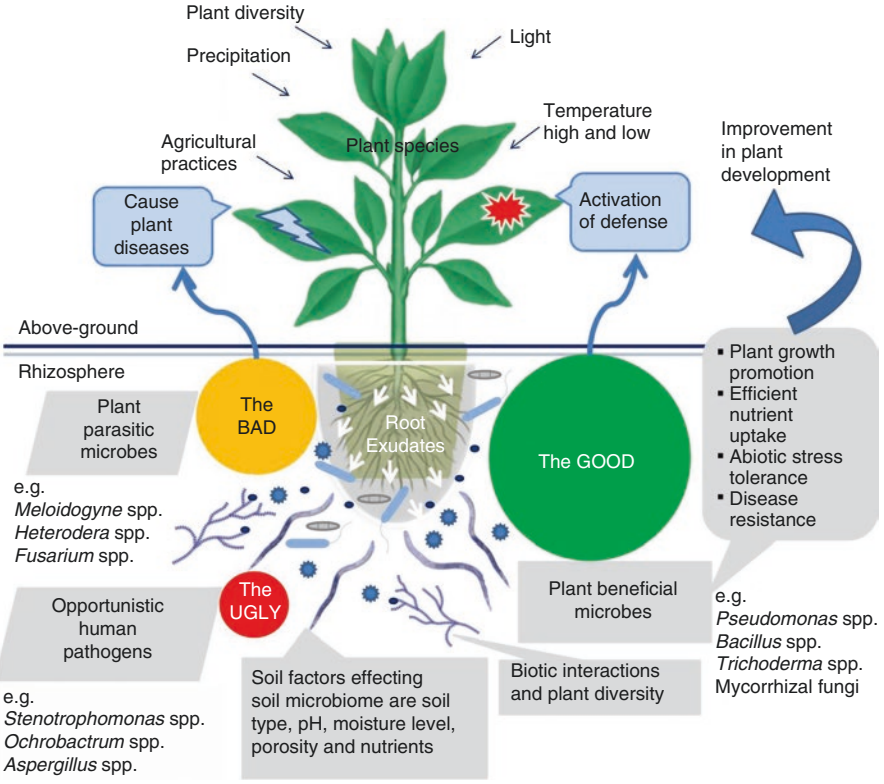

Fig. 11.1 Systematic diagram of rhizosphere microbiome; the good, the bad, and the ugly microbes from the root vicinity of soil; and various factors affecting the root exudates and microflora of rhizosphere

distinctively regulated by plant root system. A wide range of compounds are exuded by the plant roots which are the governing factors for microbial interactions which may be beneficial, neutral, and/or harmful to plants (Lynch 1990; Badri and Vivanco 2009). So the root exudates are the drivers of microbial diversity in the plant rhizosphere.

The plants release a number of diverse organic and inorganic compounds as root exudates which build a distinctive environment in the rhizosphere. The compounds include sugars, fatty acids, CO_2, different anions and cations, terpenoids, thiazoles/pyrazidines, cyclic adenosine monophosphate (cAMP), esters, aliphatic acids, amino acids, and proteins (Badri et al. 2009; Rasmann et al. 2012). These various compounds start the process of chemotaxis and make the plants capable to attract microbial communities for both the symbiotic and pathogenic interactions at the rhizosphere interface (Bais et al. 2006; Luscher et al. 2004; Chapman et al. 2006). In addition to emitting compounds used to develop beneficial association, plant roots also release several compounds like inositols, cucurbitacin A, bithienyl, and its derivatives which repel the plant pathogenic microbes out of the vicinity of rhizosphere (Giebel 1982; Johnson and Nielsen 2012; Rasmann et al. 2012; Turlings et al. 2012).

The other determinants which affect the root exudate concentration and composition are age of the plant (De-la-Pena et al. 2010), plant species and/genotype (Reviewed by Berendsen et al. 2012), plant diversity (Philippot et al. 2013), environmental factors like high and/or low temperatures, amount of precipitation, light availability, and agricultural practices being performed in the ecosystem (Flores et al. 1999; Tang et al. 1995; Chaparro et al. 2012; Mendes et al. 2013; Philippot et al. 2013). Moreover, the composition of root exudates and plant microbiome is influenced by soil factors like soil type, texture, pH, moisture level, porosity, and availability of nutrients (Rovira 1969; Girvan et al. 2003; Frey et al. 2004; Fierer and Jackson 2006; Lauber et al. 2008; Faoro et al. 2010; Rousk et al. 2010; Chaparro et al. 2012; Mendes et al. 2013). Various factors involved in the determination of microbial diversity in the rhizosphere are outlined in Fig. 11.1. The interactions between various microorganisms also determine the diversity of microbial communities in the rhizosphere. For instance, different bacteria and fungi act as antagonists against several soil-inhabiting fungal or nematode plant pathogens through different mechanisms (Reviewed by Mendes et al. 2013). These mechanisms include antibiosis (Lugtenberg and Kamilova 2009; Raaijmakers and Mazzola 2012), competition for nutrients (Duffy 2001), ability to parasitize the plant pathogenic microbes (Mela et al. 2011), inhibition of virulence of plant pathogens through quorum sensing (Uroz et al. 2009; Chan et al. 2011), and induction of systemic resistance in the plants (Yang et al. 2009; Pieterse 2012; Schenk et al. 2012). From the last few decades, microbial interactions are well demonstrated for the improvement of agricultural production through well-planned and applied research using the microbes from the rhizosphere for rhizosphere engineering of crop plants. Thus, the generation evolution, importance, functioning, and modification of rhizosphere are hot issues for current research (Berg and Smalla 2009; Jones et al. 2009; Lambers et al. 2009; Hartmann et al. 2009; Dessaux et al. 2010) (Table 11.1).

Table 11.1 Observed effects of plant beneficial bacteria in regard to plant growth promotion

PGPR	Crop	Growth condition	Proposed mechanism(s)	Plant response	References
Bacillus megaterium and *Azotobacter chroococcum*	Wheat	Field	N_2-fixation and IAA production	Bacterization caused 10–20% increase in yield	Brown (1974)
Bacillus pumilus	–	Field	Auxin and siderophore synthesis	*Bacillus* sp. increased plant biomass, root length and total nitrogen and phosphorous contents in plants	Hafeez et al. (2006)
Pseudomonas fluorescens and *P. fluorescens* biotype F	–	Pot and field trials	ACC deaminase activity, Chitinase activity	Inoculation increased fresh and dry weight of roots, increased grain yield and grain weight	Shaharoona et al. (2007)
Burkholderia ambifaria MCI7	Maize	Pot	Siderophore, antifungal activity	Increased shoot fresh weight and overall plant performance as compared to control	Ciccillo et al. (2002)
Pseudomonas and *Enterobacter* spp.	–	Field	ACC-deaminase and phytohormone production	Inoculation increased plant height, biomass, grain yield, and 1000 grain mass of upto 29, 127, 60, and 17%, respectively under stress conditions	Nadeem et al. (2009)
A. brasilense Ab-V5	–	Pot and field	N_2-fixing, IAA production	Overall increase in growth in pot trials. Increase in grain yield in maize under field conditions	Ferreira et al. (2013)
Klebsiella oxytoca Rs-5 strain	Cotton	Pot	ACC-deaminase and auxin production	Strain Rs-5 increased plant height and dry weight of cotton upto 14.9 and 26.9% over control	Yue et al. (2007)

(continued)

Table 11.1 (continued)

PGPR	Crop	Growth condition	Proposed mechanism(s)	Plant response	References
Pseudomonas putida GR12-2	Canola, Lettuce, Tomato	Axenic	ACC-deaminase and ethylene inhibition	Inoculation significantly increased fresh and dry weights of selected crops over uninoculated control	Hall et al. (1996)
Achromobacter piechaudii	Tomato	Pot trial	Indole-3-acetic acid, ethylene	Increased fresh and dry weights of tomato under salt stress	Mayak et al. (2004)
P. putida UW4, *Phyllobacterium brassicacearum* STM196, *R. leguminosarum* bv. *viciae* 128C53K, *Mesorhizobium loti* MAFF303099	*Arabidopsis thaliana*	Axenic	ACC deaminase activity	Inoculation increased length of root hairs, lateral roots, root architecture and plant growth	Contesto et al. (2008)
Pseudomonas sp.	Green gram	axenic	N_2-fixation and biocontrol	Enhanced nodulation and plant growth through suppression of disease	Sindhu et al. (1999)
Bacillus sp. strains OSU-142 and M3	Raspberry	Field	N_2-fixation, P-solubilization	*Bacillus* spp. showed 33.9–74.9% increase in yield, 13.6–15.0% increase in length	Orhan et al. (2006)
Pseudomonas syringae pv.	Cucumber	Field	Biocontrol activity	Disease suppression, improved early growth, number of leaves per plant and overall yield	Wei et al. (1996)
Rhizobium leguminosarum bv. *Viciae*	Pea	Field	N_2-fixation	*Rhizobium* sp. significantly increased in nodule number and N contents	Clayton et al. (2004)
Pseudomonas fluorescens, *P. putida*	–	Pot	ACC-deaminase activity	Inoculation increased fresh-dry weight, root-shoot length and water use efficiency under drought stress	Zahir et al. (2008)

Azospirillum sp. B510	Rice	Field	N_2 fixation and IAA production	Bacterization caused significant increase in shoot length and number of tillers	Bao et al. (2013)
P. fluorescens	Peanut	Pot	ACC-deaminase, IAA, siderophore	Inoculation increased shoot length, pod yield and NP contents	Dey et al. (2004)
Rhizobium spp., *B. subtilis* OSU142, *Bacillus megaterium* M-3	Chickpea	Pot and field	N2-fixation, biocontrol activity, P-solubilization	Inoculation significantly increased nodulation and over all plant growth	Elkoca et al. (2008)
A. brasilense Ab-V5 and Ab-V6	Wheat and maize	Field	N_2 fixation and IAA production	Increased maize and wheat yield upto 27 and 31%, respectively	Hungria et al. (2010)
G. vietnamiensis MG43, *G. diazotrophicus* LMG7603, *H. seropedicae* LMG6513	Sugarcane	Pot and field	N_2 fixation	Inoculation showed upto 20% increase in biomass in field	Govindarajn et al. (2006)
Methylobacterium fujisawaense	*Brassica campestris*	Field	ACC-deaminase activity	PGPR promoted root elongation in canola by virtue of its ACC-deaminase activity	Madhaiyan et al. (2006)
B. amyloliquefaciens sks-bnj 1	Soybean	Pot	Siderophore, IAA, ACC deaminase, antifungal	Inoculation significantly increased nutrient contents in straw and seeds of soybean	Sharma et al. (2013)

11.3 The Good, the bad, and the Ugly Impacts of Microbes on Plants

This section emphasizes on the detail of beneficial microbial communities (the good), plant pathogenic microbes (the bad), and opportunistic human pathogens (the ugly) from the rhizosphere microbiome. The introduction of these the good, the bad, and the ugly has been mentioned in the previous section. Moreover, the mechanisms of growth promotion, nutrient acquisition, and induction of tolerance against biotic and abiotic stresses rendered by beneficial microorganisms along with their implications in agriculture have been given in this section. Similarly, the detail of plant pathogenic microbes from the rhizosphere has been discussed in addition to opportunistic human pathogens.

11.3.1 The Good

11.3.1.1 Growth Promotion Through Nutrient Uptake in Plants

Rhizobacteria are associated with plant growth promotion and hence are called plant growth-promoting rhizobacteria (PGPR) (Kloepper et al. 1980). Growth promotion of plants by rhizosphere-inhabiting bacteria could be the outcome of supplying essential plant nutrients that are scarcely available in the soil including phosphorus, nitrogen, iron, and zinc. The main mechanisms in enhanced nutrient uptake are phosphate solubilization, fixation of nitrogen, zinc solubilization, and iron binding through siderophore production. In addition to these mechanisms, PGPR may also enhance plant growth, by some other mechanisms, i.e., production of phytohormones, e.g., auxins, gibberellins, and cytokinins; ACC deaminase activity; biofilm formation; and exopolysaccharide production. Microorganisms in soil represent a large dynamic community reflecting source and sink of nutrients and play a vital role in cycling of plant nutrients (Cambardella and Eliott 1992; Collins et al. 1992). A diverse array of microorganisms and their role is necessitated to create soils with high fertility through complex cycles and interactions. In fact, the tiny microorganisms are responsible for recycling important nutrients such as N, P, K, Zn, and Fe, hence increasing their bioavailability to plants. PGPR may use more than one of these mechanisms to enhance plant growth and nutrient uptake in plants (Fig. 11.1).

11.3.1.2 Biological Nitrogen Fixation

Nitrogen is a central element in plant growth and development and a key issue of agriculture as well. Accession and N assimilation are the second most important phenomenon after photosynthesis for plant growth and development (Sulieman 2011). For this reason, conventional agricultural practices rely on judicious use of chemical fertilizers for higher crop yields. Unwise use of fertilizers, however, can have negative effects on the environment through vast chemical overflow in waterways (Walker et al. 2012). Legume crops gain benefit due to a symbiotic relationship with rhizobia and can obtain N through biologically fixed nitrogen (BNF).

However, most agriculturally important plants, specifically grasses, do not possess this activity, and hence there has been assisted trend in conveying the potential to fix nitrogen into grasses such as corn, rice, wheat, etc. (Charpentier and Oldroyd 2010).

Biological dinitrogen (N_2) fixation is a natural process of enormous importance in world's agriculture. In the process of BNF, inert atmospheric nitrogen is reduced to ammonia (NH_3) in the presence of nitrogenase enzymes (Newton 2000; Franche et al. 2009) and is a function of diazotrophicus microorganisms, especially bacteria and archaea (Dixon and Kahn 2004).

Nitrogen fixers are generally categorized as (i) symbiotic nitrogen-fixing bacteria which remain in symbiosis with legume plants (e.g., rhizobia) (Zahran 2001; Ahemad and Khan 2012) and nonleguminous trees (e.g., *Frankia*) and (ii) nonsymbiotic (free living, associative, and endophytes) nitrogen fixers such as cyanobacteria (*Anabaena*, *Nostoc*), *Azospirillum*, *Azotobacter*, *Azocarus*, etc. (Bhattacharyya and Jha 2012). Symbiotic nitrogen-fixing bacteria within the *Rhizobiaceae* family develop symbiosis with legume roots. This relationship requires a complex interaction between host and symbiont (Giordano and Hirsch 2004) that would result in the formation of nodules wherein the rhizobia colonize intracellularly.

Microorganisms belonging to different species are used in the cultivation of plants of agronomic interest, smoothing growth of host plant without chemical nitrogen fertilization. In a study in Brazil, soybean (*Glycine max* L.) production was a perfect example of the regulation of BNF using various strains of *Bradyrhizobium* sp. including *B. japonicum* and *B. elkanii* (Alves et al. 2004; Torres et al. 2012). The importance of endophytes in BNF has been the focus of studies in nonlegumes such as sugarcane, wheat, rice, etc. (Thaweenut et al. 2011). Different studies suggested the involvement of *Bradyrhizobia* in colonizing and expressing *nif H* gene not only in the root nodules of leguminous plants but also in the roots of sweet potatoes (*Ipomoea batatas* L.) (Terakado-Tonooka et al. 2008). Garcia de Salamone et al. (1996) described the involvement of *Azospirillum* sp. in BNF influencing the growth of maize plants positively. Several species of genus *Burkholderia* also now reported for fixing of nitrogen. A human pathogen *B. vietnamiensis* also found in colonizing rice roots and fixing N_2 (Govindarajan et al. 2008). In addition to *Burkholderia*, other endophytes of the genus *Bacillus*, *Pantoea*, *Enterobacter*, and *Klebsiella* were also found to be associated with N_2 fixation in different genotypes of maize (Ikeda et al. 2013). Another well-studied endophyte is *Gluconacetobacter diazotrophicus* that has been reported to enhance growth and N nutrition of sugarcane (Baldani et al. 1997; Oliveira et al. 2002; Muthukumarasamy et al. 2005; Bertalan et al. 2009).

11.3.1.3 Phosphorous Solubilization

Phosphorus (P) ranks second in the essential nutrient requirements of plants after nitrogen. Exceptionally, soils serve as a reservoir of total phosphorous (P), but only a small amount is usually bioavailable for plant requirement. This low availability of phosphorus to plants is due to the reason that plants can only absorb P in two

soluble forms, the monovalent ($H_2PO_4^-$) and the bivalent (HPO_4^{-2}) ions, whereas it occurs in soil as insoluble form (Glass 1989).

However, a massive amount of P present in insoluble forms is unavailable for plant needs. Low availability of P is the consequence of highly reactive nature of phosphate with other soluble components of soil (Khan et al. 2009), such as aluminum and iron in acidic soil conditions (pH < 5) and calcium in alkaline soils (pH > 7) (Holford 1997; McLaughlin et al. 2011). Organic fraction and inorganic forms, usually in the form of insoluble minerals, are main sources and sinks of available P in the soil (Rodríguez et al. 2006; Richardson and Simpson 2011).

Since P is an essential macronutrient for plant growth and has only restricted bioavailability. The availability of P is considered to be among the important plant growth-limiting factors (Feng et al. 2004). To fulfill plant's requirements, P is commonly applied to soils in the form of fertilizers manufactured through high energy consumptive processes (Goldstein et al. 1993). However, out of total P applied, plants can use only a little amount (10–25%) of it, since 75–90% of added P is precipitated by mineral complexes and rapidly becomes fixed in soil. Thus, dissolution and mineralization of fixed P by phosphate-solubilizing bacteria (PSB) is one of the most momentous bacterial functions in soil biogeochemical cycles (Jeffries et al. 2003), as well as in growth promotion of plants by PGPB (Rodriguez and Fraga 1999; Richardson 2001).

Numerous groups of phosphate-solubilizing microorganisms (PSMs) are reported to convert the insolubly fixed form of P to soluble form via acidification, production of organic acids or protons (Richardson et al. 2009), and chelation and exchange reactions (Hameeda et al. 2008). Nahas (1996) stated that phosphate solubilization occurs through microbially derived processes including organic acid synthesis and proton extrusion.

Among the most significant PSBs are different bacterial genera including *Azotobacter*, *Azospirillum*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Pantoea*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium*, and *Serratia* (Sturz and Nowak 2000; Sudhakar et al. 2000; Mehnaz and Lazarovits 2006). Ambrosini et al. (2012) reported in a study that different strains of *Burkholderia* related with sunflower plants prevail in alkaline soils solubilizing $Ca_3(PO4)_2$ and making phosphates free for plant use.

Rhizosphere acidification is a characteristic feature of rhizobia associated with solubilization of phosphate (Qin et al. 2011). To confirm the ability of endophytes as P solubilizers, Chen et al. (2014) in another study confirmed that the endophytic *Pantoea dispersa*, pre-isolated from cassava (*Manihot esculenta* C.) roots, was excellent in solubilizing $Ca_3(PO4)_2$, $FePO_4$, and $AlPO_4$, due to the production of salicylate and benzene acetic and other organic acids. Similarly, in another case, Singh et al. (2014) reported that the bacterial isolates *Advenella* are engaged in production of phytase and enhanced growth of Indian mustard (*Brassica juncea*) through increased P availability. In a similar study, Kumar et al. (2013) suggested production of phytases by bacteria belonging to *Tetrathiobacter* and *Bacillus* genera that also improved the growth of Indian mustard and significantly

aided in the plant P content. In a related study, Idriss et al. (2002) recorded that extracellularly produced phytase by *B. amyloliquefaciens* FZB45 stimulated the growth of maize seedlings.

11.3.1.4 Siderophore Production

Plants and microorganisms essentially require iron (Fe) because it is involved in several key biological processes, such as photosynthesis, respiration, synthesis of chlorophyll (Kobayashi and Nishizawa 2012), and BNF (Dixon and Kahn 2004). Iron fluctuates in different oxidation states under aerobic and anaerobic conditions. In anaerobic and flooded soils, high contents of ferrous (Fe^{+2}) resulting from the ferric (Fe^{+3}) ion reduction may lead to toxicity due to increased Fe uptake (Stein et al. 2009). While under well-aerated (aerobic) soils, Fe solubilize slowly, due to which the major prevailing form is Fe^{+3} and is likely to form insoluble hydroxides and oxy-hydroxides, thereby making Fe generally inaccessible for plants and other life forms, especially in conditions of calcareousness (Andrews et al. 2003; Lemanceau et al. 2009). To survive within a limited supply of Fe, bacteria make use of Fe-chelating agents called siderophores. Siderophores may be defined as low molecular mass compounds (< 1000 Da) with a great affinity for Fe^{+3} chelation, followed by the shift and accumulation of Fe within the cells of bacteria (Neilands 1995; Krewulak and Vogel 2008).

It is suggested by various studies that different bacterial strains associated with plants largely produce siderophores. The siderophore production by bacteria may show plant growth-promoting effects through improved Fe nutrition or hampering the development of plant pathogens through Fe chelation from the environment. Different microbial pathogens and target plants remain unaffected by bacterially mediated Fe exhaustion, and even some plants can gain and use Fe^{+3} siderophore bacterial complexes (Dimkpa et al. 2009).

Bacterial strains of *Enterobacter* and *Burkholderia* genera produced the highest amounts of siderophores in the roots of rice (Souza et al. 2013). The possible role of siderophores in plant nutrition is further assisted by the absence of Fe on deficiency symptoms (chlorosis) and by the significantly high Fe contents in roots of plants grown in non-sterile soils compared with plants grown in sterile soils (Masalha et al. 2000). In another study, Sharma et al. (2003) reported that inoculation of mung bean (*Vigna radiata* L. Wilczek) with the siderophore-producing *Pseudomonas* strain GRP3 enhanced growth, chlorophyll contents, and reduced chlorosis under Fe-restricted conditions compared with uninoculated control.

11.3.2 Growth Promotion and Plant Tolerance Against Abiotic Stresses

Plant growth is restricted by a number of abiotic stress factors including soil salinity, drought, temperature, and heavy metal contaminations. Crop growth and microbial activity is severely affected by edaphic stresses. Most of the soils in developing countries are predisposed to soil-related constraints which are another cause of

reduction in crop yields (Lal 2000). Various approaches, i.e., breeding, transgenic and agronomic practices, have been recommended to reduce the effects of climatic and soil-related constraints in crop production, but a potent and less explored option is the use of microbes in alleviating abiotic stresses. These microbes are being used to an impartial extent in alleviating the soil-related stresses, especially of heavy metals (Glick 2010). There is still a great potential to exploit these agriculturally important microbes to manage climatic and soil-related abiotic stresses.

Bacteria help plants to get rid of the ill effects of abiotic stresses through specialized functional mechanisms. Some key mechanisms in alleviating these stresses are the production of ACC deaminase and lowering of ethylene levels, induction of systemic resistance, biofilm formation and aggregation and exopolysaccharide production.

11.3.2.1 Drought Stress

Drought stress mitigation by using potential microbes has been receiving much more attention in recent times. ACC deaminase is an enzyme that catalyzes the breakdown of ACC into ammonia and ∞-ketobutyrate, hence hindering production of ethylene. Mayak et al. (a, b) recorded that the ACC deaminase production by a PGPR, *Achromobacter piechaudii* ARV8 greatly increased the seedlings fresh and dry weights of both tomato and pepper by lowering the endogenous level of ethylene production under mild drought stress. In a study by Dodd et al. (2005), bacteriozation of pea plants with *Variovorax paradoxus* 5C-2-produced ACC deaminase significantly increased seed yield, number of seeds, and leaf nitrogen contents under long-term drying condition compared to uninoculated control. Arshad et al. (2008) in two separate (pot and field) trials confirmed that the application of ACC deaminase-producing bacteria significantly increased the growth parameters and yield of pea under drought stress. Naveed et al. (2014) reported increased drought stress resilience in maize through endophytic colonization of *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17.

11.3.2.2 Temperature Stress

In nature plants are sensitive to diurnal and seasonal variations in temperature and respond to these variations accordingly. Temperature fluctuations in plants led to significant hormonal imbalances, thus severely effecting growth of plants. The capacity of some fungal agents in conferring temperature resistance is well documented (Mc Lellan et al. 2007), but we will focus our considerations on the role of bacteria in heat resistance only. Srivastava et al. (2008) work with a thermos-tolerant *P. putida* strain NBR10987 isolated from water-limited rhizosphere of chickpea. He reported that this thermotolerance was attributed to the biofilm formation that ameliorated heat stress. Similarly, Ali et al. (2009) suggested thermotolerance of strain *Pseudomonas* AKM-P6 to ameliorate the heat stress in seedlings of sorghum. Inoculation with *Pseudomonas* AKM-P6 results in accumulation of enhanced levels of proline, chlorophyll, sugars, amino acids, and increased levels of high molecular weight proteins in leaves under high temperature. The studies by Barka et al. (2006) confirmed role of microbes in cold tolerance. They reported that in vitro inoculation

with a PGPR, strain PsJN (*Burkholderia phytofirmans*) to grapes (*Vitis vinifera cv*. chardonnay) increased root growth of grape vine and biomass production and physiological activity at a low temperature. This increment in growth and physiological activity is related to the accumulation of starch, proline, and phenolics in excess in inoculated plants. In a recent study, a ACC deaminase-producing *P. putida* UW4 accelerated canola plant growth at low temperature exposed to salt stress (Cheng et al. 2007).

11.3.2.3 Salinity Stress

Soil salinity is a notorious issue for agricultural production in irrigated areas. In arid and semiarid regions of the world, the soils are mostly saline with low agricultural potential. Under such conditions, it is the prerequisite to invigorate germination and augment growth of seedlings (Lambers 2003). Although significant attention has been given to the genetically engineered plants to be more tolerant to salinity (Apse et al. 1999), another alternative and most adequate option is to use bacterial inoculants that are more tolerant to high levels of salts, equipped with certain mechanisms such as production of auxins and gibberellins, and promote plant growth in saline soils (Mayak et al. 2004b).

Due to their phosphate-solubilizing activity, PGPR may improve phosphorous availability upon inoculation to plants and hence improve growth rate (Giri and Mukerji 2004). Bacteria showing ACC deaminase activity can also be employed in ameliorating the harmful effects of salinity by reducing the endogenous ethylene levels (Glick et al. 1998).

11.4 Growth Promotion and Antagonism to Plant Pathogens

Rhizobacteria promote plant growth both by indirect and direct mechanisms. Growth promotion by direct mechanisms occur when these bacteria provide the plants with substances synthesized by the bacterium itself or enhancing the uptake of plant nutrients from the soil environment. Indirect mechanisms of growth promotion include prevention of some detrimental effects by a plant pathogenic organism (Glick 1995). Some of the mechanisms that potentiate their use as biocontrol agents are synthesis of phytohormones such as auxin and gibberellins; synthesis of antibiotic metabolties; hydrogen cyanide (HCN) production that show antifungal activity; secretion of iron-binding siderophores; production of chitinase, lipase, and protease enzymes which can hydrolyze the fungal cell walls (Chet and Inbar 1994); and production of oxidative stress enzymes such as catalase, peroxidase, super oxide dismutase, and polyphenol oxidases for combating reactive oxygen species. Plant growth benefits upon the application of PGPR. For example, in a study by Kloepper et al. (1980), the inoculation of potato seeds with PGPR increased yield and growth under field conditions. Van Peer and Schippers (1989) reported the improved root and shoot fresh weight of tomato, cucumber, lettuce, and potato as a consequence of inoculation with *Pseudomonas* strains. Siderophores sequester iron from the

rhizosphere and render the pathogen deprived of iron availability which is required by their growth and pathogenicity. Production of large range of iron-chelating compounds such as siderophores is a significant trait by some fluorescent pseudomonads.

A work by Kloepper et al. (1980) suggested the role of siderophores in the biocontrol of plant pathogens and in plant growth promotion. Abiotic edaphic factors such as bioavailability of iron and pH are believed to be the governing factors of siderophore production and their involvement in biocontrol (Loper and Buyer 1991). Recently, Loaces et al. (2010) determined the synthesis of siderophores by endophytic bacteria in rice plants (*Oryza sativa*), reflecting the presence of an isolate of *Pseudomonas* showing antibacterial activity.

Many bacteria especially members of the *Pseudomonas* genus have the ability to produce certain types of antibiotics. Howell and Stipanovic (1979a, b) were among the pioneers who evoked interest in antibiotic-producing strains and their effect on suppression of phytopathogens. Several different types of antibiotics produced by *Pseudomonas* species with antimicrobial activity, such as pyoluteorin (Plt), phenazine-1-carboxylic acid (PCA), 2,4-diacetylphloroglucinol (DAPG), pyrrolnitrin (Prn), hydrogen cyanide (HCN), pyoluteorin (Plt), and protein-type compounds (bacteriocins) (Voisard et al. 1989; Weller et al. 2002; Haas and Keel 2003; Validov et al. 2005), and their effect on pathogen suppression are well documented.

HCN is a secondary metabolite produced by many rhizosphere Pseudomonas that negatively affects root metabolism and root growth (Bakker and Schippers 1987; Lambers, 1980). Such (HCN) producing rhizobacteria depict a vast range of effects on plant growth from beneficial to harmful (Defago et al. 1990).

11.5 The Bad

11.5.1 Plant Parasitic Fungi

Soil is diverse medium which gives an action place of many microbial communities. Soil supports the growth of plants with supply of important inputs of water and nutrients in a broader scenario. Along with plants, soil is also living platform for many microbial communities. The presence of particular microbial type depends on the microenvironment of the soil. It depends on the factors like temperature, availability of oxygen, precipitation, pore size of the soil particles, presence of organic matter in the soil, pH of the soil, and many others. The microbial communities evolve in particular soil in long span of time. Microbial communities are important in the soil because they ensure availability of proper nutrition to the crop plants along with their own struggle of survival. But all of the microbes are not beneficial for growth-promoting crop plants. as some of them are notorious pathogens and result in serious reduction of yield in crop plants. Pathogenic bacteria, fungi, and nematodes are considered the worst inhabitants of rhizosphere. Among all of these, fungi are considered the most devastating soilborne plant

pathogens. Soilborne pathogens are usually evolved under hard conditions and are well adapted to rhizosphere as compared to other microbes. During the process of evolution, they have devised the ways to have hard structures i.e. resting spores, which help them to stay for a long time without host crop. Control of these pathogens through chemicals which is considered as the most applied and result-oriented pathogen management plan is not easy to execute because of the sparse distribution of inoculum of soilborne pathogens specially fungi. Additionally, alternative hosts in the form of weeds (Malcolm et al., 2013) also help to increase the prevalence of fungal inoculum in the soil. Such natural promotions of fungi in the soil to make them diverse pathogens are adverse for the crop plants. There are many fungal species which causes diseases or root rot, collar rot, wilt, seedling blight and damping off, etc., but regarding overall economics of field, the worst genera are *Pythium* (Verbeek et al., 2016), *Fusarium* (Bentley et al., 2006), *Verticillium* (Xu et al., 2010), *Rhizoctonia* (Lehtonen et al., 2008) and *Armillaria* (Cox et al., 2006).

Root rot diseases caused by soilborne fungi result in the decay of roots. Usually older roots are stiff and stronger. The pathogen targets juvenile roots in case of rot diseases. This results into the symptoms of wilting of the plant, falling of leaf, and death of branches in case of perennial crops. The effective control depends on estimation of root rot causing fungal types in the field, survival chances of pathogen, and dissemination procedure of the pathogen in the form of secondary infection. These could be managed to some degree with the help of cultural control measures by exposing masses of soil to sunlight after plowing and eradication of alternative hosts. This disease is mainly caused by *Pythium*, *Verticillium*, and or *Rhizoctonia* species of soilborne pathogenic fungi.

Fusarium is considered the most damaging pathogen for many crop plants. *Fusarium* attack results in low production. Chickpea wilt, pepper wilt, root rot, and wilt of cotton are the main diseases caused by *Fusarium* in single or in the form of complex with other pathogens. The appearance of symptoms of this pathogen is almost the same on its various host plants (Saremi et al., 2007). *Fusarium* is also involved in the production of mycotoxin which is very potent against animal and human health (Summerell et al., 2001).

Fusarium is also active in tuber crop like potato which is rich starch storage. It infects the fresh potatoes up to 60 percent under storage conditions. When the fungus pustules which are actually mycelia attack on the soft surface of potato, the tubers become hard, and it seems as mummified (Wharton et al. 2006). Optimized *Fusarium* growth which takes place at 21–25°C is coupled with high humidity. Under field conditions, the inoculum is available in the soil, while in case of tubers, it comes through wounds during harvesting, storage, or transportation (Voss et al. 1996).

These are just few examples. Other targets of *Fusarium* are pulses, flowers, wheat, and tree plants. The wilt diseases caused by this pathogen do not give much time to the plant for recovering. So under favorable environmental conditions, the plant growth is hijacked at seedling stage which results to epidemic in the seedling stage.

11.5.2 Plant Parasitic Nematodes

The diverse plant rhizosphere microbiomes also include several nematode species parasitic to the plants. Although majority of nematodes in the soil are free living, however, 7% of the total soil-dwelling nematodes cause diseases in different plant species. The nematodes belonging to phylum Nematoda have huge variation in their morphology and feeding habits. This is why the phylum Nematoda is the second most diverse phylum in the kingdom Animalia after Arthropoda. This phylum includes parasites of plants and animals as well as many free-living species. Recently, Decraemer and Hunt (2006) defined in total 4300 species (within 197 genera) of nematodes as plant parasites which can infect a broad range of crop species such as wheat, soybean, potato, tomato, and sugar beet as important examples. Nematode infection can result in different aboveground symptoms in plants such as leaf chlorosis and patchy, wilting, stunted growth, and susceptibility to other pathogens (Webster 1995). Many nematodes are obligate biotrophic parasites of plants which have a detrimental effect on agricultural production by direct damage to crops or serving as vectors for plant-invading viruses, thereby resulting in crucial economic and social impacts worldwide. Several economically important species parasitize various crop plants. Recently, top ten important plant parasitic nematodes have been listed in ascending order based on their economic importance (Jones et al. 2013). In these ten nematodes, root-knot nematodes (RKNs) and cyst nematodes (CNs) within the family *Heteroderidae* are listed at the top because they are especially dangerous and having a wide range of host plants (Jones et al. 2013). RKNs and CNs are the most destructive plant parasitic nematodes (Ali et al. 2015). They are obligate sedentary endoparasites of host plant roots and enter the plant roots as second-stage juvenile larva (J2 larva) to establish specific feeding structures (Hussey and Grundler 1998). RKNs of the genus *Meloidogyne* stimulate specialized feeding sites containing several giant cells (Jones and Payne 1978). Cyst nematodes, from the genera *Heterodera* and *Globodera*, induce specialized feeding cells called syncytia (Hussey and Grundler 1998). Another economically important category of nematodes is the migratory endoparasitic nematodes. These nematodes spend most of their time in migrating through root tissues following the destructive feeding on plant cells (Moens and Perry 2009). This results in massive plant tissue necrosis. The important examples of migratory nematodes are the genera of lesion nematode (*Pratylenchus*), burrowing nematodes (*Radopholus*), and rice root nematode (*Hirschmanniella*).

The crop losses caused by plant parasitic nematodes are enormous despite of the under-reporting and incomprehensive field data (Sasser and Freckman 1987; Chitwood 2003). It has been reported that plant parasitic nematodes cause serious reduction in crop yields resulting in annual crop losses worth over $150 billion worldwide (Abad et al. 2008). The yield losses in potato caused by potato cyst nematodes (PCNs) are reported to be over 9% of total production globally (Jones et al. 2013). Similarly, *Meloidogyne graminicola* is the most important nematode species infecting rice and causes up to 87% yield losses in well adapted to flooded conditions (Padgham et al. 2004; Soriano et al. 2000; Jabbar et al. 2015). Altogether, plant parasitic nematodes can cause up to 20% yield losses in individual crops

which can be devastating for low-income farmers in the developing countries (Atkinson et al. 1995).

The plant root exudates are very important mediators of the population of the parasitic nematodes in the plant rhizosphere. In fact the roots are the storage site for various metabolites and nutrients. These roots can be a shelter and hub of ecological and chemically mediated interactions for soil-dwelling organisms like nematodes (Rasmann et al. 2012). During the course of parasitism, the nematodes hatch from eggs as J2 larvae due to some chemical stimuli from the soil or from the root exudates of the plant and move toward the host plant root. There is a variety of chemical compounds that have been identified as nematode attractants. Most of them are involved in the attraction of nematodes toward plants, insects, and bacteria. Many of them are odor sources like atmospheric carbon dioxide gas (CO_2), alcohols, ketones, organic acids, terpenoids, thiazoles/pyrazidines, cyclic adenosine monophosphate (cAMP), esters, ions, amines, amino acids, and other aromatic compounds (Reviewed by Rasmann et al. 2012). However, the chemical compounds like carbon dioxide gas (CO_2), terpenoids, cyclic adenosine monophosphate (cAMP), ions, and amino acids are important for nematode chemotaxis toward plant roots during compatible plant-nematode interactions. In addition to these chemical compounds, light, electricity, and mechanical and temperature fluctuation affect the aboveground parts on the plants to play their role in the chemotaxis of nematode and other microorganisms toward plants. A graphical sketch of nematode chemotaxis in plant is given in Fig. 11.2.

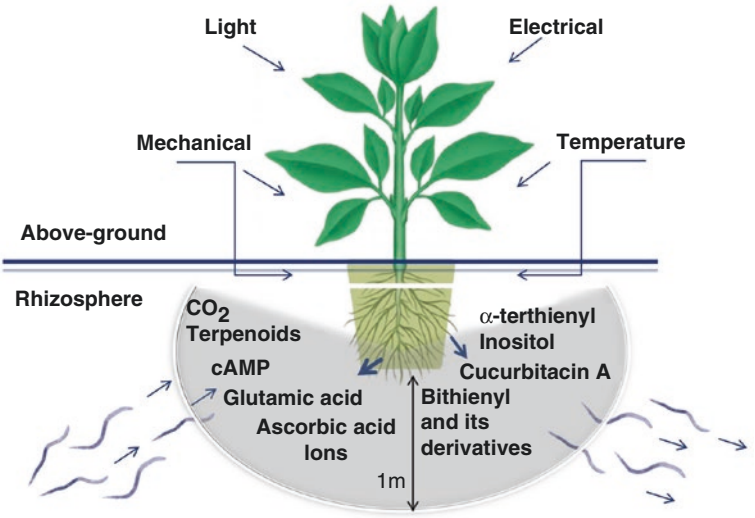

Fig. 11.2 Modulation of nematode populations by the root exudates: chemotaxis of nematodes from rhizosphere toward plant roots to commence infection process. CO_2, terpenoids, cAMPs, ions, and amino acids are the main chemical compounds involved in the nematode chemotaxis. Similarly, some nematode repellent compounds released by the roots are involved in inhibiting the nematode populations in the rhizosphere

Plant roots release several compounds in its rhizosphere to stimulate beneficial microbial communities for the enhancement and recycling of organic matter (Luscher et al. 2004; Chapman et al. 2006). However, on the other side, the plant roots secrete natural compounds which act as antagonists against soil-dwelling plant parasitic nematodes. For example, the roots of two species of marigolds (*Tagetes patula* and *T. erecta*) release bithienyl and its derivatives to minimize the populations of root-knot (*Meloidogyne* spp.) and lesion nematodes (*Pratylenchus* spp.) (Giebel, 1982). Similarly, plant roots emit chemical compounds which perform as repellents to the nematode species invading in the plant rhizosphere up to 1 m diameter of around the roots (Johnson & Nielsen, 2012; Rasmann et al. 2012; Turlings et al. 2012) (see Fig. 11.2). Moreover, the roots of plants engineered and/or having natural resistance against parasitic nematodes tend to release anti-nematodal proteins like resistance proteins, proteinase inhibitors, lectins, Bt toxins, and chemosensory disruptive peptide which repel and/or diminish the nematode populations from the plant rhizosphere (reviewed by Fuller et al. 2008, Ali et al. 2017). Thus the root exudates are the key players which modulate the population of plant parasitic nematodes in the rhizosphere vicinity.

11.6 The Ugly

11.6.1 Opportunistic Human Pathogens in the Rhizosphere

Soil is the main component in plant life which provides the basic elements for its growth and habitat for propagation and survival. Actually soil is complex of inorganic minerals and organic constituents present in solid, liquid, and gaseous states. The inorganic or mineral part of soil has an origin in rocks which is formed by the process of weathering or wear and tear of these solid stuffs. The organic part is also known as humus. Soil particles which could be sand, silt, or clay are formed by the weathering process. Their varying sizes in different soil types pack them with some pores. These pores and surface of the particles are the habituating place of soil microbial communities. Usual soil microbial communities also harbor many human pathogenic microbes, the ugly ones. These human pathogens are either indigenous to the soil and very few in number are deposited in the soil by human activities, i.e., brought to the soil via animals and bird feces, application of manures, farm machines, manure application, slaughter waste, sewerage water, and medical waste. The remains of animal dead bodies also are rich source for the colonization of saprophytic fungi which could invade humans and can cause diseases.

Most of the soil pathogenic fungi are saprophytic in nature and use the organic matter present in the soil (Pelayo Ulacia and Dafhnis 1980). The human diseases caused by pathogenic fungi are very few and have more vulnerability in people who have compromised immunity (Low and Rotstein 2011). These pathogenic fungi usually target people with little deficient immune systems and colonize more rapidly and usually respond little to antibiotic medications.

The fungal diseases in humans are a prime study of dermatology as most of them are superficial having effects on the skin, hair, nails, etc. These infections are mostly caused by *Microsporum canis*, while the athlete's foot disease which also infects human through soil is caused by fungi like *Trichophyton mentagrophytes* and *Trichophyton rubrum*. But few diseases of fungi (e.g., caused by *Aspergillus* spp.) release toxins that are food contaminants and are lethal at very low concentrations.

Extreme environmental conditions of both biotic and abiotic nature made the fungi fit to infect humans. The entry of pathogenic fungi into human body may be either directly or through wounds. Rhizosphere microbes, especially the fungal spores, are introduced into human respiratory tract via bioaerosols in the form of fine particles of dust and mud when the soil is disturbed during agricultural practices. Contaminated food is also a mode of direct entry of microbial communities into the human body (Bennet 2009; Cooney and Klein 2008). Similarly, many factors like geographic distribution of soil inhibiting human pathogenic fungi, local strain and virulence factors, active or passive nature of fungi, and most importantly the susceptibility of the host for successful invasions of these fungi are involved in the spread of fungal pathogens into the human beings (Baptista-Rosas et al. 2007).

Most of the fungal infections in human are not apparent but could be seen after lab testing of human samples. For example, *Coccidioides* fungus is dimorphic with mycelia and spherules associated with alkaline soils at extreme temperature conditions (Williams et al. 1979; Werner et al. 1972). After rain, high temperature of the soil promotes growth of the fungus and formation of arthroconidia which are sources of its dispersal and propagation. These arthroconidia are inhaled by human and result in pulmonary infections (Schneider et al. 1997; Baptista-Rosas et al. 2007). The epidemics of a disease occur when at the end of spore formation, there is some dispersal mechanism like dust storm, earthquake, excavation etc. (Sharpton et al., 2009; Flynn et al. 1979).

As compared to bacteria, fungi are complex organism and more resistant to chemicals because once they invade the human body, they become difficult and complicated to be eradicated. The reason behind this could be their existence as eukaryotic like human cells, so a lot of care and differential chemicals are required to target them by not giving any harm to human cells. In case of bacterial infections, the antibiotics are still very effective to combat most of the infections because of vulnerability of antibiotics selectively to bacteria and with least harms to human cells.

In pulmonary infections, pulmonary blastomycosis is an important disease caused by *Blastomyces dermatitidis*. This fungus is present in soil and colonizes the human respiratory system. Its infection might be mild or severe depending upon the immunity level of the patient. It leads to pneumonia which results in acute respiratory syndrome or cavitary lung disease (Chapman 2005; Baumgardner et al. 2011).

There are many pathogenic bacteria of humans which are originated from plant rhizosphere. The important are *Clostridium tetani* and *C. botulinum* which causes diseases of tetanus and botulism in human. These two bacteria are toxin producing and attack on the muscles of the body. Other symptoms include contraction of skeletal muscles, stiff neck, and adnominal rigidity. In severe cases the bones become

fragile and brittle and fracture on minute stresses (Haagsma 1991). The *C. tetani* is present in soil and also along the dust particles. The spores of these bacteria are very resistant and persist in the soil for several years. After invasion into the human body, they are brought to soil again through feces. They are mostly present in the soils of common activities, and their spread is faster during natural hazards like earthquakes. *Clostridium botulinum* is a distinct group of bacteria which produces seven types of toxins having varying degree of toxicity. Type F toxins are capable of causing disease of botulism in humans, while other toxins are specific to mammals, birds, and fish. Infections to humans occur through eating of contaminated food or they can enter into the body through wounds. Extreme cooking like at 85 °C can kill the bacteria in food (Afshar et al. 2011).

Anthrax is caused by *Bacillus anthracis*, which are gram-positive bacteria. It comes to grazing animals through soil. It is important disease of wildlife and livestock. The infections to humans are secondary in nature. The bacterial spores enter into the human body on handling wool as inhales and meat as touch. Also tillage of soil, contaminated with bacterium, exposes humans to masses of spores, and risk of primary infections increases (Blackburn et al. 2007; Van Ness 1971).

Actinomycosis is one of the important infectious diseases present in human caused by *Actinomyces israelii*, a soil origin species of bacterium found in dead organic matter. This is a group of saprophytic bacteria that are capable of infecting plants, animals, and even humans. They colonize naturally in the human gut, mouth, and vagina without any infectious disease symptoms, while very few cause disease known as actinomycosis (Roque et al. 2010). This disease involves infection of chronic nature and usually attacks the face, neck, lungs, mouth, and intestines.

Escherichia coli are widely used bacteria in the recombinant DNA technology labs and also present in human gut. All of the types of *E. coli* are not harmless. Few types of *E. coli* like enterotoxigenic bacteria cause diarrhea in infants (Guyer et al. 2000). The bacteria annually result in the millions of cases of diarrhea and thousands of deaths. *E. coli* survive in the soil for 2–3 weeks depending on the moisture contents of the soil, while the survival of pathogenic strains is for 2 months. The manipulation and operations in the soil could lead to the introduction into humans. The survival of bacteria in soil is ensured with higher moisture contents (Chandler and Craven 1980; Avery et al. 2004).

Salmonellosis disease is caused by *Salmonella* spp. the soil-dwelling bacteria which are gram negative in nature and motile and have multiple serotypes. Its presence in rhizosphere contaminates the vegetables, and then it is carried to the human body (Islam et al. 2004). The *Salmonella* has a wide range of host organisms (Grassl and Finlay 2008). The bacteria come to soil through liquid manures applied at agricultural lands (Bech et al. 2010), and the pathogenic types may persist in the soil for up to 8 months. In human it causes typhoid fever and focal diseases (Klotchko and Wallace 2011). Similarly, *Clostridium perfringens* is very common soil-inhabiting bacteria which are actually bacilli, anaerobic, and sporeforming with gram-positive staining properties (Matches et al. 1974). It causes gas gangrene disease after entry into the human body through wounds. In deeper wounds where the oxygen concentration is low, this bacterium grows faster. After surgery or trauma, the bacterial

spores get entry from soil and results in severe pain which is not pain of postsurgery period but due to this pathogenic growth and toxin productions which results in delayed healing of wound and pus formation (Revis 2008). Furthermore, *Listeria monocytogenes*, a gram-negative bacterium, gets entry into the human body through contaminated food and causes disease of listeriosis in immune-compromised patients and infants. This pathogenic bacterium stays longer in the soil having high moisture contents; usually 6- to 8-month persistency is reported in case of this bacterium if the moisture contents of the soil are high. It is also common in the soils which are near to water reservoirs (Weinstein 2011). Symptoms of listeriosis include vomiting and attack on the nervous system.

Another widely distributed soil species of human pathogenic bacteria is *Pseudomonas aeruginosa* which is also present in plants and humans. It is involved in the blood infection, heart infection, and gastrointestinal and urinary tract infections. The central nervous system is highly affected in case of higher density of this bacterium in the body during disease period (Hirulkar and Soni 2011). This bacterium is resistant to the most of the existing antibiotics.

11.7 Rhizosphere Engineering for Crop Improvement

Plants are the key determinants of microbial communities in the rhizosphere. Plants have developed a variety of functions and strategies for modification of rhizosphere to avoid environment-related stresses. The comprehension of the actions involved will propose the techniques in which the rhizosphere can be modified (engineered) for better plant health and soil productivity (Ryan et al. 2009). Some of the techniques and models of rhizosphere engineering are discussed under.

11.7.1 Models of Rhizosphere Engineering

11.7.1.1 Engineering Plants for Root Exudates

One of the important mechanisms in which the plants can modify rhizosphere is the generic term called as rhizodeposition in which plant roots secret some compounds of organic and inorganic nature (Ryan et al. 2009). Actively growing plants release these root exudates in their rhizosphere which are of significant importance and are determinants of plant-microbe interactions (Badri and Vivanco 2009). These exudates comprise of amino acids, proteins, carbohydrates, and fatty acids (Badri and Vivanco 2009) which regulate growth of plants and suitable microbial components of the rhizosphere (Bais et al. 2006; Rasmann et al. 2012). Composition of the root exudates differs mainly depending on the type of plant species and cultivar within a given species (Kowalchuk et al. 2002; Högberg et al. 2006), due to which rhizospheric microbial community may also vary accordingly (Grayston et al. 1998; Kuklinsky-Sobral et al. 2004; Salles et al. 2004). This variation in root exudate chemistry and composition among different species of plants and even within different genotypes of the same species propose the ways in which

these exudates can be manipulated for agricultural cultivars in order to create species-specific effects on rhizospheric microbiome. Several examples are available on root exudate modification, for example, plants contribute to acidification of rhizosphere through uptake of essential nutrients generating electrochemical gradient potential across plasma membrane of root cells that is resulted from efflux of H^+. This acidification can enhance plant's access to Fe^{+3} and P which are fixed with soil matrix otherwise (Hinsinger et al. 2003). Iron uptake in some monocots and dicots is another example which involves the release of organic acids that results in the reduction of Fe^{+3} into Fe^{2+} before uptake which sometimes consequences in the production of phytosiderophore (Neumann and Römheld 2007). These phytosiderophores are mainly produced early in the morning (Takagi et al. 1984; Ma and Nomoto 1994) and are released only from the root tips (Marschner et al. 1987). Release of organic anions like citrate and malate as well as phytases and phosphatases enzymes plays a crucial role in P availability in some species in the same manner (Richardson et al. 2001; Ryan et al. 2001; Vance et al. 2003). Thus, the growth of the neighboring plants may be facilitated through phytosiderophore production, and this understanding can be applied in intercropping Fe-efficient crops with calciphobe crops, i.e., maize (*Zea mays*) with peanuts (*Arachis hypogaea*) (Zhao et al. 2000). The organic anions release processes which are significant to plant growth and nutritional requirements. For instance, the organic anion efflux protects some crops from the damaging effects of Aluminium (Al) toxicity with root apices through chelating Al^{3+} ions (Ma et al. 2001; Ryan et al. 2001). Many of the genes that regulate the release of exudates have now been recognized, and it is the need of the hour to modify the rhizosphere by altering the gene expression patterns through genetic engineering. In agricultural production systems, the plant's genotypic behavior is a controlled mechanism through plant breeding and selection of a suitable cultivar. In the current scenario, rhizosphere engineering has a tremendous potential to facilitate agricultural productivity (Oger et al. 2004; Ryan et al. 2009).

Manipulation of H^+ and organic anion efflux (manipulation of rhizospheric pH) from roots of transgenic plants is the second possibility to engineer the rhizosphere. Different studies on increasing H^+ efflux have been reported till now. For example, a study where *Nicotiana tabacum* and *Arabidopsis* were transplanted with cDNA that encodes for H^+-ATPase genes (Gévaudant et al. 2007; Young et al. 1998; Zhao et al. 2000) showed increased H^+ efflux, i.e., more acidic conditions in the rhizosphere (Gévaudant et al. 2007; Yang et al. 2007), and improved growth was observed in transgenic (having foreign DNA) lines (Young et al. 1998).

Engineering the plants having greater capability to produce organic anions and engineering plants that show greater capacity for transporting organic anion out of the plant cell are two solutions to increase organic anion efflux (Ryan et al. 2009). The first study on organic anion efflux was carried out by de la Fuente et al. (1997). They reported that the citrate efflux from tobacco roots was enhanced when a citrate synthase gene from *Pseudomonas aeruginosa* was transformed in tobacco plants. The citrate synthase activity was enhanced in transgenic cultivars by threefold which is associated with three- to tenfold increase in citrate concentrations of root

tissue and fourfold increment in anion efflux activity in sterile water suspension. Similarly, in another study by Koyama et al. (1999), transplanted mitochondrial citrate synthase gene was transformed from *Arabidopsis* into carrot (*Daucus carota*), and the results showed fourfold higher citrate efflux.

11.7.1.2 Microbiome Manipulation

A more feasible way to engineer the rhizosphere is to manipulate the rhizospheric microbiome directly. Inoculation of the microbes is a right choice for such an approach. However, there exist some new approaches that would enhance the efficiency and persistence of the introduced microbe into the soil (Bakker et al. 2012). Consciously disturbing the soil before the application of beneficial plant growth-promoting rhizobacteria (Fliessbach et al. 2009), mycorrhizal fungi (Gosling et al. 2006), and other suitable microbes in compost or bioformulations (Pérez-Piqueres et al. 2006) may increase the adaptability and success of introduced novel microbe. The disturbance may be in the form of tillage, pesticide application, or cropping rotation.

Addition of the beneficial microorganisms to those that are native to the environment can have the potential to increase nutrient uptake by the plants (Kirankumar et al. 2008), boost growth of the plants (Cummings 2009; Guiñazú et al. 2009), and increase resistance to biotic and abiotic stresses (Selvakumar et al. 2012). PGPR colonization efficiency is effective in fitting the vacant niches in the soil; thus, microbial inoculations are more likely to be successful in soils of low microbial activity (Fliessbach et al. 2009).

An innovative technique for microbiome modification is to apply fungus and bacteria in consortium. In such conditions consortia of microbes might be helpful in lessening the time period for niche saturation and pathogen exclusion from the environment (Bakker et al. 2012). We have recently shown that consortium application of *Bacillus* spp. with *Trichoderma* spp. enhances plant growth promotion and grain yield along with higher ability of the plants to sustain moisture level in the plants (Din et al. Unpublished data).

Hybrid models including mechanisms such as improvement of both the plants and microbial traits is another attractive option for microbial engineering in the rhizosphere. Several novel approaches are in consideration nowadays to produce plants in association with particular microbes which produce different carbon compounds in the rhizosphere (Savka et al. 2002).

Aggressive colonization and competition among microbes in the root-soil interface often shares a common function, i.e., they are regulated. Different regulatory mechanisms have been identified. One best known mechanism is the ability of the microbes to sense signals called as “quorum sensing” (Savka et al. 2002). Several important mechanisms of microbes which are regulated by quorum sensing include formation of biofilm, pathogenicity, ability to swarm, transfer of plasmid, and uptake of iron (Fuqua et al. 2001; Miller and Bassler 2001; Whitehead et al. 2001). Rhizosphere engineering in terms of regulated mechanisms of microbial origin could be employed in biocontrol through interruption in quorum sensing (Rice et al. 1999; Robson et al. 1997).

11.7.1.3 Soil Amendment for Rhizosphere Engineering

Rhizosphere modification is often an ignored aspect of human activity. Developing new approaches to study microbial behavior and microbial ecology has paved the way for researchers toward the modification of rhizosphere (rhizosphere engineering) (Ryan et al. 2009). Many different species of the plants produce alkaloid calystegyns (Asano et al. 1994, 1996), and in parallel many rhizospheric bacteria, e.g., *Sinorhizobium* sp., also have the ability to degrade the produced calystegyns in the rhizosphere (Tepfer et al. 1988). Classical experiments using extrinsic substances as soil amendments with potent microbes have been well illustrated. The prior experiments were based on a molecule having a dual role both working as an antibacterial agent and a presumptive carbon source. Sodium salicylate is one of the selective molecules. Colbert et al. (1993a, b) in a field study gave a comparison of the population dynamics of metabolically active strains of *Pseudomonas putida (PpG7)* and *PpG7 (pNAH7)*, the latter one having the ability to degrade sodium salicylate in amended or non-amended soil. The population densities of salicylate-degrading strains were increased up to 100-fold within 2 weeks of salicylate application in amended soils compared to non-amended bulk soils. Similarly colonization by salicylate-degrading *P. putida* strains increased up to 20-fold. In a similar study, Devliegher et al. (1995) studied bacterial strains that have the ability to degrade detergents Igepal and dioctyl sulfosuccinate in amended soils. Of these selected strains, a PGPR (*Pseudomonas*) strain persists well in amended bulk as well as rhizosphere soils. Recent studies rely on favoring the plant growth through amendments. In a study *Sinorhizobium* sp. modified the soil microflora to much extent to promote growth of *Brassica juncea* (Di Gregorio et al. 2006).

Several amendments of chemical and biological origin can be used to engineer microbial community of the soil. Brussaard et al. (2007) reported that the organic amendments are among the most important ways to promote biodiversity in soils. Reproduction rates of different microbes show a gradual increase in soils amended with biochar (Pietikäinen et al. 2000; Steiner et al. 2004). The abundance rates of microbes may vary according to the groups of microorganisms. The growth of the mycorrhizal fungi (arbuscular and ectomycorrhizal) is positively influenced in biochar-amended soil conditions (reviewed in Warnock et al. (2007). In a study biochar addition increased colonization of AM in wheat roots up to 20–40% in amended soil compared with 5–20% in non-amended soil (Solaiman et al. 2010).

11.8 Implications of Rhizosphere Engineering in Agriculture

Agronomic practices can be exploited in rhizosphere engineering over short periods of plant growth cycle (Bowen and Rovira, 1999). Root-associated environment of the crops and pasture species is influenced by the farmers when they irrigate or apply fertilizer to their fields. Soil acidification is the result of ammonium-based

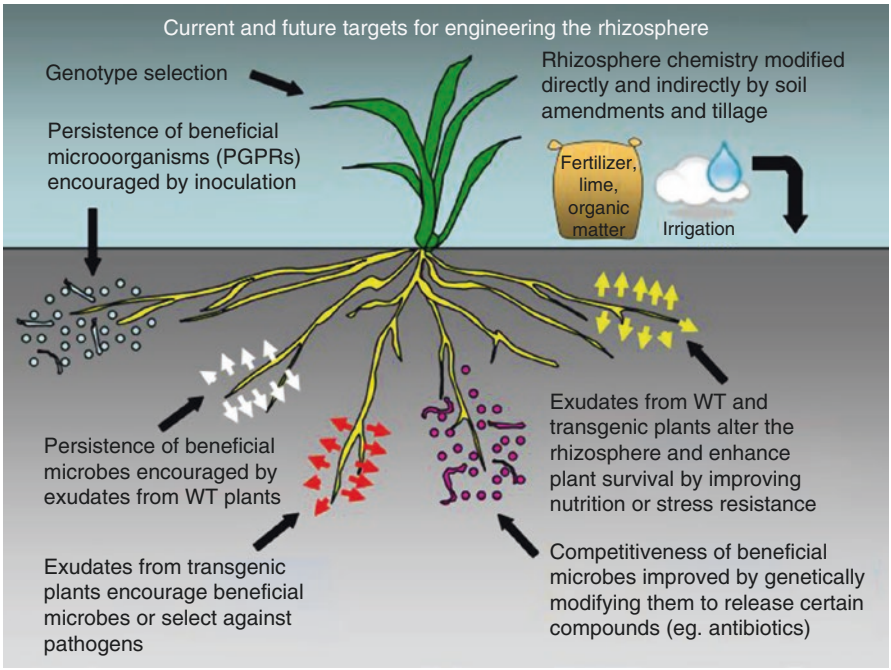

Fig. 11.3 The current and future targets of rhizosphere engineering (Adopted from Ryan et al. 2009 with permission)

fertilizers, whereas nitrate-based fertilizers tend to have more alkaline conditions in the rhizosphere. Thus, the growth, structure, and composition of the rhizospheric microbial communities are affected by the pH shifts. Breeding the plants and the use of biotechnological tools are another attractive approaches to alter rhizosphere (Ryan et al. 2009), as the supremacy of rhizosphere is an untold mystery why we must have to create conditions that are best suited for plant growth through soil amending, breeding, and engineering of feasible plants and by manipulation of beneficial plant-microbe interactions (Ryan et al. 2009). In Fig. 11.3, several strategies have been revealed to target the manipulation and engineering of rhizosphere microbiome. The selection of genotypes which could be most responsive to the beneficial microbes is the prime strategy to work with. Similarly, artificial inoculation of the rhizosphere with PGPR will enhance the populations of these beneficial microbes in the vicinity of plant roots which in turn will suppress the harmful microbial communities.

The chemistry of the rhizosphere could be modified directly and indirectly by tillage and soil amendments. Agricultural interventions like application of fertilizer, soil tillage, and schedule of irrigation may alter the chemistry of the rhizosphere by altering soil aeration, root function, or microbial communities (Ryan et al. 2009; Chaparro et al. 2012; Mendes et al. 2013). So these practices could be

used to regulate the rhizosphere microbiome. Moreover, the plants could be genetically modified to encourage beneficial microbes by exudates and suppress the populations of harmful microbes. For instance, site-specific promoters could be used to deliver defense-activating proteins in the nematode feeding structures (i.e., syncytia in cyst nematodes) to suppress the populations of plant parasitic nematodes (Ali et al. 2013, 2014, 2015, 2017). Moreover, the competitiveness of the beneficial microbes could be enhanced by genetically engineering of the microbes with compounds like antibodies which will increase their communities in the rhizosphere and which in turn will result into the betterment of agricultural plants (Ryan et al. 2009).

11.9 Conclusion and Future Prospects

In spite of decent amount of work done on rhizosphere microbiome and its engineering in the last two decades, the current state of knowledge about complex plant-microbe interactions occurring in the rhizosphere is still underdeveloped (Bisseling et al. 2009). Plants can construct their endosphere and rhizosphere microbiomes by using root exudates in their own favor which help them to withstand harsh environmental conditions and to cope with different plant pathogens. The symbiotic associations with beneficial microorganisms help the plants to acquire nutrients efficiently from the soil which improves plant growth and development that ultimately leads to high crop yields in case of crop plants. Similarly, some members of plant microbiome act antagonistically to plant pathogens and exclude or suppress their activity in the rhizosphere. These facts make the rhizosphere microbial communities a very rich source for engineering the root zones of economically important crop and orchard plants. The emerging next-generation sequencing technologies could definitely be employed to uncover the complex association between plants and the rhizosphere microbes (Schenk et al. 2012). Likewise, the rhizosphere microbiomes could be characterized using high-throughput technologies like metagenomics and transcriptomics to sort out the good ones of the microbiome. These beneficial microbes, i.e., PGPRs, could prove very dynamic entities of the ecosystem which will not only promote growth and development of the agricultural plants but also will improve the agricultural productivity (Berendsen et al. 2012). The rhizosphere engineering by using these microorganisms will opening new horizons of biological control of deleterious plant diseases. Moreover, the good ones of rhizosphere microbiome will enhance climate resilience of crop plants in the present scenario of climate change and global warming. In the era of globalization, high energy prices, climate change, and ever-increasing food requirements for ever-increasing world population, the beneficial microbiomes will be engineered. This will lead to cost-effective, durable, eco-safe, and biosafe approach to ameliorate the effect of biotic and abiotic stresses in crop plants which in turn will result in the sustainability of agricultural production and socioeconomic betterment of the society.

References

Abad P, Gouzy J, Aury JM, Castagnone-Sereno P, Danchin EGJ, Deleury E, Perfus-Barbeoch L, Anthouard V, Artiguenave F, Blok VC, Caillaud MC, Coutinho PM, Dasilva C et al (2008) Genome sequence of the metazoan plant-parasitic nematode Meloidogyne Incognita. Nat Biotechnol 26(8):909–915

Afshar M, Raju M, Ansell D, Bleck TP (2011) Narrative review: tetanus–a health threat after natural disasters in developing countries. Ann Intern Med 154:329–335

Ahemad M, Khan Ms (2012) Effects of pesticides on plant growth promoting traits of Mesorhizobium stain MRC4. J Saudi Soc Agric Sci 11:63–71

Ali SZ, Sandhya V, Grover M, Rao LV, Kishore VN, Venkateswarlu B (2009) Pseudomonas sp. strain AKM-P6 enhances tolerance of sorghum seedlings to elevated temperatures. Biol Fertil Soils 46:45–55

Ali MA, Plattner S, Radakovic Z, Wieczorek K, Elashry A, Grundler FM, Ammelburg M, Siddique S, Bohlmann H (2013) An Arabidopsis ATPase gene involved in nematode-induced syncytium development and abiotic stress responses. Plant J 74:852–866

Ali MA, Wieczorek K, Kreil DP, Bohlmann H (2014) The beet cyst nematode *Heterodera schachtii* modulates the expression of WRKY transcription factors in syncytia to favour its development in Arabidopsis roots. PLoS One 9:e102360

Ali MA, Abbas A, Azeem F, Javed N, Bohlmann H (2015) Plant-nematode interactions: from genomics to metabolomics. Int J Agric Biol 17:1071–1082

Alves BJR, Boddey RM, Urquiaga S (2004) The success of BNF in soybean in Brazil. Plant Soil 252:1–9

Ambrosini A, Beneduzi A, Stefanski T, Pinheiro FG, Vargas LK, Passaglia LMP (2012) Screening of plant growth promoting rhizobacteria isolated from sunflower (*Helianthus annuus* L.) Plant Soil 356:245–264

Andrews SC, Robinson AK, Rodríguez-Quinõnes F (2003) Bacterial iron homeostasis. FEMS Microbiol Rev 27:215–237

Apse MP, Aharon GS, Snedden WA, Blumwald E (1999) Salt tolerance conferred by overexpression of a vacuolar Na+/H+ antiport in Arabidopsis. Science 285:1256–1258

Arshad M, Sharoona B, Mahmood T (2008) Inoculation with Pseudomonas spp. containing ACC deaminase partially eliminate the effects of drought stress on growth, yield and ripening of pea (*Pisum sativum* L.) Pedosphere 18:611–620

Asano N, Oseki K, Tomioka E, Kizu H, Matsui K (1994) N containing sugars from *Morus alba* and their glycosidase inhibitory activities. Carbohydr Res 259:243–255

Asano N, Kato A, Kizu H, Matsui K, Watson AA, Nash RJ (1996) Calystegine B4, a novel trehalase inhibitor from Scopolia Japonica. Carbohydr Res 293:195–204

Atkinson HJ, Urwin PE, Hansen E, Mcpherson MJ (1995) Designs for engineered resistance to root-parasitic nematodes. Trends Biotechnol 13(9):369–374

Avery LM, Hill P, Killham K, Jones DL (2004) *Escherichia coli* O157 survival following the surface and sub-surface application of human pathogen contaminated organic waste to soil. Soil Biol Biochem 36:2101–2103

Badri DV, Vivanco JM (2009) Regulation and function of root exudates. Plant Cell Environ 32:666–681

Badri DV, Weir TL, van der Lelie D, Vivanco JM (2009) Rhizosphere chemical dialogues: plant–microbe interactions. Curr Opin Biotechnol 20:642–650

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Bakker AW, Schippers B (1987) Microbial cyanide production in the rhizosphere in relation to potato yield reduction and Pseudomonas spp. mediated plant growth stimulation. Soil Biol Biochem 19:451–457

Bakker MG, Manter DK, Sheflin AM, Weir TL, Vivanco JM (2012) Harnessing the rhizosphere microbiome through plant breeding and agricultural management. Plant Soil 360:1–13

Baldani JI, Caruso L, Baldani VLD, Goi SR, Döbereiner J (1997) Recent advances in BNF with non-legume plants. Soil Biol Biochem 29:911–922

Bao Z, Sasaki K, Okubo T, Ikeda S, Anda M, Hanzawa E, Kakizaki K, Sato T, Mitsui H, Minamisawa K (2013) Impact of Azospirillum Sp. B510 Inoculation on Rice-Associated Bacterial Communities in a Paddy Field. Microbes Environ 28(4): 487–490.

Baptista-Rosas RC, Hinojosa A, Riquelme M (2007) Ecological niche modeling of *Coccidioides* spp. in western north American deserts. Ann N Y Acad Sci 1111:35–46

Barka EA, Nowak J, Clement C (2006) Enhancement of chilling resistance of inoculated grapevine plantlets with a plant growth-promoting rhizobacterium Burkholderia phytofirmans strain PsJN. Appl Environ Microbiol 72:7246–7252

Baumgardner DJ, Temte JL, Gutowski E et al (2011) The differential diagnosis of pulmonary blastomycosis in Wisconsin: a Wisconsin network for Health Research (WiNHR) study. Wis Med J 110:68–73

Bech TB, Johnsen K, Dalsgaard A, Laegdsmand M, Jacobsen OH, Jacobsen CS (2010) Transport and distribution of Salmonella enterica serovar Typhimurium in loamy and sandy soil monoliths with applied liquid manure. Appl Environ Microbiol 76:710–714

Bennet JW (2009) Aspergillus: a primer for the novice. Med Mycol 47:5–12

Bentley AR, Cromey MG, Farrokhi-Nejad R, Leslie JF, Summerell BA, Burgess LW (2006) *Fusarium* crown and root rot pathogens associated with wheat and grass stem bases on the South Island of New Zealand. Aust Plant Pathol 35(495):502

Berendsen RL, Corné MJ, Pieterse AHM, Bakker P (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486

Berg G, Smalla K (2009) Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiol Ecol 68:1–13

Bertalan M, Albano R, de Pádua V, Rouws L, Rojas C, Hemerly A, Teixeira K, Schwab S, Araujo J, Oliveira A et al (2009) Complete genome sequence of the sugarcane nitrogen fixing endophyte Gluconacetobacter diazotrophicus PAl5. BMC Genomics e450:10

Bhattacharyya PN, Jha DK (2012) Plant-growth promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Bisseling T, Dangl JL, Schulze-Lefert P (2009) Next-generation communication. Science 324:691

Blackburn JK, McNyset KM, Curtis A, Hugh-Jones ME (2007) Modeling the geographic distribution of bacillus anthracis, the causative agent of anthrax disease, for the contiguous United States using predictive ecologic niche modeling. AmJTrop Med Hyg 77:1103–1110

Bowen GD, Rovira AD (1999) The rhizosphere and its management to improve plant growth. Adv Agron 66:1–102

Brown ME (1974) Seed and root bacterization. Annu Rev Phytopatol 12:181–197

Brussaard L, De Ruiter PC, Brown GG (2007) Soil biodiversity for agricultural sustainability. Agric Ecosyst Environ 121:233–244

Buée M, Reich M, Murat C, Morin E, Nilsson RH, Uroz S, Martin F (2009) 454 pyrosequencing analyses of forest soils reveal an unexpectedly high fungal diversity. New Phytol 184:449–456

Cambardella LA, Eliott ET (1992) Particulate soil organic matter changes across a grassland cultivation sequence. Soil Sci Soc Am J 46:777–783

Chan KG, Atkinson S, Mathee K et al (2011) Characterization of N-acylhomoserine lactone-degrading bacteria associated with the *Zingiber officinale* (ginger) rhizosphere: co-existence of quorum quenching and quorum sensing in Acinetobacter and Burkholderia. BMC Microbiol 11:51

Chandler DS, Craven JA (1980) Relationship of soil moisture to survival of *Escherichia coli* and *Salmonella typhimurium* in soils. Crop Pasture Sci 31:547–555

Chaparro JM, Sheflin AM, Manter DK, Vivanco JM (2012) Manipulating the soil microbiome to increase soil health and plant fertility. Biol Fertil Soils 48:489–499

Chapman SW (2005) Blastomyces dermatitidis. In: Mandell GL, Bennett JE, Dolin R (eds) Mandell, Douglas, and Bennett's principles and practice of infectious diseases, 6th edn. Elsevier, Philadelphia, pp 3026–3040

Chapman SK, Langley JA, Hart SC, Koch GW (2006) Plants actively control nitrogen cycling: uncorking the microbial bottleneck. New Phytol 169:27–34

Charpentier M, Oldroyd G (2010) How close are we to nitrogen-fixing cereals? Curr Opin Plant Biol 13:556–564

Chen Y, Fan JB, Du L, Xu H, Zhang QY, He YQ (2014) The application of phosphate solubilizing endophyte Pantoea dispersa triggers the microbial community in red acidic soil. Appl Soil Ecol 84:235–244

Cheng Z, Park E, Glick BR (2007) 1-Aminocyclopropane-1-carboxylate (ACC) deaminase from Pseudomonas putida UW4 facilitates the growth of canola in the presence of salt. Can J Microbiol 53:912–918

Chet I, Inbar J (1994) Biological control of fungal pathogens. Appl Biochem Biotechnol 48:37–43

Chitwood DJ (2003) Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture-Agricultural Research Service. Pest Manag Sci 59:748–753

Ciccillo F, Fiore A, Bevivino A, Dalmastri C, Tabacchioni S, Chiarini L (2002) Effects of two different application methods of Burkholderia ambifaria MCI7 on plant growth and rhizospheric bacterial diversity. Environ Microbiol 4:238–245

Clayton GW, Rice WA, Lupwayi NZ, Johnston AM, Lafond GP, Grant CA, Walley F (2004) Inoculant formulation and fertilizer nitrogen effects on field pea: nodulation, N2 fixation and nitrogen partitioning. Can J Plant Sci 84:79–88

Colbert SF, Hendson M, Ferri M, Schroth MN (1993a) Enhanced growth and activity of a biocontrol bacterium genetically engineered to utilize salicylate. Appl Environ Microbiol 59:2071–2076

Colbert SF, Schroth MN, Weinhold AR, Hendson M (1993b) Enhancement of population densities of Pseudomonas putida ppg7 in agricultural ecosystems by selective feeding with the carbon source salicylate. Appl Environ Microbiol 59:2064–2070

Collins CH, Lyne PM, Granze JM (1992) Microbiological methods. Read Educational and Professional, Gauteng, p 117

Contesto C, Desbrosses G, Lefoulon C, Bena G, Borel F, Galland M, Gamet L, Varoquaux F, Touraine B (2008) Effects of rhizobacterial ACC deaminase activity on Arabidopsis indicate that ethylene mediates local root responses to plant growth-promoting rhizobacteria. Plant Sci 175:178–189

Cooney NM, Klein BS (2008) Fungal adaptation to the mammalian host: it's a new world, after all. Curr Opin Microbiol 11:511–516

Cox K, Scherm H, Riley MB (2006) Characterization of Armillaria spp. from peach orchards in the southeastern United States using fatty acid methyl ester profiling. Mycol Res 110:4414–4422

Cummings SP (2009) The application of plant growth promoting rhizobacteria (PGPR) in low input and organic cultivation of graminaceous crops; potential and problems. Environ Biotechnol 5:43–50

Curtis TP, Sloan WT, Scannell JW (2002) Estimating prokaryotic diversity and its limits. Proc Natl Acad Sci U S A 99:10494–10499

de la Fuente JM, Ramírez-Rodríguez V, Cabrera-Ponce JL, Herrera-Estrella L (1997) Aluminum tolerance in transgenic plants by alteration of citrate synthesis. Science 276:1566–1568

Decraemer W, Hunt DJ (2006) Structure and classification. Plant nematology. CABI, Oxfordshire

Defago G, Berling C, Burger U, Haas D, Kahr G, Keel C, Voisard C, Wirthner P, Withrich B (1990) Suppression of black root rot of tobacco and other diseases by strains of *Pseudomonas fluorescens*: potential applications and mechanisms. In: Hornby D (ed) Biological control of soil-borne plant pathogens. CAB International, UK, pp 93–108

De-la-Pena C, Badri DV, Lei Z, Watson BS, Brandao MM, Silva-Filho MC et al (2010) Root secretion of defense-related proteins is development-dependent and correlated with flowering time. J Biol Chem 285:30654–30665

Dessaux Y, Hinsinger P, Lemanceau P (2010) Rhizosphere: achievements and challenges. Springer, New York

Devliegher W, Arif M, Verstraete W (1995) Survival and plant growth promotion of detergent-adapted *Pseudomonas fluorescens* ANP15 and Pseudomonas Aeruginosa 7NSK2. Appl Environ Microbiol 61:3865–3871

Dey R, Pal KK, Bhatt DM, Chauhan SM (2004) Growth promotion and yield enhancement of peanut (Arachis hypogaea L.) by application of plant growth-promoting rhizobacteria. Microbiol Res 159:371–394

Di Gregorio S, Barbafieri M, Lampis S, Sanangelantoni AM, Tassi E, Vallini G (2006) Combined application of triton X-100 and Sinorhizobium sp. Pb002 inoculum for the improvement of lead phytoextraction by *Brassica juncea* in EDTA amended soil. Chemosphere 63:293–299

Dimkpa CO, Merten D, Svatos A, Büchel G, Kothe E (2009) Siderophores mediate reduced and increased uptake of cadmiumby Streptomyces tendae F4 and sunflower (*Helianthus annuus*), respectively. J Appl Microbiol (5):687–1696

Dixon R, Kahn D (2004) Genetic regulation of biological nitrogen fixation. Nat Rev Microbiol 2:621–631

Dodd IC, Belimov AA, Sobeih WY, Safronova VI, Grierson D, Davies WJ (2005) Will modifying plant ethylene status improve plant productivity in water-limited environments? In: 4th international crop science congress

Duffy BK (2001) Competition. In: Maloy OC, Murray TD (eds) Encyclopedia of plant pathology. John Wiley & Sons, Inc, New York, pp 243–244

Duffy BK, Schouten A, Raaijmakers JM (2003) Pathogen self-defense: mechanisms to counteract microbial antagonism. Annu Rev Phytopathol 41:501–538

Egamberdieva D, Kamilova F, Validov S, Gafurova L, Kucharova Z, Lugtenberg B (2008) High incidence of plant growth-stimulating bacteria associated with the rhizosphere of wheat grown on salinated soil in Uzbekistan. Environ Microbiol 10:1–9

Elkoca E, Kantar F, Sahin F (2008) Influence of nitrogen fixing and phosphorus solubilizing bacteria on the nodulation, plant growth, and yield of chickpea. J Plant Nutr 31:157–171

Faoro H, Alves AC, Souza EM, Rigo LU, Cruz LM, Al-Janabi SM, Monteiro RA, Baura VA, Pedrosa FO (2010) Influence of soil characteristics on the diversity of bacteria in the southern Brazil- ian Atlantic forest. Appl Environ Microbiol 76:4744–4749

Feng K, HM L, Sheng HJ, Wang XL, Mao J (2004) Effect of organic ligands on biological availability of inorganic phosphorus in soils. Pedosphere 14:85–92

Ferreira AS, Pires RR, Rabelo PG, Oliveira RC, Luz JMQ, Brito CH (2013) Implications of Azospirillum brasilense inoculation and nutrient addition on maize in soils of the Brazilian Cerrado under greenhouse and field conditions. Appl Soil Ecol 72:103–108

Fierer N, Jackson RB (2006) The diversity and biogeography of soil bacterial communities. Proc Natl Acad Sci U S A 103:626–631

Fliessbach A, Winkler M, Lutz MP, Oberholzer HR, Mäder P (2009) Soil amendment with *Pseudomonas fluorescens* CHA0: lasting effects on soil biological properties in soils low in microbial biomass and activity. Microb Ecol 57:611–623

Flores HE, Vivanco JM, Loyola-Vargas VM (1999) 'radicle' biochem- istry: the biology of root-specific metabolism. Trends Plant Sci 4:220–226

Flynn NM, Hoeprich PD, Kawachi MM, Lee KK, Lawrence RM, Goldstein E, Jordan GW, Kundargi RS, Wong GA (1979) An unusual outbreak of windborne coccidioidomycosis. N Engl J Med 301:358–361

Franche C, Lindström K, Elmerich C (2009) Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. Plant Soil 321:35–59

Frey SD, Knorr M, Parrent JL, Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. Forest Ecol Manag 196:159–171

Fuller VL, Lilley CJ, Urwin PE (2008) Nematode resistance. New Phytol 180(1):27–44

Fuqua C, Parsek MR, Greenberg EP (2001) Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu Rev Genet 35:439–468

Gams W (2007) Biodiversity of soil-inhabiting fungi. Biodivers Conserv 16:69–72

Garcia de Salamone IE, Döbereiner J, Urquiaga S, Boddey RM (1996) Biological nitrogen fixation in Azospirillum strain maize genotype associations as evaluated by 15N isotope dilution technique. Biol Fertil Soils 23:249–256

Gévaudant F, Duby G, von Stedingk E, Zhao RM, Morsomme P, Boutry M (2007) Expression of a constitutively activated plasma membrane H+−ATPase alters plant development and increases salt tolerance. Plant Physiol 144:1763–1776

Giebel J (1982) Mechanism of resistance to plant nematodes. Annu Rev Phytopathol 20:257–279

Giordano W, Hirsch AM (2004) The expression of *MaEXP*1,a *Melilotus alba* expansin gene, is upregulated during the sweet clover-*Sinorhizobium meliloti* interaction. Mol Plant-Microbe Interact 17:613–622

Giri B, Mukerji KG (2004) Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake. Mycorrhiza 14:307–312

Girvan MS, Bullimore J, Pretty JN, Osborn AM, Ball AS (2003) Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Appl Environ Microbiol 69:1800–1809

Glass ADM (1989) Plant nutrition: an introduction to current concepts. Jones and Bartlett Publishers, Boston, p 234

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117

Glick BR (2010) Using soil bacteria to facilitate phytoremediation. Biotechnol Adv 28:367–374

Glick BR, Penrose DM, Li J (1998) A model for the lowering of plant ethylene concentrations by plant growth promoting bacteria. J Theor Biol 190:63–68

Goldstein AH, Rogers RD, Mead G (1993) Mining by microbe. Biotechnology 11:1250–1254

Gosling P, Hodge A, Goodlass G, Bending GD (2006) Arbuscular mycorrhizal fungi and organic farming. Agric Ecosyst Environ 113:17–35

Govindarajan M, Balandreau J, Muthukumarasamy R, Revathi G, Lakshminarasimhan C (2006) Improved yield of micropropagated sugarcane following inoculation by endophytic Burkholderia vietnamiensis. Plant Soil 280:239–252

Govindarajan M, Balandreau J, Kwon SW, Weon HY, Lakshminarasimhan C (2008) Effects of the inoculation of Burkholderia vietnamensis and related endophytic diazotrophic bacteria on grain yield of rice. Microb Ecol 55:21–37

Grassl GA, Finlay BB (2008) Pathogenesis of enteric Salmonella infections. Curr Opin Gastroenterol 24:22–26

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plantbacterium signaling processes. Soil Biol Biochem 37:395–412

Grayston SJ, Wang SQ, Campbell CD, Edwards AC (1998) Selective influence of plant species on microbial diversity in the rhizosphere. Soil Biol Biochem 30:369–378

Guiñazú LB, Andrés JA, Del Papa MF, Pistorio M, Rosas SB (2009) Response of alfalfa (*Medicago sativa* L.) to single and mixed inoculation with phosphate-solubilizing bacteria and Sinorhizobium meliloti. Biol Fertil Soils 46:185–190. doi:10.1007/s00374-009-0408-5

Guyer DM, Henderson IR, Nataro JP, Mobley HL (2000) Identification of sat, an autotransporter toxin produced by uropathogenic *Escherichia coli*. Mol Microbiol 38:53–66

Haagsma J (1991) Pathogenic anaerobic bacteria and the environment. Rev Sci Tech Off Int Epiz 10:749–764

Haas D, Keel C (2003) Regulation of antibiotic production in root-colonizing Pseudomonas spp. and relevance for biological control of plant disease. Annu Rev Phytopathol 41:117–153

Hafeez FY, Yasmin S, Ariani D, Mehboob-ur-Rahman ZY, Malik KA (2006) Plant growthpromoting bacteria as biofertilizer. Agron Sustain Dev 26:143–150

Hall JA, Peirson D, Ghosh S, Glick BR (1996) Root elongation in various agronomic crops by the plant growth promoting rhizobacterium Pseudomonas putida GR12-2. Isr J Plant Sci 44:37–42

Hameeda B, Harini G, Rupela OP, Wani SP, Reddy G (2008) Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. Microbiol Res 163:234–242

Hartmann A, Schmid M, van Tuinen D, Berg G (2009) Plant-driven selection of microbes. Plant Soil 321:235–257

Hinsinger P, Plassard C, Tang CX, Jaillard B (2003) Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review. Plant Soil 248:43–59

Hirulkar NB, Soni B (2011) Incidence of antibiotic-resistant Pseudomonas Aeruginosa isolated from drinking water. Int J Pharm Biol Arch 2:724–733

Högberg MN, Högberg P, Myrold DD (2006) Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three. Oecologia 150:590–601

Holford ICR (1997) Soil phosphorus,its measurements and its uptake by plants. Austr J Soil Res 35:227–239

Howell CR, Stipanovic RD (1979a) Control of Rhizoctonia solani on cotton seedlings with *Pseudomonas fluorescens* and with an antibiotic produced by the bacterium. Phytopathology 69:480–482

Howell CR, Stipanovic RD (1979b) Suppression of pythium ultimum-induced damping-off of cotton seedlings by *Pseudomonas fluorescens* and its antibiotic, pyoluteorin. Phytopathology 70:712–715

Hungria M, Campo RJ, Souza EM, Pedrosa FO (2010) Inoculation with selected strains of Azospirillum brasilense and A. Lipoferum improves yields of maize and wheat in Brazil. Plant Soil 331:413–425.

Hussey RS, Grundler FM (1998) Nematode parasitism of plants. The physiology and biochemistry of free-living and plant-parasitic nematodes, vol 1. CAB International.

Idriss EE, Makarewicz O, Farouk A, Rosner K, Greiner R, Bochow H, Richter T, Borriss R (2002) Extracellular phytase activity of bacillus amyloliquefaciens FZB45 contributes to its plant-growth-promoting effect. Microbiology 148:2097–2109

Ikeda AG, Bassani LL, Adamoski D, Stringari D, Cordeiro VK, Glienke C, Maria Steffens BR, Hungria M, Galli Terasawa LV (2013) Morphological and genetic characterization of endophytic bacteria isolated from roots of different maize genotypes. Microb Ecol 65:154–160

Islam M, Morgan J, Doyle MP, Phatak SC, Millner P, Jiang X (2004) Persistence of Salmonella enterica serovar typhimurium on lettuce and parsley and in soils on which they were grown in fields treated with contaminated manure composts or irrigation water. Foodborne Pathog Dis 1:27–35

Jabbar A, Javed N, Khan SA, Ali MA (2015) *Meloidogyne graminicola* an emerging threat to rice and wheat in Punjab province in Pakistan. Pak J Nematol 33:227–228

Jeffries P, Gianinazzi S, Perotto S, Turnau K (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37:1–16

Johnson SN, Nielsen UN (2012) Foraging in the dark –chemically mediated host plant location by belowground insect herbivores. J Chem Ecol 38:604–614

Jones MGK, Payne HL (1978) Early stage of nematode-induced giant-cell formation in roots of *Impatiens balsamina*. J Nematol 10(1):70–84

Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant Soil 321:5–33

Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG, Kikuchi T, Manzanilla-Lopez R, Palomares-Rius JE, Wesemael WM, Perry RN (2013) Top 10 plant-parasitic nematodes in molecular plant pathology. Mol Plant Pathol. doi:10.1111/mpp.12057

Khan MS, Zaidi A, Wani PA (2009) Role of phosphate solubilizing microorganisms insustainable agriculture - a review. Agron Sustain Dev 27:29–43

Kirankumar R, Jagadeesh KS, Krishnaraj PU, Patil MS (2008) Enhanced growth promotion of tomato and nutrient uptake by plant growth promoting rhizobacterial isolates in presence of tobacco mosaic virus pathogen. Karnataka J Agric Sci 21:309–311

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. Nature 286:885–886

Klotchko A, Wallace MR (2011) Salmonellosis clinical presentation. http://emedicine.medscape.com/article/228174-clinical#a0218
Kobayashi T, Nishizawa NK (2012) Iron uptake, translocation, and regulation in higher plants. Annu Rev Plant Biol 63:131–152
Kowalchuk GA, Buma DS, de Boer W, Klinkhamer PGL, van Veen JA (2002) Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. Antonie Van Leeuwenhoek 81:509–520
Koyama H, Takita E, Kawamura A, Hara T, Shibata D (1999) Over expression of mitochondrial citrate synthase gene improves the growth of carrot cells in al-phosphate medium. Plant Cell Physiol 40:482–488
Krewulak HD, Vogel HJ (2008) Structural biology of bacterial iron uptake. Biochim Biophys Acta 1778:1781–1804
Kuklinsky-Sobral J, Araujo WL, Mendes R, Geraldi IO, Pizzirani-Kleiner AA, Azevedo JL (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1251
Kumar V, Singh P, Jorquera MA, Sangwan P, Kumar P, Verma AK, Agrawal S (2013) Isolation of phytase-producing bacteria from Himalayan soils and their effect on growth and phosphorus uptake of Indian mustard (*Brassica juncea*). World J Microbiol Biotechnol 29:1361–1369
Lal R (2000) Soil management in the developing countries. Soil Sci 165:57–72
Lambers H (1980) The physiological significance of cyanide-resistant respiration in higher plants. Plant Cell Environ 3:293–302
Lambers H (2003) Dryland salinity: a key environmental issue in southern Australia. Plant Soil 218:5–7
Lambers H, Mougel C, Jaillard B, Hinsinger P (2009) Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. Plant Soil 321:83–115
Lauber CL, Strickland MS, Bradford MA, Fierer N (2008) The influence of soil properties on the structure of bacterial and fungal communities across land-use types. Soil Biol Biochem 40:2407–2415
Lehtonen MJ, Somervuo P, Valkonen JPT (2008) Infection with *Rhizoctonia solani* induces defense genes and systemic resistance in potato sprouts grown without light. Phytopathology 98:1190–1198
Lemanceau P, Bauer P, Kraemer S, Briat JF (2009) Iron dynamics in the rhizosphere as a case study for analyzing interactions between soils, plants and microbes. Plant Soil 321:513–535
Loaces I, Ferrando L, Scavino AF (2010) Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. Microbial Ecol 61:606–618
Loper JE, Buyer JS (1991) Siderophores in microbial interactions on plant surfaces. Molec Plant Microbe Int 4:5–13
Low C, Rotstein C (2011) Emerging fungal infections in immunocompromised patients. F1000 Med Rep 3:14
Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556
Luscher A, Daepp M, Blum H, Hartwig UA, Nosberger J (2004) Fertile temperate grassland under elevated atmospheric CO2—role of feed-back mechanisms and availability of growth resources. Eur J Agron 21(3):379–398
Lynch JM (1990) The rhizosphere. Wiley-Interscience, Chichester, p 458
Ma JF, Nomoto K (1994) Biosynthetic pathway of 3epihydroxymugineic acid and 3-hydroxymugineic acid in gramineous plants. Soil Sci Plant Nutr 40:311–317
Ma JF, Ryan PR, Delhaize E (2001) Aluminium tolerance in plants and the complexing role of organic acids. Trends Plant Sci 6:273–278
Madhaiyan M, Poonguzhali S, Ryu J, Sa T (2006) Regulation of ethylene levels in canola (*Brassica campestris*) by 1-aminocyclopropane-1-carboxylate deaminase-containing Methylobacterium fujisawaense. Planta 224:268–278
Malcolm GM, Kuldau GA, Gugino BK, Jiménez-Gasco MM (2013) Hidden host plant associations of soilborne fungal pathogens: an ecological perspective. Phytopathology 103:538–544

Marschner H, Römheld V, Kissel M (1987) Localization of phytosiderophores release and of iron uptake along intact barley roots. Physiol Plant 71:157–162
Masalha J, Kosegarten H, Elmaci O, Mengel K (2000) The central role of microbial activity for iron acquisition in maize and sunflower. Biol Fertil Soils 30:433–439
Matches JR, Liston J, Curran D (1974) *Clostridium perfringens* In the environment. Appl Microbiol 28:655–660
Mayak S, Tirosh T, Glick BR (2004a) Plant growth-promoting bacteria that confer resistance to water stress in tomatoes and peppers. Plant Sci 166:525–530
Mayak S, Tirosh T, Glick BR (2004b) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572
Mc Lellan CA, Turbyville TJ, Wijeratne EMK, Kerschen EV, Queitsch C, Whitesell L, Gunatilaka AAL (2007) A rhizosphere fungus enhances Arabidopsis thermotolerance through production of an HSP 90 inhibitor. Plant Physiol 145:174–182
McLaughlin MJ, McBeath TM, Smernik R, Stacey SP, Ajiboye B, Guppy C (2011) The chemical nature of P accumulation in agricultural soils-implications for fertilizer management and design: an Australian perspective. Plant Soil 349:69–87
Mehnaz S, Lazarovits G (2006) Inoculation effects of Pseudomonas putida, Gluconacetobacter azotocaptans, and Azospirillum lipoferum on corn plant growth under greenhouse conditions. Microb Ecol 51:326–335
Mela F, Fritsche K, de Boer W, van Veen JA, de Graaff LH, van den Berg M, Leveau JHJ (2011) Dual transcriptional profiling of a bacterial/fungal confrontation: Collimonas fungivorans versus Aspergillus niger. ISME J 5:1494–1504
Mendes R, Kruijt M, de Bruijn I, Dekkers E, van der Voort M, Schneider JHM, Piceno YM, DeSantis TZ, Andersen GL, Bakker PAHM, Raaijmakers JM (2011) Deciphering the rhizosphere microbiome for disease-suppressive bacteria. Science 332:1097–1100
Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663
Miller MB, Bassler BL (2001) Quorum sensing in bacteria. Annu Rev Microbiol 55:165–199
Moens M, Perry RN (2009) Migratory plant endoparasitic nematodes: a group rich in contrasts and divergence. Annu Rev Phytopathol 47:313–332
Muhammad A. Ali, Farrukh Azeem, Amjad Abbas, Faiz A. Joyia, Hongjie Li, Abdelfattah A. Dababat, (2017) Transgenic Strategies for Enhancement of Nematode Resistance in Plants. Frontiers in Plant Science 8
Muthukumarasamy R, Cleenwerk I, Revathi G, Vadivelu M, Janssens D, Hoste B, Gum KU, Park KD, Son CY, Sa T et al (2005) Natural association of Gluconacetobacter diazotrophicus and diazotrophic Acetobacter peroxydans with wetland rice. Syst Appl Microbiol 28:277–286
Nadeem SM, Zahir ZA, Naveed M, Arshad M (2009) Rhizobacteria containing ACC-deaminase confer salt tolerance in maize grown on salt-affected fields. Can J Microbiol 55(11):1302–1309
Nahas E (1996) Factors determining rock phosphate solubilization by microorganisms isolated from soil. World J Microbiol Biotechnol 12:567–572
Naveed M, Mitter B, Reichenauer TG, Krzysztof W, Sessitsch A (2014) Increased drought stress resilience of maize through endophytic colonization by *Burkholderia phytofirmans* PsJN and *Enterobacter* sp. FD17. Environ Exp Bot 97(1):30–39
Neilands JB (1995) Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 270:26723–26726
Neumann G, Römheld V (2007) The release of root exudates as affected by the root physiological status. In: Pinton R, Varanini Z, Nannipieri P (eds) The rhizosphere: biochemistry and organic substances at the soil-plant interface. CRC, Boca Raton, pp 23–72
Newton WE (2000) Nitrogen fixation in perspective. In: Pedrosa FO, Hungria M, Yates MG, Newton WE (eds) Nitrogen fixation: from molecules to crop productivity. Kluwer Academic Publishers, Dordrecht, pp 3–8

Oger P, Mansouri H, Nesme X, Dessaux Y (2004) Engineering root exudation of lotus toward the production of two novel carbon compounds leads to the selection of distinct microbial populations in the rhizosphere. Microb Ecol 47:96–10

Oliveira ALM, Urquiaga S, Döbereiner J, Baldani JI (2002) The effect of inoculating endophytic N2 fixing bacteria on micro propagated sugarcane plants. Plant Soil 2:205–215

Orhan E, Esitken A, Ercisli S, Turan M, Sahin F (2006) Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. Sci Hortic 111:38–43

Padgham JL, Duxbury JM, Mazid AM, Abawi GS, Hossain M (2004) Yield loss caused by Meloidogyne Graminicola on lowland rainfed rice in Bangladesh. J Nematol 36(1):42–48

Pelayo Ulacia S, Dafhnis D (1980) Isolation of the causative agent and histopathology of athlete's foot (preliminary report). Rev Cubana Med Trop 32:227–232

Pérez-Piqueres A, Edel-Hermann V, Alabouvette C, Steinberg C (2006) Response of soil microbial communities to compost amendments. Soil Biol Biochem 38:460–470

Philippot L, Raaijmakers JM, Lemanceau P, van der Putten WH (2013) Going back to the roots: the microbial ecology of the rhizosphere. Nat Rev Microbiol 11:789–799

Pieterse CMJ (2012) Prime time for transgenerational defense. Plant Physiol 158:545

Pietikäinen J, Kiikkilä O, Fritze H (2000) Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos 89:231–242

Qin L, Jiang H, Tian J, Zhao J, Liao H (2011) Rhizobia enhance acquisition of phosphorus from different sources by soybean plants. Plant Soil 349:25–36

Raaijmakers J, Mazzola M (2012) Diversity and natural functions of antibiotics produced by beneficial and pathogenic soil bacteria. Annu Rev Phytopathol 50:403–424

Rasmann S, Ali JG, Helder J, van der Putten WH (2012) Ecology and evolution of soil nematode chemotaxis. J Chem Ecol 38:615–628

Revis DRJ (2008) Closridial gas gangrene clinical presentation. http://emedicine.medscape.com/article/214992-clinical

Rice SA, Givskov M, Steinberg P, Kjelleberg S (1999) Bacterial signals and antagonists: the interaction between bacteria and higher organisms. J Mol Microbiol Biotechnol 1:23–31

Richardson A (2001) Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. Aust J Plant Physiol 28:897–907

Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability. Plant Physiol 156:989–996

Richardson AE, Hadobas PA, Hayes JE (2001) Extracellular secretion of Aspergillus phytase from Arabidopsis roots enables plants to obtain phosphorus from phytate. Plant J 25:641–649

Richardson AE, Barea JM, McNeill AM, Prigent-Combaret C (2009) Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321:305–339. doi:10.1007/s11104-009-9895-2

Robson ND, Cox AR, McGowan SJ, Bycroft BW, Salmond GPC (1997) Bacterial N-acylhomoserine lactone-dependent signalling and its potential biotechnological applications. Trends Biotechnol 15:458–464

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rodríguez H, Fraga R, Gonzalez T, Bashan Y (2006) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Plant Soil 287:15–21

Roque MR, Roque BL, Foster CS (2010) Actinomycosis in ophthalmology. http://emedicine.medscape.com/article/1203061-overview

Rousk J, Baath E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME J 4:1340–1351

Rovira AD (1969) Plant root exudates. Bot Rev 35:35–57

Ryan PR, Delhaize E, Jones DL (2001) Function and mechanism of organic anion exudation from plant roots. Annu Rev Plant Physiol Plant Mol Biol 52:527–560

Ryan PR, Dessaux Y, Thomashow LS, Weller DM (2009) Rhizosphere engineering and management for sustainable agriculture. Plant Soil 321:363–383
Salles JF, van Veen JA, van Elsas JD (2004) Multivariate analyses of Burkholderia species in soil: effect of crop and land use history. Appl Environ Microbiol 70:4012–4020
Saremi H, Mohammadi J, Okhovvat SM (2007) Naz, a resistant cultivar on bean root rot disease in Zanjan province, northwest Iran. Commun Agric Appl Biol Sci 72:757–764
Sasser JN, Freckman DW (1987) A world perspective on nematology: the role of the Society. Vistas on Nematology Hyatssville: Society of Nematologists
Savka MA, Dessaux Y, Oger P, Rossbach S (2002) Engineering bacterial competitiveness and persistence in the phytosphere. Mol Plant-Microbe Interact 15(9):866–874
Schenk ST, Stein E, Kogel KH, Schikora A (2012) Arabidopsis growth and defense are modulated by bacterial quorum sensing molecules. Plant Signal Behav 7:178–181
Schneider E, Hajj RA, Spiegel RA et al (1997) A coccidioidomycosis outbreak following the Northridge, Calif, earthquake. JAMA 277:904–908
Selvakumar G, Panneerselvam P, Ganeshamurthy AN, Maheshwari DK (2012) Bacterial mediated alleviation of abiotic stress in crops. In: Maheshwari DK (ed) Bacteria in agrobiology: stress management. Springer, New York, pp 205–224
Shaharoona B, Jamro GM, Zahir ZA, Arshad M, Memon KS (2007) Effectiveness of various Pseudomonas spp. and Burkholderia caryophylli containing ACC-deaminase for improving growth and yield of wheat (Triticum aestivum L.) J Microbiol Biotechnol 17:1300–1307
Sharma A, Johria BN, Sharma AK, Glick BR (2003) Plant growth-promoting bacterium Pseudomonas sp. strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). Soil Biol Biochem 35:887–894
Sharma SK, Ramesh A, Johri BN (2013) Isolation and characterization of plant growth promoting bacillus amyloliquefaciens strain sks_bnj_1 and its influence on rhizosphere soil properties and nutrition of soybean (Glycine max L. Merrill). J Virol Microbiol 2013:1–19
Sharpton TJ, Stajich JE, Rounsley SD et al (2009) Comparative genomic analyses of the human fungal pathogens *Coccidioides* and their relatives. Genome Res 19:1722–1731
Sindhu SS, Gupta SK, Dadarwal KR (1999) Antagonistic effect of Pseudomonas spp. on pathogenic fungi and enhancement of plant growth in green gram (*Vigna radiata*). Biol Fertil Soils 29:62–68
Singh P, Kumar V, Agrawal S (2014) Evaluation of phytase producing bacteria for their plant growth promoting activities. Int J Microbiol 2014:426483
Solaiman ZM, Blackwell P, Abbott LK, Storer P (2010) Direct and residual effect of biochar application on mycorrhizal root colonisation, growth and nutrition of wheat. Soil Res 48:546–554
Soriano IR, Prot JC, Matias DM (2000) Expression of tolerance for Meloidogyne Graminicola in rice cultivars as affected by soil type and flooding. J Nematol 32(3):309–317
Souza R, Beneduzi A, Ambrosini A, Costa PB, Meyer J, Vargas LK, Schoenfeld R, Passaglia LMP (2013) The effect of plant growth-promoting rhizobacteria on the growth of rice (*Oryza sativa* L.) cropped in southern Brazilian fields. Plant Soil 366:585–603
Srivastava S, Yadav A, Seema K, Mishra S, Choudhary V, Nautiyal CS (2008) Effect of high temperature on Pseudomonas putida NBRI0987 biofilm formation and expression of stress sigma factor Rpo S. Curr Microbiol 56:453–457
Stein RJ, Duarte GL, Spohr MG, Lopes SIG, Fett JP (2009) Distinct physiological responses of two rice cultivars subjected to iron toxicity under field conditions. Ann Appl Biol 154:269–277
Steiner C, Teixeira WG, Lehmann J, Zech W (2004) Microbial response to charcoal amendments of highly weathered soils and Amazonian dark earths in Central Amazonia-preliminary results. In: Amazonian dark earths: explorations in space and time. Springer, Berlin, pp 195–212
Sturz AV, Nowak J (2000) Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. J Appl Soil Ecol 15:183–190
Sudhakar P, Chattopadhyay GN, Gangwar SK, Ghosh JK (2000) Effect of foliar application of Azotobacter, Azospirillum and Beijerinckia on leaf yield and quality of mulberry (*Morus alba*). J Agric Sci 134:227–234
Sulieman S (2011) Does GABA increase the efficiency of symbiotic N2 fixation in legumes? Plant Signal Behav 6:32–36

Summerell B A, Leslie JF, Backhouse D, Bryden WL, Burgess LW (2001) *Fusarium*: paul E. Nelson memorial symposium. APS Press. The American Phytopathology Society, St. Paul, p 392
Takagi S, Nomoto K, Takemoto T (1984) Physiological aspect of mugineic acid, a possible phytosiderophore of graminaceous plant. J Plant Nutr 7:469–477
Tang CS, Cai WF, Kohl K, Nishimoto RK (1995) Plant stress and allelopathy. In: Inderjit KMMD, Einhellig FA (eds) Allelopathy: organisms, processes, and applications, vol 582. Acs Symposium Series Amer Chemical Soc, Washington, pp 142–157
Tepfer D, Goldmann A, Pamboukdjian N, Maille M, Lepingle A, Chevalier D, Dénarié J, Rosenberg C (1988) A plasmid of rhizobium meliloti 41 encodes catabolism of two compounds from root exudate of *Calystegia sepium*. J Bacteriol 170:1153–1161
Terakado-Tonooka J, Ohwaki Y, Yamakawa H, Tanaka F, Yoneyama T, Fujihara S (2008) Expresses nifH genes of endophytic bacteria detected in field-growth sweet potatoes (Ipomoea Batata L.) Microb Environ 23:89–93
Thaweenut N, Hachisuka Y, Ando S, Yanagisawa S, Yoneyama T (2011) Two seasons' study on nifH gene expression and nitrogen fixation by diazotrophic endophytes in sugarcane (Saccharum spp. hybrids): expression of nifH genes similar to those of rhizobia. Plant Soil 338:435–449
Torres AR, Kaschuk G, Saridakis GP, Hungria M (2012) Genetic variability in Bradyrhizobium japonicum strains nodulating soybean *Glycine max* (L.) Merrill. World J Microbiol Biotechnol 28:1831–1835
Torsvik V, Øvreås L, Thingstad TF (2002) Prokaryotic diversity – magnitude, dynamics, and controlling factors. Science 296:1064–1066
Turlings TCJ, Hiltpold I, Rasmann S (2012) The importance of root-produced volatiles as foraging cues for entomopathogenic nematodes. Plant Soil 358:47–56
Uroz S, Dessaux Y, Oger P (2009) Quorum sensing and quorum quenching: the yin and yang of bacterial communication. Chembiochem 10:205–216
Validov S, Mavrodi O, De La Fuente L, Boronin A, Weller D, Thomashow L, Mavrodi D (2005) Antagonistic activity among 2,4-diacetylphloroglucinol-producing fluorescent Pseudomonas spp. FEMS Microbiol Lett 242:249–256
Van Ness GB (1971) The ecology of anthrax. Science 172:1303–1307
Van Peer R, Schippers B (1989) Plant growth res ponses to bacterization and rhizosphere microbial development in hydroponic culture. Can J Microbiol 35:456–463
Vance C, Uhde-Stone C, Allan DL (2003) Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. New Phytol 157:423–447
Verbeek RE, Banaay CG, Sikder M, De Waele D, Vera Cruz CM, Gheysen G, Höfte M, Kyndt T (2016) Interactions between the oomycete Pythium arrhenomanes and the rice root-knot nematode Meloidogyne Graminicola in aerobic Asian rice varieties. Rice 9:36
Voisard C, Keel C, Haas D, De ´f G (1989) Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. EMBO J 8:351–358
Voss KA, Bacon CW, Meredith FI, Norred WP (1996) Comparative subchronic toxicity studies of nixtamalized and water-extracted *Fusarium moniliforme* culture material. Food Chem Toxicol 34:623–632
Walker V, Couillerot O, Von Felten A, Bellvert F, Jansa J, Maurhofer M, Bally R, Moenne-Loccoz Y, Comte G (2012) Variation of secondary metabolite levels in maize seedling roots induced by inoculation with Azospirillum, Pseudomonas and Glomus consortium under field conditions. Plant Soil 356:151–163
Warnock DD, Lehmann J, Kuyper TW, Rillig MC (2007) Mycorrhizal responses to biochar in soil–concepts and mechanisms. Plant Soil 300:9–20
Waters V, Gómez M, Soong G, Amin S, Ernst R, Prince A (2007) Immunostimulatory properties of the emerging pathogen *Stenotrophomonas maltophilia*. Infect Immun 75(4):1698–1703
Webster JM (1995) The host-parasite relationships of plant-parasitic nematodes. Advances in parasitology, vol 36. Academic Press Inc.
Wei G, Kloepper JW, Tuzun S (1996) Induced systemic resistance to cucumber diseases and increased plant growth by plant growth promoting rhizobacteria under field conditions. Phytopathology 86:221–224.

Weinstein KB (2011) *Listeria monocytogenes* treatment & management. http://emedicine.medscape.com/article/220684-treatment

Weller DM, Raaijmakers JM, McSpadden Gardener BB, Thomashow LS (2002) Microbial populations responsible for specific soil suppressiveness to plant pathogens. Annu Rev Phytopathol 40:309–348

Werner SB, Pappagianis D, Heindl I, Mickel A (1972) An epidemic of coccidioidomycosis among archeology. N Engl J Med 286:507–512

Wharton PS, Tumbalam P, Kirk WW (2006) First report of potato tuber sprout rot caused by *Fusarium sambucinum* in Michigan. Plant Dis 90:1460–1464

Whitehead NA, Barnard AM, Slater H, Simpson NJ, Salmond GP (2001) Quorum-sensing in gram-negative bacteria. FEMS Microbiol Rev 25:365–404

Williams PL, Sable DL, Mendez P, Smyth LT (1979) Symptomatic coccidioidomycosis following a sever natural dust storm. Chest 76:566–570

Xu RQ, Wang JN, Chen JY, Dai XF (2010) Analysis of T-DNA insertional flanking sequence and mutant phenotypic characteristics in Verticillium dahliae. Sci Agric Sin 43:489–496

Yang H, Knapp J, Koirala P, Rajagopal D, Peer WA, Silbart LK, Murphy A, Gaxiola RA (2007) Enhanced phosphorus nutrition in monocots and dicots over-expressing a phosphorus-responsive type IH+−pyrophosphatase. Plant Biotechnol J 5:735–745

Yang J, Kloepper JW, Ryu CM (2009) Rhizosphere bacteria help plants tolerate abiotic stress. Trends Plant Sci 14:1–4

Young JC, DeWitt ND, Sussman MR (1998) A transgene encoding a plasma membrane H+−ATPase that confers acid resistance in *Arabidopsis thaliana* seedlings. Genetics 149:501–507

Yue H, Mo W, Li C, Zheng Y, Li H (2007) The salt stress relief and growth promotion effect of Rs-5 on cotton. Plant Soil 297:139–145

Zahir ZA, Munir A, Asghar HN, Shaharoona B, Arshad M (2008) Effectiveness of rhizobacteria containing ACC deaminase for growth promotion of peas (Pisum sativum) under drought conditions. J Microbiol Biotechnol 18:958–963

Zahran HH (2001) Rhizobia from wild legumes: diversity, taxonomy. Ecology, nitrogen fixation and biotechnology. J Biotechnol 91:143–153

Zhao RM, Dielen V, Kinet JM, Boutry M (2000) Cosuppression of a plasma membrane H+−ATPase isoform impairs sucrose translocation, stomatal opening, plant growth, and male fertility. Plant Cell 12:535–546

Carriers and Their Role in Plant Agrosystem

12

Pragati Sahai and Vivek Kumar

Abstract

The population explodes and the concerns of biomagnifications by the use of synthetic pest control methods are two major problems that have created the major food crop crises in the world. To eradicate the problem, various green practices like bioformulations, mixed cropping, etc. have been designed and implicated, but almost all of them had delivery constraints, and to minimize this, effective delivery model was needed. The researchers in the quest designed a model that was harmless, stable, and inert and that did not interfere with biocontrol activity against pest which can be used at time of harvesting and postharvesting as well as to increase the shelf life; such models were called as carriers. Various types of carriers have been studied and applied, but the rate of biocontrol is still yet to reach the optimum. So it becomes necessary to gain an insight into the constraints in effective biocontrol and retrospect the best practices to minimize the constraints.

This chapter throws light on carriers, their types, their formation and inoculation, and finally their role in plant agrosystem which will further help the researchers in designing the cost-effective and efficient carrier with minimum delivery constraints and eliciting maximum biocontrol to finally eradicate the use of synthetic pest control practices from the system.

12.1 Introduction

The plant diseases affecting the cultivation and production of crops are serious concerns in agriculture as they largely affect the quality and quantity of the crops. The human population in the world has now passed 7 billion, and it is

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expected to reach up to 11 billion by 2100 with a prediction of a 70% chance of a continuous increase in population (EEA 2015). Such population increases lead to a greater demand for food. Since food is the basic necessity for life, the human population cannot compromise on food security, not even at the cost of earth's sustainability. It has been estimated in 2015 that the current global population is two to three times higher than can be sustained by current food production levels and is already utilizing 50% more resources than the earth is producing (http://www.worldpopulationbalance.org).

Moreover, the high pest population in developing countries is a complex problem with rapid increase of 1.2% annually in the human population adding to the ecological burden (Reece et al. 2011).

Consequently, our overburdened resources are declining very rapidly. There are 50,000 species of bacterial and fungal phytopathogens and 8000 species of weeds which largely reduce crop yield and quality (Ortiz-Hernandez et al. 2013). According to several studies, it has been suggested that specific crop losses due to pests may vary between 10% and 90% (Youdeowei 1989). In India, Singh and Shekhawat (1999) stated that crop losses due to pests may be as high as 80% if the crop is not well protected.

12.1.1 Major Outbreaks of the World

The major devastating effect on crops by pests worldwide is still the basis for the development of effective pest control policies, and so it should always be referred to study the nature of the outbreak (Table 12.1).

Table 12.1 Major Outbreaks of the world

Wheat and barley head scab	One of the most devastating plant diseases in the world and is ranked by the United States Department of Agriculture (USDA) as the worst plant disease to hit the United States after rust epidemics in the 1950s (Schmale and Bergstrom 2003). During the twentieth century, wheat and barley crops in the United States were largely attacked by a fungus *Fusarium graminearum* which led to serious loss of 60–70% in most susceptible cultivars (Zhang and Ye 1993). Since 1990, wheat and barley farmers in the United States have lost over $3 billion due to Fusarium head blight epidemics (Schmale and Bergstrom 2003)
Southern corn leaf blight epidemic (1970)	In 1970, a newly emerged race *Cochliobolus heterostrophus* (race T) attacked the hybrid corn plants with T cytoplasm which constituted 80% of the corn grown in the United States at that time (Hooker 1972)
The Great Bengal famine (1943)	One of the most tragic famines due to "brown spot disease" of rice caused by *Helminthosporium oryzae* which resulted in the loss of three million lives due to starvation and malnutrition (Sen 1981)
Irish potato famine (1845–1850)	One of the most devastating epidemics from Ireland resulted in a massive crop failure due to "potato late blight" caused by the fungus *Phytophthora infestans*. At present, late blight of potato accounts for the loss of US$3.75 billion annually in developing countries (Singh and Singh 2005)

12.2 Disease Management Practices and Failures

In India, disease management practices (Lamichhane et al. 2015) including the heavy use of synthetic pesticides to prevent the crop loss of 30–40% due to insect, pests, weeds, and diseases were estimated to be approximately US$2 billion in 1995 (Gautam and Mishra 1995), and worldwide crop loss due to pests in 1996 was estimated to be approximately $500 billion per year even after the annual application of 2.5 million metric tons of pesticides and synthetic chemicals which approximately were valued at $31.25 billion (Pimentel 1997).

At present worldwide, various synthetic pesticides worth 5.6 billion pounds are used in the agriculture sector (Grube et al. 2011), but in the history of agriculture, the use of pesticides has exerted a selection pressure on pests and pathogens which forced them to adapt according to their chemically modified habitats, and a consequence has been the evolution of "pesticide-resistant" varieties (Gould 1991) that was first documented by Melander in 1914. At least 447 pesticide-resistant arthropod species have been reported in the world (Callaghan et al. 1998). For example, resistance in the Colorado potato beetle (*Leptinotarsa decemlineata*) costs Michigan potato producers $16 million for crop losses in 1991 (Duchesne et al. 2001). In Brazil, the increased use of 234% in insecticides, 548% in fungicide, and 5414% in herbicides over a period of 15 years, from 1964 to 1979, resulted in an increase of only 16.8% in the production of 15 major crops (FAO 1986) that shows pest resistance against the disease management practices. Second failure of the practices was deposition of pesticide residue in food crops that eventually entered the food chain leading to biomagnifications of the pesticide. The total number of pesticide poisonings in the United States alone was 300,000 per year as estimated by EPA (1992). The studies on fruit samples of ber, grapes, and guava detected DDT, endosulfan, and HCH pesticides in almost all the samples as reported by Kumari et al. (2006). Chen et al. (2011) evaluated the residues of organophosphates and pyrethroids in fruits and vegetables collected from Xiamen, China, and found that out of 1135 samples, 37.7% contained pesticide residues. Dureja et al. (2015) stated that even the Crop Care Federation of India (CCFI) in organic farms uses chemical pesticides to protect their crops.

Thus, the current agricultural practices are not only contributing toward ecological degradation, but as the issue of food security is of prime importance, researchers are concerned to find better and safe alternatives to synthetic agrochemicals as food crops are highly susceptible to be attacked by many pathogens not only at all stages of their growth but also during postharvest storage which is largely controlled by pesticides (Gasic and Tanovic 2013).

The use of chemicals as pesticides is a common practice however with environmental concerns, and health safety biocontrol has been found to be the best practice in controlling the plant pathogens (Fig. 12.1). The **bacterial antagonism** is also an effective pest management practice (Chen et al. 2013). Plant symbionts or mutualists possess strong biocontrol potential as well as plant growth-enhancing capabilities (Fig. 12.2) (Tronsmo and Dennis 1977; Wilson and Pusey 1985; Cook 1990; Barkai-Golan 2001; Compant et al. 2005; Kavitha et al. 2003; Tewari and Arora 2014). In this context, bacterial populations in the soil which have the capability to

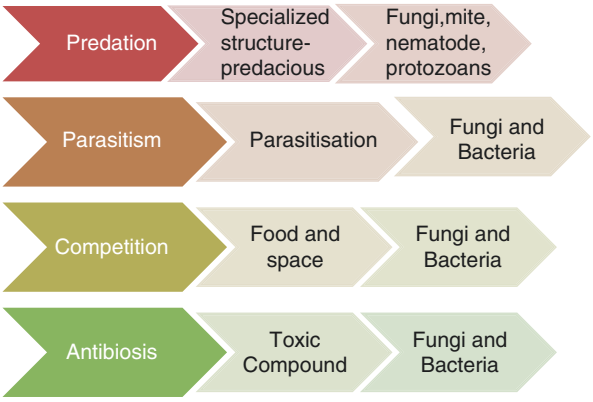

Fig. 12.1 Four mechanism of biocontrol

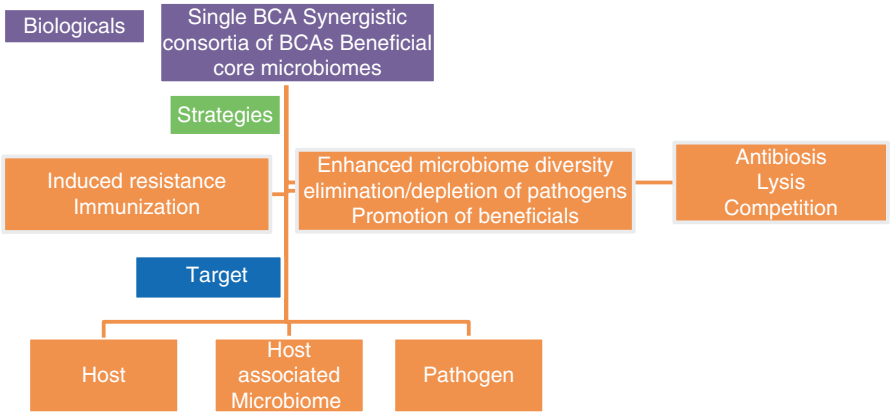

Fig. 12.2 Green revolution approach in agriculture

aggressively colonize the plant root system (i.e., rhizobacteria) and internal plant tissues (i.e., endophytic bacteria) are of considerable interest (Haas and Defago 2005; Backman and Sikora 2008; Lugtenberg and Kamilova 2009). Successful applications of antagonistic bacteria under field conditions have been evidenced from various case studies all over the world (Table 12.2). In Costa Rica, the use of dieldrin pesticide (over 12,000 ha) was stopped, and thereafter, the outbreak of six major pest infestations was suppressed by their natural enemies which started to colonize the area after cessation of pesticide use (Stephens 1984). Other examples illustrating the impact of natural enemies of plant pathogens are the use of *Bacillus thuringiensis* and the release of natural enemies like *Trichoderma* sp. on tomato crops in Colombia which over an area of 2000 ha have reduced the pesticide application from 20–30 times to 2–3, saving $650 per hectare (Belloti et al. 1990). In Sudan and Egypt, the total cost to protect the cotton crop from bollworm and white-fly reduced from 33.3% (in 1985–1986) to 19.3% (in 1988–1989) by using

Table 12.2 Some bacterial biocontrol agents against different pests of food crops

Bacteria	Target pest	Crop	References
Pseudomonas fluorescens	*Erwinia carotovora* *Gaeumannomyces graminis var. tritici* *Fusarium glycinia* *Sarocladium oryzae* *Puccinia ultimum*	Potato Wheat Wheat Soybean Sugar beet	Shaikh and Sayyed (2015), De Souza et al. (2003), and Shaikh and Sayyed (2015)
Pseudomonas putida	*Fusarium solani* *E. carotovora*	Beans Potato	Shaikh and Sayyed (2015)
Pseudomonas cepacia	*Fusarium oxysporum* *Bipolaris maydis*	Onion Maize	Shaikh and Sayyed (2015)
Azospirillum brasilense	*Pseudomonas syringae* *Fusarium* sp. *Rhizoctonia* sp. *Pythium* sp. *Sclerotinia* sp. *Pythium aphanidermatum* *Colletotrichum acutatum*	Tomato Cucumber	Bashan and Bashan (2002) and Hassouna et al. (1998)
Azospirillum lipoferum	*Heterodera avenae* (nematode)	Wheat	Bansal et al. (1999)
Azospirillum spp. *Bacillus pumilus* *Mesorhizobium loti*	*Striga hermonthica* (witchweed) *G. graminis* var. tritici *Sclerotinia sclerotiorum*	Wheat Mustard	Shaikh and Sayyed (2015) and Chandra et al. (2007)
Rhizobium leguminosarum bv. *phaseoli* strain RRE6	*Rhizoctonia solani*	Rice *Oryza sativa*	Mishra et al. (2006)
Rhizobium meliloti *Enterobacter* spp. *Streptomycetes*	*Macrophomina phaseolina* *R. solani* *F. solani* *Pythium* *Botrytis* *S. sclerotiorum*	Sunflower Okra Pea Apple Potato Tomato	Haque and Ghaffar (1993), Arora et al. (2001) and Shaikh and Sayyed (2015)

mechanical and biological control measures (Oudejans 1991). Sustainable agricultural practices including improved mechanical, cultural, and biological approaches could reduce pesticide application up to 50% saving $1 billion (Peschin 2002).

Among various groups of microbial biocontrol agents, bacteria are able to grow in wounds or damaged crop product but not on the undamaged surfaces of fruits, vegetables, etc. which make them suitable for their application not only in soil but also during storage or in the postharvest environment (Smilanick 1994; Bissonnette & Lalande 1988; Bouillant et al. 1997). Moreover, bacterial biopesticides are target-specific, rapidly multiplying, easy to handle, nontoxic, and economically suitable organisms with better survival and longevity (Usta 2013). Recent investigations in the search for more stable bacterial inoculants have drawn the attention of researchers toward endophytic bacteria. Endophytes remain well protected from fluctuating environmental conditions and biotic factors as they colonize the internal tissues of host plants and, therefore, have a competitive advantage over bacterial populations present in rhizosphere or

phyllosphere (Backman and Sikora 2008) and thus are promising biocontrol agents for the development of high-efficiency formulations. However, the bioformulations which exhibited potent biocontrol activity against their target pests in laboratories are not easy to use with equal efficiency under field conditions as undetermined factors in the environment as well as inter- and intraspecific competition with other organisms in their niche affect their growth, physiology, metabolism, and gene expression in several ways (Khare and Arora 2015), so well-formulated preparations of bacteria are done to increase the possibility of their optimum performance and commercial success in agro-food production (Bashan et al. 2014; Mari et al. 2003).

12.3 Commercial Bioformulation in the Market

As a part of green revolution and taking of a holistic approach, bioformulation can be defined as a ready-to-use formulation, containing living cells or their metabolites (of one or more strains), supported by nontoxic and inert compounds to maintain the viability and efficiency of cells or metabolites and to increase their shelf life.

Listed below are some of the important commercially available bioformulation (Table 12.3).

The percentage of application of biocontrol products still represents only 1% of the agricultural control measures to manage plant diseases, while chemical fungicide takes up the 15% stake in plant disease management.

The reason behind is the inefficacy in application of effective biocontrol. The various bioformulation types like **liquid formulation** (Singleton et al. 2002; Knowles 2005), **emulsions** (Brar et al. 2006; Gasic and Tanovic 2013), **dry formulations** (Gasic and Tanovic 2013; Brar et al. 2006; Knowles 2008), **dust formulations** (Knowles 2001), **powder seed treatment** (Woods 2003), **granules** (Tadros 2005; Knowles 2005; Lyn et al. 2010), **wettable powders** (Brar et al. 2006; Knowles 2005), and **water-dispersible granules** (Knowles 2008) also exhibited constraints in delivery, so as per Malusa et al. 2012, there are two widely applied methods which are **seed inoculation** and **soil inoculation**.

Seed coating methods have been relatively successful when applied to small volumes of soil under greenhouse conditions, but these are limited by failure of the biocontrol agents. In addition, antibiotic-producing biocontrol agents may have deleterious effects upon the seed if applied directly to the seed coat.

The field use of bioinoculation or bioformulation is largely hampered by the lack of suitable carrier. The scientists have been in process of finding effective carrier to introduce bioformulation in to the soil.

Table 12.3 Commercial bioformulation in the market

Bioinoculant used	Target pest	Food crop	References
Pseudomonas syringae	*Botrytis cinerea*, *Mucor piriformis*, *Geotrichum*, *Penicillium* sp.	Citrus and pome fruit	Shaikh and Sayyed (2015)
P. syringae ESC 11	*B. cinerea*, *Penicillium* spp., *M. piriformis*, *Geotrichum candidum*	Pome fruits and sweet potatoes	Mari et al. (2003)
Pseudomonas fluorescens	*Erwinia amylovora*	Almond, cherry, apple, potato, and tomato	Shaikh and Sayyed (2015)
Bacillus subtilis	Phytopathogenic fungi	Cotton and legumes	Shaikh and Sayyed (2015)
Streptomyces sp.	*Fusarium*, *Alternaria*, *Pythium*	Vegetable crops	Shaikh and Sayyed (2015)
Agrobacterium radiobacter	*Agrobacterium tumefaciens*	Fruit, nut, and ornamental	Shaikh and Sayyed (2015)
Streptomyces lydicus WYEC 108	*Pythium*, *Phytophthora*, *Fusarium*, *Rhizoctonia*	Food crops susceptible to root rot and damping-off fungi	Mishra et al. (2015)
Bacillus pumilus QST 2808	Powdery mildew, downy mildew, and rust fungi	Food crops susceptible to powdery mildew, downy mildew, and rust fungi	Mishra et al. (2015)
P. fluorescens A506	*E. amylovora*	Pome fruits	Stockwell and Stack (2007)
Streptomyces griseoviridis K61	*Fusarium*, *B. cinerea*, *Rhizoctonia*, *Phytophthora*	Vegetable crops	Mishra et al. (2015)

Biofertilizers prepared as carrier-based inoculants contain effective microorganisms which include rhizobia, nitrogen-fixing rhizobacteria, plant growth-promoting rhizobacteria, phosphate-solubilizing bacteria, and so on. Incorporation of microorganisms in carrier material enables easy handling, long-term storage, and high effectiveness of biofertilizers. Basically, the carrier-based inoculant of these bacteria can be prepared by a common procedure. In this chapter, type of carrier materials available for biofertilizers and preparation in general of carrier-based inoculants will be described. Various researchers as Arora et al. (2010) have defined bioformulations in diverse ways as biologically active products containing one or more beneficial microbial strains in easy-to-use and economical carrier materials.

12.4 Carriers in Modern Agricultural Practices

The vehicle that is used to deliver the live microorganism from in vitro conditions (laboratory) to in vivo conditions (Field) is known as **carrier.**

According to the *Handbook for Rhizobia* (Somasegaran and Hoben 1994), the properties of a good carrier material for seed inoculation are (1) nontoxic to inoculant bacterial strain, (2) good moisture absorption capacity, (3) easy to process and free of lump-forming materials, (4) easy to sterilize by autoclaving or gamma-irradiation, (5) available in adequate amounts, (6) inexpensive, (7) good adhesion to seeds, and (8) good pH buffering capacity. Needless to say, (9) nontoxic to plant is another important property (Fig. 12.3).

Properties of a Good Carrier

1. It should be stable.
2. It should be able to deliver.

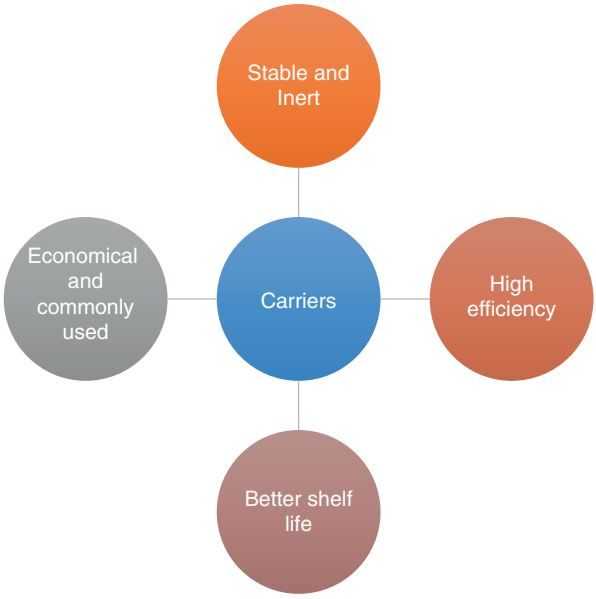

Fig. 12.3 Ray diagram to illustrate the properties of a carrier

3. It should be inert so that it does not interfere with microbial flora.
4. The bioformulation stabilized should be delivered with highest efficiency, that is, the carrier should be able to deliver the right number of viable cells under the right physiological condition at the right time (also defined as specific efficiency of the carrier).
5. It should provide better shelf life to the bioformulation.
6. It should be easily available and economical.

12.5 Types of Carriers

There are four types of carriers (Fig. 12.4):

1. Soils (peat, clay, silt, and inorganic soil) (Singh and Sharma 1973; Chao and Alexander 1984; Kotb and Angle 1986)
2. Plant waste material (mulch, sawdust, and compost), composts, farmyard manure, soybean and peanut oil (Kremer and Peterson 1982), wheat bran (Jackson et al. 1991), agricultural waste material (Sadasivam et al. 1986), sawdust (Arora et al. 2008), spent mushroom compost (Bahl and Jauhri 1986), and plant debris (Richter et al. 1989)

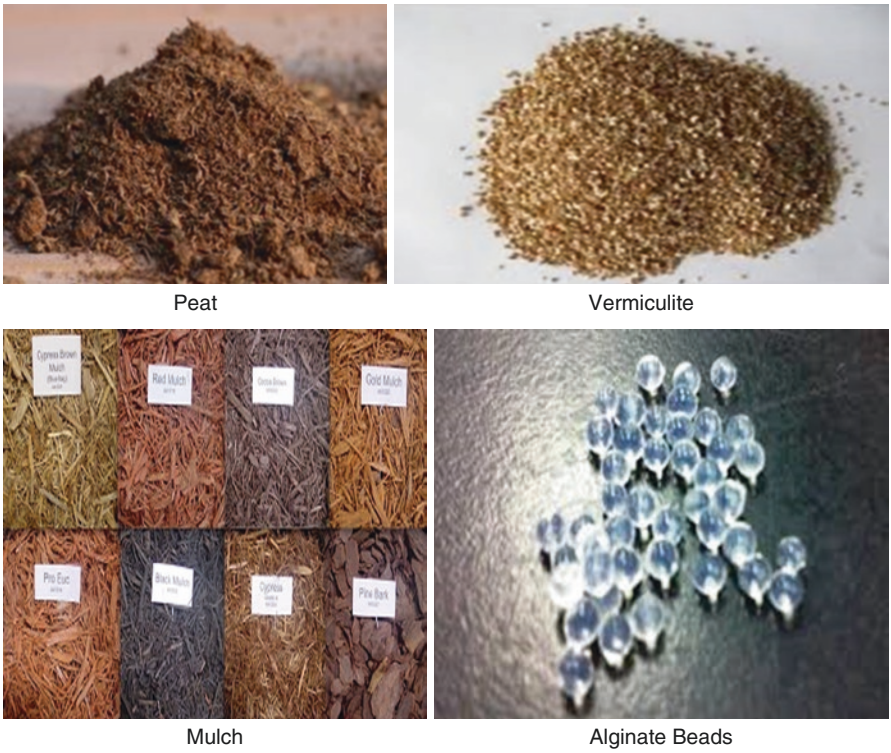

Fig. 12.4 Different types of Carriers in common use

3. Inert materials (polyacrylamide gels, alginate beads, talc)
   Vermiculite (Paau 1988; Sparrow and Ham 1983a, b), perlite (Daza et al. 2000), ground rock phosphate, calcium sulfate, polyacrylamide gels (Dommergues et al. 1979), and alginate beads (Aino et al. 1997; Sougoufara et al. 1989)
4. Plain Lyophilized Microbial Cultures

The carrier along with inoculants comes in four dispersal forms as in powders, slurries, liquids, and granules.

However in 1984, Taber et al. told about lignite-stillage carrier system for biocontrol of fungal pathogen. This carrier system was not only easy and economical but it acted as nutrient culture for biocontrol agent and was unique in the study as carrier and substrate system for impregnation of biocontrol agent to soil. After this study, many carrier-substrate systems were made for application of biocontrol agent.

Various types of material are used as carrier for seed or soil inoculation (Singh et al. 2014). For preparation of seed inoculant, the carrier material is milled to fine powder with particle size of 10–40 μm.

12.6 Sterilization of Carrier Material

Sterilization of carrier material is essential to keep high number of inoculant bacteria on carrier for long storage period.

Gamma-irradiation is the most suitable way of carrier sterilization, because the sterilization process makes almost no change in physical and chemical properties of the material. Briefly in the process of sterilization of carrier material, it is packed in thin-walled polyethylene bag and then gamma-irradiated at 50 kGy (5 Mrads).

12.6.1 The Necessity of Radiation Sterilization

The purpose of sterilization of carrier materials for biofertilizer can be for two reasons:

- To offer nutrient and place to the inoculant bacteria against the occupation by the contaminated and/or native bacteria so that the number of inoculant bacteria on carrier during the storage period before use can be kept.
- To prevent undesirable dispersion of pathogenic bacteria to agricultural field thus radiation sterilization is essential to reduce the risk of field contamination and infection.

Another way of carrier sterilization is autoclaving. Carrier material is packed in partially opened, thin-walled polypropylene bags and autoclaved for 60 min at 121 °C. It should be noted that during autoclaving, some materials change their properties and produce toxic substance to some bacterial strains. So before inoculation, the properties should be thoroughly screened.

12.7 Different Process of Formation of Carrier-Based Bioformulation

Most of the bacteria in biofertilizer have close relationship with plant roots. *Rhizobium* has symbiotic interaction with legume roots, and rhizobacteria inhabit on root surface or in soil rhizosphere. To achieve the successful inoculation of *Rhizobium* or rhizobacteria, large population of the bacterial strain must be placed close to the emerging root, so that the majority of nodules are formed by the inoculated rhizobial strain and that the inoculated rhizobacterial strain occupies the rhizosphere as a major member of rhizobacteria. If the population is not large enough, the native rhizobia/rhizobacteria will occupy most of the root nodules/rhizosphere, leading to unsatisfactory effect of inoculation. Therefore for effective inoculation, different techniques are employed with help of carriers.

12.7.1 Seed Inoculation

The most common way of inoculation is "seed inoculation" (Brockwell 1977; Bashan et al. 2014), in which the inoculant (bacteria-carrier mixture) is mixed with water to make a slurry form and then mixed with seeds. In this case, the carrier must be a form of fine powder. To achieve the tight coating of inoculant on seed surface, use of adhesive, such as gum, ethyl methyl cellulose, sucrose solutions, and vegetable oils, is recommended. Any locally available sticky material, which is nontoxic to bacteria and seeds, can be used as adhesive.

Peat is the most frequently used carrier material for seed inoculation (Bashan 1998). Peat-based rhizobial inoculant is already used in many countries, and a number of information are available on the properties and effect of the inoculants. However, seed inoculation may not always be successful, i.e., the inoculation resulted in low nodule occupancy of the inoculated rhizobial strain or low establishment of the inoculated rhizobacterial strain. This might be due to low population and/or low survival of the inoculated bacterial strain on the seed surface and in the soil.

12.7.2 Soil Inoculation

Seed inoculation may not always be successful, that is, inoculation resulted in low nodule occupancy of the inoculated rhizobial strain or low establishment of the inoculated rhizobacterial strain. This might be due to low population and/or low survival of the inoculated bacterial strain on the seed surface and in the soil. In such instance, "soil inoculation" will be adopted (Bashan et al. 2014), whereby a large population of a bacterial strain can be introduced into the soil. For soil inoculation in general, granular inoculant is placed into the furrow under or alongside the seed. This enhances the chance for the inoculated strain to be in contact with plant roots.

For soil inoculation, carrier material with granular form (0.5–1.5 mm) is generally used. Granular forms of peat, perlite, charcoal, or soil aggregates are suitable for soil inoculation.

12.8 Preparation of Carrier Material for Sterilization and Inoculation of Microorganism to Carrier

For this the following steps are followed:

- Prepare the appropriate amount of carrier material (10 kg is recommended).
- Divide into ten polyethylene packages (thickness, approx. 0.1 mm; size, approx. 20 cm × 30 cm with 1 kg carrier).
- Seal the packages using a heat sealer.
- If the carrier is a highly dry material, wet with an appropriate amount of water (to increase the indirect effect of radiation).
- If the presence of spore-forming bacteria is suspected in the carrier, add an appropriate amount of nutrient liquid medium (to promote the germination of spore).
  - Then irradiation is done by the following steps:
    Divide the carrier packages into two dose groups.
    Irradiate each group by 25 kGy or 50 kGy of γ-rays at room temperature in the atmosphere.
    In almost all cases, radiation sources are cobalt-60 or cesium-137.
    Irradiation dose can be controlled by changing the distance from the radiation source. The total irradiation time is dependent on the source activity. (option: instead of γ-rays, electron beams can be used for radiation sterilization).
    A margin of error of plus or minus 10% is allowed for irradiation dose. No limit for dose rate. A short interruption of irradiation during the total time for required dose can be allowed.
    After irradiation, preserve the irradiated packages at room temperature under the sealed condition until the inoculation of microorganisms.
  - Then confirmation of sterilization effect is done by the following methods:
    Prepare 1 g of carrier samples (nonirradiated, 25 kGy and 50 kGy irradiated samples).
    Mix with 9 ml of sterile water to make suspension.
    Dilute the suspension by serial tenfold dilutions using sterile water and spread on nutrient agar plates.
    Incubate (at 30 °C in general) and count bacterial colony number.
    Prepare 1 g of carrier samples (nonirradiated, 25 kGy and 50 kGy irradiated samples).
  - Finally inoculation of microorganisms to carrier is done by the following ways:
    Prepare starter culture for inoculation. Optionally, appropriately dilute with sterile water for moisture and cell number adjustment.

Inject the culture to the carrier package using a sterile disposable plastic syringe with a needle. Seal the needle hole with a waterproof tape.

– Keep the package at appropriate temperatures for maturation and storage as the temperatures suitable for maturation and storage are dependent on the inoculants microorganisms; however 30 °C for maturation and 20 °C–30 °C for storage will be suited for inoculants in most cases.

12.9 The Role of Carrier in Plant Disease Management

The essential criteria to be considered for carrier selection relating to survival of the inoculant bacteria are the following:

- Survival of the inoculants bacteria on seed. Seeds are not always sown immediately after seed coating with the inoculant bacteria. The bacteria have to survive on seed surface against drying condition until placed into soil.
- Survival of the inoculants bacteria during the storage period.
- Survival of the inoculant bacteria in soil. After being introduced into the soil, the inoculant bacteria have to compete with native soil microorganisms for the nutrient and habitable niche and have to survive against grazing protozoa. Such carrier materials that offer the available nutrient and/or habitable micropore to the inoculant bacteria will be desirable. In this sense, materials with microporous structure, such as soil aggregate and charcoal, will be good carrier for soil inoculants.

12.10 The Role of Carriers in Effective Delivery and Commercial Success of Bioformulation

In bioformulation preparation, carriers are the main ingredients that help to deliver bioinoculant to the field in good physiological condition and are crucial for the commercial success of bioformulations (Marjan et al. 2011). Since carrier materials play an important role in bioinoculant performance and survival in the field, they must be chosen carefully to assure easy field applicability at a minimum cost (Table 12.4). A carrier material must be easy to use, compatible with the seeding equipment at the time of seeding, stable under different field conditions and types of soil, able to help prolong the survival of the inoculated bacteria, have a long shelf life, and be harmless to nontarget organisms (Malusa et al. 2012; Bashan et al. 2014; Einarsson et al. 1993). Easy applicability of bioformulations is largely dependent on their physical form which is determined by the carrier material used in these preparations. Where various kinds of soil and organic materials like peat, clay, compost, agricultural waste, sawdust, wheat bran, etc. are used in solid formulations, liquid inoculants can be based on broth cultures, minerals or organic oils, or oil-in-water suspensions.

Table 12.4 Carriers materials used for biofertilizers

Carrier material	Inoculant bacterium	Characteristics
Sterilized oxalic acid industrial waste	*Rhizobium*	– Seed inoculation (Kaushal et al. 1996) – *Rhizobium* multiplication in carrier in ambient temperature up to 90 days – Carrier sterilization contributed significant increase in grain yield, nodule number, and nitrogen content
Alginate-perlite dry granule	*Rhizobium*	– Soil inoculation – *Rhizobium* strains survived in dry granules beyond 180 days – The inoculants can be stored in a dry state without losing much viability
Composted sawdust	*Bradyrhizobium*, *Rhizobium*, and *Azospirillum*	– Seed inoculation (Kostov & Lynch 1998) – Good growth and survival of the inoculant strains
Agriperlite, expanded clay, kaolin, Celite, diatom, porosil MP, Micro-cel, vermiculite	Agrobacterium radiobacter K84	– Crown gall control (Pesenti-Barili et al. 1991) – Screening was performed to find improved formulation of K84 cells – Effect of carrier storage temperature and carrier water content on survival of K84 was examined
Cheese whey grown cells in peat	*Rhizobium meliloti*	– Seed inoculation – Better survival at various temperatures during storage, even under desiccation
Mineral soils	*Rhizobium*	– Seed inoculants – *Rhizobium* survived better at 4 °C than at higher temperature
Coal	*Rhizobium*	– Seed inoculants (Paczkowski & Berryhill 1979) – Seven among eight tested coals supported the growth and survival of *R. phaseoli* strains. Most contained more than 107 rhizobia per g after 12 months
Granular inoculants amended with nutrients	*Bradyrhizobium japonicum*	– Soil inoculants (Fouilleux et al. 1996) – Bentonite granules, illite and smectite granules, or silica granules amended with glycerol and Na glutamate and inoculated with either peat or liquid *Bradyrhizobium japonicum* inoculants – Enhanced early nodulation of soybean and increased N content of grain
Soybean oil or peanut oil added with lyophilized cells	*Rhizobium*	– Seed inoculants – Provide more protection than peat-based inoculant when rhizobia are inoculated on seeds and exposed to condition of drought and high temperature
Perlite	*Rhizobium*, *Bradyrhizobium*, *Bacillus*	– Seed inoculants – Combination of a sucrose adhesive with the perlite carrier gave better survival of bacteria on seeds – Produced similar number of nodules, nodule dry weight, crop yield, and nitrogen content as peat-based inoculants

Table 12.4 (continued)

Carrier material	Inoculant bacterium	Characteristics
Wastewater sludge	*Sinorhizobium meliloti*	– Seed inoculants – Result showed the suitability of using sludge as a carrier because it had the same or a higher potential than peat to support survival of *S. meliloti*
Wheat bran, sugarcane bagasse	*Rhizobium*, *Bradyrhizobium*, and rock-phosphate-solubilizing fungus *Aspergillus niger*	– Soil inoculants (Hedge & Brahmaprakash 1992) – The number of cultured microorganisms was the highest with peat, followed by bran and sugarcane bagasse
Nutrient-supplemented pumice	*Rhizobium*	– Seed inoculants – Good storage and handling properties and could be mixed directly with the seeds during the sowing process

12.11 The Role of Carrier in Plant Agrosystem

12.11.1 As an Important Component of Bioformulation

There have been many articles stating the use of carriers and its roles in modern practices of plant disease management. The harmful effect of chemical pesticides is evident, and from the last two decades, efforts are being made to replace them with biopesticides, and for this, the isolates of plant growth-promoting bacteria with fungicidal property have to be successfully delivered to the soil, expressing maximum activity. To achieve this, isolates of biocontrol agents are formulated by using different organic and inorganic carriers by process of solid or liquid fermentation. The isolates are then applied as seed treatment, matrix priming, foliar spray, sucker treatment, soil treatment, seedling dip, and fruit spray (Bhattacharjee and Dey 2013).

12.11.2 To Increase the Shelf Life of Biocontrol Agent

One of the tables mentioned in *African Journal of Microbiology Research*, 2013 by R. Bhattacharjee and Utpal Dey shows the shelf life of different biocontrol agents in presence of different carriers (Table 12.5).

This chart clearly states how two bacterial strains formulated in different carriers have shown different shelf lives like *B. subtilis* formulated in talc had shelf life of only 45 days whereas when formulated in peat supplemented with chitin had shelf life of 6 months.

Even fly ash was found to be good carriers for biofertilizer strains, and it is comparatively cheaper than other carriers available in the market as stated by Kumar (2014), in his paper on fly ash as carrier to study the biocontrol, characterization, and shelf life of a locally isolated biofertilizer strains.

Table 12.5 The shelf life of different biocontrol agents in presence of different carriers

Formulation	Shelf life	Bacteria	Reference
Talc	12 months	*P. fluorescens* (p7nf tl3)	Ceaser and Burr (1991)
Talc	8 months	*P. fluorescens* (pf1)	Vidhyasekaran et al. (1997)
Talc	45 days	*B. subtilis*	Amer and Utkhede (2000)
Talc	6 months	*P. putida*	Bora et al. (2004)
Lignite	4 months	*P. fluorescens* (pf1)	Vidhyasekaran et al. (1997)
Peat with chitin	6 months	*B. subtilis*	Manjula and Podile (2001)

12.11.3 As a Facilitator in Microbial Activity

Arjomandzadegan et al. (2013), in their paper "Evaluation of Appropriate Carriers for Bio-control Agents of Apple Fire Blight," have mentioned about the carrier as an important role in biocontrol for survival of microorganisms. The aim of this study was to evaluate different compounds as carriers for *Pseudomonas fluorescens* and *Erwinia herbicola* that are used as biocontrol agents in Iran. Different compositions were prepared as carriers including peat, bagasse, bagasse-perlite, and bagasse-charcoal. The carrier was found to be of a good composition that could significantly retain bacteria viable for 6 months, and according to these criteria, all the formulae were suitable as carriers at 4 °C; however, bagasse was the best carrier at room temperature, because the numbers of bacteria were changed from 8.7×10^7 CFU/g after inoculation to 1.5×10^9 CFU/g after 6 months for *P. fluorescens* and from 2.53×10^8 CFU/g after inoculation to 1.13×10^8 CFU/g after 6 months for *E. herbicola*, and even the pH variation was not sensible in bagasse. These findings were suggestive for application of bagasse as a suitable carrier as it is nature friendly, cheap, and easily available in Iran.

12.11.4 As a Sole Source of Carbon and Energy

Vanvuurde et al. (2010) in their paper used processed manure as carrier to introduce *Trichoderma harzianum* to study population dynamics and biocontrol effect on *Rhizoctonia solani*. The antagonistic fungi could grow and sporulate on the processed manure that acted as the sole source of carbon and nutrients; thus, the incorporation of conidia in pellets of the processed manure was shown to be feasible on a laboratory scale that led to the survival of the fungus in the pellets during storage. At times the best carrier after evaluation from the rest is enriched to provide the maximum field efficiency of bioformulation. Such study was done by Naveen Arora et al. (2014) where they enriched the best carrier sawdust with molasses from the rest of the six carriers including talc, fuller's earth, rice husk, sugarcane bagasse, charcoal, and wheat bran that were also evaluated for the production of bioformulation. Molasses-enriched sawdust-based formulation showed 48.43%, 52.02%, and 57.41% enhancement in dry weight with *Rhizobium* sp., *Pseudomonas* sp., and their co-inoculant, respectively, after 60 days of sowing. Results showed that enrichment

of carrier is expected to permit the retention of cell viability thus increasing the effectiveness of the active material. In 2011, the similar growth studies were done on sugar beet by development of bioformulation of *Pseudomonas fluorescens* and *Bacillus coagulans* using organic and inorganic carriers by Jorjani et al.

12.11.5 As Single Carrier for Multiple Bioinoculants

The researchers have been in continuous process of identifying the best carrier with high efficiency and also identifying a single delivery base for multiple bio-inoculants. Naveen Arora et al. (2008) suggested sawdust as the most powerful carrier to deliver single as well as in combination bio-inoculant. The study was done on five carriers including alginate beads, charcoal, sand, sawdust, and sugarcane bagasse that were evaluated for the production of bio-inoculants. Sawdust proved to be the best carrier in maintaining the bacterial population for both individual and co-inoculation. The co-inoculants containing both rhizobial and pseudomonad population proved much better in enhancing the seedling biomass and the nodule number. The sawdust-based co-inoculant and mono-inoculant were much better than any other carrier-based inoculants taken in the study.

Similar study was done by Arora et al. (2014) by co-inoculation of PGPR (*Rhizobium* and *Pseudomonas*). The aim of this study was to determine potential five different carrier materials for survival of PGPR (*Rhizobium* and *Pseudomonas* strain) isolated from *Trigonella foenum-graecum* at room temperature for 8 weeks. Samples from the carrier materials (sterilized and non-sterilized) were taken every week and tested for the survivability and sustainability of the two different PGPR in it by determining viable cell count (CFUg-1). The result showed that after 8 weeks of storage treatment of carrier coriander husk, sawdust, and bagasse stored at room temperature (25–28 °C) was able to sustain the highest viable cell number of co-inoculation of *Rhizobium* and *Pseudomonas* followed by their individual inoculation in the carrier and determination of individual CFUg-1. These two carriers also had acceptable changes in pH value and moisture content followed by wood ashes and sand.

12.11.6 For Treatment of Seed and Enrichment of Seedling

The carriers have also helped in treatment and enrichment of seedling. The study done on the enrichment of cotton seedling and its damping off by the development of new bioformulations by Ardakani et al. (2010b) stated that formulations included a talc-based powder and bentonite-based powder as mineral carriers and peat and rice bran as organic carriers for increasing stability in interaction between PGPR and cotton plants. The results of a greenhouse experiment, where these products were applied to cotton seeds, showed that all treatments except TAL-B2 were effective (up to 62.5% control) as compared to untreated seeds. The efficacy of mineral carriers and organic carriers' treatments was much higher than that of the standard carboxin-thiram fungicide treatment at all stages.

12.11.7 Carriers as Nanoparticles and Use of Nanotechnology

In case of living microbial cells or biopesticides, nanotechnology is a newly emerging field with potent agricultural implication that includes nanocides which are encapsulated pesticide/biopesticide nanoparticles (Ghormade et al. 2011) or nanomaterial-immobilized microbial enzymes/metabolites (Kim et al. 2006). Nanoparticles of microbial metabolites or whole cell formulations induce systemic activity due to smaller particle size, higher mobility, and lower toxicity in comparison to conventionally used pesticides (Sasson et al. 2007). Integration of biomolecules (e.g., enzymes, bioactive compounds, secondary metabolites, etc.) or whole microbial cells with nanostructures leads to hybrid systems that have numerous applications in agriculture (Bailey et al. 2010).

12.12 Conclusion and Future Prospects of Existing Green Practices

The above findings clearly state that formulations containing live bacterial cells need utmost care during production, packaging, storage, and until the end use which adds extra cost to the product (Arora et al. 2010); therefore for cost-effective green revolution, there is an important role of carriers in plant agrosystem. Secondly careful selection of a biocontrol agent prior to the development of a commercial product is necessary to avoid any possible threat so that public acceptance, adoption, and registration of bacterial formulations would become easier (Handelsman 2002).

Tewari and Arora (2014) studied bio-preparations containing exopolysaccharides (EPS) derived from fluorescent pseudomonads against *Macrophomina phaseolina*, causing charcoal rot in sunflower. They found that EPS-based formulation not only effectively controlled charcoal rot but also enhanced crop yield under saline conditions. Fluorescent pseudomonads are also known to produce bioactive secondary metabolites such as antibiotics and biosurfactants that are inhibitory to phytopathogens.

The use of **biosurfactants** is also gaining importance in green practices due to their effective biocontrol potential and nontoxic nature. Raaijmakers et al. (2006) studied *Pseudomonas putida* 267 which provides excellent biocontrol activity against Phytophthora damping-off of cucumber by producing putisolvin-like cyclic lipopeptides (CLPs), biosurfactants similar to the efficacy of biosurfactants produced by *Pseudomonas koreensis*, as a crude extract was investigated successfully against *Pythium ultimum* in hydroponic tomato cultivation by Hultberg et al. (2009).

The use of nanofactories is an emerging technique in bioformulation development in which engineered bioinoculants are used to enhance communication with plants through quorum sensing that leads to **biofilm formation**. Biofilm formation not only maintains sufficient bacterial population in soil but also protects the bioinoculant from fluctuating environmental conditions and provides them a competitive advantage. N-Acyl-L-homoserine lactones, quinolone produced by genus *Pseudomonas*, and autoinducer-2 produced by *Bacillus* are examples of signaling

molecules which not only trigger biofilm formation but also enhance antibiotic production and biocontrol activity of bacterial inoculants in soil (Tewari and Arora 2013; Ryan and Dow 2008; McNab et al. 2003).

Similarly the application of **selected carrier materials** for the bacterial inoculants proves to be beneficial to protect the bacteria and have long been practiced (Ardakani et al. 2010a).

In view of safe agricultural practices and high yield, incorporation of carrier system to bioformulation is very necessary (Abd-Alla MH and Omar SA 2001). Most of the bacteria included in biofertilizer have close relationship with plant roots. *Rhizobium* has symbiotic interaction with legume roots, and rhizobacteria inhabit on root surface or in rhizosphere soil. To achieve the successful inoculation of *Rhizobium* or rhizobacteria, large population of the bacterial strain must be placed close to the emerging root, so that the majority of nodules are formed by the inoculated rhizobial strain and that the inoculated rhizobacterial strain occupies the rhizosphere as major member of rhizobacteria. If the population is not large enough, the native rhizobia/rhizobacteria will occupy most of the root nodules/rhizosphere, leading to unsatisfactory effect of inoculation, and so the carrier-based inoculation becomes a good alternative. The success of microbial inoculation to promote growth of plant is vastly influenced by the number of introduced bacteria into the soil (Catroux et al. 1999).

Therefore it is important to find out the duration of the bacterial survivability in the respective carrier materials to ensure the desired level of bacterial population remains viable for the inoculants to sustain efficient. Simultaneously the selected carrier materials must also have the properties such as cost-effectiveness, dissolve well in water so that bacteria can be released, and able to tolerate harsh environmental conditions (FAO 1998).

The studies done on carrier system and in process will one day lead to development of advanced agricultural practices of biocontrol that will completely eradicate the use of chemical pesticides and fertilizers (Reban 2002). Use of certain waste and industrial by-products as carrier materials in bacterial formulations has been studied for their significant role in bacterial formulations, and they were found quite promising (Bashan et al. 2014).

The preparation of biofertilizers is usually carrier-based containing effective microorganism. This enables easy handling, long-term storage, and high effectiveness of biofertilizer. These biofertilizers consist of majorly rhizobia, nitrogen-fixing rhizobacteria, plant growth-promoting bacteria, phosphate-solubilizing bacteria, and so on, and their carrier-based inoculants are prepared by very simple procedures. According to the results of previous studies (Shah-Smith and Burns 1997), when PGPR are formulated using inorganic or organic carriers, their stability and durability are increased. In addition, their application particularly as seed treatment becomes easier and more practical.

However it is yet to be stated that from the existing green practices which one is the best. It is the emerging agricultural need that decides the green practice that has to be implemented. Thus if every time even one of the green practices is used for pest management then it will completely replace synthetic pest control practices one day leading ecological stability.

References

Abd-Alla MH, Omar SA (2001) Survival of Rhizobia/Bradyrhizobia and a rock phosphate-solubilizing fungus *Aspergillus niger* on various carriers from agro-industrial wastes and their effects on nodulation and growth of faba bean and soya bean. J Plant Nutr 24(2):261–272

Aino M, Maekaua Y, Mayama S, Kato H (1997) Biocontrol of bacteria wilt of tomato by producing seedlings colonized with endophytic antagonistic pseudomonads. In: Ogoshi A, Kobayashi K, Homma Y, Kodama F, Kondo N, Akino S (eds) Plant growth-promoting rhizobacteria-present status and future prospects. Faculty of Agriculture, Hokkaido University, Sapporo, pp 120–123

Amer GA, Utkhede RS (2000) Development of formulations of biological agents for management of root rot of lettuce and cucumber. Can J Microbiol 46:809–816

Ardakani SS, Hedari A, Tayebi L, Mohammadi M (2010a) Promotion of cotton seedlings growth characteristics by development and use of new bioformulations. Int J Bot 6:95–100

Ardakani SS, Heydari A, Khorasani N, Arjmandi R (2010b) Development of new bioformulations of *Pseudomonas fluoroscens* and evaluation of these products against damping-off of cotton seedlings. J Plant Pathol 92(1):83–88

Arjomandzadegan M, Salimi H, Fatemi AZ, Owlia P (2013) Evaluation of appropriate carriers for bio-control agents of apple fire blight. Egypt J Pest Control 23(1):31–34

Arora NK, Kumar V, Maheshwari DK (2001) Constraints development and future of the bioinoculants with special reference to rhizobial inoculants. In: Maheshwari DK, Dubey RC (eds) Innovative approaches in microbiology. Singh and Singh, Dehradun, pp 241–254

Arora NK, Khare E, Naraian R, Maheshwari DK (2008) Sawdust as a superior carrier for production of multipurpose bioinoculant using plant growth promoting rhizobial and pseudomonad strains and their impact on productivity of *Trifolium repens*. Curr Sci 95(1):90–94

Arora NK, Khare E, Maheshwari DK (2010) Plant growth-promoting rhizobacteria: constraints in bioformulation, commercialization, and future strategies. In: Maheshwari DK (ed) Plant growth and health promoting bacteria. Springer, Berlin, pp 97–116

Arora NK, Tiwari S, Singh R (2014) Comparative study of different carriers inoculated with nodule forming and free living plant growth promoting bacteria suitable for sustainable agriculture. J Plant Pathol Microbiol 5:229

Backman PA, Sikora RA (2008) Endophytes: an emerging tool for biological control. Biol Control 46:1–3

Bahl N, Jauhri S (1986). Spent compost as a carrier for bacterial inoculant production. In: Proceedings of the international symposium on scientific and technological aspects of cultivating edible fungi. The Pennsylvania State University, University Park, PA, pp 63–68

Bailey RC, Kindt JT, Qavi AJ (2010) Sizing up the future of microRNA analysis. Anal &Bioanal Chem, 398(6):2535–2549

Bansal RK, Dahiya RS, Lakshminarayana K, Suneja S, Anand RC, Narula N (1999) Effect of rhizospheric bacteria on plant growth of wheat infected with *Heterodera avenae*. Nematol Mediterr 27:311–314

Barkai-Golan R (2001) Postharvest diseases of fruits and vegetables. Dev Control:183–245

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16(4):729–770

Bashan Y, Bashan LE (2002) Protection of tomato seedlings against infection by *Pseudomonas syringae pv*. *tomato* by using the plant growth-promoting bacterium *Azospirillum brasilense*. Appl Environ Microbiol 68:2637–2643

Bashan Y, Bashan LE, Prabhu SR, Hernandez J (2014) Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013). Plant Soil 378:1–33

Belloti AC, Cardona C, Lapointe SL (1990) Trends in pesticide use in Colombia and Brazil. J Agric Entomol 7:191–201

Bhattacharjee R, Utpal D (2013) Biofertilizer: a way towards organic agriculture; a review. Af J Micro Res 8(24):2332–2343

Bissonnette N, Lalande R (1988) High survivability of cheese Whey-Grown *Rhizobium meliloti* cells upon exposure to physical stress. Appl Environ Microbiol 54(1):183–187
Bora T, Ozaktan H, Gore E, Aslan E (2004) Biological control of *Fusarium oxysporum f. sp. melonis* by wettable powder formulations of the two strains of *Pseudomonas putida*. J Phytopathol 152:471–475
Bouillant ML, Miché L, Ouedraogo O, Alexandre G, Jacoud C, Sallé G, Bally R (1997) Inhibition of Striga seed germination associated with sorghum growth promotion by soil bacteria. C R Acad Sci Paris Sci de la vie 320:159–162
Brar SK, Verma M, Tyagi RD, Valero JR (2006) Recent advances in downstream processing and formulations of *Bacillus thuringiensis* based biopesticides. Process Biochem 41(2):323–342
Brockwell J (1977) Application of legume seed inoculants. In: Hardy RWF, Gibson AH (eds) A treatise on dinitrogen fixation section 4 agronomy and ecology. Wiley, New York, pp 277–309
Callaghan A, Guillemaud T, Makate N, Raymond M (1998) Polymorphisms and fluctuations in copy number of amplified esterase genes in *Culex pipiens* mosquitoes. Insect Mol Biol 7(3):295–300
Catroux G, Richard G, Chenu C, Duquenne P (1999) Effect of carbon source supply and its location on competition between inoculated and established bacterial strains in sterile soil microcosm. FEMS MicrobiolEcol 29:331–339
Ceaser AJ, Burr TJ (1991) Effect of conditioning, betaine and sucrose on rhizobacteria in powder formulations. Appl Environ Microbiol 57:168–172
Chandra S, Choure K, Dubey RC, Maheshwari DK (2007) Rhizosphere competent *Mesorhizobium loti* MP6 induces root hair curling, inhibits *Sclerotinia sclerotiorum* and enhances growth of Indian mustard *Brassica campestris*. Braz J Microbiol 38:128–130
Chao WL, Alexander M (1984) Mineral soil as carriers for rhizobium inoculants. Appl Environ Microbiol 47(1):94–97
Chen C, Qiana Y, Chenb O, Taoc C, Lib C, Lia Y (2011) Evaluation of pesticide residues in fruits and vegetables from Xiamen China. Food Control 22(7):1114–1120
Chen KN, Chen CY, Lyn YC, Chen MJ (2013) Formulation of a novel antagonistic bacterium based biopesticide for fungal disease control using microencapsulation techniques. J Agric Sci 5(3):153–163
Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71(9):4951–4959
Cook RJ (1990) Twenty-five years of progress towards biological control. In: Horny D (ed) Biological control of soil-borne plant pathogens. CAB International, Wallingford, pp 1–14
Daza A, Santamaria C, Rodriguez-Navarro DN, Camacho M, Orive R, Temprano F (2000) Perlite as a carrier for bacterial inoculants. Soil Biol Biochem 32:567–572
De Souza JT, Weller DM, Raaijmakers JM (2003) Frequency, diversity and activity of 2,4-diacetylphloroglucinol producing fluorescent Pseudomonas spp. in Dutch take-all decline soils. Phytopathology 93:54–63
Dommergues YR, Diem HG, Divies C (1979) Polyacrylamide entrapped Rhizobium as an inoculant for legumes. Appl Environ Microbiol 37:779–981
Duchesne RM, Laguë C, Khelifi M, Gill J (2001) Thermal control of Colorado potato beetle. In: Vincent C, Panneton B, Fleurat-Lessard F (eds) Physical control methods in plant protection. Springer, Berlin, pp 61–73
Dureja P, Singh SB, Parmar BS (2015) Pesticide maximum residue limit (MRL): background, Indian scenario. Pestic Res J 27(1):4–22
EEA (2015) European Environment: State and Outlook 2015: Assessment of Global Megatrends. ISBN: 978–92–9213-534-8. http://dx.doi.org/10.2800/126936. Ehteshamul
Einarsson S, Gudmundsson J, Sverrisson H, Kristjansson JK, Runolfsson S (1993) Production of *Rhizobium* inoculants for *Lupinus nootkatensis* on nutrient-supplemented pumice. Appl Environ Microbiol 59(11):3666–3668
EPA (1992) Regulatory impact analysis of worker protection standard for agricultural pesticides. Washington

FAO (1986) Pests in agricultural environmental protection and productivity: conflicting goals. Series: Plant Production and Protection. FAO Regional Office for Latin America and the Caribbean, Chile

FAO (1998) World reference base for soil resources. World Soil Resources Report 84, Food and Agriculture Organization of the United Nations, Rome: 88

Fouilleux G, Revellin C, Hartmann A, Catroux G (1996) Increase of Bradyrhizobium japonicum numbers in soils and enhanced nodulation of soybean (*Glycine max* (L) merr.) using granular inoculants amended with nutrients. FEMS Microbiol Ecol 20:173–183

Gasic S, Tanovic B (2013) Biopesticide formulations, possibility of application and future trends. Pestic Fitomed 28(2):97–102

Gautam KC, Mishra JS (1995) Problems, prospects and new approaches in weed management. Pestic Inf 21(1):7–19

Ghormade V, Deshpande MV, Paknikar KM (2011) Perspectives for nanobiotechnology enabled protection and nutrition of plants. Biotechnol Adv 29:792–803

Gould F (1991) The evolutionary potential of crop pests. Am Sci 79(6):496–507

Grube A, Donaldson D, Kiely T, Wu L (2011) Pesticides industry sales and usage 2006 and 2007 market estimates. Biological and Economic Analysis Division, Office of Pesticide Programs, Office of Chemical Safety and Pollution Prevention U.S. Environmental Protection Agency, Washington, DC 20460

Haas D, Defago G (2005) Biological control of soil borne pathogens by fluorescent pseudomonads. Nat Rev Microbiol 3:307–319

Handelsman J (2002) Future trends in biocontrol. In: Gnanamanickam SS (ed) Biological control of crop diseases. Marcel Dekker, New York, pp 443–449

Haque S, Ghaffar A (1993) Use of rhizobia in the control of root rot diseases of sunflower, okra, soybean and mung bean. J Phytopathol 138:157–163

Hassouna MG, El-Saedy MAM, Saleh HMA (1998) Biocontrol of soil-borne plant pathogens attacking cucumber *Cucumis sativus* by rhizobacteria in a semiarid environment. J Arid Soil Res Rehabil 12:345–357

Hedge SV, Brahmaprakash G (1992) A dry granular inoculant of Rhizobium for soil application. Plant Soil 144:309–311

Hooker AL (1972) Southern leaf blight of corn-present status and future prospects. J Environ Qual 1(3):244–249

Hultberg M, Alsberg T, Khalil S, Alsanius B (2009) Suppression of disease in tomato infected by *Pythium ultimum* with a biosurfactant produced by *Pseudomonas koreensis*. Biol Control 54:10526–10537

Jackson AM, Whipps JM, Lynch JM (1991) Production, delivery systems, and survival in soil of four fungi with disease biocontrol potential. Enzym Microb Technol 13:636–642

Kaushal A, Rawat AK, Verma LN, Khare AK (1996) Oxalic acid industrial waste as a carrier for Rhizobium inoculants and its effect on soybean. J Indian Soc Soil Sci 44(2):249–252

Kavitha K, Meenakumari KS, Sivaprasad P (2003) Effect of dual inoculation of native arbuscular mycorrhizal fungi and Azospirillum on suppression of damping off in chilli. Ind Phytopathol 56:112–113

Khare E, Arora NK (2015) Effects of soil environment on field efficacy of microbial inoculants. In: Arora NK (ed) Plant microbes symbiosis: applied facets. Springer, India, pp 37–75

Kim J, Grate JW, Wang P (2006) Nanostructures for enzyme stabilization. Chem Eng Sci 61:1017–1026

Knowles A (2001) Trends in pesticide formulations. PJB Publications Ltd, Agrow Reports UK, pp 89–92. D215

Knowles A (2005) New developments in crop protection product formulation. T and F Informa UK Ltd, Agrow Reports UK, pp 153–156

Knowles A (2008) Recent developments of safer formulations of agrochemicals. Environmentalist 28(1):35–44

Kostov O, Lynch JM (1998) Composted sawdust as a carrier for Bradyrhizobium, Rhizobium and Azospirillum in crop inoculation. World J Microbiol Biotechnol 14:389–397

Kotb SI, Angle JS (1986) Survival of blue-green algae in various carrier media. Trop Agric 63:113–116
Kremer RJ, Peterson HL (1982) Effects of carrier and temperature on survival of Rhizobium spp. legume inocula: development of an improved type of inoculant. Appl Environ Microbiol 45:1790–1794
Kumar V (2014) Characterization, bioformulation development and shelf life studies of locally isolated bio-fertilizer strains. Octa J Environ Res 2(1):32–37
Kumari B, Madan VK, Kathpal TS (2006) Monitoring of pesticide residues in fruits. Environ Monit Assess 123(1):407–412
Lamichhane JR, Barzman M, Booij K, Boonekamp P (2015) Robust cropping systems to tackle pests under climate change: a review. Agron Sustain Dev 35:443–459
Lugtenberg B, Kamilova F (2009) Plant growth promoting rhizobacteria. Annu Rev Microbiol 63:541–556
Lyn ME, Burnett D, Garcia AR, Gray R (2010) Interaction of water with three granular biopesticide formulations. J Agric Food Chem 58(1):1804–1814
Malusa E, Sas-Paszt L, Ciesielska J (2012) Technologies for beneficial microorganisms inocula used as biofertilizers. ScientificWorldJournal:1–12
Manjula K, Podile AR (2001) Chitin supplemented formulations improve biocontrol and plant growth promoting efficiency of *Bacillus subtilis* AF1. Can J Microbiol 47:618–625
Mari M, Bertolini P, Pratella GC (2003) Non-conventional methods for the control of post-harvest pear diseases. J Appl Microbiol 94:761–766
Marjan J, Asghar H, Hamid RZ, Saeed R, Laleh N (2011) Development of *Pseudomonas fluorescens* and *Bacillus* coagulans based bioformulations using organic and inorganic carriers and evaluation of their influence on growth parameters of sugar beet. J Biopest 4(2):180–185
McNab R, Ford SK, El-Sabaeny A, Barbieri B, Cook GS, Lamont RJ (2003) LuxS-based signaling in Streptococcus gordonii: autoinducer 2 controls carbohydrate metabolism and biofilm formation with *Porphyromonasgingivalis*. J Bacteriol 185:274–284
Mishra RPN, Singh RK, Jaiswal HK, Kumar V, Maurya S (2006) Rhizobium-mediated induction of phenolics and plant growth promotion in rice *Oryza sativa L.* Curr Microbiol 52:383–389
Mishra J, Tewari S, Singh S, Arora NK (2015) Biopesticides: where we stand? In: Arora NK (ed) Plant microbes symbiosis: applied facets. Springer, India, pp 37–75
Ortiz-Hernandez ML, Sánchez-Salinas E, Dantán-González E, Castrejón-Godínez ML (2013) Pesticide biodegradation: mechanisms, genetics and strategies to enhance the process. http://dx.doi.org/10.5772/56098
Oudejans JH (1991) Agro pesticide properties and functions in integrated crop protection. United Nations Economic and Social Commission for Asia and Pacific, Bangkok, p 329
Paau AS (1988) Formulations useful in applying beneficial microorganisms to seeds. Trends Biotechnol 6:276–279
Paczkowski MW, Berryhill DL (1979) Survival of *Rhizobium phaseoli* in coal-based legume inoculants. Appl Environ Microbiol 38(4):612–615
Peschin R (2002) Economic benefits of pest management. In: Pimentel D (ed) Encyclopedia of pest management. Marcel Dekker, New York, pp 224–227
Pesenti-Barili B, Ferdani E, Mosti M, Degli-Innocenti F (1991) Survival of agrobacterium radiobacter K84 on various carriers for crown gall control. Appl Environ Microbiol 57(7):2047–2051
Pimentel D (1997) Pest management in agriculture. In: Pimentel D (ed) Techniques for reducing pesticide use: environmental and economic benefits. Wiley, Chichester, pp 1–12
Raaijmakers JM, Bruijn I, de Kock MJD (2006) Cyclic lipopeptide production by plant-associated Pseudomonas spp.: diversity, activity, biosynthesis and regulation. Mol Plant-Microbe Interact 19:699–710
Reban FB (2002) Wastewater sludge as a substrate for growth and carrier for rhizobia: the effect of storage conditions on survival of Sinorhizobium Meliloti. Bioresour Technol 83:145–151
Reece JB, Urry LA, Cain ML, Wasserman SA, Minorsky PV, Jackson RB (2011) Campbell biology, ninth edn. Pearson Benjamin Cummings, San Francisco, pp 1170–1193

Richter E, Ehwald R, Conitz C (1989) Immobilization of yeast cells in plant cell wall frameworks. Appl Microbiol Biotechnol 32:309–312
Ryan RP, Dow JM (2008) Diffusible signals and interspecies communication in bacteria. Microbiology 154:1845–1858
Sadasivam KV, Tyagi RK, Ramarethinam S (1986) Evaluation of some agricultural wastes as carriers for bacterial inoculants. Agric Wastes 17:301–306
Sasson Y, Levy-Ruso G, Toledano O, Ishaaya I (2007) Nanosuspensions: emerging novel agrochemical formulations. In: Ishaaya I, Nauen R, Horowitz AR (eds) Insecticides design using advanced technologies. Springer, Berlin, pp 1–39
Schmale DG, Bergstrom GG (2003) Fusarium head blight in wheat. Plant Health Instr. http://dx.doi.org/10.1094/PHI-I-2003-0612-01
Sen A (1981) Poverty and famines: an essay on entitlement and deprivation. Oxford University Press, London, p 203
Shah-Smith DA, Burns RG (1997) Shelf-life of a biocontrol Pseudomonas putida applied to the sugar beet seeds using commercial coatings. Biocontrol Sci. Technol 7(1): 65–74
Shaikh SS, Sayyed RZ (2015) Role of plant growth-promoting rhizobacteria and their formulation in biocontrol of plant diseases. In: Arora NK (ed) Plant microbes symbiosis: applied facets. Springer, India, pp 337–351
Singh A, Sharma PB (1973) Growth and survival of rhizobia in commercial bacterial inoculants. J Res 10:95–98
Singh BP, Shekhawat GS (1999) Potato late blight in India. Tech. Bull. No. 27 (Revised). CPRI, Shimla, p 27
Singh DP, Singh A (2005) The value of disease and insect resistance. In: Disease and insect resistance. Plants Science Publishers, Enfield, pp 1–6
Singh S, Gupta G, Khare E, Behal KK, Arora NK (2014) Effect of enrichment material on the shelf life and field efficiency of bioformulation of *Rhizobium* sp. and P-solubilizing *Pseudomonas fluorescens*. Sci Res Rep 4(1):44–50
Singleton P, Keyser H, Sande E (2002) Development and evaluation of liquid inoculants. In: Herridge D (ed) Inoculants and nitrogen fixation of legumes in Vietnam, ACIAR Proceedings 109e, pp 52–66
Smilanick JL (1994) Strategies for the isolation and testing of biocontrol agents. In: Wilson CL, Wisniewski ME (eds) Biological control of postharvest diseases-theory and practice. CRC Press, Boca Raton, pp 25–41
Somasegaran P, Hoben JH (1994) Methods in Legume- Rhizobium Technology, Handbook of Rhizobia,1:16:450: ISBN:9781461383758, Springer Publ. New York.
Sougoufara B, Diem HG, Dommergues YR (1989) Response of field-grown *Casuarina equisetifolia* to inoculation with *Frankia strain ORS 021001* entrapped in alginate beads. Plant Soil 118:133–137
Sparrow SD, Ham GE (1983a) Nodulation, N2 fixation, and seed yield of navy beans as influenced by inoculant rate and inoculant carrier. Agron J 75:20–24
Sparrow SD, Ham GE (1983b) Survival of *Rhizobium phaseoli* in six carrier materials. Agron J 75:181–184
Stephens CS (1984) Ecological upset and recuperation of natural control of insect pests in some Costa Rican banana plantations. Turrialba 34:101–105
Stockwell VO, Stack JP (2007) Using Pseudomonas spp. for integrated biological control. Phytopathology 97(2):244–249
Tadros F (2005) Applied surfactants, principles and applications. Wiley-VCH Verlag GmbH and Co. KGaA, pp 187–256
Tewari S, Arora NK (2013) Transactions among microorganisms and plant in the composite rhizosphere habitat. In: Arora NK (ed) Plant microbe symbiosis: fundamentals and advances. Springer, India, pp 1–50
Tewari S, Arora NK (2014) Multifunctional exopolysaccharides from *Pseudomonas aeruginosa* PF23 involved in plant growth stimulation, biocontrol and stress amelioration in sunflower under saline conditions. Curr Microbiol 69(4):484–494

Tronsmo A, Dennis C (1977) The use of Trichoderma species to control strawberry fruit rots. Netherland J Plant Patho 83:449–455

Usta C (2013) Microorganisms in biological pest control: a review (bacterial toxin application and effect of environmental factors). http://dx.doi.org/10.5772/55786

Vanvuurde JWL, Roozen NJM, Postma J, Mass PWT, Hageman PEJ, Kok CJ (2010) Processed manure as carrier to introduce *Trichoderma harzianum*: population dynamics and biocontrol effect on *Rhizoctonia solani*. Biocontrol Sci Tech 6(2):147–162

Vidhyasekaran P, Sethuraman K, Rajappan K, Vasumathi K (1997) Powder formulation of *Pseudomonas fluorescens* to control pigeonpea wilt. Biol Control 8:166–171

Wilson CL, Pusey PL (1985) Potential for biocontrol of post-harvest plant diseases. Plant Dis 69:375–378

Woods TS (2003) Pesticide formulations. In: AGR 185 in encyclopedia of agrochemicals. Wiley, New York, pp 1–11. World Population Balance, 2015. http://www.worldpopulationbalance.org/3_times_sustainable

Youdeowei A (1989) Major arthropod pests of food and industrial crops of Africa and their economic importance. In: Yaninek JS, Harren HR (eds) Biological control: a sustainable solution to crop pest problems in Africa. IITA, Ibadan

Zhang YH, Ye HZ (1993) A study of histology of resistance of wheat to head scab *Fusarium graminearum*. J Sichuan Agric Univ 11:444–445

Microbial Plant Probiotics: Problems in Application and Formulation

13

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Abstract

Probiotics are live microbial cells or cultures which promote host growth and vigour. The study of the role of microorganisms in soil and plant health and plant-microbe interactions has been the area of interest for plant pathologists and soil microbiologists since beginning of microbiology. Plant probiotics have garnered a considerable amount of attention due to success of human probiotics in improved human health. Plant probiotics are microorganisms or group of microorganisms which by virtue of their potential role in improved nutrient acquisition and/or biocontrol activities can promote soil health, plant growth and enhance plant tolernace or immunity against various abiotic and biotic stresses. Plant growth-promoting bacteria (PGPBs) are good examples of plant probiotics which augment crop production by different activities like nitrogen fixation, growth hormones production, and phosphorus- and mineral- solubilization, enhancing water and nutrient use efficiency, and act as biocontrol and biopesticides. The growing concern over negative implications of excess use of chemi-

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cals in agriculture has led to the search for environmental-friendly alternative plant growth-promoting agents. Plant probiotics (PGPBs) are hence being employed in sustainable agriculture methods across the globe. Although several kinds of biofertilizer and biopesticide formulations are available in the markets and are being used in agricultural practices, their commercial potential is not yet being realized owing to various issues associated with formulations and applications. Therefore, there is a dire need to employ methods and means to enhance the efficacy, safety and shelf-life of the formulations related to plant probiotics. In this chapter, we try to highlight the glitches related with formulations, safety and applications of plant probiotics and also suggest the ways to improve the existing lacunae in formulations and applications of plant probiotics.

13.1 Introduction

Probiotics are live microbial cultures used to promote host health and vigour. Microbial cells used for the probiotic purposes are present in the natural ecosystem, but their correct identification, isolation and study of pathogenicity and probiotic potential are needed before their formulation and commercial use (Soccol et al. 2010). Immense amount of work has been done on human probiotics (LeBlanc et al. 2011; Soccol et al. 2010). They contain the common flora of dairy products and fermented foods and are generally used to treat digestive disorders, but in the current scenario, the application of probiotics is expanding. *Lactobacillus* and *Bifidobacterium* are good examples of human probiotics. Similar to human probiotics, the concept of plant probiotics has gained attention recently. Plant probiotics are microbial culture which shows plant growth-promoting and/or biocontrol potential by virtues of their diverse activities including nitrogen fixation, phosphate solubilization, siderophore production and improved plant immunity against diseases (Sharma et al. 2012; Compant et al. 2010; Nadeem et al. 2015). They also improve soil structure by aggregating the soil particles together by secretion of extracellular metabolite, increasing the breakdown of complex organic material and insoluble nutrient into simpler forms to make them available for plant growth and inducing resistance against stress and diseases (Maheshwari et al. 2012; Song et al. 2012; Nakkeeran et al. 2004). It has been found that adequate populations of beneficial soil microbes are essential for healthy soil structure and better plant growth. But the excess use of agricultural fertilizers and pesticides have imparted adverse effects on soil microflora and consequently disturbed the natural structure of microbial community of the soil and impacted soil health. In addition, the use of excess pesticides and chemical fertilizers not only disturbed the soil health and natural microbial community structure but also added the problem of chemical contamination and pollution of soil, air and water, thereby affecting human health and hygiene. Formulation of plant probiotic can be a single microbial culture or consortium of more than one beneficial microbe with plant growth-promoting potential. Plant growth-promoting rhizobacteria (PGPR) are well established and studied in

agricultural microbiology and plant pathology, and immense amount of work has been done on its different aspects like ability of colonization and survival in plant rhizosphere, nature of the compound produced and involved in plant growth promotion, competition with natural microbial population, packing and formulation, survival during storage and transportation, etc. (Herrmann and Lesueur 2013). Bacteria involved in phosphate solubilization, nitrogen fixation and biological control of plant disease are some of the good examples of plant probiotics (Prakash et al. 2015, 2016; Sharma et al. 2012; Dardanelli et al. 2010). In addition to rhizobacteria, research on endophytic bacteria and fungi have indicated that bacteria residing in the internal environment also help plants in terms of nutrient acquisition, stress tolerance and growth of their leaves, stem and roots (Jorjani et al. 2011). Thus, the use of plant probiotics for plant health, growth and better productivity is an environmental-friendly alternative of chemical fertilizers which maintain soil health and simultaneously promote the concept of organic farming. As discussed above, immense amount of work has been done on other aspects of probiotics, and in this chapter, we are mainly focusing on the issues associated with problems related to formulations and applications of plant probiotics.

13.2 What Are Microbial Plant Probiotics?

Plant fitness is dependent on various components including environmental, climatic and edaphic factors. Research in the past decade has been focussed on identifying the role of soil microbes on overall plant health and productivity. Results obtained through such extensive investigations have revealed that microbes are an essential component of plant well-being and can even improve resistance, tolerance and resilience of plants under biotic and abiotic stress conditions.

Microbial interactions with plants have been grouped into various categories depending upon the site and mode of interaction.

13.2.1 Root Interactions

In the rhizosphere, also designated as “microbial hot spot” (Whipps 2001), a number of diverse, significant and exhaustive interactions transpire between microorganisms, plants and soil organisms (Antoun and Prévost 2005). These connections are established on intricate conversations among roots and microorganisms; however, the positive, negative and neutral nature of these interactions are altogether delimited via multifaceted molecular signalling (Dardanelli et al. 2010). These positive advantageous collaborations considerably impact plant development and growth and consequently result in a progressive impact on crop yields and production.

Root exudates comprehend sufficient quantity of carbon and energy that are copiously accessible to microbes for their growth and development and provide substrates for physiological functions of microbial cells (Carvalhais et al. 2013).

Combinations of numerous organic compounds like auxins, sugars, vitamins, flavonoids, etc. are present in the root exudates (Raaijmakers et al. 2009; Compant et al. 2010). Owing to the presence of such varied organic components in the root exudates, bacteria colonize the rhizosphere, utilizing the root exudates for their growth and development (Bertin et al. 2003; Nadeem et al. 2015). Among these root-inhabiting bacteria, some promote plant growth and development through direct or indirect mechanisms. Such bacteria have been categorized as plant growth-promoting rhizobacteria (PGPR) and have been extensively studied for their inherent potential to act as biofertilizers or plant probiotics.

One gram of soil approximately contains 5000 bacterial operational taxonomic units (OTUs) (Roesch et al. 2007) even though the OTU population is dependent on soil conditions and environment. According to Bodelier et al. (1997), rhizosphere is about 19–32 times more contiguous than bulk soil, i.e. free of roots. Even though there have estimated that owing to the root exudates, the number of microbes in the exists a huge quantity of microbial population in the rhizosphere, only 7–15% of the entire root surface is by and large occupied by microbial cells (Berg et al. 2005). Bacteria, the most abundant microorganisms in the rhizosphere, are exceedingly competitive and aggressively inhabiting the plant roots (Antoun and Prévost 2005; Bouizgarne 2013). The rhizosphere microorganisms with the ability to metabolize chitin, cellulose and root and seed exudates play a major role in shaping the structure of rhizosphere microbiomes (Nadeem et al. 2015).

13.2.2 Endophytes

Endophytic bacteria inhabit plant tissues minus any fundamental detrimental impact. Bultman and Murphy (2000) presented a comprehensive as well as extensively recognized description of endophytes, that is, “microbes that colonize living, internal tissues of plants without carrying any immediate overt negative effects”.

Endophytes move into the plant tissue largely via the roots, though parts of plants which are above the ground, for instance, flowers, stems and cotyledons, may possibly also be used for entrance. Explicitly, the bacteria come into the plant tissues by way of sprouting radicals, secondary roots and stomata or as a consequence of foliar injury. Endophytes inside a plant may perhaps either develop while being contained at the point of entrance or proliferate all the way through the plant (Hardoim et al. 2015).

13.3 Why Plant Probiotics?

Unscrupulous and unprecedented usage of chemical fertilizers and pesticides on the crops in contemporary agriculture has damaged the sustainability of agriculture systems leading to increased expense of farming thereby escalating the average farmer’s income in turn ensuring food security and safety into a formidable task. Excessive usage of chemical fertilizers, along with chemical pesticides and

inaccessibility or less use of organic manures, has steered to extensive decline in soil health and vigour. Continued reliance on chemical fertilizers for imminent agronomic progress would result in more damage to soil quality and risks of water pollution and contamination leading to untenable impediment on the economic structure. Biological-based agriculture is an all-inclusive production management structure which encourages and augments agroecosystem vigour, together with biodiversity, biological cycles as well as soil biological activity.

Consistent with the present stance, the government targets not only to boost their usage in routine agriculture but also to correspondingly endorse private ingenuity and marketable feasibility of manufacture. In India the accessibility of fossil fuel-centred chemical fertilizers ensured simply via imports and subsidizations at the farm level has been a major factor in less popularity and use of biofertilizer in routine agricultural practices. The Government of India has been taking efforts and promoting awareness through education and legislation to help farmers adapt improved and sustainably viable agriculture practices encompassing usage of biofertilizers along with conventional chemical fertilizers. These efforts and ideas have manifold constructive bearings on the soil and can be comparatively economical and expedient to use.

13.4 Commercial Potential

The market potential for biofertilizer is extensive not only in the technologically advanced countries but also in the developing countries mainly relying on agriculture for their economic growth and stability (Bhattacharyya and Jha 2012; Malusá et al. 2012). Herridge (2008) assessed that replacement of chemical fertilizers with rhizobial inoculants would reduce the yearly cost of N fertilization to about US$1 million from US$30 million per annum. Recently, a lot of interest has been observed in endorsing biofertilizers for eco-efficient amplification of agricultural systems in sub-Saharan Africa (SSA).

Around 170 organizations in 24 countries are engaged in the commercial production of biofertilizers. Many industries have been involved in production, marketing and dissemination of microbe-based fertilizers or growth enhancers at both large and small scales. In such conditions, the expense of biofertilizers with the risk and responses will be weighed with those of chemical fertilizers, and promotion of technology for environmental reasons would demand for some amount of fortification to curtail the inter-fertilizer price bias. Australia has occupied the prime role in the quality control of various viable microbial products and biofertilizers. The present international market for organically raised agricultural produces is prized to approximately US$30 billion with a growing rate of nearly 8%. Approximately 37.2 million hectares of land is being cultivated by employing organic-based agriculture technology (Willer 2011); however, organic agriculture embodies a smaller amount of approximately 1% of the world's conservative farming production and almost 9% of the overall agricultural region. This simply indicates the remarkable prospective and capacity in the growth of biofertilizers.

13.5 Forms and Formulations of Plant Probiotics

In spite of the several means available for plant protection, most of the crops are destroyed by diseases causing fungal pathogens, pests and frost. Depending on their role in the rhizosphere, PGPR are classified as biofertilizers, phytostimulators, rhizoremediators and biopesticides (Somers et al. 2004). Microorganisms used as bioinoculants are divided into three main categories, namely, biofertilizers, biocontrol agents and organic decomposers (Sharma et al. 2012). The term biofertilizer refers to formulations based on beneficial microbes and/or biological product that either fix atmospheric nitrogen or enhance the solubility of soil nutrients and have potential to increase the yield of crops (Bhardwaj et al. 2014). Biocontrol agents are ecofriendly alternative of chemical pesticides to protect the plant against a variety of disease-causing agents, activate the host resistance mechanism and increase biomass production and yield (Nakkeeran et al. 2004). Organic decomposers which include certain fungal species, bacterial genera and actinobacteria accelerate the decomposition of organic compounds and make them available to plant as nutrients (Sharma et al. 2012). Plant probiotic formulations available in the market currently can further be improved to obtain better efficiency in terms of plant disease protection and enhanced crop production. Development of formulations with increased shelf-life and broad spectrum of action with consistent performance under field conditions could pave the way for commercialization of the technology at a faster rate (Nakkeeran et al. 2004).

Among the biocontrol agents, bacterial antagonists including *Pseudomonas* spp. and *Bacillus* spp. have shown activity in suppressing the fungal infection and promoting plant growth (Chen et al. 2000). To evaluate these strains, a study conducted on *Pseudomonas fluorescens* (B1) and *Bacillus coagulans* (B2) isolated from rhizosphere of sugar beet were procured from Microbial Culture Collection, Beneficial Microorganisms Research Laboratory and Iranian Research Institute. Talc and bentonite powders were used as inorganic carriers and peat and rice bran as organic carriers for preparation of eight bioformulations. The efficacy of bioformulations were evaluated in pots under greenhouse conditions after 60 days of sowing on growth characteristics like seedling height, seedling dry weight, root length and root weight. Results showed that the above-mentioned growth characteristics except root length were significantly increased by all test bioformulations but with different ratio except root length. In the above study, although all bioformulations significantly increase the growth of sugar beet seedlings, *P. fluorescens*-based formulations were relatively more effective. Perhaps, it is due to the more diverse metabolites such as siderophore, hydrolytic enzymes, phytohormones and/or other volatile extracellular metabolites produced by this bacterium. Among the carrier peat, rice bran, talc and bentonite performed well and effective in their respective formulation developed for this purpose (Jorjani et al. 2011). In another finding, Bharathi et al. (2004) evaluated the potential of 13 plant growth-promoting rhizobacterial strains against chilli fruit rot and dieback provoked by *Colletotrichum capsici*. Similarly, they also found that *P. fluorescens* and *B. subtilis* were more effective in increasing seed germination and seedling vigour. It was also concluded that the PGPR-mixed

bioformulation (*P. fluorescens* + *B. subtilis* + neem + chitin) was the most effective in reducing fruit rot incidence, apart from increasing plant growth and yield parameters under both greenhouse and field conditions (Bharathi et al. 2004).

Arbuscular mycorrhizal fungi (AMF) live in mutual relationship with plants. AMF have interactions with wide range of soil microorganisms including rhizobacteria, mycorrhiza, helper bacteria and beneficial or deleterious bacteria. Such interactions are important for sustainable agriculture (Miransari 2011). Interactions between rhizotrophic microorganisms can influence their activities, soil conditions and hence plant growth (Zaidi et al. 2003). In recent findings, microbial inoculants made from AMF *Funneliformis mosseae* and *Bacillus sonorensis* revealed strong synergistic relationships and significant improvement of growth, yield and nutrition content of chilli under pot culture studies (Thilagar et al. 2016).

13.6 Method of Formulation of Plant Probiotics

Large-scale commercial formulations of plant probiotics for storage and application purposes require suitable carrier materials that support the survival and applications of live microbes for a considerable length of time and at field site, respectively. Carriers may be organic or inorganic, but they should be economical, non-reactive, ecofriendly and easily available in bulk for commercial exploitation and exploration. Several kinds of carrier materials such as peat, turf, talc, lignite, kaolinite, pyrophyllite, zeolite, montmorillonite, alginate, press mud, sawdust, and vermiculite, etc. are currently used for formulation purposes (El-Fattah et al. 2013; Zayed 2016). Carriers increase the survival rate of bacteria by protecting it from desiccation and death of cells (Trivedi et al. 2005). In addition to solid and powdered formulation, several different types of liquid formulations of microbes with probiotic potentials are also available in the market. Liquid inoculants or formulations are based on aqueous (broth cultures), mineral or organic oils and oil-in-water or polymer-based suspensions. Due to easy application, storage and longer viability liquid products have been promoted than formulations based on solid organic and inorganic carriers. Different kinds of stickers and additive materials are used for formulation purposes in order to increase the adhesion of microbes with host and to increase the effectiveness of the formulations, respectively. The common additives are macro- and micronutrients, carbon or mineral sources, hormones, fungicides, etc.

13.6.1 Formulations Based on Solid, Liquid and Microencapsulation Methods

The shelf-life of microbes in any formulations depends on several factors including the particle size and nature of carrier materials, type of organisms (genera used for formulations) and their physiology, storage temperature, skill and expertise of handling scientist and technicians and type of formulation. For example, *P. fluorescens*

(2-79RN10, W4F393) showed more survival in smaller particle size carrier materials like montmorillonite, zeolite and vermiculite than kaolinite, pyrophyllite and talc which have bigger particle size (Dandurand et al. 1994). Carriers with smaller particle size provides more surface area in comparison to bigger particle size carrier materials, which provide sufficient micro-niches to the inoculated organisms and increase resistance to desiccation and death (Dandurand et al. 1994). Several formulations of fluorescent *Pseudomonas* have been developed using liquid fermentation technology in which the fermenter biomass was mixed with different carrier materials like talc, peat, kaolinite, lignite, vermiculite and stickers (Vidhyasekaran and Muthamilan 1995). On the other hand, Krishnamurthy and Gnanamanickam (1998) developed talc-based formulation of *P. fluorescens* for the management of rice blast caused by *Pyricularia grisea*, in which methyl cellulose and talc was mixed at 1:4 ratio and blended with equal volume of bacterial suspension at a concentration of 10^{10}cfu ml^{-1}. Similar to Vidhyasekaran and Muthamilan (1995), Nandakumar et al. (2001) developed talc-based formulation of fluorescent pseudomonads mixing equal volume of cultured biomass of the strains with talc powder. It was found that talc-based strain mixtures were more effective against rice sheath blight and increased plant yield under field conditions than the application of individual strains. Nakkeeran et al. (2004) also developed and used the talc- and peat-based formulations of *P. chlororaphis* and *B. subtilis* for the management of turmeric rhizome rot. In some formulations, carboxymethyl cellulose (CMC) is added as a sticker at 1:4 ratios to talc, while others suggested that CMC and talc should be used at 1:100 ratios to reduce the cost. Though it is effective in disease management, high production cost prevents the growers to adopt the technology. Hence, feasibility of the technique and shelf-life of the product have to be evaluated to make the technology a viable component in disease management so as to promote organic farming.

Microbes with survivable potential in a wide range of temperature, pH and salinity show a longer shelf-life and activity during formulation and application in comparison to those organisms which prefer narrow range of pH, temperature and salinity. Furthermore, most of the carrier materials used for commercial preparation contain their own native microbiota, and some formulations used them as such without sterilization in order to cut the cost of formulation. Unskilled handling and use of non-sterile carrier materials promote the growth of unwanted native population of carrier materials which override the growth of desired population and lead to low-quality product and increase the risk of spread of environmentally risky group of microorganisms in the field. Most of the powered biofertilizers require low-cost carriers like cow dung, coal and peat mass for the formulation and application in the field, but due to several reasons, nowadays, it has become very difficult to get such material in large quantity for formulation, and scientists should continue to seek for another low-cost alternative. Recommended moisture content of the final formulation must be below 30% because due to high moisture content, powdered packets become like bricks in winter and swell in the summer which creates difficulty in transportation and application. The utilization of many adjuvants, surfactants and oil used in the product by bacteria and some fungi as a source of carbon and energy during storage and transportation leads to the formation of gas in bottles thereby

causing explosion. Storage of the formulation at room temperatures does not stop microbial growth and metabolic activities of the microorganisms which leads to pH change and death of the microorganisms and loss of the viability of the cells and shelf-life of the products. It has been observed that liquid formulation of fungal-based probiotics or bioinoculants produces thick mats of fungal mycelium due to continuous growth of fungal strains during the storage and transportation. Formation of thick mats creates difficulty in pouring mixing and homogenous distribution of the products at the time of application and need more reserach in this area. To alleviate this kinds of problem during the time of formulations.

13.6.2 Microencapsulation

Microencapsulation is another way of formulation of microbial cells for use and applications as bioinoculants. Microcapsules of rhizobacteria consist of a cross-linked polymer deposited around a liquid phase, where bacteria are dispersed. Microparticles are characterized based on the distribution of particle size, morphology and bacterial load. The process of microencapsulation involves mixing of gelatin polyphosphate polymer pair (81:19 w/w) at acidic pH with rhizobacteria suspended in oil (Charpentier et al. 1999). Though rhizobacteria has been formulated through microencapsulation method, it has been found that the shelf-life of the product declines at a faster rate, because polymers serve as a barrier for oxygen. This was later improved by developing microcapsules by spray drying. The release of *P. fluorescens* and *P. putida* from the microencapsulated pellets occurred after 15 min immersion in aqueous buffer. It showed that water served as triggering material for the bacterial release (Charpentier et al. 1999). Though microencapsulation aids in formulating bacteria, still the technology has to be well refined for early release of bacterial cells and for the establishment in the infection court to counter attack the establishment of pathogens. Most of the experiments on microencapsulation have been restricted only to lab. The technology should be standardized for the industrial application so that the technical feasibility could be assessed to popularize the same for field use.

13.7 Methods of Delivery of Plant Probiotics or Bioinoculants

Several different methods have been applied and adopted for the delivery of plant probiotics or plant growth promoting bacteria (PGPB) based on survival potential and mode of invasion of the pathogens. It is delivered through seed, soil, foliage, rhizomes and setts or through the combinations of more than one method. Seed coating, seedling root dip, main field application and foliar spray are some of the common methods of the application. In addition, biopriming, soil treatment, sucker treatment and sett treatments are other methods for applications of biofertilizer, biopesticides and plant probiotics.

Seed treatment is the most common method adopted for all types of inoculants. In one report, talc-based formulation of *P. fluorescens*strain-Pf1 was coated on the seed surface at the rate of 4 g/kg (10^7cfu/g) of chickpea seeds (cv. Shoba) for the management of chickpea wilt, and sowing of treated chickpea seeds resulted in establishment of rhizobacteria on chickpea rhizosphere (Vidhyasekaran and Muthamilan 1995). Treatment of cucumber seeds with strain mixtures comprising of *Bacillus pumilus* INR7, *B. subtilis* GB03 and *Curtobacterium flaccumfaciens* ME1 with a mean bacterial density of 5×10^9 cfu/seed reduced intensity of angular leaf spot and anthracnose equivalent to the synthetic elicitor Actigard, and the result was better than the seed treated with individual strains (Raupach and Kloepper 1998). Seedling root dip method is generally used for transplanted crops. For seedling root dip, two packets of inoculants are mixed in 40 L of water, and the root portion of the seedlings required for an acre is dipped in the mixture for 5–10 min and then transplanted. In main field application processes, generally four packets of inoculant formulations are mixed with 20 kg of dried and powdered farm yard manure and then applied in one acre of main field just before transplanting. For all legumes, preparation of *Rhizobium* is applied as seed inoculant. Foliar spray is another way of application of bacterial formulation on leaf surface.

It has been found that delivery of *Pseudomonas* on beet leaves actively compete for amino acids present on the leaf surface and inhibited spore germination of *Botrytis cinerea*, *Cladosporium herbarum* and *Phoma betae* (Blakeman and Brodie 1977). In another instance, application of *B. subtilis* to bean leaves decreased incidence of bean rust (*Uromycesphaseoli*) by 75% equivalent to weekly treatments with the fungicide mancozeb (Baker et al. 1983). Application of *P. fluorescens* onto foliage (1 kg of talc based formulation/ha) on 30, 45, 60, 75 and 90 days after sowing reduced leaf spot and rust of groundnut under field conditions (Meena et al. 2002). Preharvest foliar application of talc-based fluorescent *Pseudomonas* sp. strain FP7 supplemented with chitin at fortnightly intervals(5 g/l; spray volume 20 l/tree) onto mango trees from pre-flowering to fruit maturity stage induced flowering to the maximum and reduced the latent infection by *C. gloeosporioides* besides increasing the fruit yield and quality.

13.8 Quality Control

One of the most vital issues resulting in their success or failure and acceptance or rejection by end user, the farmers, is the quality of biofertilizer. Herrmann et al. (2013) analysed 65commercially available biofertilizers and further indicated that merely 37% of these may possibly be rated as “pure” and approximately 63% were adulterated or contaminated with one or more bacterial strains. Additionally, 40% of the assessed commercial biofertilizers contrary to the claims comprised only of impurities. These findings emphasize the absolute prerequisite of superior quality control coordination, to warrant that effective products reach the farmers. The dearth of information (or lack of technical expertise) is a crucial predicament in the production of high-quality inoculants.

The Government of India has taken measures to ensure the safety and quality control of microbial biofertilizers. BIS standards were proposed and followed for assessment of efficacy and quality for four categories of biofertilizers, but it was deliberate in nature and not bound by legislature. *Rhizobium*, *Azotobacter*, *Azospirillum* and PSB-based biofertilizers were categorized in the sphere of fertilizer (Control) Order 1985 (FCO) by the Government of India during 2006. With the surge in mycorrhiza-based biofertilizer manufacture via tissue culture procedure, it was also catalogued under the FCO with discrete terms and conditions. Lately, potash mobilizing and zinc solubilizing biofertilizers have also been branded and incorporated in FCO.

13.9 Regulatory Frameworks Regarding Microbial Applications

Biofertilizers have not been amply assessed for quality and efficiency because of weaknesses or absence of regulatory frameworks. Consequently, a proliferation of low-quality and inefficient biofertilizer products has been reported (Herrmann et al. 2013). Currently, there is a dearth of international agreement on the standards for microbial inoculants. To date, existing guidelines and recommendations largely focus on rhizobial inoculants only which differ cross countries and are relatively obligatory (Jenkins and Grzywacz 2000; Lupwayi et al. 2000; Stephens and Rask 2000). The guidelines simply correspond to the lowest quantity of active rhizobia cells to be inoculated per seed or per unit of weight of inoculant and the highest level of impurities allowed in the finished biofertilizer (Catroux and Amarger 1992; Lupwayi et al. 2000). Some countries have a strict regulation over the selection of the microbial component exploited in the products prior to their acceptance as commercial biofertilizers (Hungria et al. 2005).

France presently maintains the most stringent regulation through standards and legislation vis-à-vis rhizobial inoculant quality.

The two major guidelines laid for rhizobia to fulfil prior to their acceptance and applications in natural conditions are as follows:

(a) All rhizobial inoculants ought to ascertain to be safe to the environment, plants, animals and humans to legalize their registration.
(b) Inoculants must be prepared of pure cultures and sterilized carriers, provide no less than 10^6 active rhizobial cells per seed at implanting and remain void of impurities and contaminants while storing (Bashan 1998; Catroux and Amarger 1992).

This instruction is obligatory as before each season an independent lab checks the microbial samples to attest the products that qualify the criteria. Canada follows a moderately strict quality regulation followed by a pertinent legislation that necessitates product cataloguing and recurring arbitrary sampling and quality assessment (Herridge 2008). Though the minimal quantity of viable cells per seed is relatively small and ranges from 10^3 to 10^5 subject to the size of the seeds,

no particular guidelines have been issued with respect to the type and degree of contamination in the product (Olsen et al. 1994). In countries like Thailand, Australia or South Africa, the quality management program is not binding. In Australia, a high count of rhizobia (up to 10^5 rhizobia/seed) is mandatory which is acceptable with a small degree of impurities (0.1% of the total microbial population) (Bashan 1998). However, in Thailand, non-sterile carriers are generally employed where the rhizobial cells per seed is high (10^5–10^6 rhizobia/seed) (Herridge 2008). Few countries permit the manufacturers to decide to apply their specific internal quality control system or whether they deem it unwarranted (Herridge 2008). In the USA or UK, no regulation is hence implemented, and the quality of microbial biofertilizers emanating from such regions is significantly variable (Date 2000). In nearly all of the developing countries, no legislations or criteria are binding or implemented. A majority of the microbe-based growth promoters from Asia, Latin America and Africa are prepared using non-sterile carrier, and in some countries (such as Argentina, Brazil, Rwanda), where standards and regulations exist, they are either not imposed or inappropriately enforced to encourage the requisite amendment in producer's procedures (Bashan 1998; Hungria et al. 2005).

13.10 Problems and Limitations

13.10.1 Screening the Strain

Conservative cultivation of microorganisms by means of selective medium is regularly employed to screen and isolate bacteria demonstrating plant growth-promoting qualities. Desirable PGP traits for a microbial strain (bacterial or fungal) take account of its genetic stability and its capacity to positively affect the target crops and potential and resilience to compete with native microbial populations, to migrate from inoculation site to target sites on host plants and to endure hostile soil conditions in the lack of the host.

13.10.1.1 Identifying Appropriate Carrier

The living inoculant must also be competent to tolerate the innumerable technological routes throughout the manufacture and retain its desired qualities (Xavier et al. 2004). A fundamental aspect involved in the failure is the swift deterioration of the dimensions of the population of active cells. The active cells decline to a level at which the formulation becomes ineffective when introduced into the soil and the desired objective is not achieved. A proficient inoculation entails over and above 1000 rhizobia per gram of soil (Ben Rebah et al. 2002). To attain this number, it is imperative to adopt appropriate carrier ingredients, whose key features are superior water-holding capacity, good aeration and competent maintenance of microbial growth and persistence. Additionally, the carrier ought to be low priced, effortlessly used, mixable, packageable and accessible as powder or granules (Bashan 1998; Ben Rebah et al. 2002).

Liquid formulations employ liquid ingredients in place of solid carriers, which are customarily water, oil or few solvents formulated as suspension, concentrates or emulsions. Most prevalent liquid inoculant preparations comprise specific organism's broth 10–40%, suspender ingredient 1–3%, dispersant 1–5%, surfactant 3–8% and carrier liquid (oil and/or water) 35–65% by weight. Viscosity is equivalent to the setting rate of the particles, which is accomplished by the utilization of colloidal clays, polysaccharide gums, starch, cellulose or synthetic polymers (Kalra et al. 2010).

13.11 Biosafety of Strains

To be contemplated as plant probiotics, it is obligatory for the microorganisms to express a positive role in plant development, but the microorganisms to be employed in biofertilization must also be harmless for humans. A polyphasic approach comprising phenotypic, chemotaxonomic and genotypic classifications ought to be engaged to catalogue and categorize the microorganisms, which can be used to develop into potential and economically viable plant probiotic (Herrmann et al. 2013). The strain can be identified and characterized at taxonomic level using molecular methods such as 16S rDNA sequencing (genus verification) and DNA-DNA hybridization analysis (species verification) (Young et al. 2012), followed by the rules and regulations of the safety assessment charter laid down by the American Biological Safety Association (ABSA). The risk assessment process can be arranged established on the risk group level of the microorganisms referenced in the classification database for infectious agents (http://www.absa.org/riskgroups/). In several countries including the USA, infectious agents are classified in risk groups on the basis of their comparative risk. Conditional to the country and/or organization concerned, this classification structure accounts several different factors, which comprises pathogenicity of the organism, method of transmission and host range, accessibility to operational preventive processes (e.g. vaccines) and availability of effective management (e.g. antibiotics).

13.12 Lack of Awareness

Effective commercialization of novel inoculants largely depends on the associations concerning the exploration and research (to formulate the best inoculant, using the right strain for the right crop in the right conditions), the private sector (to increase and improve the making, institute an economically acceptable and supportable market chain) and the performance of the inoculants in fields and subsequent response of farmers. There is an enormous necessity for developing programs aimed at farmers' training for both contemporary and less industrialized cultivation practices to convince them through education and proper demonstartion at field level. This approach might persuade them to be more willing to buy and employ biologicals-based fertilizers as an alternative to costly chemical fertilizers. To accomplish that,

the development and upgradation of the biofertilizer quality are significantly obligatory. Demonstration trials with superior products and systematic teaching of the farmers for the usage of inoculants would initiate a bigger assurance from the farmers and a substantial escalation in the utilization of biofertilizers (Bhattacharyya and Jha 2012; Kannaiyan 2003; Okon and Hadar 1987). Organized training and awareness programs can help farmers to be cognizant about the know-how of biofertilizers and subsequently developing their insight based on the agronomic conditions of their areas, the awareness acquired from response to biofertilizer applications by farmers around them and including themselves and the information provided by different distributing agents and form their individual conclusions regarding the adoption of biofertilizer technology (Sanap et al. 2009).

13.13 Distribution

The prospective demand of biofertilizers is relatively great and goes beyond the existent manufacturing degrees (Wiesman 2009; Mazid and Khan 2014). Although all the agriculture experts advocate the benefits of microbial inoculants as biofertilizers and have professed its use in cultivation methods, the dissemination of biofertilizers and its acceptance rate has consistently failed to grow in time (Dennis et al. 2010). Secondly, even though there have been new participants in the manufacturing and dissemination market, the average production and distribution capacity declined. In spite of the increase in the number of small manufacturing units, it would be a matter to review whether the smaller units will have the obligatory proficiency and enticement for meeting farm requirements or synergistic links with larger manufacturers or distribution representatives or local bodies as supply of an agro-input also necessitates extensive sales networking and a profound understanding of the field reality in agriculture (Ghosh 2004; Mazid and Khan 2014). In states like Maharashtra and other states of the west and south, in spite of the central government's policies and legislation in promoting biofertilizers, there has virtually been no diffusion of the technology. The mean capacity consumption has been meagre, but the downscaling may have halted the negative drift.

13.14 Government Intervention

The Government of India and the many state governments have endorsed the emerging biofertilizer market similarly both at the level of the user-farmer and the producer-investor through the following action plan:

1. Farm level extension and promotion programmes
2. Financial aid to stakeholders in planning and developing functionally viable units
3. Subsidizations on sales
4. Direct manufacture in public sector and cooperative organizations and in agriculture research universities and institutions.

The Government of India initiated a central sector scheme entitled "National Project on Development and use of Biofertilizers" (NPDB) as a part of the Ninth Five-year Plan for the manufacture, dissemination and advancement of biofertilizers. The National Biofertilizer Development Centre, a subordinate agency of the Department of Agriculture and Cooperation, was instituted at Ghaziabad with regional centres at Hisar, Jabalpur, Bengaluru, Bhubaneswar, Nagpur and Imphal.

The accompaniment of minor new units with the major ones has improved the variability in trade (Ghosh 2004). The stake in supply, however, has been comparatively steadier in spite of a slim decreasing tendency in the past few years seemingly. This is only valid to a certain degree as divisions with huge circulation grids mete out to larger extents. The colossal public sector fertilizer industry IFFCO's MLN Farmers' Training Institute manufactures all strains of biofertilizers and distributes in various states. In the case of biofertilizers, the ingenuity engaged by the public sector alongside with many universities and research entities should gradually lead to marketable accomplishment once the technology is transmitted to the field.

13.15 Conclusion and Future Prospects

According to published data, most of the work in the area of plant probiotics has been conducted in the area of isolation, of pure culture with plant growth-promoting potentials, study on their physiology and finally on formulation and their commercial exploitation. In brief the application of plant probiotics for their plant growth-promotion potentials and for the other activities is mainly based on the concept of bio-augmentation: augmenting the cell number of desired organism using several approaches like seed coating, foliar spray or inoculating them during the time of showing. But despite the availability of a wide range of efficient fungal and bacterial strains with efficient probiotic potentials, their formulation and applications are still facing lots of problems due to several inherent reasons. Long-term viability and multiplication of applied cells in the natural climatic condition is still a major problem of this technology due to variability in climatic as well as geographical conditions like pH, temperatures, aeration, moisture, nutrients, soil organic contents and soil microbial community structure. In addition, the generation of huge amount of inoculum and their formulation is commercially an expensive approach for using the probiotics on the concept of bio-augmentation. Due to the biological nature of inoculum, survival of formulated cells at room temperature for longer storage and their further revival is a persistent problem. Another concern is partial taxonomic characterization of microorganism used for formulation and application which gives biased information about their taxonomic status and biosafety classes and create further risk to human and environment after application. Thus considering the current caveats in the application and formulation processes of the plant probiotics, we need to do more serious efforts in order to resolve them to increase the window of its applicability, storage and commercial viability. In addition to bio-augmentation, the concept of biostimulation of beneficial natural population should be equally

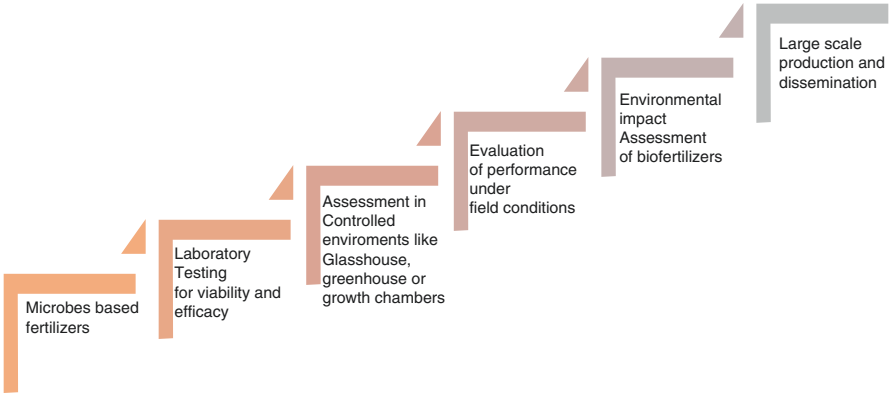

Fig. 13.1 Process of development of plant probiotics (biofertilizer/biocontrol)

promoted. More serious work is required on detection and physiology of external and internal colonization processes. Instead of just relying on 16S rRNA-based phylogeny and taxonomy species and strain level characterization and study of their biosafety classes should be traced out using polyphasic approcahes of microbial systematics before starting their formulation processes from personal as well as environmental safety point of view. Furthermore, we need to isolate and select physiologically robust strains which survive in a wide range of geographical and climatic conditions for formulation and application purposes to get the better result (Fig. 13.1).

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References

Antoun H, Prévost D (2005) Ecology of plant growth promoting rhizobacteria. In PGPR: Biocontrol and biofertilization. Springer, Dordrecht, pp 1–38

Baker CJ, Stavely JR, Thomas CA (1983) Inhibitory effect of Bacillus subtilis on Uromyces phaseoli and on development of rust pustules on bean leaves. Phytpopathology 73:11481152

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16:729–770

Ben Rebah F, Tyagi RD, Prévost D, Surampalli RY (2002) Wastewater sludge as a new medium for Rhizobial growth. Water Qual Res J Can 37:353–370

Berg G, Eberl L, Hartmann A (2005) The rhizosphere as a reservoir for opportunistic human pathogenic bacteria. Environ Microbiol 7:1673–1685

Bertin C, Yang X, Weston LA (2003) The role of root exudates and allelochemicals in the rhizosphere. Plant Soil 256:67–83

Bharathi R, Vivekananthan R, Harish S, Ramanathan A, Samiyappan R (2004) Rhizobacteria-based bioformulations for the management of fruit rot infection in chilies. Crop Prot 23:835–843

Bhardwaj D, Ansari MW, Sahoo RK, Tuteja N (2014) Biofertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. Microb Cell Fact 13:66–76

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Blakeman JP, Brodie IDS (1977) Competition for nutrients between epiphytic microorganisms and germination of spores of plant pathogens on beetroot leaves. Physiol Plant Pathol 10:29–34

Bodelier PLE, Wijlhuizen AG, Blom CWPM, Laanbroek HJ (1997) Effects of photoperiod on growth of and denitrification by *Pseudomonas chlororaphis* in the root zone of *Glyceria maxima*, studied in a gnotobiotic microcosm. Plant Soil 190:91–103

Bouizgarne B (2013) Bacteria for plant growth promotion and disease management. In: Bacteria in agrobiology: disease management. Springer, Berlin, pp 15–47

Bultman TL, Murphy JC (2000) Do fungal endophytes mediate wound induced resistance ? In: Bacon CW, White J (eds) Microbial endophytes. CRC Press, New York

Carvalhais LC, Dennis PG, Fan B, Fedoseyenko D, Kierul K, Becker A, von Wiren N, Borriss R (2013) Linking plant nutritional status to plant-microbe interactions. PLoS One 8(7):e68555

Catroux G, Amarger N (1992) Rhizobia as soil inoculants in agriculture. In: Fry JC, Day MJ (eds) Release of genetically engineered and other micro-organisms. Cambridge University Press, Cambridge, pp 1–13

Charpentier CA, Gadille P, Benoit JP (1999) Rhizobacteria microencapsulation: properties of microparticles obtained by spray drying. J Microencapsul 16:215–229

Chen C, Belanger RR, Benhamou N, Paullitz TC (2000) Defence enzymes induced in cucumber roots by treatment with plant-growth promoting rhizobacteria (PGPR). Physiol Mol Plant Pathol 56:13–23

Compant S, Clément C, Sessitsch A (2010) Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol Biochem 42:669–678

Dandurand LM, Morra MJ, Chaverra MH, Orser CS (1994) Survival of *Pseudomonas* spp. in air dried mineral powders. Soil Biol Biochem 26:1423–1430

Dardanelli MS, Carletti SM, Paulucci NS, Medeot DB, Cáceres ER, Vita FA, Bueno M, Fumero MV, Garcia MB (2010) Benefits of plant growth-promoting rhizobacteria and rhizobia in agriculture. In: Plant growth and health promoting bacteria. Springer, Berlin, pp 1–20

Date RA (2000) Inoculated legumes in cropping systems of the tropics. Field Crop Res 65:123–136

Dennis PG, Miller AJ, Hirsch PR (2010) Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? FEMS Microbiol Ecol 72:313–327

El-Fattah DAA, Eweda WE, Zayed MS, Hassanein MK (2013) Effect of carrier materials, sterilization method, and storage temperature on survival and biological activities of *Azotobacter chroococcum* inoculant. Ann Agric Sci 58:111–118

Ghosh N (2004) Promoting biofertilisers in Indian agriculture. Econ Pol Wkly:5617–5625

Hardoim PR, van Overbeek LS, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, Sessitsch A (2015) The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiol Mol Biol Rev 79:293–320

Herridge DF (2008) Inoculation technology for legumes. In: Dilworth MJ, James EK, Sprent JI, Newton WE (eds) Nitrogen-fixing leguminous symbioses. Springer, Dordrecht, pp 77–115

Herrmann L, Lesueur D (2013) Challenges of formulation and quality of biofertilizers for successful inoculation. Appl Microbiol Biotechnol 97:8859–8873

Herrmann L, Atieno M, Brau L, Lesueur D (2013) Microbial quality of commercial inoculants to increase BNF and nutrient use efficiency. In: de Bruijn FJ (ed) Molecula microbial ecology of the rhizosphere. Wiley-Blackwell, Hoboken

Hungria M, Loureiro MF, Mendes IC, Campo RJ, Graham PH (2005) Inoculant preparation, production and application. In: Werner D, Newton WE (eds) Nitrogen fixation in agriculture forestry, ecology, and the environment. Kluwer, Dordrecht, pp 223–253

Jenkins NE, Grzywacz D (2000) Quality control of fungal and viral biocontrolagents assurance of product performance. Biocontrol Sci Technol 10:753–777

Jorjani M, Heydari A, Zamanizadeh HR, Rezaee S, Naraghi L (2011) Development of *Pseudomonas fluorescens* and Bacillus coagulans based bioformulations using organic and inorganic carriers and evaluation of their influence on growth parameters of sugar beet. J Biopest 4:180–185

Kalra A, Chandra M, Awasthi A, Singh AK, Khanuja SPS (2010) Natural compounds enhancing growth and survival of rhizobial inoculants in vermicompost-based formulations. Biol Fertil Soils 46:521–524

Kannaiyan S (2003) Inoculant production in developing countries problems, potentials and success. In: Hardarson G, Broughton WJ (eds) Maximising the use of biological nitrogen fixation in agriculture. Kluwer, Dordrecht, pp 187–198

Krishnamurthy K, Gnanamanickam SS (1998) Biocontrol of rice sheath blight with formulated *Pseudomonas putida*. Indian Phytopathol 51:233–236

JG LB, Laiño JE, del Valle MJ, Vannini V, Van Sinderen D, Taranto MP, Sesma F (2011) B-Group vitamin production by lactic acid bacteria–current knowledge and potential applications. J Appl Microbiol 111:1297–1309

Lupwayi NZ, Olsen PE, Sande ES, Keyser HH, Collins MM, Singleton PW, Rice WA (2000) Inoculant quality and its evaluation. Field Crop Res 65:259–270

Maheshwari DK, Saraf M, Aeron A (2012) Bacteria in agrobiology: crop productivity. Springer, Berlin, pp 127–165

Malusá E, Sas-Paszt L, Ciesielska J (2012) Technologies for beneficial microorganisms inocula used as biofertilizers. ScientificWorldJournal 2012:491206

Mazid M, Khan TA (2014) Future of bio-fertilizers in Indian agriculture: an overview. Int J Agric Food Res 3:10–23

Meena B, Radhajeyalakshmi R, Marimuthu T, Vidyasekran P, Velazhahan R (2002) Biological control of ground nut late leaf spot and rust by seed and foliar applications of powder formulation of Pseudomonas fluorescence. Biocontrol Sci Technol 12:195–204

Miransari M (2011) Interactions between arbuscularmycorrhizal fungi and soil bacteria. Appl Microbiol Biotechnol 89:917–930

Nadeem SM, Naveed M, Ahmad M, Zahir ZA (2015) Rhizosphere bacteria for crop production and improvement of stress tolerance: mechanisms of action, applications, and future prospects. In: Plant microbes symbiosis: applied facets. Springer India, pp 1–36

Nakkeeran S, Kavitha K, Mathiyazhagan S, Fernando WGD, Chandrasekar G, Renukadevi P (2004) Induced systemic resistance and plant growth promotion by *Pseudomonas chlororaphis*strain PA-23 and *Bacillus subtilis*strain CBE4 against rhizome rot of turmeric (*Curcuma longa* L.) Can J Plant Pathol 26:417–418

Nandakumar R, Babua S, Viswanathanb R, Raguchandera T, Samiyappan R (2001) Induction of systemic resistance in rice against sheath blight disease by *Pseudomonas fluorescens*. Soil Biol Biochem 33:603–612

Okon Y, Hadar H (1987) Microbial inoculants as crop-yield enhancers. Crit Rev Biotechnol 6:61–85

Olsen PE, Rice WA, Bordeleau LM, Biederbeck VO (1994) Analysis and regulation of legume inoculants in Canada: the need for an increase in standards. Plant Soil 161:127–134

Prakash O, Sharma R, Rahi P, Karthikeyan N (2015) Role of microorganisms in plant nutrition and health. In: Rakshit A, Singh HB, Sen A (eds) Nutrient use efficiency: from basics to advances. Springer India, pp 125–161

Prakash O, Sharma R, Singh P, Yadav A (2016) Strategies for taxonomical characterisation of agriculturally important microorganisms. In: Microbial inoculants in sustainable agricultural productivity. Springer India, pp 85–101

Raaijmakers JM, Paulitz TC, Steinberg C, Alabouvette C, Moënne-Loccoz Y (2009) The rhizosphere: a playground and battlefield for soil borne pathogens and beneficial microorganisms. Plant Soil 321:341–361

Raupach GS, Kloepper JW (1998) Mixtures of plant growth-promoting rhizobacteria enhance biological control of multiple cucumber pathogens. Phytopathology 88(11):1158–1164

Roesch LF, Fulthorpe RR, Riva A, Casella G, Hadwin AK, Kent AD, Daroub SH, Camargo FA, Farmerie WG, Triplett EW (2007) Pyrosequencing enumerates and contrasts soil microbial diversity. The. ISME J 1:283–290

Sanap DJ, Satpute TG, Dudhate DG, Babar AP, Nagure DV (2009) Constraints and suggestions in soybean crop under organic and inorganic system. Int J Agric Sci 5:608–610

Sharma S, Gupta R, Dugar G and Srivastava AK (2012) Impact of application of biofertilizers on soil structure and resident microbial community structure and function. In: Maheswari DK (ed) bacetria in agrobiology: plant probiotics. Springer, Heidelberg, pp 65–77

Soccol CR, Vandenberghe LPDS, Spier MR, Medeiros ABP, Yamaguishi CT, Lindner JDD, Thomaz-Soccol V (2010) The potential of probiotics: a review. Food Tech Biotechnol 48:413–434

Somers E, Vanderleyden J, Srinivasan M (2004) Rhizosphere bacterial signalling: a love parade beneath our feet. Crit Rev Microbiol 30:205–240

Song D, Hayek S, Ibrahim S (2012) Recent application of probiotics in food and agricultural science. In: Rigobelo EC (ed) Immunology and microbiology "Probiotics". INTECH Publisher, pp 123–141

Stephens JHG, Rask HM (2000) Inoculant production and formulation. Field Crop Res 65:249–258

Thilagar G, Bagyaraj DJ, Rao MS (2016) Selected microbial consortia developed for chilly reduces application of chemical fertilizers by 50% under field conditions. Sci Hortic 198:27–35

Trivedi T, Pandey A, Palni LMS (2005) Carrier-based preparations of plant growth-promoting bacterial inoculants suitable for use in cooler regions. World J Microbiol Biotechnol 21(I6): 941–945

Vidhyasekaran P, Muthamilan M (1995) Development of formulations of Pseudomonas fluorescens for control of chickpea wilt. Plant Dis 79:782–786

Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511

Wiesman Z (2009) Desert olive oil cultivation: Advanced biotechnologies. Academic, London, pp 67–89

Willer H (2011) The world of organic agriculture: statistics and emerging trends. In: Willer H, Kilchar L (eds) Research Institute of Organic Agriculture (FiBL), Frick and International Federation of Organic Agriculture Movements (IFOAM), Bonn

Xavier IJ, Holloway G, Leggett M, Bios P (2004) Development of rhizobial inoculant formulations. Online Crop Manag. doi:10.1994/CM-2004-0301-03-RV

Young CC, Shen FT, Singh S (2012) Strategies for the exploration and development of biofertilizer. In Bacteria in agrobiology: plant probiotics. Springer, Berlin pp 127–139

Zaidi A, Khan MS, Amil MD (2003) Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (Cicerarietinum L.) Eur J Agron 19:15–21

Zayed MS (2016) Advances in formulation development technologies. In: Microbial inoculants in sustainable agricultural productivity, Springer India, pp 219–237

Modulation of Plant Micronutrient Uptake by Arbuscular Mycorrhizal Fungi

14

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Abstracts

Plants require light, water, and nutrients for better growth and reproduction. Arbuscular mycorrhizal (AM) fungi associate with root systems of most land plants and improve plant growth by enhancing the uptake of soil nutrients, including micronutrients. Contradictory influence of mycorrhizal plants in micronutrient uptake may be due to different edaphic conditions, which affect AM fungal root colonization and extraradical hyphal development. The micronutrient uptake of plants is influenced by different factors like availability of macronutrient like phosphorus (P) and micronutrients themselves in soil. AM fungal hyphal growth and root colonization are suppressed by high levels of micronutrients in soil. In soils the mobility of Cu, Zn, Mn, and Fe is low, and uptake by roots is restricted by low diffusion rates and root depletion zones created by plant roots. AM plants overcome this by exploring large volume of soil compared to roots and minimize the diffusion distance to enhance the availability of these immobile nutrients. Uptake of Cu and Zn or Mn and Fe is quite different. The uptake of Cu and Zn is affected by amount of plant and soil P levels, whereas the uptake of Mn and Fe is affected by indirect reduction of oxidation-reduction potential and availability of Mn and Fe in mycorrhizosphere. Under stress conditions, AM fungi help plants to increase their nutrient uptake, thereby imparting tolerance to prevailing stress. This is seen especially under saline conditions where AM fungal application limits the Na^+ and Ca^{2+} ion concentration in plants by enhancing Mg^{2+} uptake, thereby increasing chlorophyll concentration,

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photosynthetic efficiency, and plant growth. AM fungi are potential tool for improving plant health and rhizosphere for better uptake of micronutrients under various edaphic conditions.

14.1 Introduction

All plants in natural and seminatural ecosystems are colonized by AM fungi and form mycorrhizosphere in addition to rhizosphere (Johansson et al. 2004). Root and mycorrhizal fungi both influences the mycorrhizosphere region, whereas more particularly the term hyposphere refers to the region surrounding individual hyphae (Johansson et al. 2004). Soil microbes play a vital role on maintaining soil fertility and plant health (Gianinazzi and Schuepp 1994). Arbuscular mycorrhizal (AM) fungi are mutualistic symbiotic fungi, a major microbial population in soil, which influence the nutrient uptake and plant productivity (Johansson et al. 2004). Arbuscular mycorrhizal fungi are associated with more than 250,000 of plants worldwide (Smith and Read 1997). The formation of AM fungal symbiosis started with the penetration of host root cortical cells by AM fungi which form arbuscules (treelike), vesicles (saclike), arbusculate coils, and hyphal coils that interface with host cytoplasm (Fig. 14.1) (Smith and Read 1997).

These structures provide increased surface area for exchange of metabolites between plants and fungi. Arbuscular mycorrhizal fungi produce inter- and

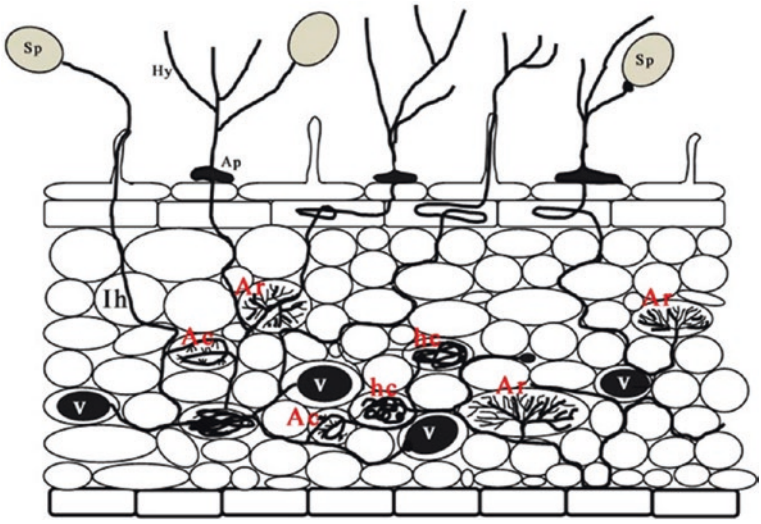

Fig. 14.1 Arbuscular mycorrhizal fungal interactions with host plant showing different functional structures. *Ap* arbuscular mycorrhizal fungal spore, *Hy* arbuscular mycorrhizal fungal hyphae, *Ap* appressorium, *V* vesicles, *Ar* arbuscules, *Ac* arbusculate coils, *Hc* hyphal coils, *Ih* intercellular hyphae

intracellular hyphae which are also connected with soils in rhizosphere regions beyond several centimeters away from the soil (Rhodes and Gerdemann 1975). The total surface areas of hyphae are higher in several orders of magnitude than that of roots which increase the nutrient uptake potentially. Moreover, AM fungal hyphae play an important role in soil stabilization through the formation of soil aggregates and mobilize the organically bound N from plant litter (Tisdall and Oades 1979; Hodge et al. 2001). Arbuscular mycorrhizal associations influence the mineral nutrient acquisition of colonized plants by various ways such as high spatial availability of nutrients and mobilization of sparingly available nutrients and protect the host plants against pathogens and abiotic stress (drought, salinity, metal toxicity, low temperature) (Marschner 1995). Arbuscular mycorrhizal (AM) fungi enhance plant growth by enhancing the absorption of N, P, K, Ca, S, Cu, Zn, Fe, and Mn through increase in the absorbing surface areas. AM fungal hyphae provide a greater absorptive root surface, capable of exploring greater volume of soil, thus limiting nutrients and water depletion zones (Clark and Zeto 1996). Plants colonized by AM fungi reduce toxicity of Al and Mn ions and pH of the rhizosphere, and these effects depend on edaphic and climatic conditions and compatibility between plant-fungus interactions. The mechanisms involved in better acquisition of Zn and Cu by colonized roots is thought to be similar to that of P (Lambert et al. 1979). In various reports, AM colonization in the concentration of potassium, calcium, magnesium (Lambert et al. 1979), iron (Liu et al. 2000), manganese (Eivazi and weir 1989; Lu and Miller 1989), and boron (Lu and Miller 1989) in plants was at various levels, low to low or unchanged compared to non-mycorrhizal plants.

Arbuscular mycorrhizal fungal colonization may alter the root morphology, which is responsible for high to low levels of nutrients uptake by plants. In addition to root morphology, rhizosphere microorganisms play a key role in nutrient uptake. The decrease or increase of the level of Mn to plant roots depends on the range and effectiveness of Mn-oxidizing and Mn-reducing microorganisms in the rhizosphere (Marschner 1988). Highly variable edaphic factors are crucial for inconsistent responses of mycorrhizal plants in micronutrient uptake, extraradical hyphal development and root colonization are influenced by soil conditions, and AM fungi in turn influence the uptake of these metals (Liu et al. 2000). In varied stress conditions like drought and salinity (Audet and Charest 2006), AM fungi improve the uptake of nutrients to enhance the survival of host plants. In wheat plants, both well-watered and water-stressed conditions aboveground mineral nutrient contents (P, Zn, Mn, Cu, and Fe) had been considerably high, compared to non-mycorrhizal plants. In saline conditions, high levels of Fe, Cu, and Zn concentration and total accumulation occurred in mycorrhizal host compared to non-mycorrhizal plants (Al-Karaki 2000).

14.2 Copper

Copper plays a vital role in photosynthetic and electron transport systems, activity of various oxidative enzymes, and pollen formation (Marschner 1995). Mycorrhizal peach seedlings show root copper concentration of 321% by inoculation of

G. mosseae and 178% by *G. versiforme*, whereas leaves show less than that of non-mycorrhizal plants, which implies that AM fungi play an important role in uptake instead of translocation (Wu et al. 2011). Arbuscular mycorrhizal fungal species shows different ranges of Cu uptake in different plant species. *G. etunicatum* and *G. mosseae* inoculated in wheat plant under well-watered and water-stressed conditions increased the shoot Cu concentrations (Al-Karaki et al. 2004). Spores of G. *etunicatum*, *G. macrocarpum*, and *Gigaspora margarita* inoculated in *Desmodium cinereum* showed increased Cu concentrations in root and shoot (Adiova et al. 2013). Tomato seedlings inoculated with *G. fasciculatum* and *G. intraradices* showed high tissue Cu concentration than non-inoculated tomato plants (Ramakrishnan and Selvakumar 2012). Arbuscular mycorrhizal fungal inoculation significantly increased the Cu concentrations in tea plants (Kahneh et al. 2006). In AM-inoculated cassava plants, the micronutrient uptake was high compared to non-inoculated plants. Most of the micronutrients were partitioned to roots, and Cu was consistently partitioned more to roots than shoots. This partition effect was due to AM fungal colonization, and the partitioning pattern could be attributed to the trace element toxicity. The level of micronutrient reduced to toxic, and the element could be diverted to the areas where it could be stored with less injury to plant. Copper is in toxic level, and roots are the sites of preferential when external supply is large (Simwambana and Ekanayake 2001). In AM-colonized citrus, stem and leaf accumulated 50 and 500% more Cu compared with non-mycorrhizal plants. But colonized citrus roots acquired 2–10 times more Cu compared with non-mycorrhizal plants. In maize, the concentration was 6 times higher in mycorrhizal roots; its concentration in shoots did not vary much between mycorrhizal and non-mycorrhizal treatments (Kothari et al. 1990). It is not clear whether the increased amount of Cu in roots of mycorrhizal plants is available to the plants, as it may be bound to fungal polyphosphate granules as has been shown for a Cu, Fe, and Mn by White and Brown (1979) or sequestered in fungal structures. In *Cicer arietinum*, increase in Cu uptake with P application may be due to increased root growth, which resulted in better exploration of soil volume. However, an antagonistic effect of Cu and P in rice was observed, where one of the nutrients were applied in large quantity (Tandon 2001). Higher rate of P application was found to have no influence on Cu concentration in red kidney beans, tomato, or sweet corn (Tandon 2001). This difference between genus and species of plants might be attributed to the genetic composition of plant species (Tandon 2001). Havlin et al. (2007) reported reduced uptake of Cu due to high rate of P application result in formation of copper phosphate, which is not readily available to plants. In calcareous soil, white clover with restricted rooting space, the delivery of Cu from the hyphal compartment ranged from 52 to 62% of the total uptake (Li et al. 1991). Increasing P supply to the hyphal compartment enhanced hyphal delivery of P and slightly depressed that of Cu with corresponding increase in the P in molar ration from 37 to 912 (25%). Thus, hyphal uptake and transport of P and Cu appear to be rather independent. In contrast, partitioning of Cu between roots and shoots was strongly affected by P. Phosphorus enhanced not only the content but also the concentration of Cu in the shoot dry matter indicating that the enhancement effect of P on Cu translocation was not exclusively regulated by

the shoot demand. These results also demonstrate that particularly with Cu the role of AM in uptake cannot properly be evaluated from only the shoot content or concentration. According to Liu et al. (2000), micronutrient and P level in soil significantly influence the uptake of Cu in maize. The micronutrient amount and P levels are not only factors which determine the uptake but also on which metal to be considered. The plants grown in low P regime have high extraradical hyphal growth and potentially explore large volume of soil and absorb large amount of micronutrients. Increased shoot P content in plants grown at high soil P levels can increase Cu sink size. This may stimulate uptake and translocation of Cu to plant shoots. Micronutrient uptake by plant roots are diffusion limited (Tisdale et al. 1993) and plants colonized by AM fungi uptake more metal nutrients via extraradical hyphae.

14.3 Zinc

Zinc is considered as a key element in maintaining cellular membrane integrity; acts as an essential enzyme metal constituent and functional, structural, or regulatory cofactor; and is associated with saccharine metabolism, photosynthesis, and protein synthesis in plants (Val et al. 1987), formation of pollen grains, and disease resistance potential (Marschner 1995). In addition, Zn plays a vital role in regulating gene expression and stress tolerance such as high solar radiation and temperature (Broadley et al. 2007). Arbuscular mycorrhizal fungi enhanced the uptake of Zn (Guo et al. 1996), although significantly smaller quantities compared to P. It is because Zn may not be as readily translocated from roots to shoots as P, since Zn distribution in roots and shoots is determined by soil P levels. But, Zn acquisition was decreased when P was increased in soil (Lambert and Weidensaul 1991), and enhanced acquisition of Zn occurred in high soil P levels (Raju et al. 1990). The increased Zn content was observed in various studies using different AM fungal species. *Plantago ovata* inoculated with *G. mosseae*, *Gigaspora margarita*, *Acaulospora morrawae*, and *G. deserticola* showed increased Zn concentrations (Mathur et al. 2006). Mycorrhizal-inoculated watermelon, cucumber, maize, cotton, horse bean, chick pea, and soybean showed high Zn concentration under non-fumigated conditions than fumigated conditions, because fumigation process eradicates the other beneficial organisms (Ortas 2012). Increased Zn concentrations were observed in alfalfa plants inoculated with *G. etunicatum*, *G. intraradices*, and *G. mosseae* under pot culture conditions (Zaefarian et al. 2011). Cucumber plants inoculated with *G. etunicatum*, *G. clarum*, and *G. caledonium* showed higher Zn tissue concentration than non-inoculated controls (Ortas 2010; Wang et al. 2008; Lee and George 2005). *Glomus versiforme*, *G. intraradices*, and *G. etunicatum* increased the uptake of Zn in apple root stocks in calcareous soils (Hosseini and Gharaghani 2015). In general view, the elements (Zn) with low mobility in the soil can be absorbed in higher levels by mycorrhizal plants (Yano-melo et al. 1999). The *Pistacia vera* inoculated with *G. mosseae* and *G. intraradices* showed higher Zn concentration than non-inoculated controls under greenhouse conditions (Bagheri et al. 2012). Inoculation of wheat with *G. mosseae* increased Zn uptake in wheat

tissues under calcareous soil conditions (Ghasemi-Fasaei et al. 2012). Higher Zn concentration (350%) was observed in *Euterpe oleracea* seedlings inoculated with mycorrhizal fungi *Scutellospora gilmorei*, *Acaulospora* sp., and *G. margarita* (Chu 1999). The higher uptake efficiency of Zn was observed in *Vitis vinifera* under pot experiment (Schreiner 2007). After P, Cu and Zn are second most important nutrients that are promoted by AM fungal colonization (Lee and George 2005). A comparative observation between mycorrhizal and non-mycorrhizal plants showed 32% higher Zn concentrations in roots (Lehmann et al. 2014). Extraradical hyphae contributed more in Zn uptake (Kothari et al. 1990), whereas in total zinc uptake, 48% is by fungal hyphae (Kothari et al. 1990). Extraradical hyphal growth of AM fungi has negative (Liu et al. 2000), positive (Seres et al. 2006), and neutral (Toler et al. 2005) impacts upon soil zinc additions. Decreased hyphal density of *G. intraradices* inoculated in maize plants with increasing soil Zn addition was observed (Liu et al. 2000). However, increased hyphal length density and intraradical colonization were found in soils added with zinc (Seres et al. 2006). The differences are likely due to complex interactions between edaphic and environmental conditions and difference in Zn addition. Moreover, plant and fungal identity is an important factor for responses of AM fungi to soil Zn addition. Increased Zn addition decreases the root colonization of AM fungi (Bi et al. 2003; Chen et al. 2004). This response results when at low toxic level, AM fungi improve Zn nutrition and at above toxic level, they protect plant tissues from Zn accumulation. The studies mostly focused on Zn effects on intraradical colonization have focused on Zn inputs in excess of this toxic level (Cavagnaro 2008). But few experiments on low level of Zn addition decreased AM fungal colonization in onion inoculated with *G. mosseae* (Gildon and Tinker 1983). The percentage of root length colonization was decreased from 74 to 47 to 0% over a range of Zn additions (0, 10, and 75 mg Zn/kg soil as $ZnSo_4$) and from 55 to 42 to 0% with Zn additions of 0, 10, 20, 40, and 75 mg Zn/kg soil. The reduction in colonization of sections of the root systems nor directly exposed to increased Zn (Gildon and Tinker 1983). In other studies, slight increase in colonization (40–46%) of white clover was observed in an unamended soil and high Zn addition treatment (400 mg/kg), and root biomass was similar in all treatments. Wild tobacco inoculated with *Glomus intraradices* increases colonization from 14 to 82% over a range of Zn addition (0–250 mg Zn/kg as $ZnSo_4$) (Audet and Charest 2006). In conclusion Zn does not necessarily result in a significant reduction in colonization, because AM fungal colonization was observed in plants growing in Zn-contaminated soils (Hildebrandt et al. 2006). These effects are due to selection of AM fungal species and for strains that can withstand high Zn concentrations. *Phaseolus vulgaris* colonized by *Glomus etunicatum* increased 24–92% with the addition of 5 mg/kg, whereas *G. mosseae* was not effective (88–90%) (Ortas and Akpinar 2006). These effects are provided by edaphic factors. A diverse range of responses of mycorrhizal colonization to Zn addition was reported by various authors (Vivas et al. 2006; Whitefield et al. 2004). Arbuscular mycorrhizal fungi and plant identity, edaphic factors, and other environmental conditions play an important role in modulating nutrient uptake and colonization responses in extraradical phase of colonization (Cavagnaro 2008). Arbuscular mycorrhizal fungi translocate the nutrients from

nutrient depletion zones formed around the roots. Burkert and Robson (1994) elucidate that AM fungi take up Zn 40 mm apart from the root surface. In maize *G. intraradices* increase the uptake of both P and Zn of plants, and almost 9% of the added Zn was transported to the plants from a distance of 50 mm within 25 days (Jansa et al. 2003). The development of large mycelia network that can enhance the potential of AM fungi to locate and utilize heterogeneously distributed Zn in the soil would likely provide a competitive advantage to plants.

14.4 Manganese

Nutrient uptake by plants depends on availability of nutrients and effectiveness of root systems for absorption (Liu et al. 2000). Difference in manganese concentration between mycorrhizal and non-mycorrhizal plants was higher in roots than in shoots; this is due to mycorrhizal fungi altering the distribution of the nutrient (Arines et al. 1989). Mn acquisition was decreased in AM plant (Kothari et al. 1991). Mycorrhizal plants will uptake lower Mn which may be explained by the presence of some hyphal mechanism controlling microorganisms (Arines et al. 1989). Increased or decreased uptake of Mn has been shown to depend on the presence of Mn-oxidizing microorganisms or the accumulation of root-derived nutrients that increase the formation of complexes of the element (Merckx et al. 1983). Both chemical and microbial processes determine the chemical equilibrium between reduced and oxidized forms of Mn (Sparrow and Uren 1987). It is possible that AM fungi play an indirect role in the uptake of Mn and the effects depend on soil chemical and microbial characteristics (Arines et al. 1989). Soil pH and oxidation-reduction potential determine the Mn availability in soils. Higher Mn uptake has been observed in plants grown in acidic soil conditions, because Mn is more soluble in acidic than alkaline conditions (Habte and Soedarjo 1995). Arbuscular mycorrhizal fungi were found to reduce the number of Mn-reducing bacteria (Posta et al. 1994) or increase the number of Mn-oxidizing bacteria in the rhizosphere (Arines et al. 1992). Therefore, AM fungi indirectly reduce oxidation-reduction potential and Mn availability in mycorrhizosphere (Liu et al. 2000). Reduced forms of these elements are more available to plants (Marschner 1988). External hyphae are responsible for the effectiveness of mycorrhizal root absorption (Burkert and Robson 1994). Increased or decreased uptake of Mn may depend on which of the two functions prevails under given soil conditions (Liu et al. 2000). AM-colonized plants have low Mn levels compared to non-mycorrhizal plants under high micronutrient level. This is due to more reduced availability of Mn than increased absorption efficiency by AM fungi in high micronutrient level. Arbuscular mycorrhizal fungal hyphae contain polyphosphates which sequester Mn by polyphosphate granules and minimize transfer to roots of the mycorrhizal plants, and these are considered as filter mechanisms (Turnau et al. 1993). The enhancement or alleviation of Mn toxicity in mycorrhizal plants is not exclusively attributed to the AM fungal species, but may be the result of several interactions attributed to changes in host physiology, with reflection on the microbial community in the mycorrhizosphere (Filion et al. 1999) and on the biological

processes of Mn oxidation (Nealson et al. 1988) and reduction (Kothari et al. 1991). Nogueira and Cardoso (2003) reported that *Glycine max* associated with *G. etunicatum* and *G. intraradices* also presented higher P concentrations in the tissues, to support the higher Mn concentration in the tissues, suppressing the Mn toxicity symptoms. Bethlenfalvay and Farson (1989) observed that, although mycorrhizal plants presented greater Mn concentration, there were no toxicity symptoms. This might have occurred because of an increase of internal tolerance to Mn (Foy et al. 1978) by plants' better accumulation with P. The lower Mn concentration in mycorrhizal plant was proportional to increase the plant biomass (Nogueira and Cardoso 2003). A positive equilibrium between the oxidizing and reducing microorganisms for AM plants decreased Mn acquisition in plants (Clark and Zeto 2000). The root exudates and microbial population in rhizosphere regions are also important for low acquisition of Mn by plants (Posta et al. 1994).

14.5 Ferrous

Arbuscular mycorrhizal fungi altered (increase or decrease) the Fe acquisition (Clark and Zeto 1996; Caris et al. 1998). Mycorrhizal-colonized plants grown under low pH uptake higher Fe content compared to AM plants grown in higher pH (Medeiros et al. 1993). Moreover, mycorrhizal plants grown in alkaline soil showed increased Fe uptake than those plants grown in acidic conditions (Clark and Zeto 1996). Reduced Fe is more available to plants (Marschner 1998). Arbuscular mycorrhizal fungi increase or decrease the uptake of Fe which may depend on the oxidation-reduction potential and effectiveness of root systems for absorption, of which these two functions prevail under given soil conditions (Liu et al. 2000). Under conditions of low nutrient level, AM fungal hyphae enhanced uptake of Fe by improved scavenging of this element. Mycorrhizal-inoculated maize grown in Fe-deficient soils showed improved Fe uptake (Clark and Zeto 1996). In mycorrhizal maize and soybean (Pacovsky et al. 1986) plants, the shoot Fe concentrations were low (Kothari et al. 1990). In general iron acquisition has been related with the presence of root exudates such as phytosiderophores (Marschner and Romheld 1994) and organic acids like citric, oxalic, and phenolics in mycorrhizosphere regions (Marschner 1998). In addition AM fungal species, host plant, and edaphic and various stress conditions determine the iron acquisition (Al-Karaki et al. 1998; Caris et al. 1998).

14.6 Arbuscular Mycorrhizal Fungal Hyphae in Nutrient Uptake

The mobility of micronutrients in soil is very much low, and AM fungal hyphae aid in the uptake of more micronutrients, which gives more absorptive area compared to root alone and minimizes the distance of diffusion, thereby enhancing the absorption by immobile micronutrients (Jakobsen et al. 1992). In soils contained high

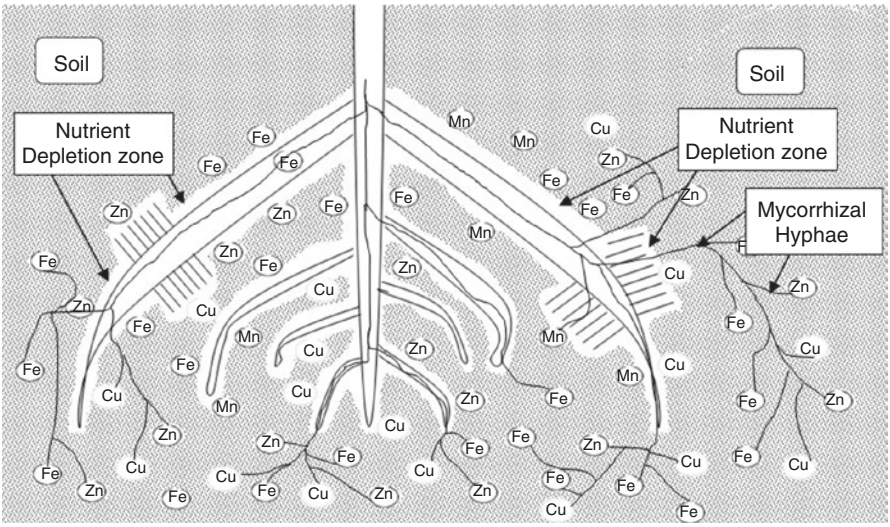

Fig. 14.2 Micronutrient absorption strategy of arbuscular mycorrhizal fungi through extraradical fungal hyphae in rhizosphere regions

density of extraradical hyphae which had higher absorption surface and metal diffusion distance will be low (Fig. 14.2). Therefore, AM plants effectively absorb the low-mobility metal nutrients (Burkert and Robson 1994). Arbuscular mycorrhizal fungal hyphae are more efficient in nutrient absorption than non-colonized roots. The higher uptake of mycorrhizal plants is due to fungal hyphae, and mycorrhizal plants should have a hyphal surface area at least equal to the total root surface area of non-mycorrhizal plants. In maize plants, hyphal surface area is 19% of the root surface area of non-mycorrhizal plants (Kothari et al. 1990), which shows P absorption efficiency per unit surface area basis of hyphae is at least five times higher than roots.

14.7 Difference Between Mycorrhizal and Non-mycorrhizal Plants

Arbuscular mycorrhizal fungal colonization changes host plant morphology. Fungal hyphae provide efficient surface with subsequent transfer to the host, capacity of the mycorrhizal or hyphae to utilize micro- and macronutrients not available to non-mycorrhizal roots, and increased viability of mycorrhizal roots than non-mycorrhizal. Non-mycorrhizal plant and mycorrhizal plants are compared in growth and nutrient uptake in various pot experiments using sterile soils. When compared to non-AM plants, AM plants acquire more phosphate (P) from the rhizosphere and attain better growth. In so many cases, the uptake of other elements also differs between mycorrhizal and non-mycorrhizal plants. However, it is impossible to determine the direct effect of AM fungi to plant micronutrient uptake by simply comparing the uptake of

non-mycorrhizal plants from the nutrient uptake of mycorrhizal plants. Arbuscular mycorrhizal fungi contribute directly or indirectly to plant growth. Many of these are related to better P uptake of mycorrhizal plants from low P soils, leading to greater shoot growth and root length, in particular, are less increased (Gnekow and Marschner 1989). Soil with adequate P levels morphology of shoot and root differed between AM and non-AM plants. Approximately 40% of the total root length was reduced in non-mycorrhizal maize plants when compared to mycorrhizal inoculated maize plants approximately 40%, in the presence or absence of mycorrhiza. AM-colonized or uncolonized plants differ in so many aspects, and the difference in the micronutrient content of plants does not necessarily reflect (George et al. 1992).

14.8 Impact of Micronutrient by AM and Non-AM Plants

Mycorrhizal and non-mycorrhizal plant comparison is problematic, but most of the current studies regarding AM fungal effects on plant micronutrient uptake are determined from such comparisons. In AM plants, concentration and total content of Zn and Cu are increased (Sharma et al. 1994). This becomes especially clear, and fertilization with additional P is needed when compared to mycorrhizal and non-mycorrhizal plants, to achieve similar P uptake in both treatments (Pacovsky and Fuller 1988). Arbuscular mycorrhizal fungal colonization does not influence the micronutrient uptake in few studies (George et al. 1992). Plant species and cultivar, fungal species, soil pH, soil physical conditions, soil temperature, soil P availability, and the levels of nutrient supply all influence the mycorrhizal effect on micronutrient uptake (Kilham and Firestone 1983; Liu et al. 2000). The broad generalizations are not possible for plants colonized by AM fungi. Direct or indirect effects of mycorrhizal colonization and more detailed investigations are required for the uptake of extraradical hyphae in order to determine the changes in micronutrient uptake resulting from mycorrhizal colonization (George et al. 1992).

14.9 Implications for AM Functioning in Nutrient Uptake

The amount of AM fungi which is active in nutrient transfer does not necessarily depend on the length of root colonized by AM fungi (Smith and Gianinazzi-Pearson 1990). Only during periods of high P demand do AM fungi contribute to the necessary rate of uptake (Sanders and Fitter 1992). The plants are in the stage of flowering and seed production which have highest rates of photosynthesis and respiration which needs high P demand. This is when AM fungi are more effectively involved in nutrient uptake (Sanders and Fitter 1992). *Phaseolus lanceolata* and *Rumex acetosa* showed highly irregular patterns of nutrient uptake which cannot be attributed to a specific period in the growth season (Sanders and Fitter 1992). From this it is impossible to determine exact periods of nutrient uptake from soil, because plant nutrient content was measured in shoots only, whereas in pot experiment, number of plants and replication were limited, so roots were used for mycorrhizal

assessment. It is possible that the timing of nutrient uptake into the roots could occur sometime before the transfer to the shoots. This creates more complexity in detecting a relationship between colonization levels and nutrient uptake (Sanders and Fitter 1992).

14.10 Consequence in Mycorrhizosphere

AM fungal colonization not only modulates the morphophysiology of the host root, and colonization also changes the conditions in mycorrhizosphere (Linderman 1992). There is a considerable difference of root exudation between AM-colonized and non-colonized root (Schwab et al. 1983). Root exudations are energy sources for microorganisms in rhizosphere (George et al. 1992). The suitable example is plant Mn uptake; when compared to non-AM plants, either decreased or increased concentration was observed in AM plants (Liu et al. 2000). The mechanisms responsible for this contrasting behavior are different root exudations of AM fungal-colonized roots and a lower number of Mn-reducing bacteria in mycorrhizosphere, so Mn uptake (Kothari et al. 1991), when the Mn-oxidizing bacterial population will be high in the mycorrhizosphere which causes to less soil Mn availability (Arines et al. 1992). In addition exudation of Mn-chelating exudates may be decreased in AM-colonized plants (Bethlenfalvay and Farson 1989). Decreased root exudation reduced the population of siderophore producing bacteria, thereby reducing their role in plant Fe supply (Crowley et al. 1992). Alternatively fungal siderospheres could compete with the plant for soil Fe, or fungi could decrease direct plant Fe uptake by degradation of plant-borne Fe (III) chelators as bacteria (Crowley et al. 1992).

14.11 Effect of Nutrient Uptake by AM Fungi in Saline Soils

Arbuscular mycorrhizal fungi alleviate salt stress, shown to promote plant growth and tolerate salinity by employing a variety of mechanisms, one of which is enhancing nutrient acquisition (Al-Karaki and Al-Raddad 1997). Arbuscular mycorrhizal colonization strongly affects Ca^{2+} concentration in plant. In lettuce, Ca^{2+} uptake was increased; roots are colonized by AM fungi (Cantrell and Linderman 2001). Yanomelo et al. (2003) reported high Ca^{2+} concentration in mycorrhizal than in non-mycorrhizal banana plants. High Ca^{2+} has a beneficial effect on toxic effects of NaCl by facilitating higher K^+/Na^+ selectively leading to salt adaptation (Cramer et al. 1985; Rabie and Almadini 2005). Jarstfer et al. (1998) reported that AM fungal colonization and sporulation are enhanced by Ca^{2+} ions. But in *Acacia auriculiformis*, when compared to mycorrhizal and non-mycorrhizal, there are no changes in the concentration of Ca^{2+} in shoot tissues. This indicates that AM fungi may not be so important to the nutrients moving to plant roots by mass flow as compared with nutrients moving by diffusion (Tinker 1975); when compared to P, Ca^{2+} is not translocated to onion roots through mycorrhizal hyphae as readily and effectively (Rhode

and Gerdemann 1978). In addition AM fungal inoculation depressed the Ca:P ratio by increased production of oxalate in the mycorrhizosphere, which is able to scavenge Ca^{2+} from the solution (Azcon and Barea 1992). Arbuscular mycorrhizal fungi improving Mg^{2+} can support a higher chlorophyll concentration (Giri et al. 2003). This suggests that salt interferes less with chlorophyll synthesis when compared to non-mycorrhizal plants (Giri and Mukerji 2004). Improved plant growth by increasingly chlorophyll concentration is due to effective uptake of Mg^{2+} ions by AM fungi.

Conclusion

In minimum micronutrient levels, mycorrhizal plant acquires increased quantities of micronutrient either by direct uptake from the soil by extraradical hyphae and translocate to the plant or by mycorrhizal effects on root and mycorrhizosphere effects. Although AM fungi are ubiquitous in agricultural and natural forests, predictions about mycorrhizal effects on plant microelement balance are not possible. The influence of AM fungi depends on the specific element, soil conditions, and plant and fungal type. In agronomic practices, fertilization of crops by chemical fertilizers is cost-effective and causes various problems in soil conditions and quality of agricultural products. In large scale manipulation of AM fungi will be useful for framers by decreasing fertilization cost. Including micronutrient uptake, AM fungi play a multifunctional role, protecting the crops from metal toxicity, various stress conditions (e.g., drought, salinity, etc.), and pathogens and uptake of other macronutrients like P, N, and K. Through AM fungal technology, crop plants attain benefit, and world plant production can be improved through enhanced nutrient uptake especially micronutrient.

References

Adiova JM, Pampolina NM, Aggangan NS (2013) Effect of arbuscular mycorrhizal fungi inoculation on growth and Cu uptake and toxicity of *Desmodium cinereum* (Kunth) DC. Philipp J Sci 142:87–96

Al-Karaki GN (2000) Growth of mycorrhizal tomato and mineral acquisition under salt stress. Mycorrhiza 10:51–54

Al-Karaki GN, Al-Raddad A (1997) Effect of arbuscular mycorrhizal fungi and drought stress on growth and nutrient uptake of two wheat genotypes differing in drought resistance. Mycorrhiza 7:83–88

Al-Karaki GN, Al-Raddad A, Clark RB (1998) Water stress and mycorrhizal –isolate effects on growth and nutrient acquisition of wheat. J Plant Nutr 21:891–902

Al-Karaki G, McMichael B, Zak J (2004) Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14:263–269

Arines JA, Vilarijo K, Sainz P (1989) Effect of different inocula of VAM fungi on manganese content and concentration in clover plants. New Phytol 112:2150–2219

Arines J, Porto ME, Vilarino A (1992) Effect of manganese on vesicular arbuscular mycorrhizal development in red clover plants and on soil Mn-oxidizing bacteria. Mycorrhiza 1:127–131

Audet P, Charest C (2006) Effects of AM colonization on 'wild tobacco' grown in zinc contaminated soil. Mycorrhiza 16:277–283

Azcon R, Barea JM (1992) The effect of vesicular arbuscular mycorrhizae in decreasing Ca acquisition by alfalfa plants in calcareous soils. Biol Fertil Soils 13:155–159

Bagheri V, Shamshiri VH, Shirani H, Roosta HR (2012) Nutrient uptake and distribution in mycorrhizal *Pistachio* seedlings under drought stress. J Agric Sci Technol 14:1591–1604
Bethlenfalvay GJ, Farson RL (1989) Manganese toxicity alleviated by Mycorrhizae in soyabean. J Plant Nutr 12:953–970
Bi YL, Li XL, Christie P (2003) Influence of early stages of arbuscular mycorrhiza on uptake of zinc and phosphorus by red clover from a low-phosphorus amended with zinc and phosphorus. Chemosphere 50:831–837
Broadley M, White PJ, Hammond JP, Zelko I, Lux A (2007) Zinc in plants. New Phytol 173:677–702
Burkert B, Robson A (1994) 65 Zn uptake in subterranean clover (*Trifolium subterraneum* L.) by 3 vesicular arbuscular mycorrhizal fungi in a root-free sandy soil. Soil Biol Biochem 26:1117–1124
Cantrell IC, Linderman RG (2001) Preinoculation of lettuce and onion with VA mycorrhizal fungi reduces deleterious effects of soil salinity. Plant Soil 233:269–281
Caris C, Hördt W, Hawkins HJ, Römheld V, George E (1998) Studies of iron transport by arbuscular mycorrhizal hyphae from soil to peanut and sorghum plants. Mycorrhiza 8:35–39
Cavagnaro TR (2008) The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: a review. Plant Soil 304:315–325
Chen BD, Shen H, Li XL, Feng G, Christie P (2004) Effects of EDTA application and arbuscular mycorrhizal colonization on growth and zinc uptake by maize (*Zea mays* L.) in soil experimentally contaminated with zinc. Plant Soil 261:219–229
Chu EY (1999) The effects of arbuscular mycorrhizal fungi inoculation on *Euterpe oleracea* mart. (açaí) seedlings. Pesq Agropec Bras Brasília 34:1019–1024
Clark RB, Zeto SK (1996) Iron acquisition by mycorrhizal maize grown on alkaline soil. J Plant Nutr 19:247–264
Clark RB, Zeto SK (2000) Mineral acquisition by arbuscular mycorrhizal plants. J Plant Nutr 23:867–902
Cramer GR, Lauchli A, Polito VS (1985) Displacement of Ca2þ by Naþ from the plasmalemma of root cells: a primary response to salt stress? Plant Physiol 79:207–277
Crowley DE, Römheld V, Marschner H, Szaniszlo PJ (1992) Root-microbial effects on plant iron uptake from siderophores and phytosiderophores. Plant Soil 142:1–7
Eivazi F, Weir CC (1989) Phosphorus and mycorrhizal interaction on uptake of P and trace elements by maize. Fertil Res 21:19–22
Filion M, St-Arnaud M, Fortin JA (1999) Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. New Phytol 141:525–533
Foy CD, Chaney RL, White MC (1978) The physiology of metal toxicity in plants. Annu Rev Plant Physiol 29:511–566
George E, Haussler K, Vetterlein G, Gorgus E, Marschner H (1992) Water and nutrient translocation by hyphae of Glomus mosseae. Can J Bot 70:2130–2137
Ghasemi-Fasaei R, Jarrah M, Mayel S (2012) Dynamics of manganese adsorption onto highly calcareous soils of Southern Iran. Int J Agric Crop Sci 4:1676–1680
Gianinazzi S, Schuepp H (1994) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhauser Verlag, Basel, p 226
Gildon A, Tinker PB (1983) Interactions of vesicular arbuscular mycorrhizal infection and heavy metals in plants. I. The effects of heavy metals on the development of vesicular arbuscular mycorrhizas. New Phytol 95:247–261
Giri B, Mukerji KG (2004) Mycorrhizal inoculant alleviates salt stress in *Sesbania aegyptiaca* and *Sesbania grandiflora* under field conditions: evidence for reduced sodium and improved magnesium uptake. Mycorrhiza 14:307–312
Giri B, Kapoor R, Mukerji KG (2003) Influence of arbuscular mycorrhizal fungi and salinity on growth, biomass and mineral nutrition of *Acacia auriculiformis*. Biol Fertil Soils 38:170–175
Gnekow MA, Marschner H (1989) Influence of the fungicide pentachloronitrobenzene on VA mycorrhizal and total root length and phosphorus uptake of oats (*Avena sativa*). Plant Soil 114:91–98

Guo Y, George E, Marschner H (1996) Contribution of an arbuscular mycorrhizal fungus to uptake of Cadmium and Nickel in bean by maize plants. Plant Soil 184:195–205

Habte H, Soedarjo M (1995) Limitation of vesicular-arbuscular mycorrhizal activity in *Leucaena leucocephala* by Cain sufficiency in an acid Mn-rich oxisol. Mycorrhiza 5:387–394

Havlin JL, Beaton JD, Tisdale SL, Nelson WL (2007) Soil fertility and fertilizers, an introduction to nutrient management, 7th edn. Pearson Education Inc. Singapore, p 221

Hildebrandt U, Hoef-Emden K, Backhausen S, Bothe H, Bożek M, Siuta A, Kuta E (2006) The rare endemic zinc violets of Central Europe originate from Viola lutea Huds. Plant Syst Evol 257:205–222

Hodge A, Campbell CD, Fitter AH (2001) An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic matter. Nature 413:297–299

Hosseini A, Gharaghani A (2015) Effects of Arbuscular mycorrhizal fungi on growth and nutrient uptake of Apple rootstocks in calcareous soil. Int J Hortic Sci Technol 2:173–185

Jakobsen I, Abbott LK, Robson AD (1992) External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. New Phytol 120:371–380

Jansa J, Mozafar A, Frossard E (2003) Long–distance transport of P and Zn through the hyphae of an arbuscular mycorrhizal fungus in symbiosis with maize. Agron 23:481–488

Jarstfer AG, Farmer-Koppenol P, Sylvia DM (1998) Tissue magnesium and calcium affect mycorrhiza development and fungal reproduction. Mycorrhiza 7:237–242

Johansson JF, Paul LR, Finlay RD (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiol Ecol 48:1–13

Kahneh E, Ramezanpour H, Haghparast Tanha MR, Shirinferk A (2006) Effects of arbuscular mycorrhizal fungi and phosphorous supplement on leaf P, Zn, Cu and Fe concentrations of tea seedlings. Caspian J Environ Sci 4:53–58

Kilham K, Firestone MK (1983) Vesicular arbuscular mycorrhizal mediation of grass response to acidic and heavy metal depositions. Plant Soil 72:39–48

Kothari SK, Marschner H, Romheld V (1990) Direct and indirect effects of VA mycorrhizal fungi and rhizosphere microorganisms on acquisition of mineral nutrients by maize (*Zea mays* L.) in a calcareous soil. New Phytol 116:637–645

Kothari SK, Marschner H, Römheld V (1991) Effect of a vesicular- arbuscular mycorrhizal fungus and rhizosphere micro-organisms on manganese reduction in the rhizosphere and manganese concentrations in maize (*Zea mays* L.) New Phytol 117:649–655

Lambert DH, Weidensaul TC (1991) Element uptake by mycorrhizal soybean from sewage sludge-treated soil. Soil Sci Soc Am J 55:393–398

Lambert DH, Baker DE, Cole HJR (1979) The role of mycorrhizae in the interactions of phosphorus with zinc, copper and other elements. Soil Sci Soc Am J 43:976–980

Lee YJ, George E (2005) Contribution of mycorrhizal hyphae to the uptake of metal cations by cucumber plants at two levels of phosphorus supply. Plant Soil 278:361–370

Lehmann A, Veresoglou SD, Leifheit EF, Rillig MC (2014) Arbuscular mycorrhizal influence on zinc nutrition in crop plants—a meta-analysis. Soil Biol Biochem 69:123–131

Li XL, George E, Marschner H (1991) Phosphorus depletion and pH decreases at the root–soil and hyphae-soil interfaces of VA mycorrhizal white clover fertilizer with ammonium. New Phytol 119:397–404

Linderman RG (1992) Vesicular-arbuscular mycorrhizae and soil microbial interactions. In: Bethlenfalvay GJ, Linderman RG (eds) Mycorrhizae in sustainable agriculture. ASASpec, Madison, pp 45–70

Liu A, Hamel C, Hamilton RI, Ma BL, Smith DL (2000) Acquisition of Cu, Zn, Mn and Fe by mycorrhizal maize (*Zea mays*) growth in soil at different P and micronutrient levels. Mycorrhiza 9:331–336

Lu S, Miller MH (1989) The role of VA Mycorrhizae in the absorption of P and Z by maize in field and growth chamber experiment. Can J Soil Sci 69:97–109

Marschner H (1988) Mechanisms of manganese acquisition by roots from soils. In: Graham RDR, Hanam RJ, Uren NC (eds) Manganese in soils and plants. Kluwer Academic Publishers, Dordrecht, pp 191–204

Marschner H (1995) Mineral nutrition of higher plants, 2nd edn. Academic

Marschner H (1998) Role of root growth, arbuscular mycorrhiza, and root exudates for the efficiency in nutrient acquisition. Field Crops Res 56:203–207

Marschner H, Romheld V (1994) Strategies of plants for acquisition of iron. Plant Soil 165:261–274

Mathur N, Singh J, Bohra S, Bohra A, Vyas A (2006) Effect of soil compaction potassium and cobalt on growth and yield of moth bean. Int J Soil Sci 1:269–271

Medeiros CAB, Clark RB, Ellis JR (1993) Effects of MES [2(N-morpholino)-ethanesulfonic acid] and pH on mineral nutrient uptake by mycorrhizal and nonmycorrhizal maize. J Plant Nutr 16:2255–2272

Merckx R, Sinnaeve J, Van Ginkel JH, Cremers A (1983) The effect of growing plant roots on the speciation of Co-60, Zn-65, Mn-54 and Fe-59 in the rhizosphere. In: Proceedings of the seminar on the transfer of radioactive materials in the terrestrial environment subsequent to an accidental release to atmosphere, Commission of the European Communities, Luxembourg 1, pp 275–288

Nealson KH, Tebo BM, Rosson RA (1988) Occurrence and mechanisms of microbial oxidation of manganese. Adv Appl Microbiol 33:279–318

Nogueira MA, Cardoso EJBN (2003) Mycorrhizal effectiveness and manganese toxicity in soybean as affected by soil type and endophyte. Sci Agric 60:329–335

Ortas I (2010) Effect of mycorrhiza application on plant growth and nutrient uptake in cucumber production under field conditions. Spanish J Agric Res 8:116–122

Ortas I (2012) Mycorrhiza in citrus: growth and nutrition. In: Srivastava AK (ed) Advances in citrus nutrition. Springer, Dordrecht

Ortas I, Akpinar C (2006) Response of kidney bean to arbuscular mycorrhizal inoculation and mycorrhizal dependency in P and Zn deficient soils. Acta Agric Scand 56:101–109

Pacovsky RS, Fuller G (1988) Mineral and lipid composition of *Glycine, Glomus, Bradyrhizobium* symbiosis. *Physiol Plant* 72:733–746

Pacovsky RS, Bethlenfalvay GJ, Paul EA (1986) Comparison between P-fertilized and mycorrhizal plants. Crop Sci 26:151–156

Posta K, Marschner H, Römheld V (1994) Manganese reduction in the rhizosphere of mycorrhizal and nonmycorrhizal maize. Mycorrhiza 5:119–124

Rabie GH, Almadini AM (2005) Role of bioinoculants in development of salt-tolerance of Vicia faba plants under salinity stress. Af. J Biotechnol 4:210–222

Raju PS, Clark RB, Ellis JR, Maranville JW (1990) Effects of species of VA-Mycorrhizal fungi on growth and mineral uptake of sorghum at different temperatures. Plant Soil 121:165–170

Ramakrishnan K, Selvakumar G (2012) Influence of AM fungi on plant growth and nutrient content of Tomato (*Lycopersicum esculentum* Mill.) Int J Res Bot 2:24–26

Rhode LH, Gerdemann JW (1978) Translocation of calcium and phosphate by external hyphae of vesicular-mycorrhizae. Soil Sci 126:125

Rhodes LH, Gerdemann JW (1975) Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol 75:555–561

Sanders IR, Fitter AH (1992) The ecology and functioning of vesicular arbuscular mycorrhizas in coexisting grassland species. New Phytol 120:525–533

Schreiner RP (2007) Effects of native and nonnative arbuscular mycorrhizal fungi on growth and nutrient uptake of 'Pinot Noir' (*Vitis vinifera* L.) in two soils with contrasting levels of phosphorus. Appl Soil Ecol 36:205–215

Schwab SM, Menge JA, Leonhard RT (1983) Quantitative and qualitative effects of phosphorus on extracts and exudates of sudangrass in relation to vesicular–arbuscular mycorrhiza formation. Plant Physiol 73:761–765

Seres A, Bakonyi G, Posta K (2006) Zn uptake by maize under the influence of AM fungi and Collembola *Folsomia candida*. Ecol Res 21:692–697

Sharma AK, Srivastava PC, Johri BN (1994) Contribution of VA mycorrhiza to zinc uptake in plants. In: Manthey JA, Crowley DE, Luster DG (eds) Biochemistry of metal micronutrients in the rhizosphere. Lewis Publishers, Boca Raton, pp 111–123

Simwambana MCM, Ekanayake IJ (2001) Plant biology 2001, Plant biology abstracts no 0490. http://www.Ryconuba.com/as

Smith SE, Gianinazzi-Pearson V (1990) Phosphate uptake and arbuscular activity in mycorrhizal *Allium cepa* L.: effects of photon irradiance and phosphate nutrition. Aust J Plant Physiol 17:177–188

Smith SE, Read DJ (1997) Mycorrhizal symbiosis. Academic, San Diego, p 605

Sparrow L, Uren NC (1987) Oxidation and reduction of Mn in acidic soils: effect of temperature and soil pH. Soil Biol Biochem 19:143–148

Tandon HLS (2001) Management of nutrient interactions in agriculture. Fertilizer Development and Consultation Organization, New Delhi, p 142

Tinker PBH (1975) Effects of vesicular-arbuscular mycorrhizas on higher plants. In: Jennings DG, Lee DL (eds) Symbiosis 29th Symp Soc exp Biol. Cambridge University Press, London, p 325–349

Tisdale SL, Nelson WL, Beaton JD, Havlin JL (1993) Soil fertility and fertilizers, 5th ed. MacMillan Publishing Company, New York, p 486

Tisdall JM, Oades JM (1979) Stabilization of soil aggregates by the systems of rye grass. Aust J Soil Res 17:429–441

Toler HD, Morton JD, Cumming JR (2005) Growth and metal accumulation of mycorrhizal sorghum exposed to elevated copper and zinc. Water Air Soil Pollut 164:155–172

Turnau K, Kottke I, Oberwinkler F (1993) Elemental localization in mycorrhizal roots of *Pteridium aquilinum* (L.) Kuhn. collected from experimental plots treated with Cd dust. New Phytol 123:313–324

Val J, Monge E, Heras L, Javier A (1987) Changes in photosynthetic pigment composition in higher plants as affected by nutrition status. J Plant Nutr 10:995–2001

Vivas A, Brio B, Ruiz-Lozano JM, Barea JM, Azcon R (2006) Two bacterial strains isolated from a Zn-polluted soil enhance plant growth and mycorrhizal efficiency under Zn toxicity. Chemosphere 62:1523–1533

Wang C, Li X, Zhou J, Wang G, Dong Y (2008) Effects of arbuscular mycorrhizal fungi on growth and yield of cucumber plants. Commun Soil Sci Plant Anal 39:499–509

White JA, Brown MF (1979) Ultrastructural and X-ray analysis of phosphorus granules in a vesicular arbuscular mycorrhizal fungus. Can J Bot 57:2812–2818

Whitefield L, Richards RJ, Rimmer DL (2004) Relationships between soil heavy metal concentration and mycorrhizal colonization in *Thymus polytrichus* in northern England. Mycorrhiza 14:55–62

Wu Z, Ren H, Mc Grath SP, Wu P, Zhao FJ (2011) Investigating the contribution of the phosphate transport pathway to arsenic accumulation in rice. Plant Physiol 157:498–508

Yano-Melo AM, Maia LC, Saggin JR, Lima-Filho JM, Melo NF (1999) Effect of arbuscular mycorrhizal fungi on the acclimatization of micro propagated banana plantlets. Mycorrhiza 9:119–123

Yano-Melo AM, Saggin OJ, Maia LC (2003) Tolerance of mycorrhized banana (*Musa* sp. cv. *Pacovan*) plantlets to saline stress. Agri Ecosyst Environ 95:343–348

Zaefarian F, Rezvani M, Rejali F, Ardakani MR, Noormohammadi G (2011) Effect of heavy metals and Arbuscular mycorrhizal fungal on growth and nutrients (N, P, K, Zn, Cu and Fe) accumulation of alfalfa (*Medicago sativa* L.) Am-Eurasian J Agric Environ Sci 11:346–352

Efficacy of Probiotic Supplemented Vermicompost on Germination, Growth, and Biochemical content of *Vigna mungo* (L.) Hepper

15

Ramasamy Karthiyayini and Mani Rama Prabha

Abstract

Vermicomposting is the most important environment and ecofriendly technique which is primarily used to produce wealth from waste. The study was conducted to evaluate the vermicompost-supplemented probiotics (*Lactobacillus sporogenes* and *Saccharomyces cerevisiae*) on seedling growth and leaf biochemical parameters of *Vigna mungo* plant. This study discovered that vermicomposting is one of the novel techniques used to get rid of the menace caused by organic wastes and vermicompost along with biofertilizers and has tremendous scope to wrest the present-day agriculture out of food and nutrition crisis.

15.1 Introduction

In most of Asian countries, the drastic increase in population is the important problem, and to provide food for all the people is very difficult and becomes a challenging task. The sustainability of production of food materials from these declining agricultural land areas needs the conservation of soil health and promising yield of plants. The practice of commonly using inorganic fertilizers for increasing the productivity affects the soil nutrients and deteriorates the overall soil health. The frequent usage of inorganic mineral fertilizers raises queries about overdependence and its influence on sustaining health and the productivity of the arable soils. Many studies have proved that the regular usage of mineral fertilizers only supplies the

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plant nutrients to the soil, but it gradually destroys the physical, chemical, and biological attributes of the soil (Chattopadhyay 2005).

Nowadays, heavy dose of inorganic fertilizers, pesticides, and herbicides is used by the farmers to get a better yield of various field crops. Use of chemical fertilizers has now raised many questions related to the productivity of land and continuously increasing cost of cultivation. These chemical fertilizers and pesticides decreased soil fertility and caused health problems to the consumers. Due to adverse effects of chemical fertilizers, interest has been stimulated for the use of organic manures (Follet et al. 1981). Organic manure works as inducer in nature and generally determined in terms of biological properties of soil and crop growth. Naturally organic manure will reduce the burden of environmental pollution caused by fertilizers and maintain the soil productivity. Therefore, nutrients can be supplied to the soil as well as to the crop through various organic manures and microbial bio-inoculants (Biswas 2014).

The presence of more useful bacteria and fungi is the chief source to maintain the fertility of soil. These microbes breakdown the organic materials present in the soil into small parts, which will be observed by the plants through their roots (Kale et al. 1992). They keep the soil healthier and decrease the uses of the fertilizers. The conception is that the presence of certain probiotic microbes will provide direct benefits to act as biocontrol agents to the plant. The plant probiotic bacteria is commercially produced to be used as biological control agents of plant diseases (Berg 2009). These microbes have fulfilled all the functions for the plant growth as they antagonize different plant pathogens, induce resistance, and influence growth (Bloemberg and Lugtenberg 2001; Nelson 2004).

Vermiculture is a mixed culture which contains soil bacteria and an effective strain of earthworm. Earthworms have efficiency to converting all biodegradable waste materials to organic manure with the help of composting (Edwards and Burrows 1988; Bhawalkar 1991; Rajendran et al. 2008). Earthworms consume on decomposed plant material, and their digestive tract processes the organic matter, which is returned to the soil through castings or worm waste. Earthworms not only play an important role in the soil nutrient cycle but also help to rise the percentage of macronutrient (Umamaheswari 2005; Levinish 2011). It also improves the soil structure such as soil porosity, soil aggregation, water holding capacity, and nutrient conservation in the soil (Ellerbrock et al. 1999). In the process of vermicomposting, important nutrients like nitrogen, potassium, phosphorus, and calcium are converted to be more soluble and easily available to plants than the parent compounds (Bhawalkar and Bhawalkar 1993; Ndegwa and Thompson 2001; Hemalatha 2013). Production of vermicompost supports to recycle the organic materials, decreases production cost of the field crops, and reduces the use of costly chemical fertilizers.

15.2 Materials and Methods

15.2.1 Collection of Samples

Flower waste was collected from flower market in Coimbatore. The quality and composition of flower waste vary widely from location to location. The types of flower are, viz, marigold, rose, jasmine, champak, Nerium, etc., whereas the leaf

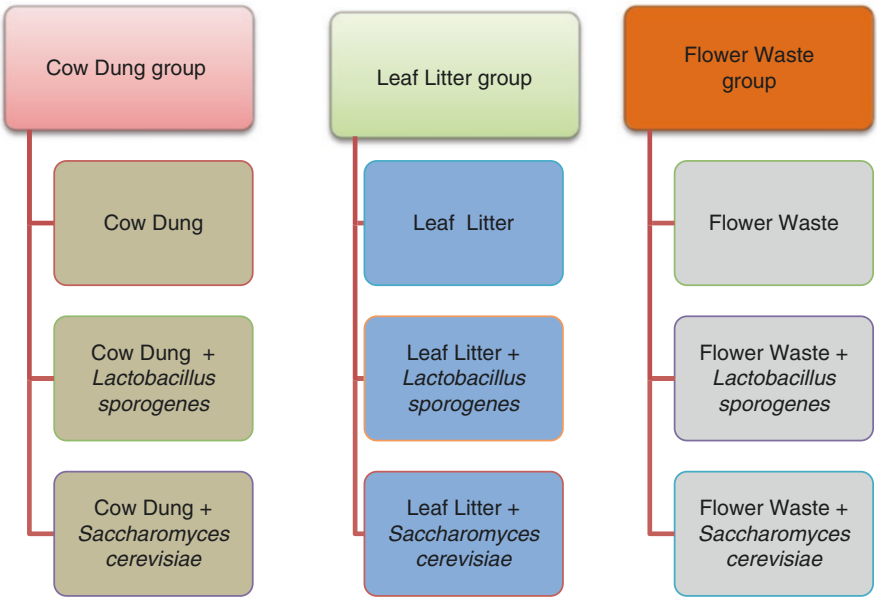
litter was collected from Western Ghats. The samples were collected, and the segregation was done to remove the unwanted materials like polythene, paper, threads, etc., and then the materials were dried and thoroughly mixed before the analysis. Cow dung was obtained from nearby cattle yard. *Vigna mungo* (L.) Hepper seeds and the samples of epigenetic earthworms—*Eudrilus eugeniae* (Kinberg)—were obtained from the Agricultural University, Coimbatore, Tamil Nadu, and maintained under laboratory conditions.

15.2.2 Preparation of Vermicomposting

Compost mixture was prepared by the ratio of 1:1 (w/w) of lead and cow dung in round plastic containers, sprinkled with dechlorinated tap water to maintain the moisture, and was allowed for predigestion. After 21 days of predigestion, the *Eudrilus eugeniae* was introduced into each container containing predigested mixture. Vermicomposting was allowed for 90 days with regular sprinkling of water to maintain the moisture content (65–70% RH) in the mixture. Similarly for the above waste, also three experimental groups were set up. At the end of 90 days of vermicomposting, the vermicompost from the container was spread separately on a polythene sheet to allow drying. Now the mixture is ready for further study.

15.2.3 Experimental Setup

The thick polythene bags of 4 kg capacity (25 cm × 22 cm) were individually filled with growth medium containing soil along with supplemented substrates. The treatment setup was divided into three major groups, and again it is subdivided into vermicompost alone and vermicompost supplemented with probiotic substance as follows.



The *V. mungo* seeds (10/bag) were sown in each bag with equal distance, kept in sunlight. Each bag was watered twice regularly throughout the study. The sampling was done after 60 days of sowing, and the growth parameter like height, leaf area, chlorophyll a and b, protein content, and carbohydrate was determined in third and fourth leaf from the top. Height was measured in centimeters, and leaf area was measured in sq/cm, whereas the chlorophyll a, b and total chlorophyll were estimated by using the Arnon method (1949). The biochemical content of the leaf was estimated by the method of Bradford (1976) for protein and anthrone reagent method for carbohydrates.

15.3 Result and Discussion

15.3.1 Germination of Seed

Germination is one of the critical phases in the life cycle of a crop that is subjected to numerous environmental factors (Copper 1979). The natural environment is favored for growth and development of the plant communities (Dixit 1994). The effect of environment on germination is quite difficult, because of the external and internal factors that modify the patterns of germination, seedling growth, as well as the yield (Rout et al. 2000).

In the present study, maximum percentage of germination was observed in (100%) four experimental groups and minimum (90%) was seen in leaf letter + *S. cerevisiae* group (Fig. 15.1). The substitution of vermicomposting in soil has often linked with increasing germination percentage and yield parameters of various crop species even at small substitution rates (Bachman and Metzger 2008). Basically the vermicompost contains humified organic matter which stimulates seed germination and plant growth (Dell'Amico et al. 1994; Garcia et al. 1992). It is also reported that the growth-regulating materials present in the vermicompost could be the possible reason for the increased germination, growth, and yield (Atiyeh et al. 2002).

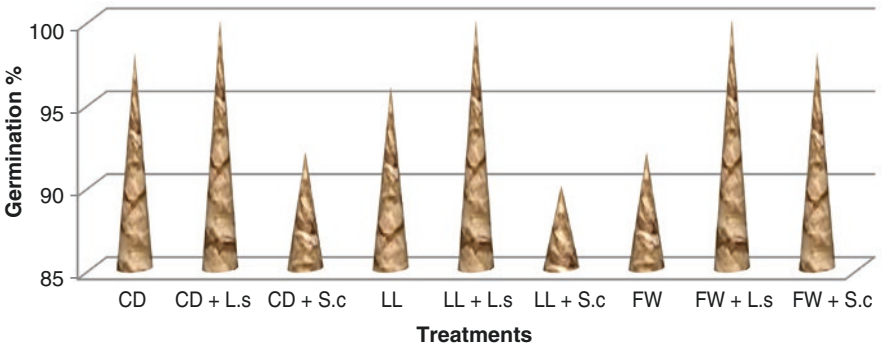


Fig. 15.1 Effects of various vermicomposts amended with probiotics on seed germination of *V. mungo* (L.) Hepper

Suthar (2006) confirmed that some crop residue + cow dung-mixed vermicompost is increasing the available phosphorus (63–105%), exchangeable potassium (45–90%), and total nitrogen (91–144%) levels in the soil. The vermicompost is having more transferable plant nutrients than other plant growth media and fertilizers. One of the important features of vermicompost is it converts the harder non-exchangeable plant growth nutrients into available simple forms taken by plants, such as nitrate or ammonium nitrate, transferable phosphorous, soluble potassium, calcium, and magnesium. Kurian et al. (2008) reported that prolonged period of vermicomposting (90 days) of leaf litter using *Eudrilus eugeniae* resulted to increased NPK.

Owa et al. (2008) stated that earthworm products are probably involved in nutrient utilization of the catabolic products of endosperm such that the bell proliferation and elongation in the embryo are facilitated. They also reported that the earthworm products must have therefore been introducing an additional factor, which may have part in causing breakdown of seed coat to facilitate germination. The present study strongly indicates the higher percentage of humic acid and growth-promoting hormones present in the probiotics-supplemented vermicompost.

15.3.2 Growth Parameters

In recent times, usage of vermicomposts as biofertilizers is rising due to its enormous nutrient content, better microbial, and antagonistic activities. Vermicompost contains most of the micro- and macronutrients in easily available forms to the plant and a large amount of useful microorganisms which impact plant growth and yield (Theunissen et al. 2010). Vermicompost contains huge amounts of humic constituents like humic acid, fulvic acid, and humin (Atiyeh et al. 2002; Masciandaro et al. 1997). It improves the growth and yield capacity of crop plants which is equal to applying plant growth regulators to the soil (Muscolo et al. 1999).

Vermicompost materials are having very rich microbial population and diversity (Edwards and Burrows 1988; Masciandaro et al. 1997). These microbial population directly influences the physiological parameters of the plant through nitrogen fixation and solubilization of nutrients (Rodriquez and Fraga 1999). The same microbial population indirectly influences the production of siderophores, chitinase, glucanase, antibiotics, and fluorescent pigments (Han et al. 2005).

In the present study, the plant growth was greatly increased with the application of probiotics-supplemented vermicompost. A maximum number of leaf and leaf length were seen in leaf litter supplemented with *L. sporogenes*. Maximum leaf width was observed in leaf litter supplemented with *S. cerevisiae*. Whereas the shoot and root length was significantly high in cow dung supplemented with *L. sporogenes*-treated group (Table 15.1). These findings support earlier reports which note that soil enriched with vermicompost additional substances and diverse microbial population which are not found in chemical fertilizers and nutrient-depleted native soils (Kale et al. 1992).

Table 15.1 Effect of various vermicomposts amended with probiotics *Lactobacillus sporogenes* and *Saccharomyces cerevisiae* on growth parameters of *Vigna mungo* (L.) Hepper

Growth parameters	Cow dung group			Leaf litter group			Flower waste group		
	CD	CD + L.s	CD + S.c	LL	LL + L.s	LL + S.c	FW	FW + L.s	FW + S.c
No. of leaves	26 ± 1.58*	32 ± 1.52**	29 ± 1.30^{NS}	26 ± 1.38*	34 ± 1.92*	29 ± 1.14 ^{NS}	26 ± 1.32*	29 ± 1.82*	28 ± 1.14**
Leaf length (cm)	7.0 ± 0.50*	8.3 ± 0.78**	7.2 ± 0.18*	7.7 ± 0.50*	8.4 ± 0.26**	6.9 ± 0.26*	7.3 ± 0.31*	7.9 ± 0.15 ^{NS}	6.7 ± 0.72*
Leaf width (cm)	5.2 ± 0.15**	6.0 ± 0.20*	4.5 ± 0.10*	4.7 ± 0.12	6.1 ± 0.22^{NS}	6.4 ± 0.31*	5.7 ± 0.21*	5.8 ± 0.21**	5.3 ± 0.20*
Shoot length (cm)	182.3 ± 3.21	244.6 ± 2.08*	211.0 ± 2.08^{NS}	183.0 ± 3.21	241.0 ± 2.65	174.3 ± 1.53*	150.6 ± 2.08*	182.2 ± 1.88*	168.3 ± 1.15 $^{NS\ NS}$
Root length (cm)	18.7 ± 0.24*	20.3 ± 0.44*	19.6 ± 0.62*	14.3 ± 0.62^{NS}	19.3 ± 0.32*	16.5 ± 0.26*	17.3 ± 0.20**	18.4 ± 0.53^{NS}	17.3 ± 0.23^{NS}

Each value represents the mean (±SD) of three observations
NS statistically no significance
*, significant; **, highly significant at 5% level/1% level

Arun Kumar (2004) had also reported the substantial increase in the growth parameters like plumule length, leaf number, and leaf length of *Amaranthus dubius* grown in soil added with vermicompost sludge when compared to sludge-amended soil. Increased seed germination percentage, shoot, and root length was reported in chili and tomato grown in vermicompost-mixed soil compared to those plants grown in normal red soil (Jose 2005). Parr and Colacicco (1987) reported about the solid and liquid vermicompost and its different active substances, which influence the germination and seedling growth of different vegetable crops.

Pathak et al. (2013) observed increased leaf number in guava plant while incorporating phosphobacteria and vermicompost. Tomati et al. (1983) observed the significant effects of vermicomposts on growth parameters of *Begonia* species and *Coleus* species, especially in root growth, lengthening of internodes, and time of flowering. Similar findings were absorbed by Arancon et al. (2003) in tomatoes (*L. esculentum*), bell peppers (*Capsicum annuum grossum*), strawberries (*Fragaria ananassa*), and peppers (*Capsicum annuum*) by the application of vermicompost prepared from different wastes (food and paper wastes).

15.3.3 Biochemical Parameters

The changes in leaf chlorophyll content will not produce the corresponding changes in photochemical efficiency of photosynthesis since these changes occur relatively late (Alonso et al. 2002). In the present study, chlorophyll a and b and total chlorophyll were high in cow dung supplemented with *L. sporogenes*-treated group when compared to other groups (Fig. 15.2). It is apparent that most biologically active components present in the vermicompost materials relatively affect the photosynthesis-related parameters in the present experiments.

Protein is a reserve food material which is utilized for the growth and development of seedlings (Lenin et al. 2012). Protein content was rich in leaf litter

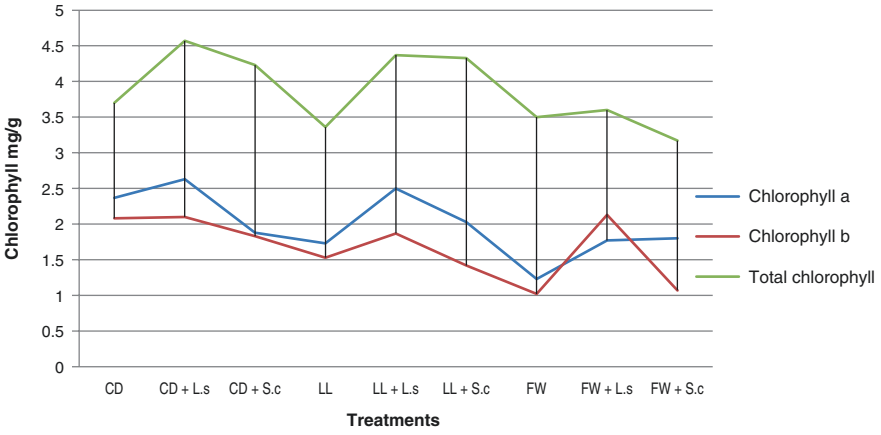


Fig. 15.2 Effects of various vermicomposts amended with probiotics on chlorophyll content

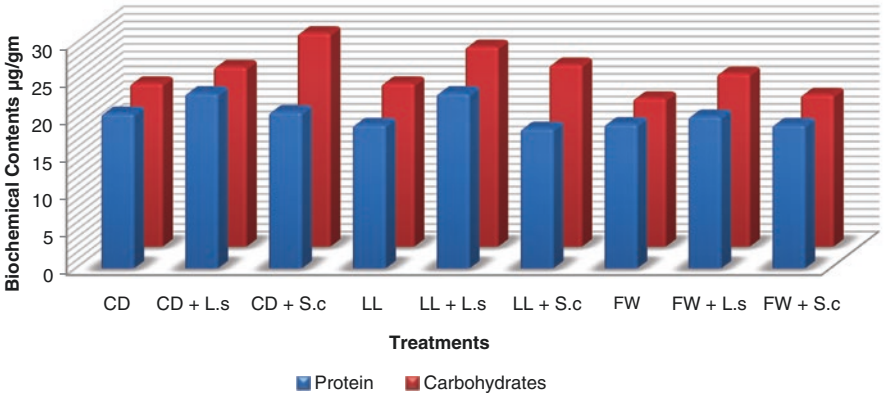


Fig. 15.3 Effects of various vermicomposts amended with probiotics on biochemical content

supplemented with *L. sporogenes*-treated seedlings when compared to other treatment groups as shown in Fig. 15.3. Increase in nitrogen content normally associated with increase in protein content and believed to stimulate plant nutrient uptake and metabolism has influenced the protein synthesis (Bago et al. 1996; Mathivanan et al. 2012).

Krishna and Bagyaraj (1984) also reported that organic acid of soils increases the plant uptake of potassium from water soluble and releases the organic acids both. The microenvironment around the roots is the major reason for potassium, manganese, iron, and zinc by plants and AM fungi. Increased levels of protein in the inoculated plants show the presence of fungal proteins or post-inflectional stimulation of protein synthesis in the host plant.

Carbohydrate is the main constituent of living organisms. In the present study, the carbohydrate content was high in cow dung + *L. sporogenes*-treated seedling (Fig. 15.3). Lenin et al. (2012) recorded the increase in carbohydrate content in the vermicompost + AMF-inoculated ground nut plants when compared to control. The enhancement of carbohydrate and the movement of essential metal ions due to mycorrhizal infection accelerate the metabolic rates (Cooper 1984).

Conclusion

Vermicompost is always having a good source of plant growth-promoting substances. A close perusal of the data obtained for the abovementioned results reveals that the vermicomposting is one of the novel techniques used to get rid of the menace caused by organic wastes and vermicompost along with biofertilizers and has tremendous scope to wrest the present-day agriculture out of food and nutrition crisis. Hence, this study confirmed that the usage of vermicompost significantly increases the plant growth. Further amendment of probiotic bacteria and fungi can enhance the nutrient level in the compost. So this compost can provide better growth and biochemical parameter of *Vigna mungo*.

References

Alonso M, Rozados MJ, Vega JA, Perez-Gorostiaga P, Cuinas P, Fonturbel MT, Fernandes C (2002) Biochemical responses of *Pinus pinaster* trees to fire- induced trunk gridling and scorch: secondary metabolites and pigments as needle chemical indicators. J Chem Ecol 28:687–700

Arancon NQ, Lee S, Edwards CA, Atiyeh RM (2003) Effects of humic acids and aqueous extracts derived from cattle, food and paper-waste vermicomposts on growth of greenhouse plants. Pedobiologia 47:744–781

Arnon DI (1949) Copper enzymes in isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. Plant Physiol 24:1–15

Arun Kumar J (2004) Effect of vermicomposted sludge on growth of *Amaranthus dubius*. J Ecotoxicol Environ Monit 14:157–160

Atiyeh RM, Lee S, Edwards CA, Arancon NQ, Metzger JD (2002) The influence of humic acids derived from earthworm-processed organic wastes on plant growth. Bioresour Technol 84(1):7–14

Bachman GR, Metzger JD (2008) Growth of bedding plants in commercial potting substrate amended with vermicompost. Bioresour Technol 99:3155–3161

Bago B, Vierheilig H, Piche Y, Azcón-Aguilar C (1996) Nitrate depletion and pH changes induced by the extra radical mycelium of the arbuscular mycorrhizal fungus *Glomus intraradices* grown in monoxenic culture. New Phytol 133:273–280

Berg G (2009) Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Bhawalkar US (1991) Vermiculture biotechnology for LEISA. Seminar on low external input sustainable agriculture, Amsterdam

Bhawalkar US, Bhawalkar UV (1993) Vermiculture biotechnology. In: Thampan PK (ed) Organics in soil health and crop production. Peekay Tree Crops Development Foundation, Cochin, pp 65–85

Biswas S (2014) Evaluation of growth, yield and nutrient content with microbial consortia combined with different organic manures in *Rumex acetosella* L. IOSR J Environ Sci Toxicol Food Technol 8(11):01–05

Bloemberg GV, Lugtenberg BJ (2001) Molecular basis of plant growth promotion and biocontrol by *rhizobacteria*. Curr Opin Plant Biol 4:343–350

Bradford MM (1976) A dye binding assay for protein. Anal Biochem 72:248–254

Chattopadhyay GN (2005) Vermicomposting as a biotechnological tool form recycling organic wastes. In: Ghosh TK, Chakrabarti T, Tripathi G (eds) Biotechnology in environmental management, vol 1. A.P.H. Publishing Corporation, New Delhi, pp 135–145

Cooper KM (1984) Physiological of VA mycorrhizal. In: Powell CL, Bagyaraj DJ (eds) VA mycorrhiza. CRC Press, Inc, Boca Raton, pp 155–186

Copper R (1979) Bacterial fertilizers in the Soviet Union. Soils Fertil 22:327–333

Dell'Amico C, Masciandaro G, Ganni A, Ceccanti B, Garcia C, Hernandez T, Costa F (1994) Effect of specific humic fractions on plant growth. In: Senesi N, Miano TM (eds) Humic substance in the global environment and implications on human health. pp 563–566

Dixit SP (1994) Effect of paddy straw management of soil fertility build up and yield of cowpea in wheat paddy cropping system in North West Himalayan soil. In: Proceedings and symposium on sustainability of rice-wheat system in India, Karnal

Edwards CA, Burrows I (1988) The potential of earthworm compost as plant growth. SPB Acedemic Publishing, The Hague, pp 211–219

Ellerbrock RH, Hoen A, Rogasik J (1999) Functional analysis of soil organic matter as affected by long-term manorial treatment. Eur J Soil Sci 50(1):65–71

Follet R, Donahue R, Murphy L (1981) Soil and Soil Amendments. New Jersey, Prentice Hall Inc.

Garcia C, Hernandez T, Costa F, Ceccanti B, Dell'Amico C (1992) Characterization of organic fractions of uncomposted and composted sewage sludge by isoelectric focusing and gel–filtration. Biol Fertil Soils 13:112–118

Han J, Sun L, Dong X, Cai Z, Yang H, Wang Y, Song W (2005) Characterization of a novel plant growth-promoting bacteria strain *Delftia tsuruhatensis* HR4 both as a diazotroph and a potential biocontrol agent various pathogens. Syst Appl Microbial 28:66–76

Hemalatha B (2013) Comparative evaluation of biodegradability of yard waste and fruit waste with industrial effluents by vermicomposting. Int J Adv Eng Technol 2(2):36–39

Jose S (2005) Effect of vermicompost on germinations and growth of tomoto and chili. M. Phil Dissertation, Bharathidasan University, Tiruchirappalli

Kale RD, Mallesh B, Hubra B, Bagyara DJ (1992) Influence of vermicompost application on the available macronutrients and selected microbial populations in a paddy field. Soil Biol Biochem 24:1317–1320

Krishna KR, Bagyaraj DJ (1984) Growth and nutrient uptake of peanut inoculated with the mycorrhizal fungus *Glomus fasciculatum* compared with non-inoculated ones. Plant Soil 77:405–408

Kurian D, Vijayakumar K, Ramaswamy M (2008) Efficacy of EM–supplemented vermicompost of leaf litter using epigenetic earthworm, *Eudrilus eugeniae* (kinberg). Seminar on sustainable Aquaculture and rural Development (Bu-Aqua2008), Bharathiar University, Coimbatore

Lenin M, Dava Sena T, Thamizhiniyan P, Ravimycin T, Indira P (2012) Interaction of AM Fungi (*Glomus intraradices)* and vermicompost and their changes on biochemical content of groundnut (*Arachis hypogaea* L.) Var. VRIGn-7. Int J Curr Sci:37–47

Levinish G (2011) Vermicompost treatment differentially affects seed germination, seedling growth and physiological status of vegetable crop species. Plant Growth Regul 65(1):169–181

Masciandaro G, Ceccanti B, Garcia C (1997) Soil agro-ecological management: fertigation and vermicompost treatments. Bioresour Technol 59:199–206

Mathivanan AL, Chidambaram A, Sundaramoorthy P, Kalaikandhan R (2012) Effect of vermicompost on germination and biochemical constituents of ground nut (*Arachis hypogaea.* L.) seedling. Int J Res Biol Sci 2(2):54–59

Muscolo A, Panuccio MR, Abenavoli MR, Concheri G, Nardi S (1999) Effect of molecular complexity acidity of earthworm faeces humic fractions on glutamate dehydrogenase, glutamine synthetase, and phosphoenolpyruvate carboxylase in *Daucus carota* II cells. Biol Fertil Soils 22:83–88

Ndegwa PM, Thompson SA (2001) Integrating composting and vermicomposting the treatment and bioconversion of biosolids. Bioresour Technol 76:107–112

Nelson LM (2004) Plant growth promoting rhizobacteria (PGPR): prospects for new inoculants. Crop Manages. doi:10.1094/CM-2004-0301-05-RV

Owa SO, Moreyibi OH, Dedeke GA, Morafa SOA, Senjobi BA, Odunbaku OA, Aladesida AA (2008) Effect over seasoned earthworm products on seed germination, implication for early rain cropping. Appl Sci Res 4:638–687

Parr F, Colacicco D (1987) Chapter 4: Organic materials as alternative nutrient sources. In: Helsel ZR (ed) Energy in plant nutrition and pest control. Elsevier Science Publishers B.V., Amsterdam, pp 81–99

Pathak DV, Singh S, Saini RS (2013) Impact of bio-inoculants on seed germination and plant growth of guava (*Psidium guajava*). Global J Wood Sci Forest Wildl 1(1):015–017

Rajendran P, Jayakumar E, Kandula S, Gunasekaran P (2008) Vermiculture and vermicomposting biotechnology for organic farming and rural economic development. http://www.eco-web.com/edi/080211.html

Rodriquez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17:319–339

Rout GR, Samantary S, Das P (2000) Effect of chromium and nickel on germination and growth in tolerant and non-tolerant populations of *Echinochloa colona* (L.) Link. *Chemosphere* 40:855–859

Suthar S (2006) Impact of vermicompost and composted farmyard manure on growth and yield of garlic (*Allium sativum* L.) field crop. Int J Plant Prod 3(1):25–38

Theunissen J, Ndakidemi PA, Laubscher CP (2010) Potential of vermicompost produced from plant waste on the growth and nutrient status in vegetable production. Int J Phys Sci 5(13):1964–1973

Tomati U, Grappelli A, Galli E (1983) Fertility factors in earthworm humus. In: Proceedings of the international. symposium on agriculture and environment: prospects in earthworm farming, Publication Min. Ricera Sci. Technol., Rome, pp 49–56

Umamaheswari S (2005) Vermicomposting of mango litters using earthworm *Perionyx excavatus*. J Ecotoxicol Monit 15(1):69–72

Range of Microbial Disease Complexes with *Meloidogyne* Species and Role of Botanicals in Management

16

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Abstract

Plant diseases are economically very important. The increasing realization of role of plant niche environment particularly the rhizosphere has triggered the application of management strategies to manage soilborne diseases below threshold. Among these regulatory strategies, one important aspect is to break the pathogenic symbioses as disease complexes. The present chapter has been divided into two parts: the first part focuses on the important soil pathogens in the vicinity with host plants with the role of edaphic climate in their association as *disease complexes*, while the second one deals with the changing strategy of soil environment using eco-friendly botanicals to discourage formation of disease complexes.

16.1 Introduction

Fear for survival drives the interactions of life forms. With reference to host crop, however, it may either be negative or positive. The ability of the parasite to interfere with one or more essential functions of the plant determines its potential to elicit disease. This potential or virulence is a decisive factor for the survival or establishment of pathogen or parasite in its host. The external climate (aerial environment and/or edaphic) strengthens disease signaling. Therefore, virulence potential of a pathogen or its inoculum potential is basically determined by the niche environment.

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Further complexity in disease pyramid is brought by ecosystemic dissection of interactants. The three principle components, i.e., pathogen, host, and environment, emerge through intra- and inter-ecosystemic interactions to decide the potential of interaction. The pathogen lives in a community of microbes, say, in rhizosphere with other pathogenic and nonpathogenic interactants. Potency of infection depends upon the inoculum level and virulence of pathogen which is further regulated by the environment, which in case of soil might be decided by soil texture, chemical composition, temperature, and moisture level. Host plant itself naturally grows in the community of several other weeds and plants which forms chemical (allelopathic), physical, or specificity barrier for pathogen. Interaction of microbial community may antagonistically interact (often used as biocontrol agent) or naturally may positively interact to form disease complex (Zacheo et al. 1977). The phenomenon of pathogenic interaction "one at a time" may or may not be excluded by cross-resistance or cross-sensitivity.

16.2 Soil Environment

Soil is not a pure culture of microorganisms, and it bears various potential pathogens, symbionts, and free living beneficial microbes at various stages of growth. Ecologically, soil is a very complex and still unexplored body where millions of microorganisms continuously interact negatively and positively or remain neutral. Most of these microorganisms which belong to kingdom Monera (prokaryotes) and Protista (simple eukaryotes) are r-selected species with high adaptive value. Fluctuating environment of soil causes successive alterations in microbial communities which include soil moisture, temperature, oxygen availability, and nutrient status. However, selective buildup or subsidence of one or some microbial species/strain among inhabiting microorganisms is aided by the efficiency to metabolize host exudates facing stressful regime in vicinity which provides the opportunity to competitively exploit the available feast. Several abiotic factors (environment) that are responsible for the stress of host include high or low temperature stress, water logging or hypoxic stress, draught, salinity, mechanical stress (injury), or natural senescence. A set of microclimatic or niche factors may determine the natural selection of species or strain build inoculums, for example, primary inoculum level, composition of host exudates (allelochemicals, volatile organic compounds), availability of antagonists, soil pH, and physical structure of soil. Among soilborne pathogens, a significant role of nematodes and fungi has been demonstrated in the development of plant diseases in crop plants around the globe which includes lentils, cotton, peanuts, brinjal, chickpea, soybean, potato, tomato, etc. (Back et al. 2002; Koike et al. 2003).

16.3 Important Soilborne Plant Pathogens

Soil is a good medium for the rich culture of plethora of microorganisms. The plant is said to be healthy when it carry out its physiological functions to the best of its genetic potential. Under natural conditions, there are numerous

microorganisms and environmental factors which alter the normal physiological functions of the plant that compromise its genetic potential and disease development. The series of invisible and visible responses of plant cells and tissues to pathogenic microorganisms or environmental factor imparts adverse changes that lead to partial impairment or death of the plant or its part. The plant pathogenic microorganisms, such as viruses, fungi, protozoa, and nematodes, usually cause diseases in plants by disturbing the metabolism of plant cells through enzymes, toxins, growth regulators, and other substances they produce. Soil consists of nutritional availability for host growth, presence of niche microbes and their secretions, host exudates, and other abiotic factors. These environmental conditions may favor one or more pathogens which further cooperate or antagonize each other. The hidden half of plant, i.e., roots, is more prone to negative interaction of microorganisms than shoots which cooperatively assists each other to develop host pathogenesis. An array of microbes could interact negatively with host crop in its rhizosphere region. Often herbaceous plants with soft root tissues infested by a number of soilborne pathogenic bacteria, fungi, nematodes, and insects are generally vegetable crop plants. These include bacteria, actinomycetes, mollicutes, protozoans, fungi, nematodes, and crustaceans. In a natural soil environment, there lie several microorganisms, i.e., nearly 10^6–10^8 bacteria, 10^6–10^7 actinomycetes, 5×10^4–10^6 fungi (cfu), 10^5–10^6 protozoa, and 10^4–5×10^5 algae in 1 g of field surface soil (Gottlieb 1976), whereas c. 1×10^7 nematodes in $1m^2$ of fertile soil (Richards 1976). Most of these microorganisms are saprophytic with little or no disease potential on plant; most others under favorable soil conditions initiate plant diseases, for instance, pathogenic fungi or root-knot nematodes.

A significant development of disease complex formation in plant pathology has occurred after the 1960s including nematodes with fungi, bacteria, and viruses. Nematode has been seen to facilitate disease development under normal conditions caused by pathogenic fungi and bacteria through synergistic or additive relations. Thus, two pathogens are required to induce disease, where primary pathogen induces changes in host inviting secondary pathogen to participate actively to exacerbate the pathogenesis. Interactions involving bacteria as secondary host are few as compared to fungi. Among bacteria, likewise fungi, wilt- and rot-causing bacteria are studied in more detail.

16.4 Root-Knot Nematodes

Nematodes are ubiquitous and cosmopolitan parasites of vascular plants, causing substantial crop damage. Although various species exploit all parts of the plant, roots are the major target. Nematodes deploy a broad spectrum of feeding strategies, ranging from simple grazing to the establishment of complex cellular structures (including galls) in host tissues (Bird and Kaloshian 2003). Plant parasitic nematodes are capable of producing recognizable disease symptoms on suitable susceptible hosts (Agrios 2005). These were first reported in roots of

greenhouse-grown cucumbers by Berkeley in 1855, England. Plant parasitic nematodes belonging to 15 genera have been reported to cause heavy losses on okra (Bhosle et al. 2004). Root-knot nematode is one of the most harmful nematode pests of crop production in tropical and subtropical regions causing extensive economic damage worldwide (Sikora and Fernandez 2005; Hussain et al. 2011; Mukhtar et al. 2013). These nematodes are obligate root parasites of more than 2000 plant species comprising herbaceous and woody plants of mono- and dicotyledons (Hussey 1985).

The primary inoculum level of nematode population in soil is regarded as key determinant of root infestation and is phloem/cell sap herbivory if host is available in vicinity. Availability of host attracts juveniles through chemotaxis through the exudation of root secretions. Possible effector molecules are also released by nematodes to discourage surface-induced defense activation in host. Soil temperature, moisture, pH, aeration, and plant exudates are other determining factors of nematode fecundity, life span, and activity in soil. For different crops and their respective varieties, the threshold nematode population required to initiate pathogenicity has been worked out by several workers (Khanna and Jyoti 2004; Chand 2004; Ekenma and Chidera 2005; Ansari and Azam 2005; Khan et al. 2006; Khan et al. 2008; Kankam and Adomako 2014). Increasing the nematode inoculum level resulted in corresponding increase in number of galls and nematode population buildup. The reduction in growth parameters and nematode infestations was found to be proportional to the inoculum level. Besides abovementioned factors, availability of heavy metals in soil also has adverse effect on soil nematode population.

16.5 Root-rot Fungi

High moisture and temperature of soil environment increase root respiration and rapidly deplete the rhizospheric oxygen. Such reducing environment with high biological oxygen demand supports the perpetuation of necrotrophic fungi especially rotting fungi. Among the soilborne fungal diseases, damping off of seedlings, root rot, and wilt of adult plants are caused by several species of *Fusarium*, *Pythium*, *Rhizoctonia*, and *Verticillium* (Kuprashvili 1996; Jacobsen 2006; Lucas et al. 1997) and are widely distributed throughout the world. *Rhizoctonia solani* is one of the most widely distributed and destructive soilborne plant pathogenic fungi, originally described by Kuhn, 1858 on potato. Occurrence and virulence of *R. solani* depend upon various factors like soil texture, moisture, and temperature (Gill et al. 2000; Gill et al. 2001). It flourishes through vegetative hyphae and sclerotia to cause serious plant diseases (Sneh et al. 1996), for instance, leaf blight, leaf spots, root rot, shoot rot, fruit rot, damping off etc., and has broad host range (Anderson 1982; Lemanczyk 2010). Fungal sclerotia are the structures which survive under adverse environmental conditions for many years. According to a survey, contribution of fungal diseases toward total yield loss of important crops in India is 18–31% (Grover and Gowthaman 2003).

16.6 Bacterial Associations with Nematodes

Nematodes play significant role in carrying pathogenic bacteria and development of disease complex. Nematode predisposes the host to these bacterial diseases providing wounds as entry points for bacteria. For instance, root-knot nematode *M. incognita*-induced wounds in host facilitate the disease complex formation inviting the bacteria *Pseudomonas solanacearum* and *M. hapla* to *Agrobacterium tumefaciens*. The association produces disease symptoms in host different than those produced by either of the pathogen alone. Nematode attaches to bacteria, its body surface binding to cuticle. The nematode *Anguina tritici* in wheat and *A. funesta* in ryegrass produces black seed galls. With bacterial species *Clavibacter*, the nematode causes spike blight with spikelets bearing bacterial mass rather than grains. Grains also produce toxins fatal to sheep and cattle and cause a disease called annual ryegrass toxicity in cattle. Similarly, the presence of *M. incognita* in tomato and brinjal exacerbates the bacterial wilt caused by *Pseudomonas solanacearum* even in resistant varieties. Some of the bacterial genera are specifically carried by their nematode hosts. Species of *Anguina* and *Aphelenchoides* also vector bacterial parasites to aerial parts of plant. The coinfection of nematode juveniles of *A. tritici* with bacterium *Clavibacter tritici* results into yellow ear rot of wheat. *A. tritici* causes ear cockle of wheat. The interaction and carrying of bacteria with nematode are essential steps for disease complex development. The mode of bacterial attachment to nematode juveniles and the nature of their association may differ. The detailed knowledge of mechanism of interaction, however, is still lacking; recent work for early bacterial-nematode interaction is discussed in forthcoming text.

16.7 Plant Disease Complexes

Soil is the pool of numerous diverse pathogens which could potentially infect plant. Nevertheless, these pathogens are host specific with specific host range. A successful pathogenic infection, inhabiting rhizospheric common niche, relies on host exudes which induces upsurge of inoculum density and competitive exclusion of other antagonists. Alternatively, several environmental conditions and host responses attenuates this inoculum potential resulting into partial or complete disease failure. It is now evident that several pathogenic fungi, like those of other non-pathogenic symbioses or complex organisms, undergo facilitative co-operation to overcome "'failure of nutrient acquisition" or pathogenesis to ensure their survival and growth.

16.7.1 Nematode-Fungi Disease Complex

From the primary inoculum of root-rot fungi, viz., *Pythium*, *Rhizoctonia*, *Macrophomina*, and *Fusarium*, the secondary inoculum level rapidly builds up the feeding level which results in sloughing off of root epidermal peels. Alternatively,

root herbivory of nematodes under favorable temperature and moisture conditions accelerates the infection by biotrophic fungi providing additive opportunity of infection in nematode-damaged roots. Counter-infection of root-rot fungi in nematode-infested roots contributes to the severity of the disease that adversely affects the host growth and yield output. Therefore, primarily nematode-resistant varieties were screened to discourage formation of nematode-fungi disease complex.

It was long been known that diseases in crop plants were the result of a complex interaction of host, pathogen, and prevailing environmental conditions. In the rhizosphere of a plant root, millions of opportunistic microorganisms inhabit in sharing ecological niche. A significant role of soilborne pathogens has been attributed globally in the successful development of disease. A disease complex is produced through interactions between two or more organisms. Studies have shown that under a set of environmental conditions, independent infections by root-rot fungi or nematodes have suboptimal disease response in their host plants as compared to their complexed or associative efforts (Bergeson 1972). Most common interactive associations of plant nematodes have been shown with viruses (Khan 1993), bacteria, insects (Sitaramaiah and Pathak 1993; Ryss et al. 2011), and fungi (Back et al. 2002). Many species of plant parasitic nematodes predispose the plants to fungal and bacterial infections, and thus, the plants may suffer greater damage from concomitant infection.

The association of nematode with fungi on host could fall under synergistic, additive, or antagonistic interactions with respect to negative or disease development in host. The synergistic association results into enhanced fungal infections due to adverse physiological effects on host plant by nematode parasitism (Golden and Van Gundy 1975; Starr and Aist 1977). Nematode-fungal disease complexes, especially those involving *Meloidogyne* spp., are common on many crops (Golden and Van Gundy 1975; Diomande et al. 1981; Abawi and Barker 1984; Starr et al. 1989; Safiuddin and Shahab 2012). Synergistic association of *M. incognita* and *R. solani* on okra or tomato roots was better colonized by *R. solani* in the presence of *M. incognita* compared to plants exposed to *R. solani* alone (Golden and Van Gundy 1975). Siddiqui and Husain (1991) reported a similar effect of *M. incognita* on the colonization of chickpea roots by *Macrophomina phaseolina*.

The frequency of involvement of nematodes and fungi in disease complexes is reflected in the number of crops, and the most destructive nematode species in the world is *M. incognita* that has been frequently reported in disease complexes. Brodie and Cooper (1964) reported that the mechanical wounding of cotton seedlings failed to increase the susceptibility to either *R. solani* or *P. debaryanum*. He also found that sporangial production of *P. debaryanum* was almost ten times greater in the presence of sap exuded from root-knot galls produced by *M. incognita* than in the presence of sap from healthy roots. These observations indicate that the nematodes create better environment for fungal development, perhaps by increasing the available nutrient supply. Batten and Powel (1971) observed that root rot was more extensive in prior inoculation of *M. incognita* to *R. solani* in the roots of tobacco plants than those where nematode and fungus were introduced either simultaneously or separately or even when *R. solani* was added after artificial wounding. Histological examination of galled roots after inoculation with *R. solani* revealed

Table 16.1 Disease complex of root pathogenic nematode and fungus forming complex with their host plants

Nematode	Pathogenic fungus	Host plant	Reference
Meloidogyne javanica	*F. oxysporum* f.sp. lentil	*Lens culinaris*	De et al. (2001)
Meloidogyne incognita	*Thielaviopsis basicola*	*Gossypium hirsutum*	Wheeler et al. (2000)
Meloidogyne incognita	*Rhizoctonia solani*	*Arachis hypogaea*	Abdel-Momen and Starr (1998)
Meloidogyne incognita	*Rhizoctonia solani*	*Solanum lycopersicum*	Arya and Saxena (1999)
Meloidogyne arabicida	*F. oxysporum*	*Coffea arabica*	Bertrand et al. (2000)
Heterodera glycines	*Phytophthora sojae*	*Glycine max*	Kaitany et al. (2000)
Heterodera glycines	*F. solani*	*Glycine max*	Rupe et al. (1999)
Globodera rostochiensis	*Rhizoctonia solani*	*Solanum tuberosum*	Back et al. (2000)
Pratylenchus thornei	*F. oxysporum* f.sp. ciceri	*Cicer arietinum*	Castillo et al. (1998)
Pratylenchus thornei	*Rhizoctonia solani*	*Cicer arietinum*	Bhatt and Vadhera (1997)
Pratylenchus neglectus	*Verticillium dahliae*	*Solanum tuberosum*	Hafez et al. (1999)
Pratylenchus penetrans	*Verticillium dahliae*	*Mentha arvensis*	Johnson and Santo (2001)
Rotylenchulus reniformis	*F. oxysporum* f.sp. pisi	*Pisum sativum*	Vats and Dalal (1997)
Rotylenchulus reniformis	*Phytophthora palmivora*	*Piper betle*	Jonathan et al. (1997)

extensive fungal colonization in the root-knot susceptible cultivar 'Dixie Bright 101' when *M. incognita* preceded *R. solani*. *Rhizoctonia solani* is normally non-pathogenic on mature tobacco roots but may cause severe losses when present with well-established root-knot nematode infections. Hazarika and Roy (1974) studied the interrelationship between *R. solani* and *M. incognita* on eggplants (*Solanum melongena* L.), and they showed that the number of galls on roots and the number of egg masses were significantly greater in plants inoculated with nematode and fungus together than inoculated with nematode alone. Moreover, the growths of eggplant were not affected significantly by the attack of *M. incognita* or *R. solani* alone or in combination (Table 16.1).

16.7.2 Nematode-Bacteria Disease Complex

Most of the potentials of pathogenic nematode-prey interaction were done in animal systems. *Caenorhabditis elegans* has been much used to study microbial pathogenesis (Kim 2013). This nematodes-bacteria interaction could involve the transition

from prey-predator to host-pathogen relationship. Bacteria may here work as food or pathogen or initially prey and later may become pathogenic (Garigan et al. 2002; Cabreiro and Gems 2013). Alternatively, the hologenome theory states that the two are holobiont, the evolutionary unit (Rosenberg and Zilber-Rosenberg 2011). The nematode could take up bacteria through digestion or external adherence (Ingham et al. 1985) that may facilitate it for further dispersal. In some other bacteria, surface pili or fimbriae may facilitate its adhesion to nematode surface (De Oliveira-Garcia et al. 2003). Mohan et al. (2001) identified heparin-binding domain (HBD) and gelatin-binding domain (GBD) of *M. javanica* second-stage juveniles which have important role in surface attachment of *Pasteuria penetrans* endospores to cuticle of nematode at first-stage infection.

16.8 Disease Management Through Organic Amendments

For the control of root-knot nematode (*Meloidogyne* spp.) and root-rot fungus, chemical control still remains to be one of the most outstanding methods in terms of immediate results, but there are many reports where chemicals (nematicides and fungicides) have been found to contaminate the soil and ultimately the underground water and thus are potentially toxic to human being (Alam and Jairajpuri 1990; Kookana et al. 1998; Komarek et al. 2010). Due to the hazardous effect and high cost of the chemicals, there has been a growing interest to find out the alternative and eco-friendly means for managing the disease caused by the pathogens. Organic and bioorganic amendments are generally used to increase the agricultural productivity (Abdel-Aziez et al. 2014) and their suppressive effect on plant parasitic nematodes (Khan and Haque 2011) and fungus (Dubey et al. 2007) and also for nematode and fungus both when they parasitized concomitantly (Mokbel et al. 2007; Akhtar and Siddiqui 2008). Organic soil amendments have been found effective to suppress the noxious nematodes to varying extent depending upon the nature of organic matter (Oduor-Owino 2003; Yadav et al. 2013). A number of indigenous plant products have been identified to be toxic to nematodes. The beneficial effects of organic amendments are due to certain nematicidal compounds that are released during decomposition of organic additives in soil, and similarly biological agents produce antagonistic substances against nematode and fungi (Amin and Sequeira 1966; Khan and Saxena 1997; Siddiqui et al. 2002; Ashraf and Khan 2010). Amending the soil with farmyard manure and commonly available plant parts and products of neem, mahua, castor, mustard, and linseed in the form of oil cakes and dry leaves, seeds, seed kernel, seed coat, seed powder, etc. is one of the common methods used against plant parasitic nematodes, especially in India. The organic matter is an important component of soil, and the value of decomposition of organic amendment is an important factor in reduction of nematode infection which was first demonstrated by Linford et al. (1938).

16.8.1 Through Plant Residues/Green Manures

Organic matter serves as a primary source of nutrients for nematophagous fungi and nematode as a secondary source (Nicolay and Sikora 1991). The organic matter is an important component of soil, and the value of decomposition of organic amendment is an important factor in reduction of nematode infection, which was first demonstrated by Linford et al. (1938). Infection of *M. javanica* on tomato was reduced by incorporating oil cakes and their formulations (Goswami and Vijayalakshmi 1986; Khanna et al. 1987; Ahmad 1989; Darekar et al. 1990; Goswami 1993; Tiyagi and Alam 1995; Javed et al. 2008).

The use of green manures and plant residues before planting has long been considered as an effective control (Lumsden et al. 1983). Organic amendments are not only safe to use but also have the capacity to improve soil structure and fertility. These control strategies are now directed toward the use of natural products. Bioactive products of plants are being less persistent in environment and are safe for mammals and other nontarget organisms. Amending the soil with farmyard manure and commonly available plant parts and products of neem, mahua, castor, mustard, and linseed in the form of oil cakes and dry leaves, seeds, seed kernel, seed coat, seed powder, etc. is one of the common methods used against plant parasitic nematodes, especially in India. The increased efficacy of organic matter in the form of oil cakes in combination with inorganic fertilizers may be attributed to the fact that organic matter could provide the required nutrients such as zinc, iron, copper, manganese, etc. which help in plant metabolism through the supply of important micronutrients in the early growing phase of the plants. Organic matters like oilseed cakes act as a nutrient reservoir, and upon decomposition, a large number of organic products are released slowly in the soil in which root absorbs the ionic forms during their growth period leading to the higher yields. Use of different plant residues for organic amendment to decrease root pathogenic disease was well reviewed by Spadaro and Gullino (2005), Oka (2010), and Ntalli and Caboni (2012).

16.8.2 Plant-Derived Phytochemicals

Because of the adverse effect of synthetic pesticides, the interests turned toward the use of pesticides of natural origin or biopesticides. The adverse effects of chemical pesticides included negative impact on environment, toxicity to nontarget organisms including humans, and resistance development in insect population. Several plant extracts and their active constituents have been tried for the efficacy against root-knot nematodes and even root-rot fungi (Khalil 2013). Different plant parts such as leaves, seeds, flowers twigs, or stems or the residues of plants have been used for soilborne disease management. The different plant species which have been found effective were neem, castor, mahua, soybean, carnation, sunflower, sesamum, mustard, karanj, etc. (Table 16.2).

Table 16.2 Selected crop plants with phytochemicals active against root-knot nematodes

Plant species	Phytochemicals	Active against	References
Azadirachta indica	Limonoids (nimbin, azadirachtin, salannin)	*M. javanica*	Devakumar et al. (1985) and Akhtar (2000)
Tagetes species	Polythienyls, myristic, and dodecanoic acids	*Memoidohyne* spp.	Gommers and Bakker (1988) and Debprasad et al. (2000)
Artemisia species	Flavonoids	*Ditylenchus dipsaci*, *M. incognita*	Timchenko and Maiko(1989) and Dias et al. (2000)
Chrysanthemum spp.	–	*M. javanica*	Bar-Eyal et al. (2006)
Crotalaria spectabilis	Monocrotaline	*M. incognita*	Fassuliotis and Skucas (1969)
Mucuna pruriens	Alcohols	*M. incognita*,	Nogueira et al. (1996)
Ricinus species	Lectins (ricin)	*M. incognita*, *M. arenaria*	Rodrıguez-Kabana et al. (1989) and Ritzinger and McSorley (1998)
Brassica spp.	Glucosinolate, isothiocyanates, nitriles	Weeds, bacteria, fungi, nematodes	Kirkegaard et al. (1993), Matthiessen and Kirkegaard (2006), and Mumm et al. (2008)
Sorghum drummondii	Cyanogenic glycoside, dhurrin	*M. hapla*	Widmer and Abawi (2000, 2002)
Secale cereale	Hydroxamic acid	*M. incognita*	McSorley and Dickson (1995) and Zasada et al. (2005, 2007)
Quillaja saponaria	Saponins, polyphenols	*Meloidogyne* spp., *Xiphinema* spp.	San Martin and Magunacelaya (2005)
Pennisetum glaucum	S-compounds	Nematodes	Rodriguez-Kabana et al. (1965)

16.8.3 Disease Management Through Organic Amendments

Infection of *M. javanica* on tomato was reduced by incorporating oil cakes and their formulations (Goswami and Vijayalakshmi 1986; Khanna et al. 1987; Ahmad 1989; Darekar et al. 1990; Goswami 1993; Tiyagi and Alam 1995; Javed et al. 2008). Singh et al. (1990) determined the effect of neem, castor, and mustard cakes against *M. incognita* on tomato cv. Pusa Ruby under pot experiment. They reported that neem cake alone was highly effective against nematode followed by castor and mustard cakes, respectively. However, a mixture of neem + mustard and neem + castor cakes was more effective than neem cake alone. Alam (1991) studied the effect of mahua, castor, mustard, neem, and groundnut oil cakes singly and in different combinations against *M. incognita*, *T. brassicae*, *R. reniformis*, *H. indicus*, and *T. filiformis*, on tomato, brinjal, chili, okra, cabbage, and cauliflower under field conditions. He reported that all the treatments singly and in different combinations significantly reduced the population of plant parasitic nematodes. Mahua cake was

phytotoxic to all the test crops except brinjal, whereas other cakes improved plant growth significantly. Mojumdar and Mishra (1993) studied the effect of neem cake and seed kernel/seed coat as single, full dose, or split doses to soil naturally infested with *M. incognita* on chickpea under pot conditions. They found that all the treatments were effective to reduce the number of root galls significantly. However, treatment with neem seed kernels was most effective when applied as full dose. Abid et al. (1995) studied the effect of neem dry leaves powder, seed powder, and neem cake at 2, 4, and 6 g/750 g soil against *M. javanica* on okra under pot conditions. They reported that all the treatments enhanced the plant growth and reduced gall formation as compared to untreated control. Maximum reduction in root-knot index was observed with oil cake followed by seed powder. Rao and Goswami (1996) determined the efficacy of organic amendments, viz., groundnut, karanj, mahua, mustard, and neem cakes, an inorganic amendment, attapulgite-based clay dust (ABCD), and carbofuran for comparison on root-knot development caused by *M. incognita* and growth of cowpea. They observed that the reduction in root-knot development was significantly high in mustard, neem cakes, carbofuran, and ABCD treatments, the least effect being found with groundnut cake. The plant growth was greatly improved in mustard, and neem cakes amended soil followed by karanj, mahua cakes, and ABCD, respectively.

Several workers achieved success by using organic matter (Baby and Manibhushanrao 1996; Bailey and Lazarovits 2003), straw of several crops (Osunlaja 1990; Alam et al. 2002), leaves, stems, seeds (Tariq et al. 2006 & Tariq et al. 2008; Ahmed et al. 2009), seaweeds (Sultana et al. 2005), aqueous extracts of plant parts (Alam et al. 2002; Dawar et al. 2007; Emmanuel et al. 2010), oil cakes, and plant products and their formulations (Jeyarajan et al. 1987; Ehteshamul-Haque et al. 1998; Dubey et al. 2009) against root-rot fungus. Use of plant residues and organic amendment has been recognized as an effective way of achieving substantial population reduction of plant-pathogenic life forms like fungi, bacteria, nematodes, etc. (Patrick and Toussoun 1965). Plant residues or organic amendments have been reported to check the population of the pathogens through a variety of mechanisms (Sayre et al. 1964; Patrick and Toussoun 1965; Cook 1977; Sitaramaiah 1990).

References

Abawi GS, Barker KR (1984) Effects of cultivar, soil temperature, and population levels of *Meloidogyne incognita* on root-necrosis and *Fusarium* wilt of tomatoes. Phytopathology 74:433–438

Abdel-Aziez SM, Eweda WE, Girgis MGZ, Ghany BFA (2014) Improving the productivity and quality of black cumin (*Nigella sativa*) by using *Azotobacter* as N_2 biofertilizer. Ann Agric Sci 59:95–108

Abdel-Momen SM, Starr JL (1998) Meloidogyne javanica-Rhizoctonia solani disease complex of peanut. Fundam Appl Nematol 21:611–616

Abid M, Zaki MJ, Maqbool MA (1995) Neem derivatives for the control of root-knot nematode (*Meloidogyne javanica*) on okra. Pak J Phytopathol 7:212–214

Agrios G (2005) Plant pathology. Elsevier Academic Press, Burlington
Ahmad MU (1989) Effect of mustard oil-cake as organic amendment on the root-knot of brinjal. Bangladesh J Agric Sci 16:257–259
Ahmed ZM, Dawar S, Tariq M (2009) Fungicidal potential of some local tree seeds for controlling root rot disease. Pak J Bot 41:1439–1444
Akhtar M (2000) Nematicidal potential of the neem tree *Azadirachta indica* (a. Juss). Integr Pest Manag Rev 5:57–66
Akhtar MS, Siddiqui ZA (2008) *Glomus intraradices*, *Pseudomonas alcaligenes* and *Bacillus pumilus*: effective agents for the control of root-rot disease complex of chickpea (*Cicer arietinum* L.) J Gen Plant Pathol 74:53–60
Alam MM (1991) Control of plant parasitic nematodes with oil seed cakes on some vegetables in field. Pak J Nematol 9:21–30
Alam MM, Jairajpuri MS (1990) Nematode control strategies. In: Jairajpuri MS, Alam MM, Ahmad I (eds) Nematode biocontrol (aspects and prospects). CBS Publisher and Distributors, Delhi, pp 5–15
Alam S, Akhtar N, Begum MF, Banu MS, Islam MR, Chowdhury AN, Alam MS (2002) Antifungal activities (*In vitro*) of some plant extracts and smoke on four fungal pathogens of different hosts. Pak J Biol Sci 5:307–309
Amin KS, Sequeira L (1966) Phytotoxic substances from decomposing lettuce residues in relation to etiology of corky root rot of lettuce. Phytopathology 56:1054–1061
Anderson NA (1982) The geneticsa nd pathology of *Rhizoctonia solani*. Annu Rev Phytopathol 20:329–347
Ansari N, Azam MF (2005) Pathogenecity of root-knot nematode, *Meloidogyne incognita* on green gram. Bionotes 7:102
Arya A, Saxena SK (1999) Influence of certain rhizosphere fungi together with *Rhizoctonia solani* and *Meloidogyne incognita* on germination of "Pusa ruby" tomato seeds. Indian. Phytopathology 52:121–126
Ashraf MS, Khan TA (2010) Integrated approach for the management of *Meloidogyne javanica* on eggplant using oil cakes and biocontrol agents. Arch Phytopathol Plant Prot 43:609–614
Baby UI, Manibhushanrao K (1996) Influence of organic amendments on arbuscular mycorrhizal fungi in relation to rice sheath blight disease. Mycorrhiza 6:201–206
Back MA, Jenkinson P, Haydock PPJ (2000) The interaction between potato cyst nematodes and *Rhizoctonia solani* diseases in potatoes. In: Proceedings of the Brighton crop protection conference, pests and diseases. Farnham, British Crop Protection Council, pp 503–506
Back MA, Haydock PPJ, Jenkinson P (2002) Disease complexes involving plant parasitic nematodes and soil-borne pathogens. Plant Pathol 51:683–697
Bailey KL, Lazarovits GL (2003) Suppressing soil-borne diseases with residue management and organic amendments. Soil Tillage Res 72:169–180
Bar-Eyal M, Sharon E, Spiegel Y (2006) Nematicidal activity of *Chrysanthemum coronarium*. Eur J Plant Pathol 114:427–433
Batten CK, Powel NT (1971) The *Rhizoctonia – Meloidogyne* disease complex in flue–cured tobacco. J Nematol 3:164–169
Bergeson GB (1972) Concepts of nematode-fungus associations in plant disease complexes: a review. Exp Parasitol 32:301–314
Berkeley MJ (1855) Vibrio forming cysts on the roots of cucumbers. Gardener's chronicle and agricultural. Gazette 14:220
Bertrand B, Nuñez C, Sarah JL (2000) Disease complex in coffee involving *Meloidogyne arabicida* and *Fusarium oxysporum*. Plant Pathol 49:383–388
Bhatt J, Vadhera I (1997) Histopathological studies on cohabitation of *Pratylenchus thornei* and *Rhizoctonia bataticola* on chickpea (*Cicer arietinum* L.) Adv Plant Sci 10:33–38
Bhosle BB, Sehgal M, Puri SN, Das S (2004) Prevalence of phytophagous nematodes in rhizosphere of okra *(Abelmoschus esculentus* L. Moench) in Parbhani District, Maharashtra, India. Indian J Nematol 34:56–59
Bird DM, Kaloshian I (2003) Are roots special? Nematodes have their say. Physiol Mol Plant Pathol 62:115–123

Brodie BB, Cooper WE (1964) Relalion of parasitic nematodes to postemergence damping-off of cotton. Phytopathology 54:1023–1027

Cabreiro F, Gems D (2013) Worms need microbes too: microbiota, health and aging in *Caenorhabditis elegans*. EMBO Mol Med 5:1300–1310

Castillo P, Mora-Rodriguez MP, Navas-Cortes JA, Jimenez-Diaz RM (1998) Interactions of *Pratylenchus thornei* and *Fusarium oxysporum* f. sp. *ciceris* on chickpea. Phytopathology 88:828–836

Chand CD (2004) Pathogenecity of *Meloidogyne incognita* on *Allium hookerii*. J Appl Zoolog Res 15:70–71

Cook RJ (1977) Management of the associated microbiota. In: Horsfall JG, Cowling EB (eds) Plant disease. Academic Press, New York, pp 145–160

Darekar KS, Mhase NL, Shelke SS (1990) Effect of placement of non-edible oilseed cakes on the control of root-knot nematodes on tomato. International nematology network. Newsletter 7:5–7

Dawar S, Younus SM, Tariq M, Zaki MJ (2007) Use of *Eucalyptus* sp., in the control of root infecting fungi on mung bean and chickpea. Pak J Bot 39:975–979

De Oliveira-Garcia D, Dall'Agnol M, Rosales M, Azzuz ACGS, Alcantara N, Martinez MB, Giron JA (2003) Fimbriae and adherence of *Stenotrophomonas maltophilia* to epithelial cells and to abiotic surfaces. Cell Microbiol 5:625–636

De RK, Ali SS, Dwivedi RP (2001) Effect of interaction between *Fusarium oxysporum* f.sp. *lentil* and *Meloidogyne javanica* on lentil. Indian J Pulses Res 14:71–73

Debprasad R, Prasad D, Singh RP, Ray D (2000) Chemical examination and antinemic activity of marigold (*Tagetes erecta* L.) flower. Ann Plant Prot Sci 8:212–217

Devakumar C, Goswami DK, Mukerjee SK (1985) Nematicida principles from neem (*Azadirachta indica* a. Juss.). Part 1. Screening of neem kernel fractions against *Meloidogyne incognita*. Ind J Nematol 15:121–124

Dias CR, Schwan AV, Ezequiel DP, Sarmento MC, Ferraz S (2000) Efeito de extratos aquosos de plantas medicinais na sobrevivencia de juvenis de *Meloidogyne incognita*. Nematol Bras 24:203–210

Diomande M, Black MC, Beute MK, Barlœr KR (1981) Enhancement of *Cylindrociadium crotalariae* root-rot by *Meloidogyne arenaria* (race 2) on a peanut cultivar resistant to both pathogens. J Nematol 13:321–327

Dubey SC, Suresh M, Singh B (2007) Evaluation of *Trichoderma* species against *Fusarium oxysporum* f. sp. *ciceris* for integrated management of chickpea wilt. Biol Control 40:118–127

Dubey RC, Kumar H, Pandey RR (2009) Fungitoxic effect of neem extracts on growth and sclerotial survival of *Macrophomina phaseolina in vitro*. J Am Sci 5:17–24

Ehteshamul-haque S, Zaki MJ, Vahidy AA (1998) Effect of organic amendments on the efficacy of *Pseudomonas aeruginosa* in the control of root rot disease of sunflower. Pak J Bot 30:45–50

Ekenma AGWUJ, Chidera EZIGBOJ (2005) Effect of *Meloidogyne incognita* (root- knot nematode) on the development of *Abelmoschus esculentus* (okra). Anim Res Int 2:358–362

Emmanuel S, Rani MS, Sreekanth MR (2010) Antidiabetic activity of *Cassia occidentalis* Linn. In streptozotocin-induced diabetic rats: a dose dependent study. Int J Pharm Bio Sci 1:14–25

Fassuliotis G, Skucas GP (1969) The effect of a pyrrolizidine alkaloid ester and plants containing pyrrolizidine on *Meloidogyne incognita acrita*. J Nematol 1:287–288

Garigan D, Hsu AL, Fraser AG, Kamath RS, Ahringer J, Kenyon C (2002) Genetic analysis of tissue aging in *Caenorhabditis elegans*: a role for heat-shock factor and bacterial proliferation. Genetics 161:1101–1112

Gill JS, Sivasithamparam K, KRJ S (2000) Soil types with different texture affects development of *Rhizoctonia* root rot of wheat seedlings. Plant Soil 221:113–120

Gill JS, Sivasithamparam K, KRJ S (2001) Effect of soil moisture at different temperatures on *Rhizoctonia* root rot of wheat seedlings. Plant Soil 231:91–96

Golden JK, Van Gundy SD (1975) A disease complex of okra and tomato involving the nematode, *Meloidogyne incognita* and the soil-inhabiting fungus, *Rhizoctonia solani*. Phytopathology 65:265–275

Gommers FJ, Bakker J (1988) Physiological diseases induced by plant response or products. In: Poinar GO, Jansson HB (eds) Diseases of nematodes, vol 1. CRC Press, Boca Raton, Florida, pp 3–22

Goswami BK (1993) Effect of different soil amendments with neem cake on root-knot nematode and soil mycoflora in cowpea rhizosphere. Indian J Plant Prot 21:87–89

Goswami BK, Vijayalakshmi K (1986) Efficacy of some indigenous plant material and non edible oil seed cakes against *Meloidogyne incognita* on tomato. Indian J Nematol 16:280–181

Gottlieb D (1976) The production and role of antibiotics in soil. J Antibiot (Tokyo) 29:987–999

Grover A, Gowthaman R (2003) Strategies for development of fungus-resistant transgenic plants. Curr Sci 84:330–340

Hafez SL, Al-Rehiayani S, Thornton M, Sundararaj P (1999) Differentiation of two geographically isolated populations of *Pratylenchus neglectus* based on their parasitism of potato and interaction with *Verticillium dahliae*. Nematropica 29:25–36

Hazarika BP, Roy AK (1974) Effect of *Rhizoctonia solani* on the reproduction of *Meloidogyne incognita* on egg plant. Indian J Nematol 4:246–247

Hussain MA, Mukhtar T, Kayani MZ (2011) Assessment of the damage caused by *Meloidogyne incognita* on okra (*Abelmoschus esculentus*). J Anim Plant Sci 21:857–861

Hussey RS (1985) Host parasite relationships and associated physiological changes. In: Barker KR, Carter CC, Sasser JN (eds) An advanced treatise on Meloidogyne. North Carolina State University Graphics, Raleigh, pp 143–153

Ingham RE, Trofymow JA, Ingham ER, Coleman DC (1985) Interactions of bacteria, fungi, and their nematode grazers: effects on nutrient cycling and plant growth. Ecol Monogr 55:119–140

Jacobsen JB (2006) Root rot diseases of sugar beet. Proc Natl Acad Sci 110:9–19

Javed N, Anwar SA, Fyaz S, Khan MM, Ashfaq M (2008) Effects of neem formulations applied as soil drenching on the development of root-knot nematode *Meloidogyne javanica* on roots of tomato. Pak J Bot 40:905–910

Jeyarajan R, Doraiswamy S, Bhaskaran R, Jayaraj S, Draiswamy S, Schmuttere S (1987). Effect of neem (*Azadirachta indica*) and other plant products in the management of plant disease in India. In: Natural pesticides from the Neem tree, *Azadirachta indica* a. Juss and other tropical plants, pp 635–644.

Johnson DA, Santo GS (2001) Development of wilt in mint in response to infection by two pathotypes of Verticillium dahliae and co-infection by Pratylenchus Penetrans. Plant Dis 85:1189–1192

Jonathan EI, Gajendran G, Arulmozhiyan R (1997) Interaction of *Rotylenchulus reniformis* and *Phytophthora palmivora* on betelvine. Nematol Mediterr 25:191–193

Kaitany R, Melakeberhan H, Bird GW, Safir G (2000) Association of *Phytophthora sojae* with *Heterodera glycines* and nutrient stressed soybeans. Nematropica 30:193–199

Kankam F, Adomako J (2014) Influence of inoculum levels of root knot nematodes (*Meloidogyne* spp.) on tomato (*Solanum lycopersicum* L.) Asian J Agric Food Sci 2:171–178

Khalil MS (2013) Abamectin and azadirachtin as eco-friendly promising biorational tools in integrated nematodes management programs. J Plant Pathol Microbiol 4:174–183

Khan MW (1993) Mechanism of interactions between nematodes and other plant pathogens. In: Khan MW (ed) Nematode interactions. Chapman and Hall, London, pp 55–78

Khan MR, Haque Z (2011) Soil application of *Pseudomonas fluorescens* and *Trichoderma harzianum* reduces root-knot nematode, *Meloidogyne incognita*, on tobacco. Phytopathol Mediterr 50:257–266

Khan TA, Saxena SK (1997) Effect of root-dip treatment with fungal filtrates on root penetration, development and reproduction of *Meloidogyne javanica* on tomato. Int J Nematol 7:85–88

Khan TA, Ashraf MS, Hasan S (2006) Pathogenicit and life cycle of *Meloidogyne javanica* on balsam (*Impatiens balsamia)*. Arch Phytopathol Plant Prot 39:45–48

Khan TA, Ashraf MS, Dar RA (2008) Pathogenecity and life cycle of *Meloidogyne javanica* on broccoli. Arch Phytopathol Plant Prot 43:602–608

Khanna AS, Jyoti J (2004) Pathogenicity of *Meloidogyne incognita* on *Dianthus caryophillus*. Phytopathol Mediterr 32:125–126

Khanna AS, Nattuthurai N, Vaitheeswaran M, Rathinavathy J, Reuben R (1987). Control of plant parasitic nematode with organic amendments. In: Proceedings of the symposium alternatives to synthetic insecticides in integrated pest management systems, Madurai, pp 223–321.
Kim DH (2013) Bacteria and the aging and longevity of Caenorhabditis Elegans. Annu Rev Genet 47:233–246
Kirkegaard JA, Gardner PA, Desmarchelier JM, Angus JF (1993). Biofumigation using *Brassica* species to control pests and diseases in horticulture and agriculture. In: Wratten N, Mailer RJ (eds) Proceedings 9th Australian Research Assembly on Brassicas Agricultural Research Institute, Wagga, pp 77–82.
Koike ST, Subbarao KV, Davis RM, Turini TA. (2003). Vegetable diseases caused by soilborne pathogens. ANR University of California, Publication 8099.
Komarek M, Cadkova E, Chrastny V, Bordas F, Bollinger JC (2010) Contamination of vineyard soils with fungicides: a review of environmental and toxicological aspects. Environ Int 36:138–151
Kookana RS, Baskaran S, Naidu R (1998) Pesticide fate and behaviour in Australian soils in relation to contamination and management of soil and water: a review. Aust J Soil Res 36:715–764
Kühn J (1858). Die Krankheiten der Kulturgewachse, ihre Ursachen und Verhutung.
Kuprashvili TD (1996) The use phytocides for seed treatment. Zashchita-I- Karantin- Rastenii 55:31
Lemanczyk G (2010) Occurrence of sharp eyespot in spring cereals grown in some regions of Poland. J Plant Prot Res 50:505–512
Linford MB, Yap F, Oliveira JM (1938) Reduction of soil population of root-knot nematode during decomposition of organic matter. Soil Sci 45:127–141
Lucas GB, Campbell CL, Lucas LT (1997) Introduction to plant disease identification and management. CBS Publishers and Distributors, New Delhi, p 364
Lumsden RD, Lewis JA, Millner PD (1983) Effect of composted sewage sludge on several soilborne plant pathogens and diseases. Phytopathology 73:1543–1548
Matthiessen JN, Kirkegaard JA (2006) Biofumigation and enhanced biodegradation: opportunity and challenge in Soilborne Pest and disease management. Crit Rev Plant Sci 25:235–265
McSorley R, Dickson DW (1995) Effect of tropical rotation crops on *Meloidogyne incognita* and other plant-parasitic nematodes. J Nematol 27:535–544
Mohan S, Fould S, Davies G (2001) The interaction between the gelatin-binding domain of fibronectin and the attachement of *Pasteuria penetrans* endospores to nematode cuticle. Parasitology 123:271–276
Mojumdar V, Mishra SD (1993) Soil application of aqueous extracts of neem products for the management of Meloidogyne Incognita in chickpea. Curr Nematol 4:105–107
Mokbel AA, Ibrahim IKA, Shehata MRA, El-Saedy MAM (2007) Interaction between certain root-rot fungi and the root-knot nematode, *Meloidogyne incognita* on sunflower plants. Egypt J Phytopathol 35:1–11
Mukhtar T, Kayani MZ, Hussain MA (2013) Response of selected cucumber cultivars to *Meloidogyne incognita*. Crop Prot 44:13–17
Mumm R, Burow M, Bukovinszkine'kiss G, Kazantzidou E, Wittstock U, Dicke M, Gershenzon J (2008) Formation of simple nitriles upon glucosinolate hydrolysis affects direct and indirect defense against the specialist herbivore, *Pieris rapae*. J Chem Ecol 34:1311–1321
Nicolay R, Sikora RA (1991) Interrelationships between fungal egg parasitism in *Heterodera schachtii* (Schmidt) and nematode population density. Rev Nematol 14:231–249
Nogueira MA, de Oliveira JS, Ferraz S, Dos Santos MA (1996) Nematicidal constituents in *Mucuna aterrima* and its activity on *Meloidogyne incognita* race 3. Nematol Mediterr 24:249–252
Ntalli G, Caboni P (2012) Botanical nematicides: A review. J Agric Food Chem 60:9929–9940
Oduor-Owino P (2003) Control of root-knot nematodes in Kenya with aldicarb and selected antagonistic plants. Nematol Mediterr 31:125–127
Oka Y (2010) Mechanisms of nematode suppression by organic soil amendments–a review. Appl Soil Ecol 44:101–115

Osunlaja SO (1990) Effect of organic soil amendments on the incidence of stalk rot of maize caused by *Macrophomina phaseolina* and *Fusarium moniliforme*. J Basic Microbiol 30:753–757
Patrick ZA, Toussoun TA (1965) Plant residue and organic amendments in relation to biological control. In: Baker KF, Synder WC (eds) Ecology of soil-borne plant pathogens. John Murray, London, pp 440–459
Rao U, Goswami BK (1996) Comparative efficacy of soil amendments with carbofuran against root-knot nematode, *Meloidogyne incognita* on cowpea. Pest Res J 8:87–89
Richards BN (1976) Introduction to the soil ecosystem. Longman, London
Ritzinger CHSP, McSorley R (1998) Effect of castor and velvetbean organic amendments on *Meloidogyne arenaria* in greenhouse experiments. J Nematol 30:624–631
Rodriguez-Kabana R, Jordan JW, Hollis JP (1965) Nematodes: biological control in rice fields: role of hydrogen sulfide. Science 148:524–526
Rodrıguez-Kabana R, Robertson DG, Wells L, King PS, Weaver CF (1989) Crops uncommon to Alabama for the management of *Meloidogyne arenaria* in peanut. J Nematol 21:712–716
Rosenberg E, Zilber-Rosenberg I (2011) Symbiosis and development: the hologenome concept. Birth Defects Res C Embryo Today 93:56–66
Rupe JC, Robbins RT, Becton CM, Sabbe WA, Gbur EE (1999) Vertical and temporal distribution of *Fusarium solani* and *Heterodera glycines* in fields with sudden death syndrome of soybean. Soil Biol Biochem 31:245–251
Ryss AY, Kulinich OA, Sutherland JR (2011) Pine wilt disease: a short review of worldwide research. Forestry Sci China 13:1–11
Safiuddin A, Shahab S (2012) Interactive effect of root-knot nematode, *Meloidogyne incognita* and root-rot fungus, *Rhizoctonia solani*, on okra [*Abelmoschus esculentus* L.] Arch Phytopathol Plant Prot 45:660–666
San Martin R, Magunacelaya JC (2005) Control of plant–parasitic nematodes with extracts of *Quillaja saponaria*. Nematology 7:577–585
Sayre RM, Patrick ZA, Thorpe JH (1964) Substances toxic to plant parasitic nematodes in decomposing plant residue. Phytopathology 54:905
Siddiqui ZA, Husain SI (1991) Interaction of *Meloidogyne incognita* race-3 and *Macrophomina phaseolina* in a root-rot disease complex of chickpea. Nematol Mediterr 19:237–239
Siddiqui IA, Shaukat SS, Khan GH, Zaki MJ (2002) Evaluation of *Argemone mexicana* for control of root-infecting fungi in tomato. J Phytopathol 150:321–329
Sikora RA, Fernandez E (2005). Nematode parasites of vegetables. In: Luc M, Sikora RA, Bridge J (eds) Plant parasitic nematodes in subtropical and tropical agriculture, 2nd edn. CABI Publishing, p 319–392.
Singh, S.K, Khan, M.R. and Khan, A.A. (1990). Control of root-knot nematode, Meloidogyne incognita by organic amendment on tomato cv. Pusa Ruby. Indian Journal of Applied and Pure Biology, 5:21–23.
Sitaramaiah K (1990) Mechanism of reduction of plant parasitic nematodes in soils amended with organic materials. In: Saxena SK, Khan MW, Rashid A, Khan RM (eds) Progress in plant nematology. CBS Publishers and Distributors, New Delhi, pp 263–265
Sitaramaiah K, Pathak KN (1993) Nematode bacterial disease interactions. In: Khan MW (ed) Nematode interactions. Chapman and Hall, New York, pp 232–250
Sneh, B, Jabaji-Hare, S, Neate, S. and Djist, G. (1996). Rhizoctonia species: taxonomy, molecular biology, ecology, pathology and disease control. Kluwer Academic Publishiers, Dordrecht, The Netherlands, Chapter V, pp. 247–404 and Chapter VIB, pp. 445–559.
Spadaro D, Gullino LM (2005) Improving the efficacy of biocontrol agents against soilborne pathogens. Crop Prod 24:601–613
Starr JL, Aist JR (1977) Early development of *Pythium polymorphon* on celery roots infected by *Meloidogyne hapla*. Phytopathology 67:497–501
Starr JL, Martyn RD, Jeger MJ, Schilling K (1989) Effect of *Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *vasinfectum* on plant mortaliry and yield of cotton. Phytopathology 79:640–646

Sultana V, Ehteshamul-Haque S, Ara J, Akhtar M (2005) Comparative efficacy of brown, green and red seaweeds in the control of root infecting fungi and okra. Int J Environ Sci Technol 2:129–132

Tariq M, Dawar S, Mehdi FS, Zaki MJ (2006) Use of *Avicennia marina* in the control of root infecting fungi on okra and mash bean. Pak J Bot 38:811–815

Tariq M, Dawar S, Mehdi FS, Zaki MJ (2008) The effect of mangroves amendments to soil root rot and root-knot of potato (*Solanum tuberosum* L.) Acta Agrobot 61:115–121

Timchenko LS, Maiko TK (1989). Nematicidal properties of plants-antagonists of nematodes of decorative plants. Byulleten' Vsesoyuznogo Instituta Gel'mintologii im. K.I. Skryabina No 50:81–84.

Tiyagi SA, Alam MM (1995) Efficacy of oil seed cakes against plant-parasitic nematodes and soil inhabiting fungi on chickpea and mungbean. Bioresour Technol 51:233–239

Vats R, Dalal MR (1997) Interaction between *Rotylenchulus reniformis* and *Fusarium oxysporum* f.sp. *pisi* on pea (*Pisum sativum* L.) Ann Biol 13:239–242

Wheeler TA, Hake KD, Dever JK (2000) Survey of *Meloidogyne incognita* and *Thielaviopsis basicola*: their impact on cotton fruiting and producers' management choices in infested fields. J Nematol 32:576–583

Widmer TL, Abawi GS (2000) Mechanism of suppression of *Meloidogyne hapla* and its damage by a green manure of sudangrass. Plant Dis 84:562–568

Widmer TL, Abawi GS (2002) Relationship between levels of cyanide in sudangrass hybrids incorporated into soil and suppression of *Meloidogyne hapla*. J Nematol 34:16–22

Yadav SK, Babu S, Yadav MK, Singh K, Yadav GS, Pal S (2013) A review of organic farming for sustainable agriculture in northern India. Int J Agron. doi:10.1155/2013/718145

Zacheo G, Lamberti F, Arrigoni-Liso R, Arrigoni O (1977) Mitochondrial protein-hydroxyproline content of susceptible and resistant tomatoes infected by *Meloidogyne incognita*. Nematologica 23:471–476

Zasada IA, Meyer SL, Halbrendt JH, Rice C (2005) Activity of hydroxamic acids from *Secale cereale* against the plant–parasitic nematodes *Meloidogyne incognita* and *Xiphinema americanum*. Phytopathology 95:1116–1121

Zasada IA, Rice CP, Meyer SLF (2007) Improving the use of rye (*Secale cereale*) for nematode management: potential to select cultivars based on *Meloidogyne incognita* host status and benzoxazinoid content. Nematology 9:53–60

Endophytic Probiotics and Plant Health: Toward a Balanced Accost

17

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Abstract

The endophytic probiotic microorganisms have been reported to be found in virtually each plant studied, where endophytes colonize the internal tissues of the host plant and they might form a variety of dissimilar and distinct associations that include but not limited to interdependency, positive and neutral cooperative, mutualistic, commensalistic, and also trophobiotic. Most of the endophytic microbiomes appear to originate either from the plant rhizosphere or the phyllosphere. However, some of the endophytes may also be transmitted through the seed. Probable endophytic microbes can enhance and accelerate the plant growth and production and, moreover, can also act as potential biocontrol agents. There are numerous potential fungal and bacterial endophytes that make indispensable secondary metabolites such as phytohormones, siderophores, volatile organic compounds, HCN production that support the development and progression of the host plant. Certain compounds produced by endophytes act as antibiotics which have possible antibacterial, antifungal, and insecticidal properties. These compounds intensely restrain the growth of pathogenic microorganisms, including the probable plant pathogens. On the other hand, these probable endophytic microbes can also be precious to human beings by producing a variety of natural products that could be utilized for the possible employment in medication, agronomy, or commerce. Additionally, it has been shown that endophytes too have the potential to eliminate the soil contaminants by enhancing bioremediation and phytoremediation process

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and, therefore, may play a remarkable role in soil fertility augmentation through notable and striking valuable processes such as biological nitrogen fixation, phosphate solubilization, metal chelation, and potassium mobilization. There is a growing and vested interest in development of biotechnological applications of probable endophytic microbes for improving crop production, phytoremediation, and sustainable production of food crops for biomass as well as biofuel production, which is a feasible and practical step toward the sustainable form of agriculture.

17.1 Introduction

Advantageous and positive plant microbe communications that cheer and boost plant health and development have been the subject of substantial study. Maximum of these studies have been focused on bacteria isolated from the rhizosphere of crop plants (Adachi et al. 2002; Ali and Hasnain 2007; Andreote et al. 2010). The endophytic microbiome or endophytic probiotics can be defined as those microbiomes that colonize the internal tissue of the plant and show no external sign of infection or negative, bad, or undesirable effect on their host (Sturz 1995; Holliday 1989; Zinniel et al. 2002), and as per the latest research, there are nearly 300,000 plant species that exist on the earth; each individual plant is host to one or more endophytes (Thomas and Soly 2009; Lopez et al. 2011). Out of 300,000 plants, only a limited number of these plants have ever been exclusively reviewed related to their endophytic biology. Therefore, it will be beneficial to find novel, aboriginal, innovative, and beneficial endophytes that colonize an ecological niche comparable to that of potential phytopathogens, which could make them appropriate biocontrol agents (Sturz et al. 1997; Backman and Sikora 2008; Larran et al. 2016). Positively, copious reports have discovered that endophytic microbiome may have the potential to control deadly plant pathogens (Sturz and Matheson 1996; Duijff et al. 1997), insects (Azevedo et al. 2000), and nematodes (Hallmann et al. 1997, 1998; Schouten 2016). In some of the cases, the endophytes can also hasten the seedling development, endorse the plant founding under opposing conditions (Chanway 1997; Ryan et al. 2008), and enhance overall plant growth and development (Bent and Chanway 1998). The bacterial endophytes have been demonstrated to avert or check disease development through endophyte-facilitated de novo production of innovative compounds and antifungal metabolites. Examination of biodiversity of endophytic microbial strains for innovative metabolites may be utilized for new drugs for operative cure of diseases in plants, humans, and animals (Strobel and Daisy 2003). Along with the making of unusual and unique chemicals, there are many endophytes which have demonstrated a natural ability for xenobiotic biodegradation or may act as vectors to initiate degradative traits (Kang et al. 2012). The aptitude of some endophytes to exhibit resistance to certain heavy metals and antimicrobials and biodegrade difficult organic compounds possibly stems from their acquaintance to various compounds in the plant and soil nook (Ruppel et al. 1992; Varsha et al. 2011). This inherent and basic natural capability to biodegrade these xenobiotic or

difficult compounds has been investigated with regard to refining phytoremediation (Germaine et al. 2006; Porteous-Moore et al. 2006). This article on endophytes is intended to provide an impression about the potential use of microbial endophytes predominantly in the area of sustainable and natural agriculture.

17.2 Endophyte Isolation and Characterization

The probiotic endophytic microorganisms have been isolated from several plants and also from the different parts of. Endophytes have been isolated from the meristem, from resin ducts, and also from scale primordia (Prittila et al. 2000, 2003); from leaf parts including midrib, root, and root hairs (Hata et al. 2002); and from stem parts including stem bark, leaf tip and blade, leaf petiole (Hata and Sone 2008), and flower buds (Prittila et al. 2008). A sequence-based technique was used for finding the spread of varied fungal endophytes in seed and needles of Western white pine and *Pinus monticola* (Ganley and Newcombe 2006). They have been isolated around 2003 fungal endophytes from 750 surface-sterilized pine needles; on the other hand, only 16 endophytes were isolated from 800 surface-sterilized seeds. Since the studies on endophytes started, isolation of endophytic microbes from the plant tissue parts has been a challenge. Numerous scientists have reviewed widely dissimilar methods of the bacterial endophyte isolation (Hallmann et al. 1997; Reinhold-Hurek and Hurek 1998). Usually, the endophytes are isolated by initial surface sterilization process followed by the culturing from macerated plant tissue extract (Rai et al. 2007) or from direct culturing of plant tissues (Hata and Sone 2008) on any suitable media for bacteria, fungi, or actinomycete. Verma et al. (2011) studied the influence of dissimilar culture media on isolation of endophytic fungal strains from *Azadirachta indica* A. Juss plant root and fruits and also suggested that mycological agar (MCA) medium resulted in the highest number of fungal isolates, with the utmost species richness. *Enterobacter cloacae*, an obligatory endophytic bacterium, was associated with the pollen of several Mediterranean pine trees (Madmony et al. 2005). Most of the fungal endophytes isolated from plants and algae belong to the *Ascomycota*, with a few reports of endophytes from basidiomycetes. These fungal endophytes are often being orchid mycorrhizas (Jones 2006). Basidiomycetous endophytes were isolated from oil palm (*Elaeis guineensis*) leaves, rachis, and petioles, which were further typified by employing molecular tools using rDNA sequences. Interestingly, the species *Tetraploa aristata*, *Acremonium terricola*, *Penicillium glandicola*, *Phoma tropica*, and *Monodictys castaneae* were reported as endophytic fungi (Bezerra et al. 2012).

After getting endophytes on Petri plate, usually the identification of endophytes has been done based on the morphological characteristics and microscopic observation and by performing certain biochemical tests for bacteria, fungi, and actinomycetes. With the advancement in molecular biology techniques, rDNA internal transcribed spacer (ITS) sequence analysis is widely employed for identification of endophytic microbes. rDNA ITS has been proved to be a valued source of indication or sign to decide phylogenetic relationships at lower levels, that is, among genera

level or species level (Youngbae et al. 1997). It has also been reported that ITS sequence analysis is particularly actual in identification of nonsporulating fungi which lessened the impact of biased judgment (Chen et al. 2008) and moreover large subunit (LSU) and ITS data are powerful means to resolve the taxonomy problem of basidiomycetous endophytes (Rungjindamai et al. 2008). By employing the rDNA ITS analysis technique, endophytes like *Pleurostoma*, *Chaetomium*, *Coniochaeta* (*Lecythophora*), *Daldinia*, *Xylaria*, *Hypoxylon*, *Nodulisporium*, *Cazia*, and *Phellinus* have been isolated from *Huperzia serrata*.

17.3 Influence of Environment on Endophytes

The probiotic endophytic population differs from plants to plants and from species to species. Within the same plant species, the endophytic populations vary from section to section and also vary with disparity in environmental conditions of the same section. Chareprasert et al. (2006) studied the sequential changes in relative frequency of total endophytic fungi found in the host plant. The researchers observed that ripened leaves of teak plant (*Tectona grandis*) and rain tree (*Samanea saman*) exhibited larger number of fungal genera and species, with greater colonization occurrence, compared to the young leaves and, moreover, their frequency increased in leaves during rainy season. Endophytic microbial population and their occurrence tended to vary during sampling dates for all the pant parts studied, such as leaves, petiole, and twigs of *Ginkgo biloba* (Thongsandee and Matsuda 2012). Researchers concluded that occurrence of fungus *Phyllosticta* sp. in both leaves and petioles was first noticed in August and reached highest in October with no one in summer, that is, in the month of May. *Phomopsis* sp. was detected in leaf twigs all throughout the growing season. These results suggested that distribution pattern of two prevailing fungal endophytes was plant part specific and varied with change in climatic conditions.

17.4 Means of Plant Growth Stimulation

The means, methods, and procedures by which the endophytic microbiome can affect plant growth vary among species and strains, so characteristically there is no solitary means and methods or mechanism which is solely responsible for promoting plant growth. Various studies have been conducted concerning the capabilities of numerous endophytic microbiomes to endorse and encourage plant growth; among them, the endophytic bacteria prevail (Hallmann et al. 1997; Strobel 2003; Hardoim et al. 2008). The endophytes are unadventurously defined as microbes such as bacteria or fungi that colonize internal plant tissues part and can be isolated from the plant after surface disinfection and sterilization and cause no negative or harmful effects on plant growth and promotion (Fisher et al. 1992; Kuklinsky-Sobral et al. 2004; Gaiero et al. 2013). There are several endophytic microbes which promote the plant growth at various stages of the host plant life cycle by means of various methods and ways.

17.4.1 Endophytes as Biocontrol Agent

Endophytic microbes are capable and competent to lessen or avert the harmful effects of some pathogenic microorganisms. Advantageous and positive consequences of bacterial endophytes on their host plant seem to happen through comparable means and methods or mechanisms as mentioned for rhizospheric zone-associated bacteria. These means and methods have been appraised in abundant detail by Larran et al. (2016), by Backman and Sikora (2008), and also by Compant et al. (2005). The infections of bacterial, fungal, viral derivation and in some cases even harm caused by insects and nematodes can be lessened following earlier inoculation with potential endophytes (Hallmann et al. 1998; Azevedo et al. 2000; Schouten 2016).

It has been assumed that some endophyte bacteria activate or start a phenomenon known as induced systemic resistance (ISR), which is phenotypically comparable to systemic acquired resistance (SAR). The SAR progresses when plants efficaciously and fruitfully activate their own defense mechanism in response to any primary infection by a pathogen. It happens notably when the latter persuades or encourages a hypersensitive reaction through which it remains limited in a local necrotic lesion of brown desiccated plant tissue (van Loon et al. 1998; Melnick et al. 2011). The ISR is effective against diverse types of plant pathogens but varies or disagrees from SAR in that the inducing bacterium does not cause noticeable or observable signs on the host plant (Yi et al. 2013). Bacterial endophytes and their role in the ISR have been reviewed in detail by Kloepper and Ryu (2006).

Majority of probiotic endophytes isolated from host plants are known to possess antimicrobial or biocontrol activity. They help in regulating the growth of pathogenic microbes in plants or animals in situ. Potential endophytes isolated from the medicinal plants exhibited broad spectrum biocontrol activity against pathogenic microorganisms (Sette et al. 2006; Selim et al. 2011; Devaraju et al. 2011). Isolated a total of 37 endophytes only from *Tectona grandis* L. and *Samanea saman* Merr. of which 18 endophytes produced inhibitory substances which were effective against bacteria like *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus* and, on the other hand, 3 endophytes isolated also inhibited the growth of *Candida albicans* (Chareprasert et al. 2006). Kumar et al. (2011) analyzed biocontrol activity of the endophytic microbes such as *Dothideomycetes* sp., *Alternaria tenuissima*, *Thielavia subthermophila*, *Alternaria* sp., *Nigrospora oryzae*, *Colletotrichum truncatum*, and *Chaetomium* sp. Joseph and Mini Priya (2011) reported that endophytes were isolated from medicinal plant, *Tylophora indica*, and were bioactive against *Sclerotinia sclerotiorum* and *Fusarium oxysporum*.

Another good example of endophytic fungi is *Beauveria bassiana* also called as entomopathogenic fungus, and this organism has been reported to control the borer insects in coffee seedlings (Posada and Vega 2006) and sorghum (Tefera and Vidal 2009). The fungal pathogen *Botrytis cinerea* causes stern rotting of tomato fruits during storage and ultimately reduces shelf life. In another report (Wang et al. 2009), one endophytic bacterium, *Bacillus subtilis*, which was isolated from *Speranskia tuberculata* (Bge.) Baill plant has potential bioactivity against the plant pathogen *B. cinerea* under in vitro studies.

17.4.2 Biological Nitrogen Fixation

Among the leguminous plants of the family Fabaceae, the prominent soil bacteria of *Rhizobiaceae* (rhizobia) family are restricted to the root nodules only (Olivares et al. 2013). Within these root nodules, rhizobia efficiently and effectually perform biological nitrogen fixation (BNF). This activity is performed through the acceptable control of the existence of oxygen air, which is an inhibitor of enzyme nitrogenase functioning (Galloway et al. 2004; Bru et al. 2011). Many strains of microbes are employed in cultivation of plants which are of economic interest and facilitate the host plant growth and development without or with less application of nitrogenous fertilizers. For example, in Brazil, the production of soybean crop (*Glycine max* L.) is an exceptional example of the effectiveness of BNF through the application of diverse strains of *Bradyrhizobium* sp., such as *B. elkanii* and *B. japonicum* (Bohrer and Hungria 1998; Alves et al. 2003). The significance of endophytic nitrogen-fixing bacteria has also been the main objective of the findings in nonleguminous plants such as sugarcane (*Saccharum officinarum* L.) (Thaweenut et al. 2011). Other reports have advocated that bradyrhizobium bacteria colonize and express the nifH not only in root nodules of leguminous plants but also in roots of sweet potatoes (*Ipomoea batatas* L.), therefore acting as diazotrophic endophytes (Torres et al. 2008).

17.4.3 Indolic Compound Production

The influence of endophytic microbes of host plant is principally owing to the manufacture of phytohormone known as auxin (Celloto et al. 2012; Uma Maheswari et al. 2013). There are several bacterial species which can produce indolic compounds (ICs) such as indole-3-acetic acid (IAA), an auxin phytohormone, which represent a great physiological significance for bacteria plant communications, fluctuating from pathogenesis to phyto-stimulus (Jha and Kumar 2007; Uma Maheswari et al. 2013). The capability to make ICs is extensively distributed among plant-accompanying bacteria. In one study, Sturz et al. (2000) established that nearly 80% of bacteria isolated from rhizospheric zone of rice produce ICs. On the other hand, further studies have also exhibited that rhizospheric zone bacteria produce more ICs compared to the bacteria present in bulk soil (Thuler et al. 2003), and a latest study conducted by Costa et al. (2014) indicated that this effect was also detected in endophytic bacteria, establishing high IC production in the family *Enterobacteriaceae*.

The production of ICs in bacteria relies on the existence of precursors in the plant root exudates. Among the various root exudates, chemical L-tryptophan has been identified as the key precursor for the IC biosynthesis in most bacteria (Stijn et al. 2007). Description of in-between compounds has led to the identification and documentation of dissimilar pathways that use L-tryptophan as chief precursor. Diverse pathways of indole acetic acid synthesis in bacteria exhibited a high degree of similarity with the indole acetic acid biosynthesis pathways in plants (Spaepen et al. 2007). The advantageous bacteria mainly synthesize indole acetic acid thru the

indole-3-pyruvic acid pathway, another pathway which is dependent on L-tryptophan (Bianco et al. 2006; Govindarajan et al. 2007).

In one study, Xin et al. (2009) stated that from wild cottonwood (*Populus trichocarpa*), an endophytic bacteria *Burkholderia vietnamiensis*, which fixes nitrogen, also produced indole acetic acid; all these features promote the growth of the plant. This fact was recognized by observing the difference between uninoculated and *B. vietnamiensis*-inoculated plants in the nitrogen-free media. In this work, the inoculated plants demonstrated higher dry weight and more nitrogen content. In another report, a new strain of *Cladosporium sphaerospermum*, an endophytic fungus isolated from the roots of *Glycine max* (L) Merr., showed the presence of higher amounts of biomolecules such as GA3, GA4, and GA7, which influenced and promoted better plant growth in both rice and soybean plants (Hamayun et al. 2009).

17.4.4 Manufacture of Siderophore

An essential micronutrient, iron (Fe) is being utilized by plants and microbes, as it is involved in plentiful noteworthy biological progressions, such as respiration, photosynthesis and chlorophyll biosynthesis (Costa and Loper 1994; Rout et al. 2013), and biological nitrogen fixation (Thaweenut et al. 2011). In acidic and anaerobic soils, such as water-flooded soils, high amount of ferrous (Fe^{2+}) ions produced through the reduction of ferric (Fe^{3+}) ions may lead to iron toxicity owing to unnecessary Fe uptake (Bohrer and Hungria 1998). In the aerobic conditions, iron solubility is less, reflecting the prevalence of Fe^{3+} characteristically observed as oxyhydroxide polymers, thus limiting the iron supply for diverse forms of life, chiefly in the calcareous soils (Abdallah 1991; Andrews et al. 2003). The microbes have established active approaches and tactics for Fe uptake. The endophytic bacteria can overcome the micronutrient Fe limitation by employing chelator agents known as siderophores. Therefore, the siderophores are defined as the low molecular mass molecules (<1000 Da) with high affinity and specificity for binding or chelating ferric, followed by its transportation and deposition within bacterial cells (Liaqat and Eltem 2016).

The secretion of siderophores by endophytic microbes might arouse plant growth, thereby improving the nutrition (a direct effect) or preventing establishing the phytopathogens (an indirect effect) during the sequestration of iron from the ecosystem (Leong 1986). Unlike the microbial pathogens, the plants are not influenced by microbial mediated iron diminution, and even then, some plants can capture and exploit Fe^{3+} siderophore bacterial complexes (Dimkpa et al. 2009). The role of endophytic bacteria producing siderophore has been seldom studied, though the capability to produce siderophores bestows a competitive benefit to endophytic bacteria for colonization of inner plant tissues and the exclusion of other microbes from the same ecological position (Loaces et al. 2011). These previously mentioned authors observed that a group of endophytic siderophore-excreting bacteria associated with rice roots is richer than those from the soil at the tillering and grain filling stages. The endophytic bacterial strains belonging to genus *Burkholderia* exhibited

preferential localization inside the rice plants, and their role may be pertinent to avert the infection of young plants by microbes like *Sclerotium oryzae* and *Rhizoctonia oryzae*.

17.4.5 ACC Deaminase Activity

The ethylene is an endogenously manufactured gaseous phytohormone that acts at low amount and participates in regulation of all progressions of the plant growth development and senescence (Nonaka et al. 2008; Sun et al. 2009). It additionally acts as a plant growth regulator also; the ethylene has also been recognized as stress phytohormone. Under biotic and abiotic stresses (such as pathogen damage, flooding, water loss, salt, change in pH, and organic and inorganic impurities), endogenous or internal ethylene production is noticeably augmented and harmfully affects the root growth and consequently the overall growth of the plant (Grincko and Glick 2001).

A number of means and methods have been explored intending to lessen the ethylene levels in plants. One of the potential mechanisms comprises activity of bacterial enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase (Glick et al. 2007). The ACC deaminase controls the manufacture of plant ethylene by metabolizing ACC (the immediate precursor of ethylene biosynthesis in higher plants) and converts into α-ketobutyric acid and NH_3 (Onofre-Lemus et al. 2009). A noteworthy amount of plant ACC could be expelled from the roots of the plant and consequently taken up by soil microbes and hydrolyzed by enzyme ACC deaminase, therefore lessening the quantity of ACC in environment. When associated with plant roots, soil microbial populations with ACC deaminase activity may have an improved growth than other free microbes, as these microbiomes employ ACC as a source of nitrogen (Ali et al. 2014).

17.4.6 Phosphate Solubilization and Mobilization

The element phosphorus (P) is an indispensable and vital nutrient for plants. It participates as a structural constituent of nucleic acids, adenosine triphosphate (ATP), and phospholipids, as a main element of metabolic and biochemical pathways, which is imperative and predominantly significant for biological nitrogen fixation and photosynthesis (Goldstein 1986). The plants absorb phosphorus in two soluble or available forms: monobasic (H_2PO_4) and dibasic (HPO_4^{2-}) (Glass 1989), though a large quantity of phosphorus is present in insoluble or unavailable forms and is therefore not accessible for plant nutrition. Low quantities of phosphorus reflect the high reactivity of phosphate with other soluble components present in the soil (Valverde et al. 2006), such as Al in acidic soils ($pH < 5$) and Ca in alkaline soils ($pH > 7$) (Chen et al. 2006). Organic and inorganic compounds, principally in the form of insoluble mineral complexes, are main bases of accessible phosphorus in soil (Oteino et al. 2015). Consequently, the obtainability

of phosphorus relies on the solubility of this element in soil, which could be manipulated by activity of plant roots and microbes in soil. Phosphate-mobilizing bacterial and fungal strains constitute about 1–50% and 0.1–0.5%, correspondingly, of the total population of cultivable microbes in the soil (Illmer and Schinner 1992).

Among the diverse sources of phosphorus in soil, the solubilization and mobilization of inorganic phosphates (Pi) have been the chief focus of research studies. Phosphate-solubilizing and phosphate-mobilizing bacteria solubilize inorganic soil phosphates, such as calcium phosphate [$Ca_3(PO_4)_2$], iron phosphate [$FePO_4$], and aluminum phosphate [$AlPO_4$], through the production of organic acids, siderophores, and chelating and hydroxyl ions (Chen et al. 2006; Taurian et al. 2010). There are some bacteria which only solubilize calcium phosphate, while other microbes are capable of solubilizing and mobilizing other forms of inorganic phosphates (Pi) at dissimilar intensities. The bacterial isolates such as *Enterobacter*, *Pantoea*, *and Klebsiella* can solubilize $Ca_3(PO_4)_2$ (calcium phosphate) to a greater extent than $FePO_4$ (iron phosphate) and $AlPO_4$ (aluminum phosphate) (Chen et al. 2014). Production of organic acids, predominantly humic, gluconic, and carboxylic, is one of the well-studied means and methods developed by microbes to solubilize and mobilize inorganic phosphates (Pi) (Kim et al. 2003).

17.4.7 Biogeochemical Cycling

Probiotic endophytic microbes are likely to influence the decay and breakdown of plant litter and soil nutrient biotransformations generally by three ways: (a) by acting as saprotrophs in separated plant parts and facilitating in their decay and decomposition, (b) by influencing the amount and/or quality of the plant waste, and (c) by influencing the profusion, abundance, composition, and assembly of decomposer microorganisms (Fig. 17.1).

The nutrient cycling is a very significant process, that is, an ongoing process, and happens uninterruptedly to balance the prevailing nutrients and make it available for every constituent and module of ecosystem. Biodegradation of the dead and decaying matter becomes one chief step in it to recycle or bring back the utilized macro- and micronutrients to the environment. The recycled nutrients in turn again become available to the organisms. This process becomes a cyclic chain process which is ever going on. There are lots of saprophytic endophytic organisms which play a significant role in this process. Only few studies have demonstrated that endophytic microbes have imperative role in degradation of the refuse of its host plants (Muller et al. 2001; Kumaresan and Suryanarayanan 2002; Osono 2003, 2006; Korkama-Rajala et al. 2008; Fukasawa et al. 2009; Osono and Hirose 2009; Promputtha et al. 2010). During the biodegradation of the debris, the endophytic microbiomes colonize primarily within the host plants (Thormann et al. 2003) and enable the saprophytic microbiome to act on through antagonistic collaboration and consequently increase the refuse decomposition (Fryar et al. 2001; Terekhova and Semenova 2005). He et al. (2012) demonstrated that all the endophytes had the potential to

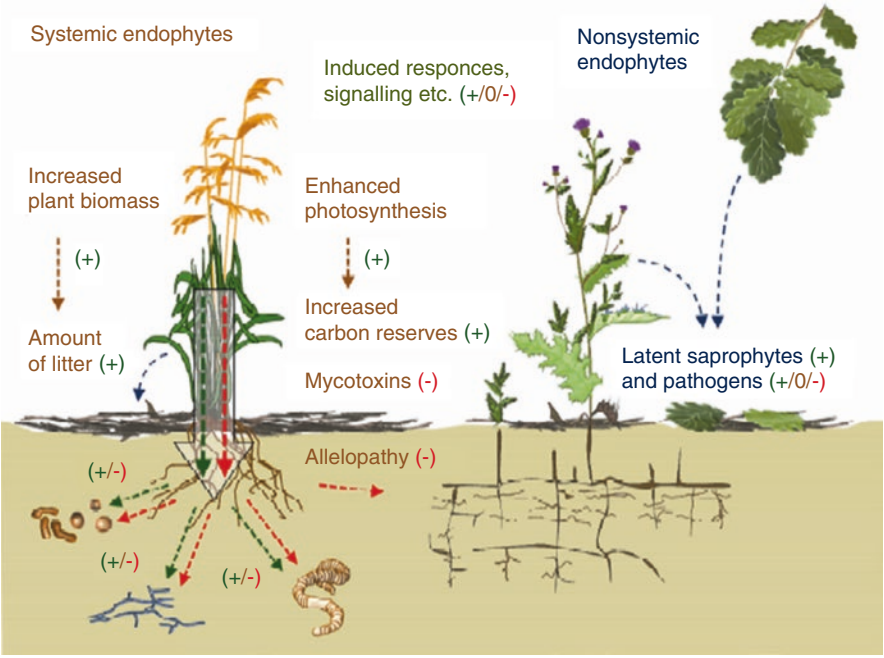

Fig 17.1 Role of endophytes in recycling of nutrients (Source: Saikkonen et al. 2015)

degrade the organic components, such as recalcitrant materials cellulose, lignin, and hemicellulose, though the favorites of numerous types of endophytic microbes with respect to organic complexes varied and diverged.

17.5 Conclusion and Future Aspects

Credit goes to the simpler molecular tools and techniques for isolation and characterization due to which we have seen tremendous development in the field of endophytic microbes and their application in various aspects of ecosystem. Recently researchers are showing great interest in isolation of novel endophytic microbes for studying bioactive compounds in agricultural, pharmaceutical, and environmental sectors, since there are many bioactive molecules produced by endophytes which are important and beneficial to mankind. Owing to their great demand and importance toward human well-being, researchers have already started exploiting endophytes for very much newer compounds and their newer roles.

Employment of various state-of-the-art micro-biotechnological and molecular techniques will help in the establishment of the perception and grasping of plant endophytic communications, producing original and new bioactive molecules; improve the growth of crop plants; and enhance biocontrol activity, reducing the debris and other wastes which are otherwise harmful to the ecosystem. Bearing in

mind all these, certainly the endophytes have proved to be an advantage and have casted virtuous impact on crop plants, ecosystem, and also the human beings in numerous promising ways.

All over the globe, effects of incessant agricultural practices such as chemical fertilization could lead to grave and stern damage to the ecosystem. Inoculation of plants or seeds with potential endophytic microbes is one of the utmost significant to maintainable agricultural practices, since endophytic microbes establish relations with crop plants and boost plant growth by many ways of plentiful precious and positive characteristics. The endophytic microorganisms are suitable for crop plant inoculation, reflecting the capability of these microbes for plant colonization internally and externally. Many studies have recognized the exact, accurate, and basic communication among endophytic microbes and the host plants of diverse species and genotypes.

The blending of different methods and technologies with these endophytic microorganisms, such as uncovering of plant growth promoting attributes documentation of probable endophytic strains, in addition to the seed inoculation assays under laboratory circumstances. After selecting potential endophytic microbes, they are generally tested for crop production experiments under field conditions. This method is part of the search for novel technologies for agricultural crop enhancement using new endophytes. Consequently, after this, research work which shows a probable endophytic bacterial inoculant will be acceptable for introduction into the environment. Based upon the research work, there are many endophytic genera such as *Pseudomonas*, *Bacillus*, *Rhizobium*, *Fusarium*, and *Alternaria* which could be the crucial contenders.

Lastly, the hunt for useful and advantageous endophytic microbiome is a significant aspect for the development of innovative, competent, and effectual inoculants for agriculture. Also other significant aspects could be investments in new means and methods that can contribute to upsurge the inoculum efficiency and survival rate of endophytic microbes adherent to the plants and seeds. Consequently, introduction of beneficial endophytes in the ecosystem tends to be less aggressive and more beneficial and causes less impact to the environment compared to chemical fertilization, which makes it a popular longtime agriculture practice and a way of lessening the production costs, which will ultimately benefit the farmer and environment.

References

Abdallah MA (1991) Pyoverdines and pseudobactins. Handbook of microbial iron chelates. CRC Press, Boca Raton, pp 139–153

Adachi K, Nakatani M, Mochida H (2002) Isolation of an endophytic diazotroph, Klebsiella oxytoca, from sweet potato stems in Japan. Soil Sci Plant Nutr 48:889–895

Ali B, Hasnain S (2007) Potential of bacterial indoleacetic acid to induce adventitious shoots in plant tissue culture. Lett Appl Microbiol 45(2):128–133

Ali S, Trevor CC, Glick BR (2014) Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. Plant Physiol Biochem 80:160–116

Alves BJR, Boddey RM, Urquiaga S (2003) The success of BNF in soybean in Brazil. Plant Soil 252:1–9

Andreote FD, Rossetto PB, Mendes R, Avila LA, Labate CA, Pizzirani-Kleiner AA, Azevedo JL, Asraful Islam SM, Math RK, Kim JM, Yun MG, Cho JJ, Kim EJ, Lee YH, Yun HD (2010) Effect of plant age on endophytic bacterial diversity of balloon flower (Platycodon grandiflorum) root and their antimicrobial activities. Curr Microbiol 61:346–356

Andrews SC, Robinson AK, Rodríguez-Quinõnes F (2003) Bacterial iron homeostasis. FEMS Microbiol Rev 27:215–237

Azevedo JL, Maccheroni J Jr, Pereira O, Ara WL (2000) Endophytic microorganisms: a review on insect control and recent advances on tropical plants. Electr J Biotechnol 3:40–65

Backman PA, Sikora RA (2008) Endophytes: an emerging tool for biological control. Biol Control 46:1–3

Bent E, Chanway CP (1998) The growth-promoting effects of a bacterial endophyte on lodgepole pine are partially inhibited by the presence of other rhizobacteria. Can J Microbiol 44:980–988

Bezerra JDP, Santos MGS, Svedese VM, Lima DMM, Fernandes MJS, Paiva LM, Souza-Motto CM (2012) Richness of endophytic fungi from Opuntia fiscus-indica Mill. (Cactaceae) and preliminary screening for enzyme production. World J Microbiol Biotechnol 28:1989–1995

Bianco C, Imperlini E, Calogero R, Senatore B, Pucci P, Defez R (2006) Indole-3-acetic acid regulates the central metabolic pathways in Escherichia coli. Microbiology 152:2421–2431

Bohrer TRJ, Hungria M (1998) Availacao de cultivares de soja quanto a fixacao biologico do nitrogeno. Pesq Agrop Bras 33:937–953

Bru D, Ramette A, Saby NPA, Dequiedt S, Ranjard L, Joliver C, Arrouays D, Philippot L (2011) Determinants of the distribution of nitrogen cycling microbial communities at the landscape scale. ISME J 5:532–542

Celloto VR, Oliveira AJB, José EG, Watanabe CSF, Graciette M, Regina ACG (2012) Biosynthesis of indole-3-acetic acid by new *Klebsiella oxytoca* free and immobilized cells on inorganic matrices. ScientificWorldJournal 7:23–28

Chanway CP (1997) Inoculation of tree roots with plant growth promoting soil bacteria: an emerging technology for reforestation. Forest Sci 43:99–112

Chareprasert S, Piapukiew J, Thienhirun S, Whalley AJS, Sihanonth P (2006) Endophytic fungi of teak leaves Tectona grandis L. and rain tree leaves Samanea saman Merr. World J Microbiol Biotechnol 22:481–486

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Chen YX, Zhang LP, Lu ZT (2008) Analysis of the internal transcribed spacer (ITS) sequences in rDNA of 10 strains of Fusarium spp. J Anhui Agric Sci 36:4886–4887

Chen Y, Fan JB, Du L, Xu H, Zhang QH, He YQ (2014) The application of phosphate solubilizing endophyte Pantoea dispersa triggers the microbial community in red acidic soil. Appl Soil Ecol 84:235–244

Compant S, Duffy B, Nowak J, Cl C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Costa JM, Loper JE (1994) Characterization of siderophore production by the biological control agent Enterobacter cloacae. Mol Plant Microbe Interact 7:440–448

Costa PB, Granada CE, Ambrosini A, Moreira F, Souza R, Passos JFM, Arruda L, Passaglia LMP (2014) A model to explain plant growth promotion traits: a multivariate analysis of 2,211 bacterial isolates. PLoS One 9:231–237

Devaraju R, Sathish S (2011) EndophyticMycoflora of Mirabilis jalapa L. and studies on antimicrobial activity of its endophytic Fusarium sp. Asian J Expt Biol Sci 2(1):75–79

Dimkpa CO, Merten D, Svatos A, Büchel G, Kothe E (2009) Siderophores mediate reduced and increased uptake of cadmium by Streptomyces tendae F4 and sunflower (Helianthus annuus), respectively. J Appl Microbiol 107(5):1687–1696

Duijff BJ, Gianinazzi-Pearsonand V, Lemanceau P (1997) Involvement of the outer membrane lipopolysaccharides in the endophytic colonization of tomato roots by biocontrol Pseudomonas fluorescens strain WCS417r. New Phytol 135:325–334
Fisher PJ, Petrini O, Scott HML (1992) The distribution of some fungal and bacterial endophytes in maize (Zea mays L.) New Phytol 122:299–305
Fryar SC, Yuen TK, Hyde KD, Hodgkiss IJ (2001) The influence of competition between tropical fungi on wood colonization in streams. Microbial Ecol 41(3):245–251
Fukasawa Y, Osono T, Takeda H (2009) Effects of attack of saprobic fungi on twig litter decomposition by endophytic fungi. Ecol Res 24(5):1067–1073
Gaiero JR, McCall CA, Thompson KA, Day NJ, Best AS, Dunfield KE (2013) Inside the root microbiome: bacterial root endophytes and plant growth promotion. Am J Bot 100(9):1738–1750
Galloway JN, Dentener FJ, Capone DG, Boyer EW, Howarth RW, Seitzinger SP, Asner GP, Cleveland CC, Green PA, Holland EA, Karl DM, Michaels AF, Porter JH, Townsend AR, Vörösmarty CJ (2004) Nitrogen cycles: past, present and future. Biogeochem 70:153–226
Ganley RJ, Newcombe G (2006) Fungal endophytes in seeds and needles of Pinus monticola. Mycol Res 110(3):318–327
Germaine K, Liu X, Cabellos G, Hogan J, Ryan D, Dowling DN (2006) Bacterial endophyte-enhanced phyto-remediation of the organochlorine herbicide 2,4-dichlorophenoxyacetic acid. FEMS Microbiol Ecol 57:302–310
Glass ADM (1989) Plant nutrition: an introduction to current concepts. Jones and Bartlett Publishers, Boston, p 234
Glick BR, Cheng Z, Czarny J, Duan J (2007) Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur J Plant Pathol 119:329–339
Goldstein AH (1986) Bacterial solubilization of mineral phosphates: historical perspective and future prospects. Am J Altern Agric 1:51–57
Govindarajan M, Kwon SW, Weon HY (2007) Isolation, molecular characterization and growth promoting activities of endophytic sugarcane diazotroph *Klebsiella* sp. GR9. World J Microbiol Biotechnol 23:997–1006
Grincko VP, Glick BR (2001) Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiol Biochem 39:11–17
Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914
Hallmann J, Quadt-Hallmann A, Rodriguez-Kabana R, Kloepper JW (1998) Interactions between Meloidogyne incognita and endophytic bacteria in cotton and cucumber. Soil Biol Biochem 30:925–937
Hamayun M, Afzal Khan S, Ahmad N (2009) Cladosporium sphaerospermum as a new plant growth promoting endophyte from the roots of Glycine max (L.) Merr. World J Microbiol Biotechnol 25(4):627–632
Hardoim PR, van Overbeek LS, van Elsas JD (2008) Properties of bacterial endophytes and their proposed role in plant growth. Trends Microbiol 16:463–471
Hata K, Atari R, Sone K (2002) Isolation of endophytic fungi from leaves of Pasania edulis and their within leaf distribution. Mycoscience 43:369–373
Hata K, Sone K (2008) Isolation of endophytes from leaves of Neolitsea sericea in broadleaf and conifer strands. Mycoscience 49:229–232
He X, Han G, Lin Y, Tian X, Xiang C, Tian Q, Wang F, He Z (2012) Diversity and decomposition potential of endophytes in leaves of a Cinnamomum camphora plantation in China. Ecol Res 27(2):273–284
Holliday P (1989) A dictionary of plant pathology. Cambridge University Press, Cambridge
Illmer P, Schinner F (1992) Solubilization of inorganic phosphates by microorganisms isolated from forest soil. Soil Biol Biochem 24:389–395
Jha PN, Kumar A (2007) Endophytic colonization of *Typha australis* by a plant growth-promoting bacterium Klebsiella oxytoca strain. J Appl Microbiol 103:1311–1320
Jones DL (2006) A complete guide to native orchids of Australia including the Island Territories. Reed New Holland Publishers, Sydney

Joseph B, Mini Priya R (2011) Bioactive compounds from endophytes and their potential in pharmaceutical effect: a review. Am J Biochem Mol Biol 1(3):291–309
Kang JW, Khan Z, Doty SL (2012) Biodegradation of trichloroethylene by an endophyte of hybrid poplar. Appl Environ Microbiol 78(9):3504–3507
Kim CH, Han SH, Kim KY, Cho BH, Kim YH, Koo BS (2003) Cloning and expression of pyrroloquinoline quinone (PQQ) genes from a phosphate-solubilizing bacterium *Enterobacter intermedium*. Curr Microbiol 47:457–461
Kloepper JW, Ryu CM (2006) Bacterial endophytes as elicitors of induced systemic resistance. In: Schulz BJE, Boyle CJC, Sieber TN (eds) Microbial root endophytes. Springer, Berlin, pp 33–52
Korkama-Rajala T, Muller MM, Pennanen T (2008) Decomposition and fungi of needle litter from slow and fast growing Norway spruce (Picea abies) clones. Microbial Ecol 56(1):76–89
Kuklinsky-Sobral J, Araújo WL, Mendes R, Geraldi IO (2004) Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. Environ Microbiol 6:1244–1251
Kumar S, Kaushik N, Edrada-Ebel R, Ebel R, Proksch P (2011) Isolation, characterization, and bioactivity of endophytic fungi of Tylophora indica. World J Microbiol Biotechnol 27(3):571–577
Kumaresan V, Suryanarayanan TS (2002) Endophyte assemblages in young, mature and senescent leaves of Rhizophora apiculata: evidence for the role of endophytes inmangrove litter degradation. Fungal Divers 9:81–91
Larran S, Simón MR, Moreno MV, Santamarina Siuranae MP, Perelló A (2016) Endophytes from wheat as biocontrol agents against tan spot disease. Biol Control 92:17–23
Leong J (1986) Siderophores: their biochemistry and possible role in control of plant pathogens. Annu Rev Phytopathol 24(1):187–209
Liaqat F, Eltem R (2016) Identification and characterization of endophytic bacteria isolated from in vitro cultures of peach and pear rootstocks. 3 Biotech 6(2):120–125
Loaces I, Ferrando L, Scavino AF (2011) Dynamics, diversity and function of endophytic siderophore-producing bacteria in rice. Microb Ecol 61:606–618
Lopez BR, Bashan Y, Bacilio M (2011) Endophytic bacteria of Mammillaria fraileana, an endemic rock-colonizing cactus of the southern Sonoran Desert. Arch Microbiol 193:527–541
Madmony A, Chernin L, Pleban S, Peleg E, Riov J (2005) Enterobacter cloacae, an obligatory endophyte of pollen grains of mediterranean pines. Folia Microbiol 50:209–216
Melnick RL, Carmen S, Bryan AB, Paul AB (2011) Isolation of endophytic endospore-forming bacteria from *Theobroma cacao* as potential biological control agents of cacao diseases. Biol Control 57:236–245
Muller MM, Valjakka R, Suokko A, Hantula J (2001) Diversity of endophytic fungi of single Norway spruce needles and their role as pioneer decomposers. Mol Ecol 10(7):1801–1810
Nonaka S, Sugawara M, Minamisawa K, Yuhashi KI, Ezura H (2008) 1-Aminocyclopropane-1-carboxylate deaminase producing *Agrobacterium* confers higher ability for gene transfer into plant cells. Appl Environ Microbiol 74:2526–2528
Olivares J, Bedmar EJ, Sanjuán J (2013) Biological nitrogen fixation in the context of global change. Mol Plant Microbe Interact 26(5):486–494
Onofre-Lemus J, Hernández-Lucas I, Girard L, Caballero-Mellado J (2009) ACC (1-aminocyclo propane-1-carboxylate) deaminase activity, a widespread trait in Burkholderia species, and its growth-promoting effect on tomato plants. Appl Environ Microbiol 75:6581–6590
Osono T (2003) Effects of prior decomposition of beech leaf litter by phyllosphere fungi on substrate utilization by fungal decomposers. Mycoscience 44(1):41–45
Osono T (2006) Role of phyllosphere fungi of forest trees in the development of decomposer fungal communities and decomposition processes of leaf litter. Can J Microbiol 52(8):701–716
Osono T, Hirose D (2009) Effects of prior decomposition of Camellia japonica leaf litter by an endophytic fungus on the subsequent decomposition by fungal colonizers. Mycoscience 50(1):52–55

Oteino N, Lally RD, Kiwanuka S, Lloyd A, Ryan D, Germaine KJ, Dowling DN (2015) Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. Front Microbiol 6:745–750

Porteous-Moore F, Barac T, Borremans B, Oeyen L, Vangronsveld J, van der Lelie D, Campbell D, Moore ERB (2006) Endophytic bacterial diversity in poplar trees growing on a BTEX-contaminated site: the characterisation of isolates with potential to enhance phytoremediation. Syst Appl Microbiol 29:539–556

Posada F, Vega FE (2006) Inoculation and colonization of coffee seedlings (Coffea arabica L.) with the fungal entomopathogen Beauveria bassiana (Ascomycota: Hypocreales). Mycoscience 47(5):284–289

Prittila AM, Lankkanen H, Pospiech H, Myllylä R, Hohtola A (2000) Detection of intracellular bacteria in the buds of Scotch pine (Pinus sylvestris L.) by in situ hybridization. Appl Environ Microbiol 66:3073–3077

Prittila AM, Pospiech H, Lankkanen H (2003) Two endophytic fungi in different tissues of Scot pine buds (Pinus sylvestris L.). Microbial Ecol 45:53–62

Prittila AM, Hohtola A, Ivanova EG (2008) Identification and localization of methylotrophic plant associated bacteria. In: Sorvari S, Prittila AM (eds) Prospects and application for plant associated microbes. A laboratory manual, Part A: bacteria. Biobien Innovationa, Turku, pp 218–224

Promputtha I, Hyde KD, McKenzie EHC, Peberdy JF, Lumyong S (2010) Can leaf degrading enzymes provide evidence that endophytic fungi becoming saprobes? Fungal Divers 41:89–99

Rai R, Dash PK, Prasanna BM, Singh A (2007) Endophytic bacterial flora in the stem tissue of a tropical maize (Zea mays L.) genotype: isolation, identification and enumeration. World J Microb Biotechnol 23:853–858

Reinhold-Hurek B, Hurek T (1998) Life in grasses: diazotrophic endophytes. Trends Microbiol 6(4):139–144

Rout ME, Chrzanowski TH, Westlie TK, DeLuca TH, Callaway RM, Holben WE (2013) Bacterial endophytes enhance competition by invasive plants. Am J Bot 100(9):1726–1737

Rungjindamai N, Pinruan U, Choeyklin R, Hattori T, Jones EBG (2008) Molecular characterization of basidiomycetous endophytes isolated from leaves, rachis and petioles of the oil palm, Elaeis guineensis, in Thailand. Fungal Divers 33:139–161

Ruppel S, Hecht-Buchholz C, Remus R, Ortmann U, Schmelzer R (1992) Settlement of the diazotrophic, phytoeffective bacterial strain *P. agglomerans* on and within winter wheat: an investigation using ELISA and transmission microscopy. Plant Soil 145:261–273

Ryan RP, Germaine K, Franks A, Ryan DJ (2008) Bacterial endophytes: recent developments and applications. FEMS Microbiol Lett 278:1–9

Saikkonen K, Mikola J, Helander M (2015) Endophytic phyllosphere fungi and nutrient cycling in terrestrial ecosystems. Curr Sci 109(1):121–126

Schouten A (2016) Mechanisms involved in nematode control by endophytic fungi. Annu Rev Phytopathol 54:121–142

Selim KA, El-Beih AA, AbdEl-Rahman TM, El-Diwany AI (2011) Biodiversity and antimicrobial activity of endophytes associated with Egyptian medicinal plants. Mycosphere 2(6):669–678

Sette LD, Passarini MRZ, Delarmelina C, Salati F, Duarte MCT (2006) Molecular characterization and antimicrobial activity of endophytic fungi from coffee plants. World J Microbiol Biotechnol 22(11):1185–1195

Spaepen S, Vanderleyden J, Remans R (2007) Indole- 3-acetic acid in microbial and microorganism-plant signaling. FEMS Microbiol Rev 31:425–448

Stijn S, Jos V, Roseline R (2007) Indole-3-acetic acid in microbial and microorganism plant Signaling. FEMS Microbiol Rev 31:1–24

Strobel G (2003) Endophytes as sources of bioactive products. Microbes Infect 5:535–544

Strobel GA, Daisy B (2003) Bioprospecting for microbial endophytes and their natural products. Microbiol Mol Biol Rev 67:491–502

Sturz AV (1995) The role of endophytic bacteria during seed piece decay and potato tuberization. Plant Soil 175:257–263

Sturz AV, Matheson BG (1996) Populations of endophytic bacteria which influence host resistance to *Erwinia*-induced bacterial soft rot in potato tubers. Plant Soil 184:265–271
Sturz AV, Christie BR, Matheson BG, Nowak J (1997) Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. Biol Fertil Soils 25:13–19
Sturz AV, Christie BR, Nowak J (2000) Bacterial endophytes: potential role in developing sustainable systems of crop production. Crit Rev Plant Sci 19:1–30
Sun Y, Cheng Z, Glick BR (2009) The presence of a 1-aminocyclopropane-1-carboxylate (ACC) deaminase deletion mutation alters the physiology of the endophytic plant growth-promoting bacterium Burkholderia phytofirmans PsJN. FEMS Microbiol Lett 296:131–136
Taurian T, Anzuay MS, Angelini JG, Tonelli ML, Luduena L, Pena D, Ibanez F, Fabra A (2010) Phosphate solubilizing peanut associated bacteria: screening for plant growth promoting activities. Plant Soil 329:421–431
Tefera T, Vidal S (2009) Effect of inoculation method and plant growth medium on endophytic colonization of sorghum by the entomopathogenic fungus Beauveria bassiana. BioControl 54(5):663–669
Terekhova VA, Semenova TA (2005) The structure of micromycete communities and their synecologic interactions with basidiomycetes during plant debris decomposition. Microbiol 74(1):91–96
Thaweenut N, Hachisuka Y, Ando S, Yanagisawa S, Yoneyama T (2011) Two seasons' study on nifH gene expression and nitrogen fixation by diazotrophic endophytes in sugarcane (*Saccharum* spp. hybrids): expression of nifH genes similar to those of rhizobia. Plant Soil 338:435–449
Thongsandee W, Matsuda Y (2012) Temporal variations in endophytic fungal assemblages of Ginkgo biloba L. J Forest Res 17(2):213–218
Thomas P, Soly TA (2009) Endophytic bacteria associated with growing shoot tips of banana (*Musa* sp.) cv. Grand Naine and the affinity of endophytes to the host. Microb Ecol 58:952–964
Thormann M, Currah RS, Bayley SE (2003) Succession of microfungal assemblages in decomposing peatland plants. Plant Soil 250(2):323–333
Thuler DS, Floh EIS, Handro W, Barbosa HR (2003) Plant growth regulators and amino acids released by *Azospirillum* sp. in chemically defined media. Lett Appl Microbiol 37:174–178
Torres AR, Araújo WL, Cursino L, Hungria M, Plotegher F, Mostasso FL, Azevedo JL (2008) Diversity of endophytic enterobacteria associated with different host plants. J Microbiol 46(4):373–379
Uma Maheswari T, Anbukkarasi K, Hemalatha T, Chendrayan K (2013) Studies on phytohormone producing ability of indigenous endophytic bacteria isolated from tropical legume crops. Int J Curr Microbiol Appl Sci 2(6):127–136
Valverde A, Burgos A, Fiscella T, Rivas R, Velázquez E, Rodríguez-Barrueco C, Cervantes E, Chamber M, Igual J-M (2006) Differential effects of coinoculations with *Pseudomonas jessenii* PS06 (a phosphate-solubilizing bacterium) and Mesorhizobium ciceri C-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. Plant Soil 287:43–50
van Loon LC, Bakker PA, Pieterse CM (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36:453–483
Varsha YM, Deepthi N, Chenna S (2011) An emphasis on xenobiotic degradation in environmental cleanup. J Bioremediat Biodegrd S11:001
Verma VC, Gond SK, Kumar A, Kharwar RN, Boulanger LA, Strobel GA (2011) Endophytic fungal flora from roots and fruits of an Indian neem plant Azadirachta indica A. Juss., and impact of culture media on their isolation. Indian J Microbiol 51(4):469–476
Wang S, Hu T, Jiao Y, Wei J, Cao K (2009) Isolation and characterization of Bacillus subtilis EB-28, an endophytic bacterium strain displaying biocontrol activity against Botrytis cinerea Pers. Front Agric China 3(3):247–252

Xin G, Zhang G, Kang JW, Staley JT, Doty SL (2009) A diazotrophic, indole-3-acetic acid-producing endophyte from wild cottonwood. Biol Fertil Soil 45(6):669–674

Yi HS, Yang ZW, Ryu CM (2013) ISR meets SAR outside: additive action of the endophyte Bacillus pumilus INR7 and the chemical inducer, benzothiadiazole, on induced resistance against bacterial spot in field-grown pepper. Front Plant Sci 4:122–126

Youngbae S, Kim S, Park CW (1997) A phylogenetic study of polygonumsect. tovara (polygonaceae) based on ITS sequences of nuclear ribosomal DNA. Plant Biol 40:47–52

Zinniel DK, Lambrecht P, Harris BN (2002) Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. Appl Environ Microbiol 68:2198–2208

18 Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth-Promoting Rhizobacteria (PGPR) Association in Potato (*Solanum tuberosum* L.): A Brief Review

Deepmala Pathak, Rafiq Lone, and K.K. Koul

Abstract

Chemical fertilizers extensively used in soil fertilization directly affect environment and indirectly human health. The way out is the use of biofertilizers, of which arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) are successfully being seen to be an effective replacement for improving both the establishment and subsequently growth of field crops. AMF and PGPRs used singly and also in combinations have shown significant improvements of various crops in the field. These and aspects related are already reviewed. Here an effort has been made to bring those of previous reviews, monographs, and other relevant literature, specifically in relation to the potato crop, to a single consolidation in brief, however, without losing the crux.

18.1 Introduction

Potato (*Solanum tuberosum* L.) from family Solanaceae is a crop grown worldwide under diverse range of altitudes, thus under diverse climatic condition. This is somewhat unique to potato when compared to other food crops. The crop distribution ranges from that of sea level to more than 4000 m elevation. The commercial part of the plant is an underground stem modification tuber, which is predominantly used as a vegetable. In certain areas of the world, it constitutes a staple food crop and competes with several other crops in those areas (Guenthner 2002; Rosenthal 2007). Besides being a rich source of vitamin C, niacin, and vitamin B_6, it is an important

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and major source of starch. Starch from potato is of superior quality with some interesting properties and, hence, is extensively used in the commercial food industry (Christensen and Madsen 1996; Jansen et al. 2001). Food characteristics of potato have been ascribed to its balanced nutrition. The tuber is in high demand for its high potassium content and is therefore used in osmotically deficient human and animal populations. This further enhances the qualitative use of potato universally (Mengel and Kirkby 2001; Fageria 2009), the production being more than 364 million tons annually.

Agriculture practices recommended use of chemicals to control pests and other pathogens along with fertilizers. These affect the soil environment and subsequently soil fertility. A search for finding an alternative/s to these chemicals is presently in a high gear mode. Microorganisms have been proven to be of high potential in directly controlling pests and diseases of plants. Their ability in increasing pest and disease resistance in hosts is based upon biopriming, a mechanism by which the plants enhance host resistance (Bloemberg and Lugtenberg 2001; Conrath et al. 2002; Nowak and Shulaev 2003). One such phenomenon is symbiosis—wherein beneficial associations occur between certain microorganisms with the roots of host plant, for example, the plant growth-promoting rhizobacteria (PGPR) and the arbuscular mycorrhizal fungi (AMF) (Bashan 1998).

The term rhizobacteria is used for rhizosphere bacteria capable of colonizing the root and promoting plant growth; these beneficial bacteria are referred to as plant growth-promoting rhizobacteria (PGPR) (Kloepper et al. 1991; Kloepper 1994) because they mobilize the immobile nutrients, accelerate nodulation and fixation of nitrogen (Zhang et al. 1996), produce microbial iron transport agents or siderophores (Kloepper et al. 1980b), synthesize phytohormones (Khalid et al. 2004; Spaepen et al. 2007), and promote antibiotic production against plant pathogens (Sandra et al. 2001; Morales et al. 2008) or suppressing pathogens or combinations of them (Somers et al. 2008). Phosphate solubilizing bacteria (PSB) transform insoluble phosphates into available soluble forms for host plant uptake. Conversion processes include acidification, chelation, exchange reactions, and gluconic acid production (Chung et al. 2005; Gulati et al. 2010). The classification of PGPRs is done according to their association with host plant and intra- and extracellular plant growth-promoting rhizobacteria (Martinez-Viveros et al. 2010). These may enhance plant growth either through direct or indirect process. In direct process, PGPR releases some plant growth-promoting substances, siderophores, or enzymes in rhizosphere. In indirect process, PGPR strains secrete antimicrobial substances like enzymes, antibiotics, and HCN which prevent the growth of pathogenic microorganisms (Deshwal et al. 2013). The PGPR other group is now added and defined mycorrhization helper bacteria (MHB). These bacteria are associated with mycorrhizal fungi and mycorrhizal roots, which collectively promote spread of establishment of mycorrhizal symbioses (Garbaye 1994). PGPRs are defined by three fundamental characteristics: (1) ability to colonize the root, (2) survive and multiply in habitats accompanying with the root surface and in competition with other microbiota, and (3) enhance plant growth. PGPRs are regarded as efficient microbial competitors in the soil-root zone. Generally these include spp. of *Pseudomonas*,

Serratia, *Burkholderia*, *Erwinia*, *Agrobacterium*, *Azospirillum*, *Xanthomonas*, *Bacillus*, *Rhizobium*, *Enterobacter*, *Arthrobacter*, *Alcaligenes*, *Acinetobacter*, *Acetobacter*, *Achromobacter*, *Azotobacter*, *Aerobacter*, *Klebsiella*, *Clostridium*, *Micrococcus*, *Rhodospirillum*, *Rhodobacter*, and *Flavobacterium* (Rodriguez and Fraga 1999; Bloemberg and Lugtenberg 2001 and Esitken et al. 2003). PGPRs are used as biofertilizer for different crop plants as a substitute source to chemical fertilizers so to improve plant root growth and nutrition uptake (Egamberdiyeva and Hoflich 2004).

The relationship between plants and microbes is now established as an essential for plant health and growth and is recommended to be considered when planning high production with farming practices which are environment-friendly (Kloepper et al. 1992).

18.2 PGPRs and Potato

Plant growth-promoting rhizobacteria (PGPR) colonize potato plant roots and enhance plant growth (Vessey 2003). *Bacillus* and *Pseudomonas* are the most commonly reported among the PGPRs (Compant et al. 2005; Vessey 2003), whereas *Pseudomonas* and *Azotobacter* are the most phosphate solubilizing genera. However, *Bacillus* strains are also phosphate solubilizing bacteria (Chatli et al. 2008; Nautiyal 1999; Vessey 2003). *Pseudomonas* strains by Deshwal et al. (2013) and *Bacillus* and *Pseudomonas* by Sati et al. (2013) were isolated from potato rhizosphere. Hanif et al. (2015) observed that by inoculation of *B. subtilis* strain, KPS-11 in potato plant showed increased shoot length and root length and shoot weight of potato as compared to control plants having no inoculation. Naqqash et al. (2016) reported that *Azospirillum* sp. TN10 has the highest potential to increase nitrogen uptake and growth of potato. It is therefore recommended as a good candidate for the production of biofertilizer in potato for integrated nutrient management. Vrany and Fiker (1984) reported that when potato seed tubers were inoculated with PGPR before planting, 4–30% increase in tuber yield and plant growth occurs. Nonfluorescent *Pseudomonas* sp. show in vitro effects on growth, enhancement and developmental modifications (Frommel et al. 1991), and yield of tuber (Sturz 1995) in potato. Nookaraju et al. (2011) reported in vitro and ex vitro tuberization influenced potato growth by lipoxygenase (LOX) associated with plant growth-promoting rhizobacteria (PGPR) isolated from potato fields, and Malboobi et al. (2009) observed that the combinations of either *Microbacterium laevaniformans* or *Pseudomonas agglomerans* with *Pseudomonas putida* give higher biomass and potato tuber in greenhouse and under field condition.

Al-Ani et al. (2013) reported that *Pseudomonas fluorescens*, *Rhodotorula* sp., and fermented neem extract can protect potato plants against potato virus Y disease, whereas Rahman et al. (2012) have demonstrated that the identified antagonistic bacterial strain E-65 (*Bacillus* sp.) can significantly inhibit the growth of potato soft rot bacteria in vitro and in storage. Potato tubers with antagonistic bacteria successfully prevented the initial infection and reduced soft rot disease and the

multiplication of rot bacteria. PGPR mutants resistant to rifampicin (rif) and nalidixic acid (nal) retained plant growth-promoting activity in the greenhouse assay. These are reported to induce mutant increase in plant weights by fivefold and develop larger root system with increased branching due to PGPR (Kloepper et al. 1980a).

In potato rhizosphere, *Bacillus* is found to be a more dominant species. The bacteria isolated also include the genera *Bacillus*, *Proteobacteria*, *Variovorax*, *Chryseobacterium*, *Agrobacterium*, *Staphylococcus*, and *Plantibacter*. These genera represent both Gram-negative (*Agrobacterium*, *Chryseobacterium*, *Variovorax*, and *Proteobacteria*) and Gram-positive (*Staphylococcus*, *Plantibacter*, *and Bacillus*) bacteria. Some of the strains of the PGPR of the above genera are reported to be useful to the potato crop plants (Banik and Dey 1982; Datta et al. 1982; Cezon et al. 2010). Employing direct PCR-DGGE based on DNA extracted from plants and fatty acid methyl ester (FAME) analysis of bacteria and/or sequencing of their partial 16S ribosomal RNA genes, different pseudomonas species were found to most frequently isolated strains (>5% of the total in potato). These were characterized as different *Pseudomonas* spp., i.e., *P. corrugata*, *P. aureofaciens*, and *P. putida*, and others such as *Agrobacterium radiobacter*, *Stenotrophomonas maltophilia*, *Flavobacterium resinovorans*, *Bacillus* sp., and *Sphingomonas paucimobilis* (Garbeva et al. 2001).

The term mycorrhiza is derived from the Greek words mykos, fungus, and rhizo, the root, and approximately 80–90% of all terrestrial plant families are associated with AMF (Trappe 1987; Bonfante 2001). AMF and soil microbial community present underground show symbiosis between members of fungal phylum *Glomeromycota* (Wang et al. 2008; Schüβler et al. 2001) and their widespread symbiotic association with plant roots of most *Bryophyta*, Pteridophyta, Angiospermophyta, and Coniferophyta. It has now been ascertained that all, but for a few vascular plant species mainly belonging to the families Chenopodiaceae, Cruciferae, Cyperaceae, Juncaceae, and Caryophyllaceae, are not able to form mycorrhiza (Harley and Smith 1983; Smith and Gianinazzi-Pearson 1988; Azcon-Aguilar and Bago 1994). Mycorrhiza can be grouped as endomycorrhiza, in which root cortex is intracellularly colonized by the fungus. They are further classified as "ericoid" type, which are restricted to some species of Ericaceae; orchid type which are restricted in family Orchidaceae; and the third group, arbuscular mycorrhizas, which are more prominent and widespread. The fungi belong to the class Zygomycotina and order Glomerales. Nearly 150 species predominantly in six genera are capable to form AMF. These genera are *Acaulospora*, *Entrophospora*, *Gigaspora*, *Glomus*, *Sclerocystis*, and *Scutellospora* (Walker 1992; Rosendahl et al. 1994 and Morton et al. 1995).

The fungi in AMF symbiosis obtain the hydrocarbon skeleton from the host plant in exchange of nutrients. These symbiosis properties are determined by (1) the ability of a plant to obtain nutrients through a fungus (mycotrophy), (2) the dependence of the fungus on the host plant to complete its life cycle, as it is a physiologically obligate symbiont (fungal dependency), and (3) the dependence of host plant on mycorrhiza for its proper development (mycorrhizal dependency of a plant). These

are having external hyphae which absorb and translocate nutrients for symbiotic plant growth by their penetration to more soil volume for such nutrients (Joner and Jakobsen 1995). Bharadwaj et al. (2008) reported AMF produce mass production of propagules in the form of extraradical hyphae. Plant species and abiotic and biotic factors play an important role in production and viability of AMF spores, and among the biotic factors included were several kinds of bacteria associated with AMF spores. Agronomically AMF is the most widespread type of plant symbiont which interacts with saprophytic soil microflora (Barea and AzcoÂn-Aguilar 1982; Meyer and Linderman 1986). The mechanism of their interaction seems to be still not completely understood. However, the AMF are now established as soil incorporate biofertilizers.

18.3 AMF and Potato

Potato plant roots are more capable in absorbing and translocating more nutrients by exploring abundant soil volume, thus enhancing the supply of slowly diffusing ions such as phosphate due to external hyphae of mycorrhiza (McArthur and Knowles 1993; Hodge et al. 2010). AMF significantly inhibited disease, due to the pathogen *Fusarium sambucinum* on potato plants (Ismail and Hijri 2012).

Lone et al. (2015) observed that the AMF had positive effect under pot culture on various growths of parameters, i.e., plant height, no. of leaves, no. of tubers, fresh weight, and dry weight were increased than control without AMF. The growth and yield of potato tubers were most affected by phosphorus (P) nutrition since P deficiency developed increased stress during the period of tuberization and bulking (MacKay et al. 1988). Ngakou et al. (2006) suggested that AMF and soil solarization enhanced growth of potato, to a degree saving N and P fertilizers, earlier considered as a remediation of agricultural soils from biopollutants. Douds David et al. (2007) revealed that with inocula of AMF and also with vermiculite mixtures, the yield of potato tubers can be increased to 20%. AMF inoculation of low P availability on prenuclear minitubers of Peruvian potato increased yield by an average of 85% (Davies Fred et al. 2005a). McArthur and Knowles (1993) observed that the growth responses of potato were different with different AMF; however, all enhanced nutrient uptake particularly P (Black and Tinker 1977). AMF are also reported to increase productivity of potatoes by increasing disease resistance (Graham et al. 1976; Niemira et al. 1995, 1996). Micropropagated plant material with AMF strains *Glomus* and *Gigaspora* inoculum improved growth both in minituber and potato seedling and tuber production (Cheng et al. 2008). Micropropagated virus-free tuber has been shown to optimize and improve the quality and tuber yield (Donnelly et al. 2003). Duffy and Cassells (2000) too report that the yield and quality of potato microplants can be influenced by mycorrhizal colonization. Mycorrhizal isolate and plant genotype determine the influence of AMF on the plant growth and yield. In order to reduce or eliminate chemical inputs, being common in present-day cultivation, the response of potato toward AMF can act as an important tool in agriculture systems. The importance of mycorrhiza as biofertilizers increases the nutrient

uptake, tuber yield, and phosphorus use efficiency (PUE) of "Yungay" variety (Davies Fred et al. 2005b; Douds David et al. 2007).

Potato microplant growth and yield of saleable potato minitubers occur significantly due to inoculation of AMF. In protected cropping, mycorrhizal inoculation can increase or decrease yield quality of microplants. This depends on the mycorrhizal isolate and host genotype (Duffy et al. 1999). Sarikhani and Aliasgharzad (2012) have shown that non-mycorrhizal treatments in comparison to mycorrhizal treatments, especially *G. etunicatum*, had higher content of potassium in shoot. Interestingly they further reported that AMF treatments had higher dry matter of tuber, percent of starch, and specific gravity even when mycorrhizal association with potato plants often forms very weak root colonization under field conditions. Bharadwaj et al. (2007) suggested that to improve efficacy of AMF inocula for potatoes, crop rotation is needed. At the field level, *G. mosseae* under monocultures is the most abundant species. Gallou et al. (2011) investigated impact of in vitro AMF on *Phytophthora infestans* where leaf infection index decreased in mycorrhiza-associated potato plants.

18.4 PGPRs + AMF and Potato

The plant health, growth, and nutritional status are positively affected by the microbial soil biodata management. Arbuscular mycorrhizal fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) are now being identified as major agricultural inputs to make less dependent on fertilizer and chemical pesticides, thus improving the sustainability of potato-growing soils. These eco-friendly biofertilizers and bio-protectors prove as cost-effective inputs for small-scale farmers and resource-poor agricultural systems (Franco et al. 2011). Baradar et al. (2015) revealed that interaction of chelating factor of iron (EDTA and EDDHA), mycorrhizal colonization, and PGPR strains had positive effects on root colonization and in consequence leads to increase fresh and dry weight and other growth factors in potato plant. In rhizosphere, the microbial communities play an important role and influence plant growth. Lynch (1990) reported that mobilization of nutrients occurs due to microbes and releases plant growth hormones which play an important role in growth and development of plant. Different combinations of two or more different AMF and PGPR species have been tried for cultivation (Yan et al. 2002; Mia et al. 2005; Domenech et al. 2006; Rodriquez-Romero et al. 2005; Vestberg et al. 2004). Further reported are those of combinations of AMF, PGPRs, *Trichoderma harzianum* (Srinath et al. 2003), and other bacteria (Bashan 1998). Vosãtka and Gryndler (1999) observed that total weight of tubers and enhancement of mycorrhization and extraradical mycelium activity of plants can be increased by mixing *Pseudomonas putida* along with AMF inoculation in potato plants. Palacios et al. (2009) have thus suggested that the inoculation of native diazotrophic bacteria and AMF in micropropagated in vitro potato plantlets can increase growth.

The biotic essential component of the soil microbiota consists of AMF and their bacterial associates. Bacteria associated with AMF referred to as AMB isolates also

stimulate mycorrhizal formation. *Paenibacillus* sp. isolated from surface-sterilized *Glomus mosseae* spores stimulates mycorrhizal formation in *Sorghum bicolor* (Budi et al. 1999), while *Bacillus pabuli* isolated from *G. clarum* spores enhances *G. clarum* colonization in pea roots (Xavier and Germida 2003). The AMB which play an important role in mycorrhizal symbiosis development are termed as mycorrhizal helping bacteria (MHB) (Garbaye 1994). It has been reported that AMB also function as PGPRs because they help the plant in nutrient uptake. (Artursson et al. 2006). The plant ISR/SAR response with the involvement of plant activators and elicitors of SAR helps the plant to control both soil-borne and foliar diseases of potato plant in presence of AMF and PGPRs. AMF or PGPRs combined with foliar spraying with an elicitor can be used to control late blight of potato caused by *Phytophthora infestans* (O'Herlihy et al. 2003).

The recent literature and details for PGPRs, AMF, and PGPRs+AMF in potato are consolidated and summarized in Tables 18.1, 18.2, and 18.3, respectively. Figures 18.1 and 18.2 represent the three different varieties, viz, KS = Kufri Sindhuri, KC-3 = Kufri Chipsona-3, KL = Kufri Luvakar, of potato plant affected by using AMF and PGPR individually and in combination for determining their effect on growth parameters of potato plant. The different AMF and PGPR species used individually or in combination in the above experiment are given in Table 18.4.

Table 18.1 PGPRs and potato cultivation

S. no.	PGPRs	Potato cultivar	Experimental site/condition	References
1.	*Azospirillum* sp. TN10, *Agrobacterium* sp. TN14, *Pseudomonas* sp. TN36, *Enterobacter* sp. TN38, and *Rhizobium* sp.	Potato	Potato rhizospheric soil samples were collected from two different areas of Punjab, Pakistan	Naqqash et al. (2016)
2.	*Bacillus subtilis* strain KPS-11	Potato	Potato rhizospheric soil samples were collected from Jhang, Pakistan	Hanif et al. (2015)
3.	PGPR isolates HB1-HB40 and HB42	Hartapel	Leksula and South Buru, Maluku, Indonesia	Kesaulya et al. (2015)
4.	*Enterobacter cloacae* strain *AB2*	Potato	Lokhandi potato field, Bilaspur C.G, India	Verma and Shahi (2015)
5.	*Pseudomonas aeruginosa*, *P. cepacia*, *P. fluorescens*, *P. putida*	Potato	Dehradun, India	Deshwal et al. (2013)
6.	*Bacillus* sp. and *Pseudomonas* sp.	Potato	Mana village, Uttarakhand, India	Sati et al. (2013)

(continued)

Table 18.1 (continued)

S. no.	PGPRs	Potato cultivar	Experimental site/condition	References
7.	*Erwinia carotovora* subsp. *carotovora* (Ecc), *Bacillus* sp. (E-65), and *Lactobacillus* sp. (E-45)	Potato	Bangladesh	Rahman et al. (2012)
8.	*Bacillus amyloliquefaciens* and *B. subtilis*	Waycha	Native Bolivian	Franco et al. (2011)
9.	Isolates of PGPRs belong to genera *Bacillus*, *Variovorax*, *Proteobacteria*, *Staphylococcus*, *Agrobacterium*, *Chryseobacterium*, and *Plantibacter*	Potato	In vitro and ex vitro tuberization	Nookaraju et al. (2011)
10.	*Bacillus pumilus*	Pamina, Claustar, Odessa, Sahel, Russet, Yukon gold, Norland	Mali, Canada	Bathily et al. (2010)
11.	*Pantoea agglomerans* strain P5, *Microbacterium laevaniformans* strain P7, and *Pseudomonas putida* strain P13	Potato	Greenhouse and field experiments	Malboobi et al. (2009)
12.	*Bradyrhizobium elkanii* BR 113, *Sinorhizobium fredii* BR 112, *Mesorhizobium plurifarium* BR 3804, and *Burkholderia* sp. BR 11340	Achat, Bintje, Agata, Monalisa, and Asterix	Seropédica, RJ, Brazil, Greenhouse experiments	Ferreira et al. (2008)
13.	*Bacillus* sp.	Spunta	in vitro and in vivo condition	Daami-Remadi et al. (2006)
14.	*Agrobacterium tumefaciens*, *Arthrobacter globiformis*, *A. ilicis*, *A. oxydans*, *Bacillus amyloliquefaciens*, *B. aquamarinus*, *B. cereus*, *B. licheniformis*, *B. mycoides*, *B. pumilus*, *B. subtilis*, *B. thuringiensis*, *Curtobacterium flaccumfaciens*, *Enterobacter taylorae*, *Erwinia amylovora*, *E. persicinus*, *E. rhapontici*, *Lysobacter antibioticus*, *Micrococcus kristinae*, *Paenibacillus peoriae*, *P. polymyxa*, *Pseudomonas chlororaphis*, *P. corrugata*, *P. fluorescens*, *P. jessenii*, *P. marginalis*, *P. migulae*, *P. orientalis*, *P. putida*, *P. reactans*, *P. savastanoi* pv. *fraxinus*, *P. straminea*, *P. syringae*, *Rhodococcus erythropolis*, *Serratia plymuthica*, *Shingobacterium spiritovorum*, *Staphylococcus epidermidis*, *S. pasteuri*, *Streptomyces halstedii*, *Variovorax paradoxus*	Cilena	Institute for Plant Diseases, Bonn University, in Bonn-Poppelsdorf, Germany	Berg et al. (2005)

Table 18.1 (continued)

S. no.	PGPRs	Potato cultivar	Experimental site/condition	References
15.	*Pseudomonas aureofaciens*, *P. corrugata*, *P. putida*, *Agrobacterium radiobacter*, *Stenotrophomonas maltophilia*, *Flavobacterium resinovorans*, *Bacillus* sp., *and Sphingomonas paucimobilis*	Desirée	–	Garbeva et al. (2001)
16.	*Pseudomonas gladioli* NCPPB 1891 (nonfluorescent species), *P. viridiflava* NCPPB 635 and *Pseudomonas cichorii* NCPPB 943 (fluorescent species), *Erwinia herbicola*	Kennebec	Plant Propagation Center of the New Brunswick Department of Agriculture, Fredericton	Frommel et al. (1991)
17.	*Pseudomonas fluorescens* and *P. putida*	Radka, Resy, and Nicola	Celery and parsley laboratory and field cond.	Vrany and Fiker (1984)
18.	*Erwinia carotovora*	White Rose and Katahdin	Shafter, Tulelake	Kloepper (1983)
19.	Unidentified PGPR strain E-10 *Rhizobacterium* and E2, E6, and E8 *Pseudomonas fluorescens*	Potato seeds	Shafter	Kloepper and Schroth (1981)
20.	*Pseudomonas* spp. A1, B10, and E6 strain	White rose, Netted gem, and Centennial	Shafter, Tulelake, and Monte Vista	Kloepper et al. (1980a)

Table 18.2 AMF vis-a-vis potato cultivars

S. no.	AMF	Potato cultivar	Experimental site/condition	References
1.	*Glomus intraradices* and *G. mosseae*	Jyoti, TPS	Greenhouse pot experiment	Lone et al. (2015)
2.	*Glomus intraradices* and *G. etunicatum*	Marfona and Draga	Greenhouse pot experiment	Sarikhani and Aliasgharzad (2012)
3.	*Glomus* sp. MUCL 41833	Bintje	In vitro	Gallou et al. (2011)
4.	*Glomus intraradices* MUCL 41833	Bintje	In vitro	Gallou et al. (2010)
5.	Basidiomycetes, ascomycete fungi, no evidence found of AMF during observation	Modena, parental cultivar (Karnico), and four additional nonmodified cultivars (Aveka, Aventra, Désirée, and Premiere)	Northeastern part of the Netherlands	Hannula et al. (2010)
6.	*Glomus intraradices*	Potato	Castelnuovo Scrivia, Italy	Cesaro et al. (2008)

(continued)

Table 18.2 (continued)

S. no.	AMF	Potato cultivar	Experimental site/ condition	References
7.	*Glomus mosseae and G. versiforme*	Micro-tuber (Hansa) Minituber (Luyin No.1)	In vitro	Cheng et al. (2008)
8.	*Acaulospora* sp., *G. geosporum*, *Glomus caledonium*, *G. mosseae*, unknown *Glomus* spp., *G. sinuosum*, *G. intraradices*, *G. microaggregatum*	King Edward	Greenhouse experiment	Bharadwaj et al. (2007)
9.	*Glomus intraradices*, G. *etunicatuni*, G. *mosseae*, *Glomus claroideum*, *G. geosporiini*, and *Gigaspora gigantea*	Superior	The Rodale Institute in Kutztown, PA	Douds David et al. (2007)
10.	*Gigaspora* spp.	Cipira	Dang Ngaoundéré, Guinea	Ngakou et al. (2006)
11.	*Glomus* spp., *Gigaspora* spp., and *Scutellospora* spp.	Andean	Central highlands of Peru	Davies Fred et al. (2005a)
12.	*Glomus intraradices*	Yungay	Under shade house conditions/ Universidad Nacional Agraria La Molina (UNALM) in Lima, Peru	Davies Fred et al. (2005b)
13.	*Glomus. etunicatum* and *G. intraradices*	Goldrush and LP89221	In vitro	Yao et al. (2002)
14.	*Glomus intraradices*	Golden Wonder	In vitro/in vivo	Duffy and Cassells (2000)
15.	*Glomus etunicatum*	Karin and Krista	–	Vosãtka and Gryndler (2000)
16.	*Glomus intraradices*	potato	Greenhouse experiment	Niemira et al. (1996)
17.	*Glomus intraradices*	potato	Field experiment	Niemira et al. (1995)
18.	*Glomus fasciculatum*	Russet Burbank	Pot experiment	McArthur and Knowles (1993)
19.	VAM fungi	SSC 1174 (highly resistant), Kufri jyoti (resistant), Up-to-date (highly susceptible)	North Eastern Hill University, Shillong	Bhattarai and Mishra (1984)
20.	*Glomus fasciculatum*	King Edward	Pot experiment	Ocampo and Hayman (1980)

Table 18.3 PGPRs and AMF in combination as used in potato

S. no.	AMF	PGPRs	Potato cultivar	Experimental site/condition	References
1.	*Glomus intraradices* and *G. mosseae*	*P. fluorescens* T17-4, *P. fluorescens* VUPf5, *P. fluorescens* F140	Potato	Greenhouse pot experiment	Baradar et al. (2015)
2.	*Glomus mosseae* and *G. fasciculatum*	Two strains of *Pseudomonas* (P116 and P173) and *Bacillus* (*Bacillus subtilis* and *B. megaterium*)	Agria and Sante	Pot experiment	Hassani et al. (2014)
3.	*Glomus intraradices*	*Azotobacter chrooccum*, DSM-281 (N2-fixing bacteria), *Bacillus polymyxa,* PTCC1020 and *Pseudomonas putida*, CHAO (phosphate solubilizing bacteria)	Agria, Arinda, and Marfona	Greenhouse condition	Otroshy et al. 2013
4.	*Glomus fasciculatum*	*Bacillus subtilis*	Waycha and Desiree	Native Bolivian	Franco et al. (2011)
5.	*Glomus claroideum* and *G. fasciculatum*	Diazotropic bacteria	Alfa	In vitro conditions	Palacios et al. (2009)
6.	*Glomus mosseae and G. intraradices*	*Pseudomonas putida* biotypes A and B, *P. fluorescens* biotype F, *Bacillus subtilis*, *Arthrobacter ilicis*, *Stenotrophomonas maltophilia* (non-PGPR)	King Edward and Matilda	In vitro conditions (pot experiment)	Bharadwaj et al. (2008)

(continued)

Table 18.3 (continued)

S. no.	AMF	PGPRs	Potato cultivar	Experimental site/condition	References
7.	Members of *Ascomycota* and *Gigaspora* cluster	*Enterobacter amnigenus*, *Clostridium pasteurianum* DSM 525, *Erwinia carotovora* DSM 30168, *Agrobacterium tumefaciens* DSM 30205, *Pseudomonas fluorescens* R2f, *Pantoea agglomerans, Nocardia asteroides* N3, *Rhizobium leguminosarum* DSM 30132, *Actinomadura viridis* DSM 43462, *Kineosporia aurantiaca* JCM 3230, *Nocardiopsis astra* ATCC 31511, and *Actinoplanes philippiensis* JCM 3001	SIBU S1 (transgenic potato line) and SIBU (non-transgenic parental cultivar line), Solana (non-transgenic cultivar)	Southern part of Germany (Oberviehhausen)	Milling et al. (2005)
8.	*Glomus mosseae*	*Pseudomonas fluorescens* strains CHA0 and IP10	Potato stock plants	In vitro	Duffy et al. (1999)
9.	*Glomus fistulosum*	*Pseudomonas putida*	Micropropagated potato	Greenhouse experiments	Vosãtka and Gryndler (1999)
10.	*Glomus etunicatum*, *G. fistulosum*	*Bacillus subtilis*	Micropropagated potato	Greenhouse or shadow house	Vosãtka and Gryndler (2000)

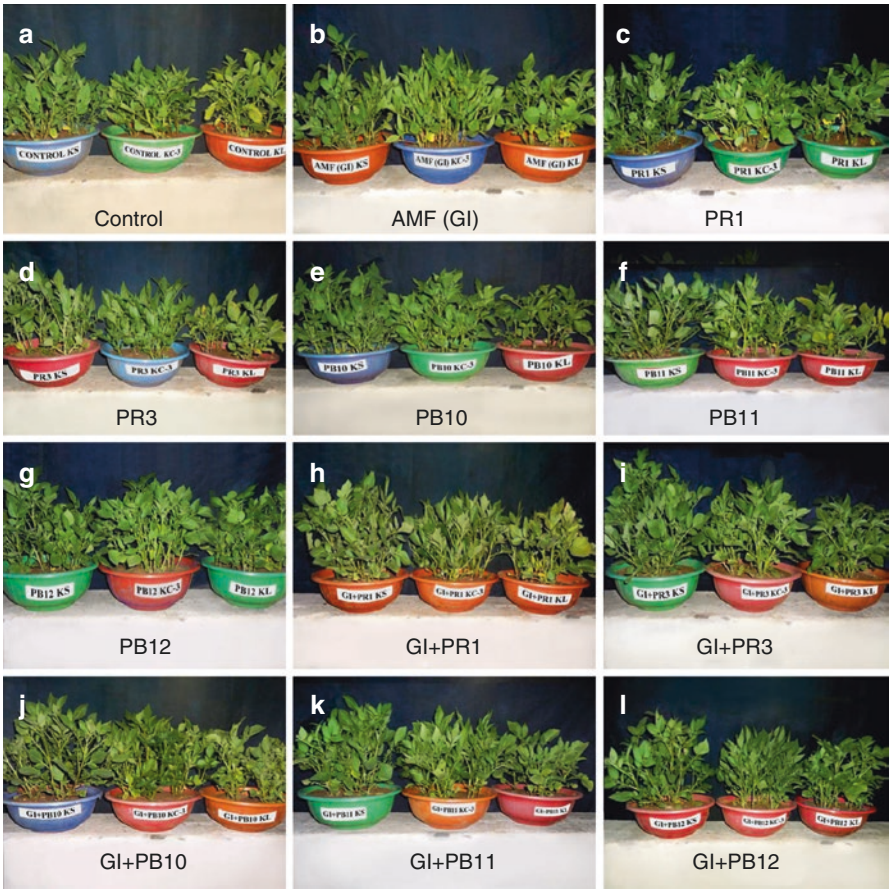

Fig. 18.1 Effect of AMF and PGPR, individually and in combination, on potato plant var. KS, KC-3, and KL at 90-day growth after seedling emergence under pot culture conditions

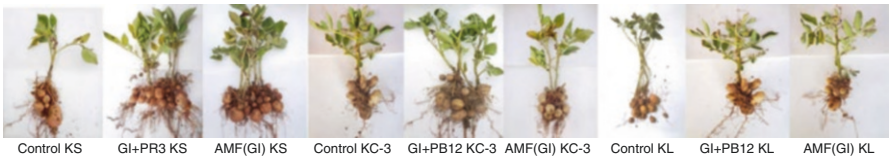

Fig. 18.2 Effect of AMF and PGPR, individually and in combination, on root length and tuber number of potato plant var. KS, KC-3, and KL at 90-day growth after emergence under pot culture conditions. *KS* Kufri Sindhuri, *KC-3* Kufri Chipsona3, and *KL* Kufri Luvakar

Table 18.4 AMF and PGPR species used in pot culture individually and in combination for determining their effect on potato plant

S. no.	Treatments	Microbial inoculants
1	Uninoculated control	Without PGPR and AMF inoculums
2	AMF	*Glomus intraradices* (GI)
3	PR1	*Bacillus amyloliquefaciens*
4	PR3	*Bacillus subtilis*
5	PB10	*Lysinibacillus boronitolerans*
6	PB11	*Pseudomonas brassicacearum*
7	PB12	*Bacillus subtilis*
8	AMF (GI)+ PR1	GI+*B. amyloliquefaciens*
9	AMF (GI)+ PR3	GI+*B. subtilis*
10	AMF (GI)+ PB10	GI+*L. boronitolerans*
11	AMF (GI)+ PB11	GI+ *P. brassicacearum*
12	AMF (GI)+ PB12	GI+*B. subtilis*

Conclusion

Soil microbes are important components of all agricultural systems. Interaction between plants and microbes and their sustained management can benefit the plant and as food consumer chain. The use of AMF and PGPRs and AMF-associated bacteria or consortia of AMF and PGPRs can enhance the growth of potato plantlets in vitro or minituber plantlets because both have direct/indirect multiple activities to promote plant growth. Further studies can also lead the replacement of chemical use in agriculture. We conclude that there is a need for more attention toward the dual inoculation effect of potato crop. This as a biofertilizer can generate an eco-friendly environmental condition and sustainable eco-agro-system beneficial for both plants and human health.

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References

Al-Ani AR, Adhab AM, Matny NO (2013) Management of potato virus Y (PVY) in potato by some biocontrol agent under field condition. Int J Microbiol Mycol 1(1):1–6

Artursson V, Finlay RD, Jansson JK (2006) Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ Microbiol 8(1):1–10

Azcon-Aguilar C, Bago B (1994) Physiological characteristics of the host plant promoting an undisturbed functioning of the mycorrhizal symbiosis. In: Gianinazzi S, Schiiepp H (eds) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. ALS. Birkhäuser, Basel, pp 47–60

Banik S, Dey BK (1982) Available phosphate content of an alluvial soil is influenced by inoculation of some isolated phosphate-solubilizing microorganisms. Plant Soil 69(3):353–364

Baradar A, Saberi-Riseh R, Sedaghati E, Akhgar A (2015) Effect of some bacteria and iron chelators on potato colonization by arbuscular mycorrhiza fungi inoculated by *Rhizoctonia*. Ind J Sci Technol 8(19):1(IPL095)

Barea JM, AzcoÂn-Aguilar C (1982) Interactions between mycorrhizal fungi and soil microorganisms. In: Gianinazzi S, Gianinazzi-Pearson V, Trouvelot A (eds) Les Mycorhizes: Biologie et Utilisation INRA. Service des Publications, Paris, pp 181–193

Bashan Y (1998) Inoculants of plant growth-promoting bacteria for use in agriculture. Biotechnol Adv 16(4):729–770

Bathily H, Babana AH, Samake F (2010) *Bacillus pumilus*, a new pathogen on potato tuber in storage in Mali. Afr J Microbiol Res 4(20):2067–2071

Berg G, Krechel A, Ditz M, Sikora RA, Ulrich A, Hallmann J (2005) Endophytic and ectophytic potato-associated bacterial communities differ in structure and antagonistic function against plant pathogenic fungi. FEMS Microbiol Ecol 51(2):215–229

Bharadwaj DP, Lundquist PO, Alström S (2007) Impact of plant species grown as monocultures on sporulation and root colonization by native arbuscular mycorrhizal fungi in potato. Appl Soil Ecol 35(1):213–225

Bharadwaj DP, Lundquist PO, Persson P, Alström S (2008) Evidence for specificity of cultivable bacteria associated with arbuscular mycorrhizal fungal spores. FEMS Microbiol Ecol 65(2):310–322

Bhattarai ID, Mishra RR (1984) Study on the vesicular–arbuscular mycorrhiza of three cultivars of potato (*Solanum tuberosum L.*) Plant Soil 79(2):299–303

Black RLB, Tinker PB (1977) Interaction between the effects of vesicular–arbuscular mycorrhizae and fertilizer phosphorus on yields of potatoes in the field. Nature 267:510–511

Bloemberg GV, Lugtenberg BJJ (2001) Molecular basis of plant growth promotion and bio-control by rhizobacteria. Curr Opin Plant Biotechnol 4(4):343–350

Bonfante P (2001) At the interface between mycorrhizal fungi and plants: the structural organization of cell wall, plasma membrane and cytoskeleton. In: Hock B (ed) Fungal associations (Vol 9: The mycota). Springer, Berlin, pp 45–91

Budi SW, Van Tuinen D, Martinotti G, Gianinazzi S (1999) Isolation from *Sorghum bicolor* mycorrhizosphere of a bacterium compatible with arbuscular mycorrhiza development and antagonistic towards soil-borne fungal pathogens. Appl Environ Microbiol 65(11):5148–5150

Cesaro P, Tuinen D, van Copetta A, Chatagnier O, Berta G, Gianinazzi S, Lingua G (2008) Preferential colonization of *solanum tuberosum* l. roots by the fungus *glomus intraradices* in arable soil of a potato farming area. Appl Environ Microbiol 74(18):5776–5783

Cezon R, Gutiérrez Mañero FJ, Probanza A, Ramos B, Lucas Garcia JA (2010) Effects of two plant growth promoting rhizobacteria on the germination and growth of pepper seedlings (*Capsicum annum*) cv. Roxy. Arch Agron Soil Sci 49(6):593–603

Chatli AS, Beri V, Sidhu BS (2008) Isolation and characterization of phosphate solubilising microorganisms from the cold desert habitat of *Salix alba* Linn. in trans Himalayan region of Himachal Pradesh. Ind J Microbiol 48(2):267–273

Cheng Y, Bai D, Sun L, Feldmann F, Feng G (2008) Utilization of arbuscular mycorrhizal fungi during production of micropropagated potato *Solanum tuberosum*. In: Feldmann F, Kapulnik Y, Baar J (eds): Mycorrhiza works, © Deutsche Phytomedizinische Gesellschaft, Braunschweig, Germany. pp 165–178. ISBN 978-3-941261-01-3

Christensen DH, Madsen MH (1996) Change in potato starch quality during growth. Potato Res 39(1):43–50

Chung H, Park M, Madhaiyan M, Seshadri S, Song J, Cho H, Sa T (2005) Isolation and characterization of phosphate solubilizing bacteria from the rhizosphere of crop plants of Korea. Soil Biol Biochem 37(10):1970–1974

Compant S, Duffy B, Nowak J, Clement C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71(9):4951–4959

Conrath U, Pieterse CMJ, Mauch-Mani B (2002) Priming in plant-pathogen interactions. Trends Plant Sci 7(5):210–216

Daami-Remadi M, Ayed F, Jabnoun-Khiareddine H, Hibar K, El Mahjoub M (2006) Effects of some *Bacillus* sp. isolates on *Fusarium* sp. *in vitro* and potato tuber dry rot development *in vivo*. Plant Pathol J 5(3):283–290

Datta M, Banik S, Gupta RK (1982) Studies on the efficacy of a phytohormone producing phosphate solubilizing *Bacillus firmus* in augmenting paddy yield in acid soils of Nagaland. Plant Soil 69(3):365–373

Davies Fred T Jr, Calderón CM, Huaman Z, Gomez R (2005a) Influence of a flavonoid (formononetin) on mycorrhizal activity and potato crop productivity in the highlands of Peru. Sci Hortic 106(3):318–329

Davies Fred T Jr, Calderón CM, Huaman Z (2005b) Influence of arbuscular mycorrhizae indigenous to peru and a flavonoid on growth, yield, and leaf elemental concentration of 'Yungay' potatoes. Hort Sci 40(2):381–385

Deshwal VK, Singh SB, Kumar P, Chubey A (2013) Isolation and characterization of *Pseudomonas* strains from potatoes Rhizosphere at Dehradun Valley, India. Int J Basic Appl Sci 2(2):53–55

Domenech J, Reddy MS, Kloepper JW, Ramos B, Gutierrez-Manero J (2006) Combined application of the biological product LS213 with *Bacillus, Pseudomonas* or *Chryseobacterium* for growth promotion and biological control of soil-borne diseases in pepper and tomato. Biocontrol 51:245–258

Donnelly DJ, Coleman WK, Coleman SE (2003) Potato microtuber production and performance: a review. Am J Potato Res 80(2):103–115

Douds David D Jr, Nagahashi G, Reider C, Hepperly PR (2007) Inoculation with arbuscular mycorrhizal fungi increases the yield of potatoes in a high p soil. Biol Agric Hortic 25(1):67–78

Duffy EM, Cassells AC (2000) The effect of inoculation of potato (*Solanum tuberosum* L.) microplants with arbuscular mycorrhizal fungi on tuber yield and tuber size distribution. Appl Soil Ecol 15(2):137–144

Duffy EM, Hurley EM, Cassells AC (1999) Weaning performance of micropropagated potato following bacterisation and mycorrhization. Potato Res 42:521–527

Egamberdiyeva D, Hoflich G (2004) Effect of plant growth-promoting bacteria on growth and nutrient uptake of cotton and pea on a semi-arid region of Uzbekistan. J Arid Environ 56(2):293–301

Esitken A, Karlıdag H, Ercisli S, Turan M, Sahin F (2003) The effects of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot (*Prunus armeniaca* L.) Aust J Agric Res 54(4):377–380

Fageria NK (2009) The use of nutrients in crop plants. CRC Press, Taylor and Francis Group, New York

Ferreira EPB, Dusi AN, Xavier GR, Rumjanek NG (2008) Rhizosphere bacterial communities of potato cultivars evaluated through PCR-DGGE profiles. Pesq Agropec Arm Brasilia 43(5):605–612

Franco J, Main G, Navia O, Ortuno N, Herbas J (2011) Imrroving productivity of traditional Andean small farmers by bio-rational soil management: 1. The potato case. PROINPA, Cochabamba

Frommel MI, Nowak J, Lazarovits G (1991) Growth enhancement and developmental modifications of in vitro grown potato (*Solanum tuberosum ssp. tuberosum*) as affected by a non fluorescent *Pseudomonas sp*. Plant Physiol 96(3):928–936

Gallou A, De Jaeger N, Cranenbrouck S, Declerck S (2010) Fast track *in vitro* mycorrhization of potato plantlets allow studies on gene expression dynamics. Mycorrhiza 20(3):201–207

Gallou A, Mosquera HPL, Cranenbrouck S, Suárez JP, Declerck S (2011) Mycorrhiza induced resistance in potato plantlets challenged by *Phytophthora infestans*. Physiol Mol Plant Pathol 76(1):20–26

Garbaye J (1994) Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol 128(2):197–210

Garbeva P, van Overbeek LS, van Vuurde JW, van Elsas JD (2001) Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. Microbiol Ecol 41(4):369–383

Graham SO, Green NE, Hendrix JW (1976) The influence of vesicular–arbuscular mycorrhizae on growth and tuberization of potatoes. Mycologia 68(4):925–929

Guenthner JF (2002) Consumer acceptance of genetically modified potatoes. Am J Potato Res 79(5):309–316

Gulati A, Sharma N, Vyas P, Sood S, Rahi P, Pathania V, Prasad R (2010) Organic acid production and plant growth promotion as a function of phosphate solubilization by *Acinetobacter* rhizosphaerae strain BIHB 723 isolated from the cold deserts of the trans-Himalayas. Arch Microbiol 192(11):975–983

Hanif MK, Hameed S, Imran A, Naqqash T, Shahid M, Van Elsas JD (2015) Isolation and characterization of a β-propeller gene containing phosphor bacterium Bacillus subtilis strain KPS-11 for growth promotion of potato (*Solanum tuberosum* L.) Front Microbiol 6:583

Hannula SE, de Boer W, Veen van JA (2010) In situ dynamics of soil fungal communities under different genotypes of potato, including a genetically modified cultivar. Soil Biol Biochem 42(12):2211–2223

Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic, New York, p 483

Hassani F, Ardakani M, Asgharzade A, Paknezhad F, Hamidi A (2014) Efficiency of mycorrhizal fungi and phosphate solubilizing bacteria on phosphorus uptake and chlorophyll index in potato plants. Int J Biosci 4(1):244–251

Hodge A, Helgason T, Fitter AH (2010) Nutritional ecology of arbuscular mycorrhizal fungi. Fungal Ecol 3(4):267–273

Ismail Y, Hijri M (2012) Arbuscular mycorrhisation with *Glomus irregulare* induces expression of potato PR homologues genes in response to infection by *Fusarium sambucinum*. Funct Plant Biol 39(3):236–245

Jansen G, Flamme W, Schuler K, Vandrey M (2001) Tuber and starch quality of wild and cultivated potato species and cultivars. Potato Res. 44(2):137–146

Joner EJ, Jakobsen I (1995) Growth and extra cellular phosphatase activity of arbuscular mycorrhizal hyphae as influenced by soil organic matter. Soil Biol Biochem 27(9):1153–1159

Kesaulya H, Zakaria B, Syaiful SA (2015) Isolation and physiological characterization of pgpr from potato plant rhizosphere in medium land of BURU island. Proc Food Sci 3:190–199

Khalid A, Arshad M, Zahir ZA (2004) Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol 96(3):473–480

Kloepper JW (1983) Effect of seed piece inoculation with plant growth-promoting rhizobacteria on populations of E*rwinia carotovora* on potato roots and in daughter tubers. Phytopathology 73:217–219

Kloepper JW (1994) Plant growth promoting Rhizobacteria (other system). In: Okon Y (ed) *Azospirillum*/plant association. CRC Press, Boca Raton, pp 111–118

Kloepper JW, Schroth MN (1981) Plant growth promoting rhizobacteria and plant growth under gnotobiotic conditions. Phytopathology 71:642–644

Kloepper JW, Leong J, Teintze M, Schroth MN (1980a) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885–886

Kloepper JW, Schroth MN, Miller TD (1980b) Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70:1078–1082

Kloepper JW, Zablotowics RM, Tipping EM, Lifshitz R (1991) Plant growth promotion mediated by bacterial rhizosphere colonizers. in the rhizosphere and plant growth. In: Keister DL, Cregan PB (eds) Kluwer Academic Publishers, USA, pp 315–326

Kloepper JW, Wie G, Sham T (1992) Rhizosphere populations dynamics and internal colonization of cucumber by plant growth promoting rhizobacteria which induce systemic resistance to *Colletotrichum orbiculare*. In: Tjamos EC, Papavizas GC, Cook J (eds) Biological control of plant diseases. Progress and challenges for the future. Plenum Press, New York, pp 185–191

Lone R, Shuab R, Sharma V, Kumar V, Mir R, Koul KK (2015) Effect of Arbuscular Mycorrhizal Fungi on growth and development of potato (*Solanum tuberosum*) plant. Asian J Crop Sci 7(3):233–243

Lynch JM (1990) The rhizosphere. Wiley, Chichester, p 458

MacKay DC, Carefoot JM, Entz T (1988) Detection and correction of midseason p deficiency in irrigated potatoes. Can J Plant Sci 68(2):523–534

Malboobi MA, Behbahani M, Madani H, Owlia P, Deljou A, Yakhchali B, Moradi M, Hassanabadi H (2009) Performance evaluation of potent phosphate solubilizing bacteria in potato rhizosphere. World J Microbiol Biotechnol 25(8):1479–1484

Martinez-Viveros O, Jorquera MA, Crowley DE, Gajardo G, Mora ML (2010) Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J Soil Sci Plant Nutr 10(3):293–319

McArthur DAJ, Knowles NR (1993) Influence of vesicular-arbuscular mycorrhizal fungi on the response of potato to phosphorus deficiency. Plant Physiol 101(1):147–160

Mengel K, Kirkby EA (2001) Principles of plant nutrition, 5th edn. Kluwer Academic Publishers, Dordrecht, p 849

Meyer JR, Linderman RG (1986) Selective influence on populations of rhizosphere or rhizoplane bacteria and *actinomycetes* by mycorrhizas formed by *Glomus fasciculatum*. Soil Biol Biochem 18(2):191–196

Mia MAB, Shamsuddin ZH, Wahab Z, Marziah M (2005) High-yielding and quality banana production through plant growth-promoting rhizobacterial inoculation. Fruits 60(3):179–185

Milling A, Smalla K, Maidl FX, Schloter M, Munch JC (2005) Effects of transgenic potatoes with an altered starch composition on the diversity of soil and rhizosphere bacteria and fungi. Plant Soil 266(1):23–39

Morales H, Sanchis V, Usall J, Ramos AJ, Marin S (2008) Effect of biocontrol agents Candida sake and *Pantoea agglomerans* on *Penicillium expansum* growth and patulin accumulation in apples. Int J Food Microbiol 122(1-2):61–67

Morton JB, Franke M, Bentivenga SP (1995) Developmental foundations for morphological diversity among endomycorrhizal fungi in *Glomales*. In: Varma A, Hock B (eds) Mycorrhiza. Springer, Heidelberg, pp 669–683

Naqqash T, Hameed S, Imran A, Hanif MK, Majeed A, van Elsas JD (2016) Differential response of potato toward inoculation with taxonomically diverse plant growth promoting rhizobacteria. Front Plant Sci 7:144

Nautiyal CS (1999) An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbiol Lett 170(1):265–270

Ngakou A, Megueni C, Nwaga D, Mabong MR, Djamba FE, Gandebe M (2006) *Solanum tuberosum* (L.) responses to soil solarization and arbuscular mycorrhizal fungi inoculation under field conditions: growth, yield, health status of plants and tubers. Middle-East J Sci Res 1(1):23–30

Niemira BA, Safir GR, Hammerschmidt R, Bird GW (1995) Production of prenuclear mini-tubers of potato with peat based arbuscular mycorrhizal fungal inoculum. Agron J 87(5):942–946

Niemira BA, Hammerschmidt R, Safir GR (1996) Postharvest suppression of potato dry rot (*Fusarium sambucinum*) in prenuclear mini-tubers by arbuscular mycorrhizal fungal inoculum. Am Potato J 73(11):509–515

Nookaraju A, Kappachery S, Yu JW, Park SW (2011) Rhizobacteria influence potato tuberization through enhancing lipoxygenase activity. Am J Potato Res 88(6):441–449

Nowak J, Shulaev V (2003) Priming for transplant stress in *in vitro* propagation. In Vitro Cell Dev Biol Plant 39(2):107–124

O'Herlihy EA, Duffy EM, Cassells AC (2003) The effects of arbuscular mycorrhizal fungi band chitosan sprays on yield and late blight resistance in potato crops from microplants. Folio Geobot 38(2):201–207

Ocampo JA, Hayman DS (1980) Effects of pesticides on mycorrhiza in field-grown barley, maize and potatoes. Trans Br Mycol Soc 74(2):413–416

Otroshy M, Naeem AH, Soleymani A, Bazrafshan AH, Khodaee SMM, Struik PC (2013) Efficiency of plant growth promoting rhizobacteria and fungi on enhancement of growth and minituber production of tissue cultured potato cultivars (*Solanum tuberosum*). World Sci J 5(2):38–52

Palacios G, Abud M, Salvador M, Adriano L, Dendooven L, Gutierrez FA (2009) Influence of arbuscular mycorrhizal fungies and a native diazotrophic bacteria in survival and tuberization of *ex-vitro* potato plants. Gayana Bot 66(2):127–133

Rahman MM, Ali ME, Khan AA, Akanda AM, Uddin MK, Hashim U, Abd Hamid SB (2012) Isolation, characterization, and identification of biological control agent for potato soft rot in Bangladesh. Sci World J 2012:6

Rodriguez H, Fraga R (1999) Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 17(4-5):319–339

Rodriquez-Romero AS, Guerra MSP, Jaizme-Vega MDC (2005) Effect of arbuscular mycorrhizal fungi and rhizobacteria on banana growth and nutrition. Agron Sustain Dev 25(3):395–399

Rosendahl S, Dodd JC, Walker C (1994) Taxonomy and phylogeny of the *Glomales.* In: Gianinazzi S, Schbepp H (eds) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. ALS. Birkhtser. Basel, pp l–12

Rosenthal E (2007) A genetically modified potato, not for eating, is stirring some opposition in Europe. New York Times

Sandra AI, Wright I, Zumoff CH, Schneider L, Beer SV (2001) *Pantoea agglomerans* strain EH318 produces two antibiotics that inhibit *Erwinia amylovora in vitro*. Appl Environ Microbiol 67:284–292

Sarikhani MR, Aliasgharzad N (2012) Comparative effects of two arbuscular mycorrhizal fungi and k fertilizer on tuber starch and potassium uptake by potato (*solanum tuberosum L.*). (ECISI). Int J Agric Res Rev 2(3):125–134

Sati P, Dhakar K, Pandey A (2013) Microbial diversity in soil under potato cultivation from cold desert Himalaya, India. ISRN Biodiversity 2013:9

Schüβler A, Schwarzott D, Walker C (2001) A new fungal phylum, the *Glomeromycota*, phylogeny and evolution. Mycol Res 105(12):1413–1421

Smith SE, Gianinazzi-Pearson V (1988) Physiological interaction between symbionts in vesicular-arbuscular mycorrhizal plants. Ann Rev Plant Physiol Plant Mol Biol 39:221–244

Somers E, Vanderleyden J, Srinivasan M (2008) Rhizosphere bacterial signalling: a love parade beneath our feet. Crit Rev Microbiol 30(4):205–240

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism–plant signaling. FEMS Microbiol Rev 31(4):425–448

Srinath J, Bagyaraj DJ, Satyanarayana BN (2003) Enhanced growth and nutrition of micropropagated *Ficus benjamina* to *Glomus mossae* co-inoculated with *Trichoderma harzianum* and *Bacillus coagulans*. World J Microbiol Biotechnol 19(1):69–72

Sturz AV (1995) The role of endophytic bacteria during seed piece decay and potato tuberization. Plant Soil 175(2):257–263

Trappe JM (1987) Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In: Safir GR (ed) Ecophysiology of VA mycorrhizal plants. CRC Press, Boca Raton, pp 5–25

Verma P, Shahi SK (2015) Isolation and characterization of bacterial isolates from potato rhizosphere as potent plant growth promoters. Int J Curr Microbiol App Sci 4(3):521–528

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Vestberg M, Kukkonen S, Saari K, Parikka P, Huttunen J, Tainio L, Devos N, Weekers F, Kevers C, Thonart P, Lemoine MC, Cordier C, Alabouvette C, Gianinazzi S (2004) Microbial inoculation for improving the growth and health of micropropagated strawberry. Appl Soil Ecol 27(3):243–258

Vosãtka M, Gryndler M (1999) Treatment with culture fractions from *Pseudomonas putida* modifies the development of *Glomus fistulosum* mycorrhiza and the response of potato and maize plants to inoculation. Appl Soil Ecol 11(2-3):245–251

Vosãtka M, Gryndler M (2000) Response of micropropagated potatoes transplanted to peat mediato post-vitro inoculation with arbuscular mycorrhizal fungi and soil bacteria. Appl Soil Ecol 15(2):145–152

Vrany J, Fiker A (1984) Growth and yield of potato plants inoculated with rhizosphere bacteria. Folia Microbiol 29(3):248–253

Walker C (1992) Systematics and taxonomy of the arbuscular endomycorrhizal fungi (Glomales)-a possible way forward. Agronomie 12(10):887–897

Wang YY, Vestberg M, Walker C, Hurme T, Zhang X, Lindstrom K (2008) Diversity and infectivity of arbuscular mycorrhizal fungi in agricultural soils of the sichuan province of mainland China. Mycorrhiza 18(2):59–68

Xavier LJC, Germida JJ (2003) Bacteria associated with *Glomus clarum* spores influence mycorrhizal activity. Soil Biol Biochem 35(3):471–478

Yan Z, Reddy MS, Ryu CM, McInroy JA, Wilson M, Kloepper JW (2002) Induced systemic protection against tomato late blight elicited by plant growth-promoting rhizobacteria. Phytopathology 92(12):1329–1333

Yao MK, Tweddell RJ, Desilets H (2002) Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by *Rhizoctonia solani*. Mycorrhiza 12(5):235–242

Zhang F, Dashti N, Hynes RK, Smith DL (1996) Plant growth promoting rhizobacteria and soybean [*Glycine max (L) Merr*] nodulation and nitrogen fixation at suboptimal root zone temperatures. Ann Bot (Lond) 77(5):453–460

Microbial Biofertilizer Interventions in Augmenting Agroforestry

19

Kumud Dubey, K.P. Dubey, A. Pandey, and P. Tripathi

Abstract

Owing to limited land resources, agroforestry in the present scenario is the proper utilization of available land resources in the development of the agriculture and forestry sector together with the protection of the environment. It is estimated that the population of India will increase to about 1.44 billion by 2030, necessitating commensurate increase in the production of food grains. Due to rise in living populations, the demand of food, fodder, and fuel wood has been increased. Agroforestry is the combinations of food crop and tree crop to make more dynamic, multipurpose, and sustainable utilization of land resources aimed to fulfill the requirement of increased living populations. For enhancing production, a wide use of chemical fertilizers is making our land resources nutrient deficient and has detrimental impacts on soil, water, environment, and crop quality and productivity. Therefore, there is an urgent need to shift from inorganic agricultural practices to organic practices, and interventions of microbial biofertilizers are required to ensure sustained crop productivity and environmental protection. These microbial biofertilizers can benefit the plant health by influencing the essential nutrient availability, releasing plant growth regulators, and providing resistance against pathogens, thereby enhancing the crop productivity. Agroforestry systems are also reported to enhance plant-beneficial bacteria. The present review emphasizes on the proper land utilization in the form of agroforestry with microbial biofertilizer interventions for sustainably coping up with the 3F (food, fodder, and fuel) production targets and problems related to environment and health hazards.

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Table 19.1 Forest and tree cover of Uttar Pradesh **(in ha)**

1	Uttar Pradesh geographical area	2,40, 92,800
2	Recorded forest area	16,58,300
3	Forest cover	14,33,800
4	Tree outside forest	7,38,200
5	Forest and tree cover	21,72,000
6	Forest and tree cover against geographical area	9%

Source: State Forest Report 2011, published by Forest Survey of India

19.1 Introduction

Increase in live population enforced to acquire more land under farming to achieve the increasing requirement of grain, silage, vegetable, firewood, timber, medicines, etc. This increasing demand for food and wood mainly has resulted in overexploitation of forests. This has made planners, foresters, and environmentalists to consider alternate system of land use for serving society (Khosla and Khurana 1987). Agroforestry is one of the most viable alternative land-use systems for maximum sustainable productivity (fuel, fodder, and food), while preserving the environment. Extension of agroforestry in Uttar Pradesh is also the need of the day because this region has only about 9.01% of area under tree cove (Table 19.1) against the figure of 33% recommended by National Forest Policy (website of ENVIS Centre: Uttar Pradesh). Agroforestry may be defined as preferred multipurpose land organization which involved planting of woody components including trees, shrubs, bamboo, etc. with agricultural food crops. This land organization fulfills the ecological as well as socioeconomic demand of the people. Agroforestry is also considered as a powerful solution to the climate crisis by harnessing the immense power of photosynthesis; it can fix atmospheric carbon, a problem, into soil carbon safely in the ground (Nair 2012). In order to achieve the goal of higher production from limited land resources, imprudent high doses of inorganic fertilizers are being applied incessantly in agriculture which has severely damaged the soil vigor and has decreased the soil fertility of large portions of land, every year. Moreover, the use of microbial biofertilizer may affect plant growth and quality. Under such conditions, microbial biofertilizer interventions in combination with agroforestry are to be opted to enhance land efficiency in a sustainable approach. This joint intervention may deal with both the soil and agroecosystems, simultaneously, and may play a major role in resolving worldwide challenges of food, fodder, and fuel requirement for the twenty-first century including climate change.

19.2 Biofertilizer

Biological soil fertility management is an ecological approach for sustainable agriculture. The soil rhizosphere treasures the microbial diversity. Microorganisms carry out various important biological roles necessary for its survival and, at the

same time, beneficial to the soil ecosphere through management of nutrient resources, removal of toxic substances, quality improvement, bioremediation, supportive in nutrient and water absorption, production of phytohormones, environmental stress relief, prevention of plant diseases, etc. (García-Fraile et al. 2015; Sengupta and Gunri 2015). This plant-allied rhizospheric microbial population, also called as rhizosphere microbiome, has crucial role in plant healthiness. Latest findings on plant-microorganism relations discovered that plant life suitably generates their own specific rhizospheric microbiome, particular to their requirements, which may also be proved by occurrence of species-specific microbiome in associated soil rhizosphere. These microbiomes consist of both beneficial and pathogenic microbes. The beneficial microbes are considered as microbial biofertilizer. It is also evinced that upon disease incidence, plants promote protective or beneficial microbes to suppress pathogens in their rhizospheric microbiome (Berendsen et al. 2012; Marasco et al. 2012; Berg et al. 2013; Mendes et al. 2013; Berg et al. 2014; Pérez-Jaramillo et al. 2016). These various interrelationships of rhizosphere microbes with roots of plant species can be advantageous for the plant health through influencing the essential nutrient availability, through secretion of plant growth regulators, and through providing resistance against pathogens. Land organizations consisting of tree with agricultural crop were also endowed with plant-useful microorganisms (Köberl et al. 2015). Different soil microorganisms play an important role in conversion and mobilization of soil nutrients for plant use. Some microorganisms are capable of fixing nitrogen, while some can increase the availability of nitrogen and phosphorus. Biofertilizers are the products containing diversity of microorganisms that have the ability to organize the availability of different soil nutrients from non-utilizable form by metabolic functions (Hayat et al. 2010). Biofertilizers also known as microbial inoculants have great prospective as additional, replenishable, and eco-friendly source of plant nutrients and are an integrated part of plant soil rhizospheric nutrient system. These microbes generally associated with rhizosphere soil and also termed as plant growth-promoting rhizobacteria (PGPR). Use of microbial biofertilizers is one of the vital parts of collective nutrient management system, as they are low-costing and self-sustainable source of plant nutritional requirements. A number of beneficial PGPR have been utilized universally as commercial biofertilizers, responsible for increase in agricultural production and land productiveness sustainably in both cases of agriculture and forestry (Khalid et al. 2009). Depending upon the nature and functions, they have been grouped (Table 19.2).

As per their relations with plants, microbial biofertilizers may be divided into symbiotic category, which resides within the plants and release/uptake bio-nutrients directly with them, as per their needs. The other is nonsymbiotic category, which resides exterior to plant (Gray and Smith 2005). In symbiotic association, they survive within the intercellular spaces of the host plant forming true mutually interdependent interactions with their host plant cells. While in case of nonsymbiotic associations, they are free-living and involved in nutrient cycling and other plant growth-promoting functions.

Table 19.2 Major important groups of biofertilizers

S. no.	Group	Examples
N2-fixing biofertilizers		
1.	Symbiotic	*Rhizobium* sp. with Leguminosae family and *Frankia* sp. with non-Leguminosae family
2.	Free-living nonsymbiotic, associative, or endophytic N2 fixer	*Azotobacter* sp., *Azospirillum* sp., *Cyanobacteria* (blue-green algae), and *Azolla*
P-solubilizing biofertilizers		
1.	Phosphate-solubilizing bacteria	*B. subtilis*, *B. megaterium*, *B. circulans*, *Pseudomonas striata*
2.	Phosphate-solubilizing fungi	*Penicillium* sp., *Aspergillus awamori*
P-mobilizing biofertilizers		
1.	Phosphate mobilizer endomycorrhiza	*Glomus* sp., *Gigaspora* sp., etc.
2.	Phosphate mobilizer ectomycorrhiza	*Laccaria* sp., *Pisolithus* sp., *Boletus* sp., *Amanita* sp.
Other plant growth-promoting rhizobacteria		
1.	Mineral solubilizers	*Bacillus* sp., *Bacillus edaphicus, Enterobacter hormaechei*, etc.
2.	Other growth-supporting and micronutrient solubilizers	*Pseudomonas* sp., *Burkholderia sp., Brevundimonas sp., Serratia sp.* etc.

19.3 Nitrogen-Fixing Biofertilizer

Nitrogen is a basic source material for the biosynthesis of amino acids and proteins and essentially required nutrient for plant growth and development. But unfortunately its most common form as atmospheric nitrogen is unavailable to the plants. Its fixed forms only, such as ammonia and nitrate, are available to the plants which are proffered only through biological nitrogen fixations (Wagner 2012; Sengupta and Gunri 2015). Globally, biological means of nitrogen (N_2) fixation are supposed to fix 180 metric tons/year. Eighty percent of this fixation is contributed from symbiotic associations and the rest from rhizospheric free-living microorganisms or other related systems (Graham 1988; Tilak et al. 2005). The capability to capture such appreciable amounts of atmospheric N_2 and supplement to soil N_2 in fixed form is credited to N_2-fixing microbes (Young 1992; Tilak et al. 2005). These include (i) symbiotic nitrogen-fixing forms, viz., *Rhizobium*, specific to the leguminous plants, and *Frankia*, specific to nonleguminous plants, and (ii) nonsymbiotic nitrogen-fixing (N_2-fixing) forms, surviving exterior to the plants such as *Cyanobacteria*, *Azospirillum*, and *Azotobacter.* Bio-fixation of nitrogen by blue-green algae and bacteria (*Rhizobium* and *Frankia*) in forest tree species and the importance of biological nitrogen fixation in afforestation were described by Santi et al. (2013).

19.4 Symbiotic Nitrogen Fixers

Under this category *Frankia* and *Rhizobia* are the most studied nitrogen-fixing bacteria. These microbes form physiologically integrated nodular structures in plant root (Gray and Smith 2005).

19.4.1 Frankia

Frankia are N_2-fixing actinomycetes. They induce nitrogen-fixing root nodules on diverse nonleguminous (actinorhizal) plants like *Casuarina equisetifolia* (Saravanan et al. 2012). Over 200 plant species belonging to eight families form symbiotic association with these bacteria (Benson and Silvester 1993; Wall 2000; Tilak et al. 2005). These bacteria fix nitrogen from the atmosphere and add large amount of the fixed nitrogen in soil. Plant-*Frankia* associations are ecologically important and valuable for land reclamation, reforestation, restoration, and soil stabilization (Joel et al. 2001). Actinorhizal plants are capable to grow rapidly on nitrogen-deficient soils even in harsh conditions. They enhance the productiveness of agroforestry land organization system for wood, fodder, and firewood production, land restoration, and multipurpose planting (Schwenke and Caru 2001).

19.4.2 Rhizobium

Rhizobium is a group of soil microbes which form an interdependent association with Leguminosae family to make nodular structure in plant roots and convert atmospheric nitrogen to usable form of nitrogen in significant amount and is responsible for increase in crop productivity. Bio-fixation of nitrogen in the soil also benefits the following crops. Inoculum of *Rhizobium* can add 50–230 kg N_2 per hectare. These nodules are considered as miniature nitrogen production factories in the field. In interdependence association, *Rhizobium* provides nitrogen to the plant, and in return the plant protects the bacteria from O_2 damage by harboring it inside nodular structure. *Rhizobia* biorelease biochemicals like auxins, cytokinins, abscisic acids, lumichrome, riboflavin, lipo-chito-oligosaccharides, and vitamins that enhance the plant growth (Dakora 2003; Matiru and Dakora 2004; Hayat and Ali 2004, 2010; Hayat et al. 2008a, b; Laranjo et al. 2014). *Rhizobia* also provide protection against disease and harsh conditions (Yagi et al. 2000; Ghosh and Basu 2002; Dakora 2003; Bardin et al. 2004; Matiru and Dakora 2004). Forest trees belonging to Leguminosae family form nodular structure with the fast-growing *Rhizobia* sp. or slow-growing *Bradyrhizobium* sp. and bio-fix N_2 by utilizing 84,000 tons of nitrogen gas in the air above each hectare of land. About 7200 of species out of 18,000 leguminous species are woody out of which only 18% forms nodular structure. Most of the plants belonging to Mimosaceae and Papilionaceae family form nodular structure, but only few of the Caesalpiniaceae plants are reported to nodulate (Allen and Allen 1981; Brewbaker et al. 1982; Dobereiner 1984). In case of agricultural crops, no strict symbiotic association with *Rhizobium* is reported. A broad array of *Rhizobium* sp. can make nodules in agricultural crops (Räsänen 2002).

Continuous use of nitrogenous fertilizers does not seem to influence the effectiveness of *Rhizobium*. *Rhizobium* can survive at low temperatures and tolerate temperatures up to 50°C for more than few hours. It is susceptible to plant protectants and other biochemicals. It can survive in soil for several years under dry storage conditions, although the mechanism of its continued existence is unknown. Several microorganisms and bacteriophages are known to reduce the growth of *Rhizobia*,

while, in nature, nodulation is rarely inhibited by the activity of these antagonistic microorganisms. *Rhizobium* is more tolerant toward salt than its host legumes and, therefore, survives in saline soils (Subbarao 1997).

Rhizobia are able to survive in harsh soil conditions even without the host plant (Barnet et al. 1985; Odee et al. 2002). *Rhizobium* has several features to cope with inhospitable environments. For instance, extracellular polysaccharides are natural products of the growth of *Rhizobium* (Dudman 1968, 1976). Polysaccharide-based encapsulation of *Rhizobium* cells (Dudman 1968) may be a mechanism for the survival of the bacteria when they are exposed to harsh conditions. This characteristic of this bacterium is important in agroforestry where shifting of leguminous to non-leguminous crop plants is a common practice.

19.5 Free-Living N_2 Fixers

Free-living microbes present in soil rhizosphere are also significantly involved in bio-fixation of N_2. Blue-green algae (BGA) or *Cyanobacteria* and *Azolla* are also known as important free-living N_2 fixers. Such N_2 fixation has important value in agriculture and mainly depends on the availability of carbon source for carrying the bio-fixation of nitrogen. Due to release of various biomolecules, plant root zone is supposed to be a rich source of carbon; therefore these free-living N_2 fixers mostly reside in soil rhizosphere, closer to the plant roots to meet their energy demands. It forms a very well-organized recycling system for nitrogen through absorption by deep roots in deep soil and through decomposition of leaf litter in upper layer. Disruption of this mineral cycle conserving important soil nutrients may affect the soil health. In the maintenance of the litter layer, common in agroforestry land organization, due to the leaf fall, nutrient cycle is maintained effectively which is very important for reviving soil health (Dubey 2010a, b).

19.5.1 Azotobacter

Azotobacter are free-living Gram-negative bacteria, dominant inhabitants in arable soils, and fix atmospheric N_2 nonsymbiotically. According to Kizilkaya (2009), about 20 kg nitrogen per hectare per year may be bio-fixed by *Azotobacter*. Excretions of plant roots consisting of various important biomolecules like amino acids, vitamins, and organic acids provide the required survival source for *Azotobacter*. They bio-reduce N_2 to ammonia in soil, which can be utilized by plants. In addition, these bacteria synthesize and secrete thiamine, riboflavin, pyridoxine, cyanocobalamin, nicotinic acid, pantothenic acid, indoleacetic acid, and gibberellins or gibberellin-like substances (Kizilkaya 2009). They also produce antifungal antibiotics, which inhibit a variety of soil fungi. It is also known to produce ether-soluble fungistatic substances that inhibit plant disease-causal microorganisms. Therefore, these microbes also naturally manage the occurrence of disease by checking the growth of pathogenic microbes. In fact, these twin attributes of

Azotobacter may also explain the positive stimulating effects of the microbes on seed germination (Subbarao 1997; Sokolova et al. 2011). This biosynthesis of the auxins, vitamins, growth-promoting substances, and antifungal antibiotics confers on it additional advantages in addition to its ability to fix nitrogen. These attributes are accountable for enhancing the seed germination and plant development and finally yield to a considerable extent.

Azotobacter is known to form cysts to withstand adverse conditions. Each cyst has a living cell with two coats. The cyst accumulates polyhydroxybutyric acid. These encapsulated cysts have improved in heat resistance and developed resistance toward desiccation and adverse conditions (Chadha et al. 2011). These cysts regenerate in favorable conditions producing microbial cells. They also release polysaccharides which is helpful in soil aggregation. The populace of *Azotobacter* is mainly affected by the presence of other microbes, and it is about 10,000 to 1 lakh per g in Indian soils. Some microbes present in the soil promote the *Azotobacter* populace, thereby enhancing the bio-reduction of N_2 by *Azotobacter*. Cellulolytic microorganisms, which degrade plant residues in soil, are known to encourage the proliferation of *Azotobacter* in soil. On the other hand, there are some rhizobacteria that negatively affect the *Azotobacter* growth and reduce nitrogen fixation process. For example, *Cephalosporium*, a prevalently present organism in soil, limits the growth of *Azotobacter* (Subbarao 1997). *Azotobacte*r inoculation has been observed for enhanced seed germination of tree species (Dubey 2010a, b).

19.5.2 Azospirillum

Microbes belonging to *Azospirillum* sp. mainly bio-fixed N2 under microaerophilic situations and are commonly coupled with root and rhizosphere of a large number of agricultural species. Due to their common presence in the rhizosphere, these are also recognized as associate diazotrophs. *Azospirillum* is proposed as a nonspecific plant growth-promoting bacterium (Bashan et al. 2004). They are called as associative endosymbiont promoting the growth of plants (Okon 1985; Tilak and Subba Rao 1987; Bashan and Holguin 1997). In spite of their N_2–fixing ability which is about 1 to 10 kg per hectare, the augmentation in product yield is chiefly due to enhanced root growth through release of growth-promoting biomolecules which improve water and mineral absorption in plant (Okon and Kapulnik 1986; Fallik et al. 1994). *Azospirillum* flourish in the soil rhizosphere of various plant species. The bacterium is known to produce indoleacetic acid, gibberellins, cytokinin, and vitamins like biomolecules that may be attributed to increase in productivity (Subbarao 1997; Ghallab and Salem 2001).

19.5.3 Cyanobacteria

Cyanobacteria or blue-green algae (BGA) are considered as an important group of microorganisms, belonging to order *Nostocales* and *Stigonematales*, and have the capabilities of carrying photosynthesis and nitrogen bio-fixation simultaneously.

They fix nitrogen nonsymbiotically through action of enzyme nitrogenase. Dominant nitrogen-fixer blue-green algae are *Anabaena*, *Nostoc*, *Aulosira*, *Calothrix*, *Plectonema*, etc. (Mishra and Pabbi 2004). Fixation of nitrogen in blue-green algae takes place in specialized cells called "heterocysts." Vegetative cells and heterocysts are collectively involved in nitrogen bio-fixation. Heterocysts get energy for nitrogen bio-fixation from photosynthetic vegetative cells, and heterocysts fulfill the nitrogen requirement of vegetative cells. The application of phosphate promotes algal multiplication in 2 weeks on clay soil and in 3–4 weeks in sandy soils (Subbarao 1997). Being photosynthesizer, cyanobacteria have extra advantage of bio-fixing of carbon in addition to the fixing of nitrogen which may nourish all intact heterotrophic soil microbes. Being photosynthesizer and nitrogen fixer, this group of microbes forms microbial mats which are self-sustaining without any requirement for survival. In conclusion, cyanobacterial cellular mats are not only metal and metalloid resistant but also take out these toxicants from the atmosphere. The possible use of these cyanobacterial microbial mats, in bioremediation of wastelands with hazardous substances, has to be investigated. Besides being a bio-fixer of nitrogen, BGA provide the following other advantageous features:

1. Algal biological mass accumulated as carbon product. Preservation of sufficient soil organic product is necessary for sustainable and increased crop productivity.
2. Biosynthesis and discharge of bioactive extracellular molecules that may affect plant health. These have been stated to be plant promoters, vitamins, amino acids, polypeptides, and antibacterial or antifungal substances important for disease control and soil fertility.
3. It gives resistance to pesticides and fungicides.
4. Secretion of biomolecules that enhance phosphorous accessibility and absorption.
5. It is useful in remediation of alkaline soils by releasing organic acid, thereby lowering of pH and improving the soil quality.
6. In arid/dry conditions, cyanobacteria and microphytes release glutinous substance that sticks or tangles clay particles in sand-forming crusts suitable for surviving the beneficial microbes (Abdel-Raouf et al. 2012).
   Cyanobacteria is considered to be the most vital and common N_2 bio-fixer microbes in agricultural systems (Rodrigo and Eberto 2007). Their potential as N_2 bio-fixer in paddy fields has been reported by various researchers. Being the chief components of wetland paddy ecosystems, cyanobacteria are considered as readily accessible and the cheapest sources of natural biofertilizers (Omar 2000; Ladha and Reddy 2003).

19.5.4 Azolla

Azolla is water fern residing in drain and stagnant water. It nurtures an algal endosymbiont named as *Anabaena azollae* that bio-fixes atmospheric N_2. The fern grows rapidly with the interdependent alliance of the *Anabaena*, and this quick

growth builds an enormous amount of biomass on the water surface. It also adds in the soil organic carbon. The *Azolla-Anabaena* symbiosis has drawn notice as a biofertilizer universally, particularly in Southeast Asia. It is extensively utilized in paddy areas and in fisheries. It can bio-fix up to 900 kg of nitrogen per hectare per year (Subbarao 1997).

19.5.5 Phosphorus-Mobilizing Biofertilizers

Phosphorous is an important plant nutrient, which is referred to as vital component in crop production. Phosphorous is found in soil in various organic and inorganic combinations, most of which is unavailable to plants. Plant takes phosphorous in the form of soluble orthophosphate ions. The most important aspect of the phosphorous cycle is microbial mineralization and solubilization; otherwise extraction of phosphorous is not handy to plant roots and immobilization. Inorganic phosphate is solubilized by microbes which are of economic significance for plant nutrition. There are mainly two categories of microorganisms involved in phosphorous nutrition. These are phosphate-solubilizing microbes (PSMs) and arbuscular mycorrhizal (AM) fungi, which are involved in mineralization and absorption of phosphorous, respectively.

19.5.6 Phosphate-Solubilizing Microbes (PSMs):

Phosphorus (P) is a very crucial nutrient for biological growth and development of plants. P in soils remains in immobilized form or turns into less available forms either by chemical process. Major portion of the phosphorous present in the soil remains unavailable to plants due to its chemical fixation and low solubility. Appliance of chemical phosphatic fertilizers is also not helpful because its major portions remain insoluble. Under such circumstances, only microbes are capable to convert this unavailable form of P to available form of P through a biological rescue system and make it accessible to the plants (Khan et al. 2006). Phosphorus is also necessary for nodulation by *Rhizobium*.

Phosphorous-solubilizing microbes (PSMs), an imperative part of rhizomicrobes, comprise mostly bacteria and fungi. Such microorganisms not only absorb P but also release a significant quantity of phosphorous in soil in usable form (Gaur 1990). They lower pH of soil by releasing a variety of bioorganic acids in soil making insoluble form of phosphate to readily available form of soluble phosphate. The most proficient PSMs belong to the genera *Bacillus* and *Pseudomonas* among bacteria and *Aspergillus and Penicillium among* fungi. PSM inoculants include species of *Aspergillus*, *Bacillus*, *Escherichia*, *Arthrobacter*, and *Pseudomonas* (Mishra 1985; Datta et al. 1982), which can put in 30–35 kg of phosphate per ha (Gaur et al. 2004). The higher population of PSMs is more in the rhizospheric zone as compared to non-rhizospheric zone, and release of P is of much benefit to plants because roots can absorb the solubilized P. The soluble P in the non-rhizospheric region, on the

other hand, cannot be utilized by the plants because of its low mobility. Biological nitrogen fixation by *Azotobacter*, *Azospirillum*, or *Rhizobium* depends appreciably on the available forms of phosphorous. As such, nitrogen fixation by N-fixing organisms is expected to improve as a consequence of PSM inoculation. There is, thus, a good scope to improve crop production through use of dual cultures involving N-fixing and PSM microorganisms (Lal 2002). There are also increasing evidences that PSMs promote plant development due to release of plant growth biomolecules rather than their action to release plant-available P. Production of plant growth-stimulating compounds like vitamins, gibberellins, auxins, vitamin B_{12}, GA_3, and IAA by PSMs has been reported by several workers (Subbarao 1997). These growth-promoting substances stimulate plant growth and thus produce greater biomass that naturally would have larger amounts of all the nutrients including P as compared to an uninoculated plant with relatively smaller biomass. In some cases the release of plant growth-stimulating substances has been found to be associated with enhanced uptake of micronutrients in addition to controlling the growth of fungal pathogens like *Fusarium* and *Alternaria* (Lal 2002).

Above-discussed microbes that are well known to be useful to plants are also termed as the plant growth-promoting rhizobacteria (PGPR). Plant growth-promoting rhizobacteria (PGPR) may support growth directly, e.g., by bio-fixation of N_2, solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron, or production of plant growth regulators (hormones) (Tilak et al. 2005). These highly productive and diversified microbial consortia of ecosystems continuously convert and biodegrade dead vegetation into sources of nitrogen, phosphorus, and other nutrients which can later be utilized by the plants. Some microbes promote plant growth indirectly by creating growth-promoting conditions either via production of antagonistic substances or by inducing disease resistance (Siddiqui et al. 2000; Siddiqui et al. 2001). Consecutively, plant root excretes provide energy source for the survival of microorganisms in the ecosystem.

19.6 Arbuscular Mycorrhizal (AM) Fungi

Mycorrhiza (meaning fungus root) are well-developed non-harming symbiotic association between plant roots and certain specialized soil fungi belonging to the family *Basidiomycetes*, *Ascomycetes*, and *Zygomycetes*. These fungi incorporate and make association with the cortical tissues of roots during active plant growth in nature (Miller and Jastrow 1994; Smith and Read 1997). Plant species gain from mycorrhizal associations is mainly due to the capability of the fungi to act as conduits for plant nutrients and improve nutrient absorption. In this symbiotic relationship, the host plant absorbs mineral nutrients from the soil, and the fungi gets photosynthetically fixed carbon as source of energy from the host for their survival through mycorrhizal hyphae (Srivastava et al. 2001; Mohan 2000a, b). Mycorrhizal links are the most common symbioses between plants and microbes (Marschner 1995; Brundrett 2002). About 83% of dicots and 79% of monocots are associated

with mycorrhizal fungi (Wilcox 1991). Recently, their occurrence was also reported in water plants (Beck-Nielsen and Madsen 2001). Mycorrhizal associations are present in varied range of habitations, including water ecosystems; arid, tropical rain forests; high altitudes; high latitudes; and in canopy epiphytes (Allen 1991). There are two main types of mycorrhizal fungal associations with plant roots: ectomycorrhiza and endomycorrhiza. Ectomycorrhiza live outside of the plant roots and are present in few families only, whereas endomycorrhiza are prevalent in most of the families of angiosperms and in conifers except Pinaceae. In endomycorrhiza, the fungal hyphae enter the cells of the root. Arbuscular mycorrhiza (AM) is the most abundant kind of mycorrhiza described as "a universal plant symbiosis" which possesses special structures known as vesicles and arbuscules, the latter helping in the transfer of nutrients from the soil into the root system (Subbarao 1997; Dubey 2010b). Studies on AM fungi performed during the last few decades envisioned their presence in a broad diversity of hosts and different habitats along with difference in quality and quantity. The fungi are obligate endosymbionts, and they need a living plant root (or equivalent structure) in order to grow and reproduce (Asghari 2004). AM fungal development in roots of host plants starts when fungal hyphae grow from spores or from colonized roots toward the uncolonized roots. After contact of the hyphae with the root surface, the fungus is stimulated to change in morphology from an original simple branching pattern to irregularly septate pattern with reduced inter-hyphal spacing (Giovannetti et al. 1993; Harrison 1998). The fungus produces swollen appressoria on the root surface and spreads between and into the root cortical cells. In general, internal hyphae branch and make four recognizable structures as intracellular hyphae that may be coiled, intercellular hyphae, arbuscules, and spherical or ovoid vesicles (Smith and Smith 1997).

Mycorrhizal plants enhance the superficial root area improving absorption of nutrients especially from nutrient-poor soil. Bidirectional movement of nutrients describes the fungus-plant symbioses, where carbon flows to the fungus and nutrients move to the plant, thereby establishing a critical linkage between the plant root and soil. This association is a survival mechanism for both the fungi and plants, allowing each to survive in different surroundings (Gupta et al. 2000). Mycorrhizal plants, in comparison with non-mycorrhizal plants, have better nutrient absorption capability because they possess an external network of hyphae (Sanders and Sheikh 1983). The hyphae are the interface between soil and plant and have a large surface area that acts as an addition of the root-absorbing area (Li et al. 1991; Asghari 2004). This not only increases the volume of soil from which nutrients are absorbed but also overcomes both the problems of depletion of nutrients (Nurlaeny et al. 1996; Smith and Read 1997) and water depletion (Marulanda et al. 2003) close to actively absorbing roots and plays a significant role in stabilizing soil structure (Asghari 2004). Arbuscular mycorrhizal fungi (AMF) have been shown to increase productivity in low fertile soil and are predominantly vital for enhancing the absorption of relatively immobilized minerals like P, Zn, and Cu and other nutrients such as cadmium which are present in very low quantity. Under dry situations, the absorption of extremely mobile nutrients such as nitrate can also be improved by mycorrhizal hyphae (Liu et al. 2002; Quilambo 2003). Mycorrhizal fungal

associations provide numerous benefits to their host plant including augmented growth and productivity due to enhanced nutrient acquisition (Diederichs 1990; Lewis and Koide 1990; Stanley et al. 1993). Mycorrhizal associations decrease the soilborne disease incidence by reducing the susceptibility to pathogens like *Phytophthora* sp., *Chalara elegans*, *Fusarium* sp., and *Pythium* sp. and nematodes (Bondoux and Parrin 1982; Bagyaraj 1984; Singh et al. 2000; Dehne 1982; Jalali and Jalali 1991; Hooker et al. 1994; Mohan 2000a). The mycorrhizal associations are involved with the scavenging and retaining of the nutrients and formation of collective system that turns as a regulator point for accumulation and mineralization of soil organic matter. At a broader perspective, the mycorrhizal association, by its participation in nutrient accrual and retention, generates a system that decreases attrition and loss of nutrient due to leaching and recovers soil structure (Thomas et al. 1986; Degens et al. 1994; Beaden and Petersen 2000). AM fungi are involved in aggregation of soil particles and its stabilization (Rillig et al. 2002). By ramifying through soil, fungal hyphae may bring soil particles together and force their contact with binding agents. Mycorrhizal hyphae differed markedly in their stabilizing abilities because of their hyphal network (Lal 2002). AM fungal (AMF) hyphae have a major role in soil equilibrium by producing a glycoprotein named glomalin which sequesters trace elements, and it should be considered for biostabilization leading to remediation of contaminated soils (Wright and Upadhyaya 1999; Franzluebbers et al. 2000; Khan 2005). It improves water relations (Allen and Allen 1986; Davies et al. 1993; Subramanian et al. 1997) and tolerance of extreme pH (Sidhu and Behl 1997; Douds et al. 2000). Abundance of AM fungi is mainly in uppermost soil layer and makes a main constituent of soil fertility. It has a substantial role in the regulation of soil biological activity. Mycorrhiza is supposed to form a significant portion of soil organic matter and belowground biomass in a range of systems. It may act as carbon sink. Due to its chitinous walls, they are relatively resistant to microbial degradation and resistant to desiccation and can survive for 2–3 years in harsh conditions. AM can tolerate a wide range of soil conditions which is evident from a worldwide distribution of VAM. This strongly implies adaptation to a whole range of soil factors. Several workers have observed enmeshment of soil or sand particles by AM hyphae that act as effective colonizer of sand dunes. They are able to increase the aggregate weight of dune sands by binding sand grains to the extensive VAM mycelium (Lal 2002). VAM symbiosis has also typically increased water-use efficiency and host growth rates during drought conditions (Augé 2001). It has been observed that the presence of ectomycorrhizal or endomycorrhizal fungi on the roots of plants reduced the heavy metal absorption by the plants and thereby augmented plant growth (Heggo et al. 1990; Tam 1995). AM colonization improves the productivity of tropical soils (Lal 2002) by improving the plant health.

AM fungi are acknowledged for some physiological changes in plants by stimulating various enzymatic activities. Peroxidase and polyphenol oxidase activities are increased during stress conditions. It is an important defense mechanism of plants against pathogens. Phenol and catechin levels are also reported to be increased by VAM inoculation during disease incidence developing resistance, thereby improving the plant health (Mathur and Vyas 1996). Increased nitrogen uptake by

mycorrhizal hyphae has been well recognized. Mycorrhizal plants access N as free amino acids and are able to absorb N from proteins and chitins as well (Boddey et al. 2000; Brockwell et al. 2005). Mycorrhizal fungi have also been reported to induce systemic resistance. As well as inducing systemic resistance, mycorrhizal fungi can also form a connecting network between plants that can convey a resistance inducing signal to adjacent plants (Pozo and Azco 2007; Song et al. 2010; Berendsen et al. 2012).

19.7 Other Plant Growth-Promoting Rhizobacteria (PGPR)

There are several microbes present in the rhizosphere. Silicate and aluminum silicates are degraded by a specific group of bacteria known as silicate-solubilizing bacteria (SSB). During the biodegradation process, numerous acidic biomolecules are produced by the microbes which have a significant role in silicate degradation. A combined application of SSB with organic siliceous residue like rice straw resulted in augmented productivity. This improvement is due to enhanced dissolution of silica and nutrients from the soil. There are various microorganisms that reside in rhizospheric soil and promote growth through suppression of disease incidence, improved nutrient attainment, and production of growth-promoting biomolecules like indoleacetic acid, cytokinins, gibberellins, and inhibitors of ethylene production. *Pseudomonas* sp. and *Bacillus* sp. can release growth-promoting biomolecules that stimulate formation of fine roots increasing the absorptive area for absorption of water and nutrients. These PGPR have biostimulant property. Some PGPR inhibit pathogenic microbial growth through antibiotic and siderophore production (TNAU Agritech Portal Information).

Potassium (K) is the third essential nutrient necessary for plant growth. Some rhizobacteria like *Bacillus edaphicus*, *Paenibacillus glucanolyticus*, *Bacillus mucilaginosus*, etc. are able to solubilize insoluble potassium (Shanware et al. 2014; Sheng and He 2006; Sangeeth et al. 2012; Basak and Biswas 2009).

PGPR in the rhizosphere prevent plant diseases and improve plant health by contending for available nutrients, decreasing the contact surface area between the pathogen and the plant root or by interfering with the mechanisms leading to plant disease; by production of antibiotics; and by synthesizing cyanogenic defense compounds (García-Fraile et al. 2015). Varied biological procedures are involved in disease resistance, which is often indirectly connected with plant growth and health.

19.8 Agroforestry in Uttar Pradesh

Agroforestry is a preferred land organization that enhances overall production from limited area. It collectively uses agricultural crops with forest tree crops and/or animals simultaneously or sequentially and applies organization practices that are well suited with cultural arrangements of local population. It utilizes agriculture and forestry tools to generate more combined, varied, fruitful, gainful, healthy, and

sustainable land organization. It implies multiple cropping consisting of at least two plant species that interact biologically; one of the plant species is a woody perennial, and the other plant species is managed for forage, annual, or perennial crop production, as per the choice of the farmer**.** In Uttar Pradesh state of India, agroforestry practices vary considerably according to the agroclimatic zones, socioeconomic conditions, land holdings of the farmers, and the marketability of tree produce. Existing agroforestry was surveyed in UP; aonla or amla (*Emblica officinalis*, a fruit tree), eucalyptus, teak, and poplar-based agroforestry are prevalent agroforestry in this region (Fig. 19.1) (Dubey 2010b).

In agroforestry, the performance of tree-crop combinations mainly depends on their ability to share various available natural growth resources. Agroforestry is advantageous due to its positive influence on production of associated component increases. Trees are capable of improving productivity of soil in many ways. A large number of trees are known to bio-fix N_2 symbiotically (Tewari 2008; Dubey 2010b).

Effect of trees in agroforestry system on soil organic carbon was ameliorative. Soil under agroforestry had higher soil organic carbon in comparison to control. Similar finding was also reported by Soni et al. (2008). Gupta and Sharma (2009) also reported that poplar plantations enriched the soils with organic carbon and

Fig. 19.1 Prevalent agroforestry in Uttar Pradesh (**a**) teak agroforestry, (**b**) aonla agroforestry, (**c**) eucalyptus agroforestry, and (**d**) poplar agroforestry

nutrients. Dubey (2010a, b) studied soil organic carbon under aonla (fruit tree), eucalyptus, teak, and poplar-based agroforestry, and a significant increase was observed. Soil moisture was also increased. This increase in soil moisture and soil organic carbon may affect positively on rhizosphere soil microbes. Considerable improvement in organic matter due to tree leaf litter and nutrient content was noted under *Prosopis cineraria* in arid regions. Thus encouraging role played by tree component could be beneficial to productivity of crop component grown along with trees (Tewari 2008). A change in soil chemical properties under agroforestry systems was studied by Kumar et al. (2006), and soil fertility status was observed to be higher under different agroforestry systems which were supposed to be due to increased activities of rhizosphere soil microbes. Yadav et al. (2010) investigated the effect of traditionally grown trees (*P. cineraria*, *D. sissoo*, *A. leucophloea*, and *A. nilotica*) on soil biological characteristics. Their results discovered significant and extensive improvement in soil biological activity in terms of microbial biomass C, N, and P and dehydrogenase and alkaline phosphatase activity under different tree-based agroforestry systems as compared to control without tree planting system. From the findings it may be concluded that agroforestry has enhanced rhizosphere microbes.

19.9 Microbial Biofertilizer Interventions in Agroforestry

Microbial biofertilizer is used for applications to seeds, plants, soil, or composting areas with the objectives of increasing the numbers of such beneficial microorganisms in soil and accelerating certain desirable microbial processes to augment the extent of availability of nutrients. Effect of microbial biofertilizers on the forest tree crops and agricultural crops, as sole crop, has been studied extensively. However, in case of agroforestry, it has not been studied much in India. Agroforestry system promoted more closed nutrient cycling than sole agricultural system by facilitating the growth of rhizosphere microbes. In agroforestry, system nutrients taken by the tree roots are rapidly recycled to the soil in the form of leaf litter. It helps to create the specific rhizosphere microbiome as per the complex integrated requirement of tree and crop plants helping in the synchronization of nutrient release. Activities of soil organisms, which determine several key processes, are also expected to be high in agroforestry (Kumar 2005). In agroforestry, mycorrhizal associations contribute much to the growth of *Acacia* species in unfertilized fields (Dart et al. 1991). Maximum diversity in population density of *Azotobacter* and *Azospirillum* was reported in soil of agroforestry in comparison to solely agricultural crop system (Maurya et al. 2012). Microbial biofertilizer interventions in agroforestry may improve the productivity as well as crop health. Seed treatment of *Emblica officinalis* (aonla) with *Azospirillum* biofertilizer increased the germination. Application of AM fungi and PSB in combination produced maximum plant health in *Emblica officinalis* (Verma et al. 2008).The growth and biomass response of *Tectona grandis* (teak) were enhanced due to microbial inoculants (Sharma and Chaubey 2015). Microbial inoculation (*Frankia*, *Azospirillum*, and phosphobacteria) resulted in

significant increase in root length, shoot length, and basal diameter in *Casuarina equisetifolia*, a fast-growing multipurpose agroforestry tree species (Saravanan et al. 2012). The relative consequence of dual application of indigenous N_2 fixer (*Rhizobium*) and AM fungi consortia with different organic fertilizers (vermicompost and farm yard manure) on fodder production and quality of two leguminous tree species (*Leucaena leucocephala* and *Sesbania sesban*) in silvi-pastoral land organization and their influence on the feedstuff production, *Panicum maximum*, were studied. The results in this study suggested that improved yield and fodder quality from silvi-pastoral land organization are possible through application with proper AM fungi species with native *Rhizobium* strain (Mishra et al. 2011). Banana plants grown in the agroforestry system with *Inga* trees of Fabaceae family were found to affect plant-associated microbiome and characterized by an increase of potential plant-beneficial bacteria, like *Pseudomonas* and *Stenotrophomonas* (Köberl et al. 2015).

19.10 Conclusions and Future Perspectives

Agroforestry as alternative land management system addresses many of the global challenges and can be applied for poverty alleviation and mitigating declining agricultural productivity and natural resources. Agroforestry improves soil health of the farm through ameliorated microclimate and nutrition level. Trees play a dual role for nutrient cycling and sites for the accrual of nutrients. Soil microbial biomass, consisting of microbiome, acts as a source and sinks, both, for the plant nutrients playing a crucial role in nutrient cycling and organic carbon dynamics (Yadav et al. 2010). These microbes are primarily involved in plant residue decomposition, nutrient conservation, and cycling processes in the soil. An increase in the size of the soil microbial biomass represents an improvement in soil fertility, thereby affecting the plant health. Therefore, increased nutrient pool and microbial activities are necessary for long-term productivity of the soil. Trees can exert positive, negative, or neutral effects on production, composition, and diversity of plant communities, depending on local environmental conditions and position in the landscape. The ameliorating effect of the trees on top soil increases with age of tree. Hence, integration of trees in farming is highly recommended (Berhe and Retta 2015). Agroforestry provides a suitable microclimate for soil microbes to grow. Application of microbial biofertilizer in agroforestry can enhance crop yield by promoting the plant growth not only by supplying nutrients to the plant but also by producing phytohormones, inducing stress resistance, or preventing pathogen-induced plant diseases, thereby affecting the plant health. To increase and sustain the productivity of agricultural lands, the combined approach to determine the most favorable plant-microorganism interaction is vital, and the effects of different microbial biofertilizers in different tree-crop combinations have to be studied for productive, efficient, and sustainable agroforestry system to ensure the food supply for an expanding world population and minimizing damage to the environment.

References

Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM (2012) Agricultural importance of algae. Afr J Biotechnol 11(54):11648–11658

Allen MF (1991) The ecology of mycorrhizae. Cambridge University Press, Cambridge

Allen ON, Allen EK (1981) The Leguminosae. A source book of characteristics, uses and nodulation. University of Wisconsin Press, Madison, Wisconsin, p 812

Allen EB, Allen MF (1986) Water relations of xeric grasses in the field: interactions of mycorrhizas and competition. New Phytol 104:559–571

Asghari HR (2004) Effects of arbuscular mycorrhizal fungal colonization on management of saline lands. Thesis submitted for the degree of Doctor of Philosophy in School of Earth and Environmental Sciences, Faculty of Sciences, University of Adelaide, Australia. p 219

Augé RM (2001) Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Bagyaraj DJ (1984) Biological interactions with VA mycorrhizal fungi. In: Bailey CL, Mansfield JW (eds) VA mycorrhizae. CRC Press, Boca Raton, pp 131–154

Bardin SD, Huang HC, Pinto J et al (2004) Biological control of *Pythium* damping-off of pea and sugar beet by *Rhizobium leguminosarum .viceae*. Can J Bot 82(3):291–296

Barnet YM, Catt PC, Hearne DH (1985) Biological nitrogen fixation and root-nodule bacteria (*Rhizobium* sp. and *Bradyrhizobium* sp.) in two rehabilitating sand dune areas planted with *Acacia* spp. Aust J Bot 33:595–610

Basak BB, Biswas DR (2009) Influence of potassium solubilizing microorganism (Bacillus mucilaginosus) and waste mica on potassium uptake dynamics by sudan grass (*Sorghum vulgare* Pers.) grown under two Alfisols. Plant Soil 317:235–255

Bashan Y, Holguin G, de-Bashan LE (2004) *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). Can J Microbiol 50(8):521–577

Bashan Y, Holguin G (1997) *Azospirillum*–plant relations: environmental and physiological advances (1990–1996). Can J Microbiol 43:103–121

Beaden BN, Petersen L (2000) Influence of arbuscular mycorrhizal fungi on soil structure and aggregate stability of a vertisol. Plant Soil 218:173–183

Beck-Nielsen D, Madsen TV (2001) Occurrence of vesiculararbuscular mycorrhiza in aquatic macrophytes from lakes and streams. Aquat Bot 71:141–148

Benson DR, Silvester WB (1993) Biology of Frankia strains, actinomycete symbionts of actinorhizal plants. Microbiol Rev 57(2):293–319

Berendsen RL, Pieterse CMJ, Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends Plant Sci 17(8):478–486

Berg G, Mahnert A, Moissl-Eichinger C (2014) Beneficial effects of plant-associated microbes on indoor microbiomes and human health? Front Microbiol 5:15. doi:10.3389/fmicb.2014.00015

Berg G, Zachow Z, Müller H et al (2013) Next-generation bio-products sowing the seeds of success for sustainable agriculture. Agronomy 3:648–656

Boddey RM, Peoples MB, Palmer B et al (2000) Use of the 15N natural abundance technique to quantify biological nitrogen fixation by woody perennials. Nutr Cycl Agroecosyst 57:235–270

Bondoux P, Parrin R (1982) Mycorrhizae et protection des plants. CR Acad Agric De France 15:1162–1167

Berhe DH, Retta AN (2015) Soil improvement by trees and crop production under tropical agroforestry systems: a review. Merit Res J Agric Sci Soil Sci 3(2):18–28

Brewbaker JL, Beldt R, MacDicken K (1982) Nitrogen-fixing tree resources: potentials and limitations. In: Graham PH, Harris SC (eds) Biological nitrogen fixation technology for tropical agriculture. CIAT, Cali, pp 413–425

Brockwell J, Searle SD, Jeavons AC et al (2005) Nitrogen fixation in acacias: an untapped resource for sustainable plantations, farm forestry and land reclamation. ACIAR Monogr No 115:132

Brundrett MC (2002) Coevolution of roots and mycorrhizas of land plants. New Phytol 154:275–304

Chadha V, Kumar V, Sharma S (2011) Growth related production of poly-ß- hydroxtbutyrate by *Azotobacter chroococcum* soil isolate/mutant using N, P and cane molasses. Res Revs J Microbiol Virol 1(2):14–23

Dakora FD (2003) Defining new roles for plant and rhizobial molecules in sole and mixed plant cultures involving symbiotic legumes. New Phytol 158:39–49

Dart PJ, Umali-Garcia M, Almendras A (1991) Role of symbiotic associations in the nutrition of tropical acacias. In: Turnbull JW (ed) Advances in tropical Acacia research. ACIAR Proceedings No 35, Canberra, pp 13–19

Datta M, Banik S, Gupta RK (1982) Studies on the efficacy of a phytohormone producing, phosphate solubilizing *Bacillus firmus* in augmenting paddy yield in acid soils of Nagaland. Plant Soil 69:365–373

Davies FT Jr, Potter JR, Linderman RG (1993) Drought resistance of mycorrhizal pepper plants independent of leaf P concentration-response in gas exchange and water relations. Physiol Plant 87:45–53

Degens BP, Sparling GP, Abbott LK (1994) The contribution from hyphae, roots and organic carbon constituents to the aggregation of a sandy loam under long-term clover-based and grass pastures. Eur J Soil Sci 45:459–468

Dehne J (1982) Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathology 72:1115–1118

Diederichs C (1990) Improved growth of Cajanus cajan (L.) Millsp. In an unsterile tropical soil by three mycorrhiza fungi. Plant Soil 123:261–266

Dobereiner J (1984) Nodulation and nitrogen fixation in legume trees. Pesqui Agropecuaria Brasiliera 19:83–90

Douds DD, Gadkar V, Adholeya A (2000) Mass production of VAM fungus biofertilizer. In: Mukerji KG, Chamola BP, Singh J (eds) Mycorrhizal biology. Kulwer Academic Publisher, New York, p 247

Dudman WF (1968) Capsulation in *Rhizobium* species. J Bacteriol 95:1200–1201

Dudman WF (1976) The extracellular polysaccharides of *Rhizobium japonicum:* compositional studies. Carbohydr Res 46:97–110

Dubey K (2010a) Bioreclamation of Silica mining area through microbial technology. Ph.D thesis, FRI University, Dehradun

Dubey K (2010b) Development of agro-forestry models for eastern Uttar Pradesh. ICFRE Project Report −2010, p 91

Fallik E, Sarig S, Okon Y (1994) Morphology and physiology of plant roots associated with *Azospirillum*. In: Okon Y (ed) Azospirillum–plant associations. CRC Press, Boca Raton, pp 77–84

Franzluebbers AJ, Wright SF, Stuedemann JA (2000) Soil aggregation and glomalin under pastures in the southern piedmont USA. Soil Sci Soc Am J 64:1018–1026

García-Fraile P, Menéndez E, Rivas R (2015) Role of bacterial biofertilizers in agriculture and forestry. AIMS Bioeng 2(3):183–205

Gaur AC (1990) Phosphate solubilizing microorganisms as Biofertilizers. Omega Scientific Publishers, New Delhi, p 176

Gaur R, Shani N, Kawaljeet et al (2004) Diacetyl phloroglucinol-producing *Pseudomonas* do not influence AM fungi in wheat rhizosphere. Curr Sci 86:453–457

Ghallab AM, Salem SA (2001) Effect of biofertilizer treatments on growth, chemical composition and productivity of wheat plants grown under different levels of NPK fertilization. Ann Agril Sci Cairo 46:485–509

Ghosh AC, Basu PS (2002) Growth behaviour and bioproduction of indole acetic acid by a *Rhizobium* sp. isolated from root nodules of a leguminous tree *Dalbergia lanceolaria*. Indian J Exp Biol 40(7):796–801

Giovannetti M, Sbrana C, Avio L et al (1993) Differential hyphal morphogenesis in arbuscular mycorrhizal fungi during pre-infection stages. New Phytol 125:587–593

Gray EJ, Smith DL (2005) Intracellular and extracellular PGPR: commonalities and distinctions in the plant-bacterium signaling processes. Soil Biol Biochem 37:395–412

Graham PH (1988) Principles and application of soil. Microbiology:322–345
Gupta MK, Sharma SD (2009) Effect of tree plantation on soil properties, profile morphology and productivity index - poplar in Yamunanagar District. Ann Forest 17(1):43–70
Gupta V, Satynarayana T, Sandeep G (2000) General aspects of mycorrhiza. In: Mukerji KG, Chamola BP, Singh J (eds) Mycorrhizal biology. Kluwer publisher, New York
Harrison MJ (1998) Development of the arbuscular mycorrhizal symbiosis. Curr Opin Plant Biol 1:360–365
Hayat R, Ali S (2004) Potential of summer legumes to fix nitrogen and benefit wheat crop under rainfed condition. J Agron 3:273–281
Hayat R, Ali S (2010) Nitrogen fixation of legumes and yield of wheat under legumes-wheat rotation in Pothwar. Pak J Bot 42(4):2317–2326
Hayat R, Ali S, Amara U et al (2010) Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol 60:579–598
Hayat R, Ali S, Siddique MT, Chatha TH (2008a) Biological nitrogen fixation of summer legumes and their residual effects on subsequent rain fed wheat yield. Pak J Bot 40(2):711–722
Hayat R, Ali S, Ijaz SS et al (2008b) Estimation of N2-fixation of mung bean and mash bean through xylem uriede technique under rainfed conditions. Pak J Bot 40(2):723–734
Heggo A, Angle JS, Chaney RL (1990) Effect of vesicular-arbuscular mycorrhyzae fungi on heavy metal uptake by soybeans. Soil Biol Biochem 22:865–869
Hooker JE, Jaizzmme Vega M, Atkinson D (1994) Biocontrol of plant pathogens using vesicular-arbuscular mycorrhizal fungi. In: Gianinazzi S, Schuepp H (eds) Impact of Arbuscular mycorrhizas on sustainable agriculture and natural ecosystem. Birkhauser Verlag Basal, Switzerland, pp 191–200
Jalali BL, Jalali I (1991) Mycorrhizae in plant disease control. In: Arora DK, Rai B, Mukerji KG, Knudsen GR (eds) Handbook of applied mycology, vol 1: soil and plants. Marcel Dekker, New York, pp 131–154
Joel G, Chapin FS, Chiariello NR, Tahyer SS, Field CB (2001) Species-specific responses of plant communities to altered carbon and nutrient availability. Glob Change Biol 7:435–450
Khalid A, Arshad M, Shaharoona B et al (2009) Plant growth promoting rhizobacteria and sustainable agriculture microbial strategies for crop improvement. Springer, Berlin, pp 133–160
Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18(4):355–364
Khan MS, Zaidi A, Wani PA (2006) Role of phosphate-solubilizing microorganisms in sustainable agriculture - a review. Agron Sustain Dev. doi:10.1051/agro:2006011
Khosla PK, Khurana DK (1987) Agroforestry for rural need, vol I. Indian Society Of Tree Scientists, Solan, pp 12–13
Kizilkaya R (2009) Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. J Environ Biol 30(1):73–82
Köberl M, Dita M, Martinuz A et al (2015) Agroforestry leads to shifts within the gammaproteobacterial microbiome of banana plants cultivated in central America. Front Microbiol 6:91. doi:10.3389/fmicb.2015.00091
Kumar BM (2005) Homegardens as harbingers of belowground biodiversity in the humid tropics. Paper presented at the national workshop on conservation and sustainable management of belowground biodiversity, KFRI, Peechi, India, 21–23 June 2005
Kumar M, Verma KS, Mishra VK (2006) Changes in soil chemical properties under agroforestry systems. Short rotation forestry for industrial and rural development. In: Proceedings of the IUFRO ISTS UHF international conference on world perspective on short rotation forestry for industrial and rural development, Nauni, Solan, India, 7–13 September 2003, p 320–325
Ladha JK, Reddy PM (2003) Nitrogen fixation in rice systems: state of knowledge and future prospects. Plant Soil 19:151–167
Lal L (2002) Phosphatic biofertilizers. Agrotech Publishing Academy, Udaipur, p 224
Laranjo M, Alexandre A, Oliveira S (2014) Legume growth-promoting rhizobia: an overview on the *Mesorhizobium* genus? Microbiol Res 169(1):2–17

Lewis JD, Koide RT (1990) Phosphorus supply, mycorrhizal infection and plant offspring vigour. Funct Ecol 4:695–702
Li X-L, George E, Marschner H (1991) Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. Plant Soil 136:41–48
Liu A, Hamel C, Elmi A et al (2002) Concentrations of K, Ca and mg in maize colonised by arbuscular mycorrhizal fungi under field conditions. Can J Soil Sci 82(3):271–278
Marschner H (1995) Mineral nutrition of higher plants. Academic Press, London, San Diego
Marasco R, Rolli E, Ettoumi B et al (2012) A drought resistance-promoting microbiome is selected by root system under desert farming. PLoS One 7(10):e48479. doi:10.1371/journal.pone.0048479
Marulanda A, Azcon R, Ruiz Lozano JM (2003) Contribution of six arbuscular mycorrhizal fungal isolates to water uptake by *Lactuca sativa* plants under drought stress. Physiol Planlarum 119:1–8
Mathur N, Vyas A (1996) Biochemical changes in *Ziziphus xylopyrus* by VA mycorrhizae. Bot Bull Acad Sin 37:209–212
Matiru VN, Dakora FD (2004) Potential use of rhizobial bacteria as promoters of plant growth for increased yield in landraces of African cereal crops. Afr J Biotechnol 3(1):1–7
Maurya BR, Kumar K, Raghuwanshi R (2012) Diversity of *Azotobacter* and *Azospirillum* in Rhizosphere of different crop rotations in eastern Uttar Pradesh of India. Res J Microbiol 7:123–130
Mendes R, Garbeva P, Raaijmakers JM (2013) The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. FEMS Microbiol Rev 37:634–663
Miller RM, Jastrow JD (1994) Vesicular-arbuscular mycorrhizae and biogeochemical cycling. In: Pfleger FL, Linderman RG (eds) Mycorrhizae and plant health. The American Phytopathlogical Society, St Paul, pp 189–212
Mishra MM (1985) Solubilization of insoluble inorganic phosphate by soil microorganisms – a review. Agric Rev 6:23
Mishra U, Pabbi S (2004) Cyanobacteria: a potential biofertilizer for rice. Resonance 9(6):6–10
Mishra S, Sharma S, Vaasudevan P (2011) Role of bioinoculants and organic fertilizers in fodder production and quality of leguminous tree species. J Environ Biol 32(1):57–64
Mohan V (2000a) Endomycorrhizal interaction with rhizosphere and rhizoplane mycoflora of forest tree species in Indian arid zone. Indian Forester 126:749–755
Mohan V (2000b) Mycorrhizae – a potential Biofertilizer for Forest trees. In: Bennet SSR, Subramanian K, James Kurian A (eds) Genetic improvement and propagation of Forest trees. Institute of Forest Genetics and Tree Breeding, Coimbatore, pp 133–137
Nair PKR (2012) Climate change mitigation: a low hanging fruit of agroforestry. In: Agroforestry-the future of global land use. Springer, Netherlands, pp 31–67
Nurlaeny N, Marschner H, George E (1996) Effects of liming and mycorrhizal colonization on soil phosphate depletion and phosphate uptake by maize (*Zea mays* L.) and soybean (*Glycine max* L.) grown in two tropical acid soils. Plant Soil 181:275–285
Odee DW, Haukka K, McInroy SG et al (2002) Genetic and symbiotic characterization of rhizobia isolated from tree and herbaceous legumes grown in soils from ecologically diverse sites in Kenya. Soil Biol Biochem 34:801–811
Okon Y (1985) *Azospirillum* as a potential inoculant for agriculture. Trends Biotechnol 3:223–228
Okon Y, Kapulnik Y (1986) Development and function of *Azospirillum* inoculated roots. Plant Soil 90:3–16
Omar HH (2000) Nitrogen-fixing abilities of some cyanobacteria in sandy loam soil and exudates efficiency of rice grain germination. Egypt J Phycol 1:157–167
Pérez-Jaramillo JE, Mendes R, Raaijmakers JM (2016) Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol Biol 90(6):635–644
Pozo MJ, Azco ´n-A C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393–398

Quilambo OA (2003) The vesicular-arbuscular mycorrhizal symbiosis. Afr J Biotechnol (12):539–546

Räsänen LA (2002) Biotic and abiotic factors influencing the development of N_2-fixing symbioses between rhizobia and the woody legumes *Acacia* and *Prosopis*. Academic Dissertation in Microbiology, Department of Applied Chemistry and Microbiology, Division of Microbiology, University of Helsinki

Rillig MC, Wright SF, Eviner VT (2002) The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation: comparing effects of five plant species. Plant Soil 238:325–333

Rodrigo V, Eberto N (2007) Seasonal changes in periphyton nitrogen fixation in a protected tropical wetland. Biol Fertil Soils 43:367–372

Santi C, Bogusz D, Franche C (2013) Biological nitrogen fixation in non-legume plants. Ann Bot. doi:10.1093/aob/mct048

Saravanan TS, Rajendran K, Santhaguru K (2012) Selection of suitable biofertilizers for production of quality seedlings of *Casuarina equisetifolia* (Forst.) using decomposed coir pith compost in root trainers. Asian J Exp Biol Sci 3(4):752–761

Sengupta A, Gunri SK (2015) Microbial intervention in agriculture: an overview. Afr J Microbiol Res 9(18):1215–1226

Sanders FE, Sheikh NA (1983) The development of vesicular-arbuscular mycorrhizal infection in plant root systems. Plant Soil 71:223–246

Sangeeth KP, Bhai RS, Srinivasan V (2012) *Paenibacillus glucanolyticus*, a promising potassium solubilizing bacterium isolated from black pepper (*Piper nigrum* L.) rhizosphere. J Spic Aromat Crops 21:118–124

Schwenke J, Caru M (2001) Advances in actinorhizal symbiosis: Hostplant–Frankia interactions, biology and applications in arid land reclamation. A review. Arid Land Res Manage 15:285–327

Shanware AS, Kalkar SA, Trivedi MM (2014) Potassium solublisers: occurrence, mechanism and their role as competent biofertilizers. Int J Curr Microbiol App Sci 3:622–629

Sharma A, Chaubey OP (2015) Biotechnological approach to enhance the growth and biomass of *Tectona grandis* Linn. F. (teak) seedlings. Int J Bio Sci Bio Technol 7(1):19–28

Sheng XF, He LY (2006) Solubilization of potassium-bearing minerals by a wild-type strain of Bacillus Edaphicus and its mutants and increased potassium uptake by wheat. Can J Microbiol 52:66–72

Siddiqui IA, Haque SE, Zaki MJ et al (2000) Effect of rhizobacteria in the control of root infecting fungi and root knot nematode on tomato. In: Plant diseases of national economic importance and their management. Proceedings of the second international conference of plant pthology. Department of Plant Pathology, University of Agriculture, Faisalabad, p 183–189

Siddiqui IA, Haque SE, Shaukat SS (2001) Use of rhizobacteria in the control of root-rot knot disease complex of mungbean. J Phytopathol 149:337–346

Sidhu OP, Behl HM (1997) Response of three Glomus species on growth of *Prosopis juliflora* Swartz at high pH levels. Symbiosis 23:23–34

Singh R, Adholeya A, Mukerji KG (2000) Mycorrhiza in control of soil borne pathogens. In: Mukerji KG (ed). Proceedings of mycorrhizal biology. New York: Kluwer Academic Publishers, p 173–196

Sokolova MG, Akimova GP, Vaishlia OB (2011) Effect of phytohormones synthesized by rhizosphere bacteria on plants. Prikl Biokhim Mikrobiol 47:302–307

Song YY, Zeng RS, JF X et al (2010) Interplant communication of tomato plants through underground common mycorrhizal networks. PLoS One 5(10):1–11. doi:10.1371/journal.pone.0013324

Soni ML, Beniwal RK, Yadava ND et al (2008) Spatial distribution of soil organic carbon under agroforestry and traditional cropping system in hyper arid zone of Rajasthan. Ann Arid Zone 47(1):103–106

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic Press, London

Smith FA, Smith SE (1997) Structural diversity in (vesicular)-arbuscular mycorrhizal symbiosis. New Phytol 137:373–388

Srivastava KK, Mohan V, Verma N (2001) Impact of VAM inoculation on some semi-arid zone tree species. Indian Forester 127:936–940
Stanley MR, Koide RT, Shumway D (1993) Mycorrhizal symbiosis increases growth reproduction and recruitment of *Abutilon theophrasti* medic in the field. Oecologia 94:30–35
Subbarao NS (1997) Biofertilizers in agriculture and forestry. Oxford & IBH publishing Co. Pvt. Ltd, New Delhi
Subramanian KS, Charest C, Dwyer LM et al (1997) Effects of arbuscular mycorrhizae on- leaf water potential, sugar content, and P content during drought and recovery of maize. Can J Bot 75:1582–1591
Tam PCF (1995) Heavy metal tolerance by ectomycorrhizal fungi and metal amelioration by *Pisolithus tinctorius*. Mycorrhiza 5:181–187
Tewari SK (2008) Farm forestry. Report G.B. Pant University of Agriculture and Technology, Pantnagar
Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Nautiyal CS, Mittal S, Tripathi AK, Johri BN (2005) Diversity of plant growth and soil health supporting bacteria. Curr Sci 89(1):136–150
Tilak KVBR, Subba Rao NS (1987) Association of *Azospirillum brasilense* with pearl millet (*Pennisetum americanum* (L.) Leeke). Biol Fertil Soils 4:97–102
Thomas RS, Dakessian S, Ames RN (1986) Aggregation of a silty clay loam soil by mycorrhizal onion roots. Soil Soc Am J 50:1494–1499
Verma RK, Jamaluddin, Thakur AK (2008) Effect of Biofertilizers on Growth of Aonla (*Emblica officinalis*) in nursery. Indian Forester 134(1):125–130
Wagner SC (2012) Biological nitrogen fixation. Nat Educ Knowledge 3(10):15
Wall LG (2000) The actinorhizal symbiosis. J Plant Growth Regul 19:167–182
Wilcox HE (1991) Mycorrhizae. In: Waisel Y, Eshel A, Kafkafi U (eds) The plant root, the hidden half. Marcel Dekker, New York, pp 731–765
Wright SF, Upadhyaya A (1999) Quantification of arbuscular mycorrhizal fungi activity by the glomalin concentration on hyphal traps. Mycorrhiza 8:283–285
Yadav RS, Yadav BL, Chhipa BR et al (2010). Soil biological properties under different tree based traditional agroforestry systems in a semi-arid region of Rajasthan, India. Springer Science Business Media B.V.
Yagi K, Matsumoto T, Chujo T (2000) Isolation and characterization of low Indole −3- acetic acid producing mutants from *Bradyrhizobium*. Biosci Biotechnol Biochem 64:1359–1364
Young JPW (1992) Phylogenetic classification of nitrogen-fixing organisms. In: Stacey G, Burris RH, Evans HJ (eds) Biological nitrogen fixation. Chapman and Hall, New York, pp 43–86

Web Reference

http://upenvis.nic.in/database/forest_838.aspx. Updated: 16/02/2016
http://agritech.tnau.ac.in/org_farm/orgfarm_biofertilizers.html

Video Reference

Center for Food Safety. (2016). Soil solutions to climate problems – narrated by Michael Pollan. Video. See: http://www.centerforfoodsafety.org/video/2519/cfs-videos/soil-solutions/4134/soil-solutions-to-climate-problems–narrated-by-michael-pollan#

Effect of Biofertilizers on Biomass Yield and Quality of *Ocimum basilicum* L.

20

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Abstract

Microbes in the natural environment are important to maintain physical and biological status of the soil. The long-term use of chemical fertilizers caused several adverse effects to the health and environment and reduced the soil microflora. Nowadays biofertilizers are used as the best alternative to chemical fertilizers. Biofertilizers are considered as a gift of modern agriculture which are eco-friendly and growth-promoting organisms. They enhance the growth of plants by producing growth hormones and by solubilizing nutrients. The present study was aimed to assess the influence of *Azophos* on growth, physiological, nutrient, and yield parameters of *Ocimum basilicum* against synthetic fertilizers. The results proved the efficiency of Azophos on growth and yield of *Ocimum basilicum*.

20.1 Introduction

Microbes are omnipresent which is the universal truth. In today's world, yet another truth is developing that hazardous chemicals are omnipresent. The chemicals enter directly or indirectly into the environment and daily life of humans. But the alarming cause is chemicals are penetrating to the human body through foods and natural

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medicine which are exposed to fertilizers. At the time of green revolution, the roles of chemical fertilizers are significant, and they are recommended for crop growth and to improve the soil fertility. Most of the developing countries, including India, import these fertilizers, which are often in limited supply and are at unaffordable cost for poor farmers. Later, these fertilizers are identified as xenobiotics and are progressively concentrated in each link of food chain, a process called biomagnifications.

Promiscuous use of chemical fertilizers causes several environmental hazards including pollution, contamination of soil and water resources, depletion of microorganism and eco-friendly insects, development of unsound crops, reduction in soil fertility (Subba Roa 2001), and eutrophication (Boy and Arcad 2013). The enormous use of synthetic fertilizers in intensive agriculture has adverse effects on physicochemical properties of the soils (Khan et al. 2009a, b) and negative effect on root growth and root colonization by mycorrhizal fungi (Smith and Read 2008). When the chemical usage to crops reaches its theoretical maximum, there will not be any expected increase in crop yields (Ahmed 1995). Therefore, the search for economical and eco-friendly fertilizer resulted in several organic fertilizers that act as natural stimulators for plant growth and development (Khan et al. 2009a, b).

One scientific approach involves microbiological enrichment of liquid plant growth promoters with consortia of beneficial microbes (Sas Paszt et al. 2015). The application of microbial inoculums has a long history that dates back to the origin of compost production and passed through generation of farmers. This is recognized when the cultures accelerate the decomposition of organic residues and agricultural by-products through various processes and give healthy harvest of crops (Abdul Halim 2009). The long-term effects of beneficial microbes on plant growth rely on their adaptation and survival in the prevailing environmental condition, and their efficiency was enhanced by the application of native mycorrhizal fungi (Regvar et al. 2003).

In developing countries like India, microbial inoculants are used as biofertilizers which are economical and environment-friendly. The biofertilizers contain live or latent cells of efficient strains of nitrogen-fixing, phosphate-solubilizing, or cellulolytic microorganisms. They are mixed with seed and soil or in a composting area with the aim to improve the microbial count and induce some microbial process for enhancing the availability of nutrients in a form which can assimilate by plants (Khosro and Yousef 2012). Malusá and Vassilev (2014) defined biofertilizers as "the formulated product containing one or more organisms that enhances the nutrient status (the growth and yield) of the plants by either replacing soil nutrients and/or by making nutrients more available to plants and/or by increasing plant access to nutrients." They are symbiotic or asymbiotic, play crucial role in atmospheric nitrogen fixation, solubilize insoluble soil phosphate, and thus stimulate the productivity by increasing the soil fertility (Venkatashwarlu 2008). They have a significant role in the adsorption of elements like P, Zn, Cu, C, S, Ca, K, Mn, Cl, and Br (Tinker 1984). For sustainable agriculture, most of the naturally occurring PGPR (plant growth-promoting rhizobacteria) are used as biofertilizers worldwide to increase the crop yield (Khalid et al. 2004). Biofertilizers aid the plants in promoting

Fig. 20.1 Mechanism of PGPR

productivity, resistance, and immunity by secreting growth substances and secondary metabolites (Subba Rao 2002) (Fig. 20.1).

Ocimum basilicum ranks among the most important aromatic and medicinal plants from the time of old civilization and belongs to Lamiaceae. The family contains economically useful herb which forms a rich source of many naturally occurring aroma chemicals, which are great perfume, flavoring, and pharmaceutical value. It has been receiving a good deal of attention as a source of valuable essential oil content primarily found in leaves. In recent years, the farmers and industrialists are interested in cultivation for its medicinal value. The production level of Indian basil oil is still short of its local demand; besides, there exist good possibilities for the export of oil in the world market. The extractable essential oils have been shown to contain biologically active constitutes which are insecticidal, nematicidal, fungicidal, and bactericidal. The research focused on the influence of *Azophos* on the growth, biomass, and nutrient uptake of *Ocimum basilicum*.

20.2 Materials and Methods

The present study was carried out in a farm in Pannimadai village, Coimbatore District, Tamil Nadu, India. The treatment includes inorganic fertilizers and biofertilizers.

20.2.1 Treatment Details

Control (C) – NPK in the ratio of 40:30:20 kg/ha
Test (T) – NPK (40:30:20) + 2 kg *Azophos* (*Azospirillum*/*Phosphobacterium*; 1:1)/ha

20.2.2 Methods

The effects of these fertilizers on growth, physiological, yield, and nutrient parameters were measured using standard methods.

20.2.3 Growth and Yield Parameters

The growth parameters, viz., plant height, primary branch, secondary branch, plant spread, number of leaves per plant, fresh weight and fresh herbage yield/ha, and dry herbage yield/ha, were measured 90 days after treatment.

20.2.4 Physiological Parameters

The physiological parameters which include leaf area (Balyan 1981), leaf area index (Williams 1946), crop growth rate (Watson 1956), chlorophyll (SPAD meter), and relative growth rate were estimated.

20.2.5 Soil and Plant Nutrient Analysis

Available nitrogen (Subbaiah and Asija 1956), available phosphorous (Olsen et al. 1954), and available potassium (Hanway and Heidal 1952) in soil and nitrogen (Humphries 1956), phosphorous (Jackson 1973), and potassium (Jackson 1973) in leaf sample were analyzed.

20.3 Results and Discussion

The present study reveals that the incorporation of biofertilizers showed increased growth, biomass, and nutrient uptake than control (Fig. 20.2).

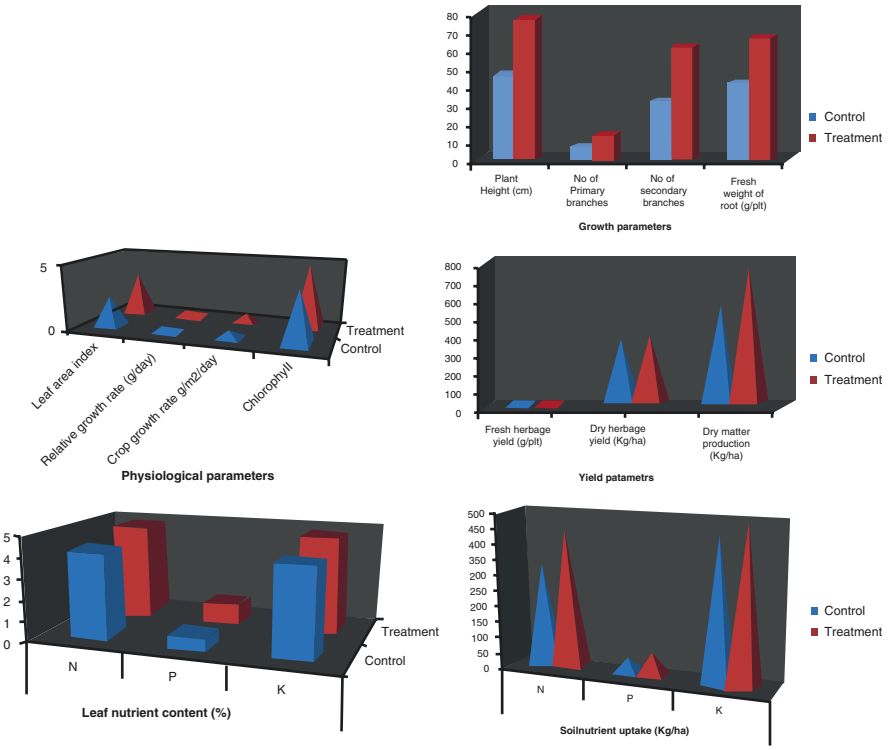

Fig. 20.2 Effect of biofertilizers on *Ocimum basilicum* L.

The plants treated with *Azophos* showed greater leaf area which decides the photosynthetic effect. This aids the crop to synthesize more metabolites exhibiting high photosynthetic rate and good source-sink relationship during the period of growth and development of crop (Nykanen 1989). The accumulation of photosynthates in sink relates with the vegetative yield of the crop (Hedge and Srinivas 1989). Nitrogen and phosphorus had a dramatic effect on photosynthetic efficiency of the crop and support the vegetative growth.

For optimum plant growth, nutrients must be available in sufficient and balanced quantities (Chen 2006). Nitrogen fixation is the second most important process following photosynthesis in crop production. Nitrogen fixation can provide for free up to 300–400 kg/N/ha/year (Adam et al. 2002). In biological nitrogen fixation, the atmospheric dinitrogen is fixed in the form of ammonia and converted to nitrate which was the available form of nitrogen to crops by several soil microbes. It was reported that in plants, up to 25% of total nitrogen came from nitrogen fixation. The activity of nitrogen-fixing microbes depends on the rich amount of available carbon and low level of combined nitrogen (Andrew et al. 2007). The conversion of nitrogen gas to ammonia is mediated by an enzyme called nitrogenase, which was produced by a group of bacteria called diazotrophs (Desbrosses and Stougaard 2011). Most of the diazotrophs are free-living or symbiotic, and some of them are

associative or endophytic such as *Azospirillum* sp., *Azoarcus* sp., and *Herbaspirillum* (Santi et al. 2013). Roots of plants release substances into the soil, which support colonization and nitrogen-fixing activity of bacteria in rhizosphere of plants (Nghia and Gyurjan 1987).

In the research based on genetic, biochemical, and applied studies, *Azospirillum* is considered one of the best-studied plant growth-promoting bacteria (Vande Broek et al. 2000). The genus *Azospirillum*, a rhizosphere bacterium, is comprised of species that are gram-negative to gram-variable, motile, curved to rod shaped, and oxidase positive and exhibits acetylene reduction activity under microaerophilic condition. The genus *Azospirillum* comprises of seven species so far, namely, *A. brasilense*, *A. lipoferum*, *A. amazonense*, *A. halopraeferens*, *A. irakens* (Bashan and Levanony 1990; Bashan and Holguin 1997), *A. largomobile* (Dekhil et al. 1997), and *A. doebereinerae* (Eckert et al. 2001).

The interaction of *Azospirillum* with plants has been studied since 1970. *Azospirillum* predominantly inhabit the root surface (Dobereiner and Baldani 1995); some are in the root interior and injured cortical cells (Baldani et al. 1986). The beneficial effect of *Azospirillum* significantly associated with its nitrogen fixation and effect on root development (Noshin et al. 2008). The growth-promoting capacities of *Azospirillum* greatly depend on its ability to produce phytohormones relatively than nitrogen fixation (Fulchieri et al. 1993; Dobbelaere et al. 2001) especially indole acetic acid (Remans et al. 2008), gibberellic acid (Bottini et al. 1989), abscisic acid (Cohen et al. 2009), cytokinin (Schmidt et al. 1988), ethylene (Perrig et al. 2007), and polyamines (Bashan et al. 2004). The adsorption of water and uptake of minerals also facilitate the production of crops (Dobbelaere et al. 2001). The inoculation of *Azospirillum* is found to be effective against drought stress in wheat (El-Komy et al. 2003) and plant pathogens in tomato (Bashan and de-Bashan 2002).

Phosphorus plays a major role in crop improvement next to nitrogen, being a component of nucleic acids, phospholipids, and adenosine triphosphate (ATP) and key factor in the metabolic process like cell division, cell development, energy transport, signal transduction, macromolecular biosynthesis, photosynthesis, and respiration (Ahemad et al. 2009; Khan et al. 2009a, b). Phosphorus is available to plants in two soluble forms, i.e., monobasic (H_2PO_4) and dibasic (HPO_4) (Glass 1989). But the large amount of P present in soil was insoluble in nature and therefore not available to plants. Predominantly P found in soil as insoluble mineral complexes in the form of soil organic matter and inorganic compounds (Richardson and Simpson 2011). Therefore, the availability of P depends on its solubility and could be influenced by PGPR in the soil. The insoluble P is made available through solubilization of inorganic P and mineralization of organic P. The phosphate-solubilizing bacteria produce organic acids, siderophores, and hydroxyl ions to solubilize inorganic soil phosphates, such as $Ca_3(PO_4)_2$, $FePO_4$, and $AlPO_4$ (Chen et al. 2006; Sharma et al. 2013).

The P-solubilizing microbes are reported to produce organic acids like citric acid, gluconic acid, malic acid (Mendes et al. 2013), lactic acid, maleic acid, acetic acid, tartaric acid, and fumaric acid (Akintokun et al. 2007). Production of organic

acid was recorded as an effective methodology of P-solubilizing microbes. These organic acids decrease the pH (Pradhan and Shukla 2005) and cause acidification of microbial cell, and the habitat resulted in release of P ions from the mineral complex (Trivedi and Sa 2008) as a result of anion exchange. The solubilization of P following the mechanism of chelation and reduction to solubilize phosphorus was reported in *Trichoderma harzianum* (Reyes et al. 2001). Other than organic acid, some inorganic acids like hydrochloric acid (Kim et al. 1997), nitric acid, and sulfuric acid (Dugan and Lundgren 1965) are involved in P solubilization.

The process of mineralization is mediated by enzymes like phosphatases (Tarafdar and Claassen 1988) and phytases (Maougal et al. 2014). The influence of phosphate-solubilizing bacteria on plant growth depends on the production of IAA, gibberellic acid (Viruel et al. 2011), and antibiotics (Lipping et al. 2008), and it acts as a biocontrol agent (Singh et al. 2010). The use of phosphate-solubilizing bacteria represents a good alternative for chemical fertilizers. *Micrococcus*, *Pseudomonas*, *Bacillus*, and *Flavobacterium* were reported to be efficient phosphate solubilizers (Pindi and Satyanarayana 2012).

The increased leaf area index of the crop might be due to increased auxin, carbohydrates, and organic compounds synthesized by biofertilizers (Arularasu 1995). The enhanced nitrogen fixation of biofertilizers leads to the secretion of amino acids and thus influences the leaf area (Sardana 1997).

The increased leaf area simultaneously influences the content of chlorophyll. The present study reveals the highest chlorophyll content in plants provided with *Azophos*. Several researchers exposed the relation between chlorophyll and nitrogen content (Evans 1983; Amaliotis et al. 2004). Chlorophyll content is proportional to the leaf nitrogen content (Evans 1983). The nitrogen availability by *Azospirillum* resulted in increased number of chlorophylls.

The better production of dry matter by the plant may be due to nitrogen fixation by microbes, regulation of nitrogen supply to the plants, and production of plant growth promoters (Krishnamoorthy and Ravikumar 1973), and the effect of such growth hormones on photosynthesis and translocation of assimilates could be the cause for the enhanced biomass (Herold 1980). Similarly, the availability of nitrogen makes the plant to synthesize protein and other enzymes, which results in highest plant height. The presence of higher optimal P is essential for numerous metabolic processes of photosynthesis and respiration, which will enhance the root proliferation and thereby plant growth (Shenoy and Kalagudi 2005).

The increase in the number of branches might be due to the enhanced vegetative growth, because of increased cell division, and meristematic cell elongation in the axillary buds in turn trigged the activity and increases the supply of photosynthates and thereby increases the laterals. Cytokinins produced by the biofertilizers might be responsible for increased laterals due to arrest of apical dominance, and therefore the plant spread was increased (Torry 1950).

The application of phosphorus increased the root growth, and this is in accordance with the studies reported by Farooqi et al. (1991) in *Artemisia pallens*, Krishnamoorthy and Madalageri (2002) in *Trachyspermum ammi*, Sundharaiya et al. (1998) in *Solanum khasianum*, and Lakshmipathiah et al. (1999) in *Psoralea*

corylifolia. The well-established root system encourages the easy availability of nutrients from the soil to the plant parts. The inoculation of *Azospirillum* also enhanced the root growth. Changes in the plant root morphology, *viz.*, root elongation (Dobbelaere et al. 1999) and development of lateral roots (Creus et al. 2005) and root hairs (Hadas and Okon 1987), was reported in several plant species and significantly improved the root system.

The favorable physical and chemical changes in soil due to the addition of inorganic nutrients along with *Azophos* primarily enhanced the plant height. The application of nitrogen and phosphorus along with *Azophos* might have promoted higher vegetative yield, which corresponds with the findings of Ramesh Babu (1996).

Application of NPK along with *Azophos* recorded the highest phosphorus and potassium content at 90 days after treatment. *Phosphobacteria* present in *Azophos* played a significant role in increasing the level of phosphate available to plants by dephosphorylating phosphorus bearing organic compounds and also influenced the soil microbiota leading to the solubilization of phosphate sources (Somani et al. 1990; Kumar and Narula 1999; Kumar et al. 2001). The inoculation of *Phosphobacteria* in Azophos made the unavailable form of phosphorus into an available form of energy supply; this has been also supported by Sharma et al. (2013). Since *Azospirillum* enhances the root surface area, phosphorus was absorbed by diffusion; the increased root surface increased the uptake of nutrients.

A linear increase in potassium content may be due to the addition of potassium fertilizer in the soil. Application of Azophos recorded the highest K content in leaf. Azophos enhanced the availability of nitrogen. Thus in turn increased the potassium content in leaf in order to maintain the potassium content to balance the plant nutrients.

Conclusion

Ocimum basilicum is a holistic medicinal plant with vast curative properties. The use of chemical fertilizers may affect the biochemical constituent of the plant and is perilous to the environment. On the other hand, biofertilizers are eco-friendly and cost-effective and secure the nature of the plant without any contamination. In the present study, result shows that the inoculation of *Azophos* enhanced the plant growth. *Azospirillum* and *Phosphobacterium* present in Azophos enhance the root proliferation, shoot growth, photosynthesis, nutrition uptake by fixing nitrogen, solubilization of minerals, and producing phytohormones. Using biofertilizers, a low-input system can be carried out, and it can be helped in achieving healthy plantation.

References

Abdul Halim NB (2009) Effects of using enhanced biofertilizer containing N-fixer bacteria on patchouli growth. Thesis, Faculty of Chemical and Natural Resources Engineering University Malaysia Pahang, p 145

Adam SM, Abdalla AM, Risk FA (2002) Effect of the interaction between the mineral and biofertilizer on the productivity of cantaloupe (*Cucumis melo* L.) under the newly reclaimed soils conditions. Egypt J Hortic 29:301–315

Ahemad M, Zaidi A, Khan MS, Oves M (2009) Biological importance of phosphorus and phosphate solubilizing microorganisms—an overview. In: Khan MS, Zaidi A (eds) Phosphate solubilizing microbes for crop improvement. Nova, New York, pp 1–4

Ahmed S (1995) Agriculture – fertilizer interface in Asia issues of growth and sustainability. Oxford and IBH publishing Co, New Delhi

Akintokun AK, Akande GA, Akintokun PO, Popoola TOS, Babalola AO (2007) Solubilization of insoluble phosphate by organic acid producing fungi isolated from Nigerian soil. Int J Soil Sci 2:301–307

Amaliotis D, Therios I, Karatissiou M (2004) Effect of nitrogen fertilization on growth, leaf nutrient concentration and photosynthesis in three peach cultivars. Acta Hortic 449:36–42

Andrew JW, Jonathan D, Andrew R, Lei S, Katsaridou NN, Mikhail S, Rodionov AD (2007) Living without Fur: the subtlety and complexity of iron-responsive gene regulation in the symbiotic bacterium *Rhizobium* and other a-proteobacteria. Biometals 20:501–511

Arularasu P (1995) Effect of graded doses of nitrogen and spacing on yield of herbage and oil in Tulsi (*Ocimum sanctum*. L) M.Sc (Hort.). Thesis, TNAU, Coimbatore, Tamil Nadu

Baldani VB, Alvarez I, Baldani I, Dobereiner J (1986) Establishment of inoculated *Azospirillum* spp. in the rhizosphere and in the roots of field grown wheat and sorghum. Plant Soil 90:35–46

Balyan SS (1981) Leaf area determination of some aromatic plants by a quick and non-destructive method. Indian Perfumer 25(1):46–50

Bashan Y, de-Bashan LE (2002) Reduction of bacterial speck (*Pseudomonas syringae* pv. tomato) of tomato by combined treatments of plant growth-promoting bacterium, *Azospirillum brasilense*, streptomycin sulfate, and chemothermal seed treatment. Eur J Plant Pathol 108:821–829

Bashan Y, Holguin G (1997) *Azospirillum*-plant relationships: environmental and physiological advances (1990–1996). Can J Microbiol 43:103–121

Bashan Y, Levanony H (1990) Current status of *Azospirillum* inoculation technology: *Azospirillum* as a challenge for agriculture. Can J Microbiol 36:591–608

Bashan Y, Holguin G, de-Bashan LE (2004) *Azospirillum*–plant relationships: physiological, molecular, agricultural, and environmental advances (1997–2003). Can J Microbiol 50:521–577

Bottini R, Fulchieri M, Pearce D, Pharis RP (1989) Identification of gibberellins A1, A3 and iso-A3 in cultures of *Azospirillum lipoferum*. Plant Physiol 90:45–47

Boy J, Arcad Y (2013) Current trends in green technologies in food production and processing. Food Eng Rev 5:1–17

Chen J (2006) The combined use of chemical and organic fertilizer and or biofertilizer for crop growth and soil fertility. International workshop on sustained management of the soil-rhizosphere system for efficient crop production and fertilizer use, October, pp 16–20

Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC (2006) Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl Soil Ecol 34:33–41

Cohen AC, Travaglia CN, Bottini R, Piccoli PN (2009) Participation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. Botany 87:455–462

Creus CM, Graziano M, Casanovas EM, Pereyra MA, Simontacchi M, Puntarulo S, Barassi CA, Lamattina L (2005) Nitric oxide is involved in the *Azospirillum brasilense*-induced lateral root formation in tomato. Planta 221:297–303

Dekhil SB, Cahill M, Stackebrandt E, Sly LI (1997) Transfer of *Conglomeromonas largomobiliz* subsp *largomobiliz* to the genus *Azospirillum* as *Azopirillum largomobile* comb nov., and elevation of *Conglomeromonas largomobiliz* subsp *parooensis* to the new type species of *Conglomeromonas*, *Conglomeromonas parooensis* sp nov. Syst Appl Microbiol 20:72–77

Desbrosses GJ, Stougaard J (2011) Root nodulation: a paradigm for how plant-microbe symbiosis influences host developmental pathways. Cell Host Microbe 10:348–358

Dobbelaere S, Croonenborghs A, Thys A, Vande Broek A, Vanderleyden J (1999) Phytostimulatory effect of *Azospirillum brasilense* wild type and mutant strains altered in IAA production on wheat. Plant Soil 212:155–164

Dobbelaere S, Croonenborghs A, Thys A (2001) Responses of agronomically important crops to inoculation with *Azospirillum*. Aust J Plant Physiol 28:871–879

Dobereiner J, Baldani VLD (1995) Endophytic diazotrophs other than rhizobia. In: Fendrik I, del gallo M, Vanderleyden J, de Zamarocy M (eds) *Azospirillum* IV and related microorganism. Springer, Berlin, pp 1–6

Dugan P, Lundgren DG (1965) Energy supply for the chemoautotroph *Ferrobacillus ferrooxidans*. J Bacteriol 89:825–834

Eckert B, Weber OB, Kirchhof G, Halbritter A, Stoffels M, Hartmann A (2001) *Azospirillum doebereinerae* sp. nov., a nitrogen-fixing bacterium associated with the C4-grass *Miscanthus*. Int J Syst Evol Microbiol 51:17–26

El-Komy HM, Hamdia MA, El-Baki GKA (2003) Nitrate reductase in wheat plants grown under water stress and inoculated with *Azospirillum* spp. Biol Plant 46:281–287

Evans JR (1983) Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L). Plant Physiol 72:297–302

Farooqi AA, Davaiah KA, Dasharatha Rao ND, Vasundhara M, Raju B (1991) Effect of nutrient of growth, yield and essential oil content in Davana. J Med Aromat Plant Sci 23:75–87

Fulchieri M, Lucangeli C, Bottini R (1993) Inoculation with *Azospirillum lipoferum* affects growth and gibberellin status of corn seedling roots. Plant Cell Physiol 34:1305–1309

Glass ADM (1989) Plant nutrition: an introduction to current concepts. Jones and Bartlett Publishers, Boston, p 234

Hadas R, Okon Y (1987) Effect of *Azospirillum brasilense* inoculation on root morphology and respiration in tomato seedlings. Biol Fertil Soils 5:241–247

Hanway J, Heidal H (1952) Soil analysis method as used in Iowa state college. Agric Bull 57:1–13

Hedge DM, Srinivas K (1989) Optimization of nutrient management in field crops. Indian J Agron 34:180–184

Herold A (1980) Regulation of photosynthesis by S and K activity the missing link. New Phytol 86:131–144

Humphries EC (1956) Mineral components and ash analysis in modern methods of plant analysis. Springer, Berlin, pp 408–502

Jackson ML (1973) Soil chemical analysis. Prentice Hall of India (pvt) Ltd., New Delhi

Khalid A, Arshad M, Zahir ZA (2004) Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. J Appl Microbiol 96:473–480

Khan MS, Zaidi A, Wani PA, Ahemad M, Oves M (2009a) Functional diversity among plant growth-promoting rhizobacteria. In: Khan MS, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, Berlin, pp 105–132

Khan W, Rayirath UP, Subramanian S (2009b) Seaweed extracts as biostimulants of plant growth and development. J Plant Growth Regul 28:386–399

Khosro M, Yousef S (2012) Bacterial biofertilizers for sustainable crop production: a review. ARPN J Agric Biol Sci 7:307–316

Kim KY, Jordan D, Krishnan HB (1997) *Rahnella aquatilis*, bacterium isolated from soybean rhizosphere, can solubilize hydroxyapatite. FEMS Microbiol Lett 153:273–277

Krishnamoorthy V, Madalageri MB (2002) Effect of nitrogen and phosphorus nutrition on growth of ajowan genotypes (*Trachyspermum ammi*). J Med Aromat Plant Sci 24(1):45–49

Krishnamoorthy KK, Ravikumar V (1973) Comparative effectiveness of organic and mineral fertilizers on Dernopodzolic soils. Soil Ferti 30:2920

Kumar V, Narula N (1999) Solubilization of inorganic phosphates by *Azotobacter chroococcum* mutants and their effect on seed emergence of wheat. Biol Fertil Soil 28:301–305

Kumar V, Behl RK, Narula N (2001) Establishment of P- solubilizing analogue resistant strains of *Azotobacter chroococcum* in rhizosphere and their effect on wheat under greenhouse conditions. Microbial Res 156(1):87–94

Lakshmipathiah OR, Farooqiand AA, Sreeramu BS (1999) Influence of nitrogen, phosphorus and potassium on growth and yield of babchi (*Psoralea corylifolia* L.) Mysore J Agric Sci 33(4):323–327

Lipping Y, Jiatao X, Daohong J, Yanping F, Guoqing L, Fangcan L (2008) Antifungal substances produced by Penicillium oxalicum strain PY-1 potential antibiotics against plant pathogenic fungi. World J Microbiol Biotechnol 24:909–915

Malusá E, Vassilev N (2014) A contribution to set a legal framework for biofertilizers. Appl Microbiol Biotechnol 98:6599–6607

Maougal RT, Brauman A, Plassard C, Abadie J, Djekoun A, Drevon JJ (2014) Bacterial capacities to mineralize phytate increase in the rhizosphere of nodulated common bean (*Phaseolus vulgaris*) under P deficiency. Eur J Soil Biol 62:8–14

Mendes GO, Dias CS, Silva IR, Junior JIR, Pereira OL, Costa MD (2013) Fungal rock phosphate solubilization using sugarcane bagasse. World J Microbiol Biotechnol 29:43–50

Nghia N, Gyurjan H (1987) Problems and perspectives in establishment of nitrogen- fixing symbioses and endosymbioses. Endocyt C Res 4:131–141

Noshin I, Asghari B, Sumera I (2008) Variation in *Rhizobium* and *Azospirillum* strains isolated from maize growing in arid and semiarid areas. Int J Agric Biol 10:612–618

Nykanen I (1989) The effect of cultivation condition on the composition of basil oil (*Ocimum basilicum*). Flavor Fragr J 4(3):125–128

Olsen SE, Cola CI, Watnable FS, Deen DA (1954) Estimation of available phosphorus in soil by extraction with sodium bicarbonate. USDA (Inc) 939

Perrig D, Boiero ML, Masciarelli OA, Penna C, Ruiz OA, Cassan FD, Luna MV (2007) Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense*, and implications for inoculant formulation. Appl Microbiol Biotechnol 75:1143–1150

Pindi PK, Satyanarayana SDV (2012) Liquid microbial consortium—a potential tool for sustainable soil health. J Biofertil Biopest 3:1–9

Pradhan N, Shukla LB (2005) Solubilization of inorganic phosphates by fungi isolated from agriculture soil. Afr J Biotechnol 5:850–854

Ramesh Babu TI (1996) Nutritional studies in Ashwagandha, M.Sc (Ag). Thesis, TNAU, Coimbatore

Regvar M, Vogel-Mikuš K, Ševerkar T (2003) Effect of AMF inoculums from field isolates on the yield of green pepper, parsley, carrot and tomato. Folia Geobot 38:223–234

Remans R, Beebe S, Blair M, Manrique G, Tovar E, Rao I, Croonenborghs A, Torres-Gutierrez R, El-Howeity M, Michiels J, Vanderleyden J (2008) Physiological and genetic analysis of root responsiveness to auxin-producing plant growth-promoting bacteria in common bean (*Phaseolus vulgaris* L.) Plant Soil 302:149–161

Reyes I, Baziramakenga R, Bernier L, Antoun H (2001) Solubilization of phosphate rocks and minerals by a wild-type strain and two UV induced mutants of *Penicillium rugulosum*. Soil Biol Biochem 33:1741–1747

Richardson AE, Simpson RJ (2011) Soil microorganisms mediating phosphorus availability. Plant Physiol 156:989–996

Santi C, Bogusz D, Franche C (2013) Biological nitrogen fixation in non-legume plants. Ann Bot:1–25

Sardana V (1997) Agronomic evaluation of bioinoculants to supplement inorganic fertilizers for sustained crop production: a critical review. Agric Res 18:69–95

Sas Paszt L, Malusá E, Sumorok B, Canfora L, Derkowska E, Głuszek S (2015) The influence of bioproducts on mycorrhizal occurrence and diversity in the rhizosphere of strawberry plants under controlled conditions. Adv Microbiol 5(1):40–53

Schmidt W, Martin P, Omay H, Bangerth F (1988) Influence of *Azospirillum brasilense* on nodulation of legumes. In: Klingmüller W (eds) Azospirillum IV, genetics, physiology, ecology. Springer, Heidelberg, pp 92–100

Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springerplus 2:587

Shenoy VV, Kalagudi GM (2005) Enhancing plant phosphorus use efficiency for sustainable cropping. Biotechnol Adv 23:501–513

Singh N, Kumar S, Bajpai VK, Dubey RC, Maheshwari DK, Kang SC (2010) Biological control of *Macrophomina phaseolina* by chemotactic fluorescent *Pseudomonas aeruginosa* PN1 and its plant growth promotory activity in chir-pine. Crop Prot 29:1142–1147

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Elsevier and Academic, New York

Somani LL, Bhandari SC, Vyas KK, Saxena SN (1990) Biofertilizers. Scientific Publishers, Jodhpur, pp 271–294

Subba Rao NS (2002) Soil microbiology. In: Soil microorganisms and plant growth, 4th edn. Oxford and IBH Publication Company, New Delhi, pp 211–228

Subba Roa NS (2001) An appraisal of biofertilizers in India. In: Kannaiyan S (ed) The biotechnology of biofertilizers. Narosa Pub. House, New Delhi

Subbaiah BV, Asija GL (1956) A rapid procedure for estimation of available nitrogen in soil. Curr Sci 25:259–260

Sundharaiya K, Nainar P, Ponuswami V, Jaya Jasmine A, Muthuswamy M (1998) Effect of Inorganic nutrients and spacing on the yield of marunthukathiri (*Solanum khasianum* Clarke). South Indian Hortic 48(1-6):168–171

Tarafdar JC, Claassen N (1988) Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. Biol Fertil Soils 5:308–312

Tinker PB (1984) Plant Soil 76:77–91

Torry JC (1950) The induction of lateral shoots by IAA and decapitation. Am J Bot 37:257–264

Trivedi P, Sa T (2008) *Pseudomonas corrugata* (NRRL B-30409) mutants increased phosphate solubilization, organic acid production, and plant growth at lower temperatures. Curr Microbiol 56:140–144

Vande Broek A, Dobbelaere S, Vanderleyden J, Van Dommelen A (2000) *Azospirillum* –plant root interactions: signaling and metabolic interactions. In*:* Triplett EW (ed) Prokaryotic nitrogen fixation: a model system for the analysis of a biological process. Horizon Scientific Press, Wymondham, pp 761–777

Venkatashwarlu B (2008) Role of bio-fertilizers in organic farming: organic farming in rain fed agriculture: Central Institute for Dry Land Agriculture*,* Hyderabad, 85–95

Viruel E, Lucca ME, Siñeriz F (2011) Plant growth promotion traits of phosphobacteria isolated from Puna, Argentina. Arch Microbiol 193:489–496

Watson DJ (1956) Comparative physiological studies on the growth of field crops. I. Variation in net assimilation rate and leaf area between species and varieties and within and between years. Ann Bot 11:41–76

Williams RF (1946) The physiology of plants growth with special response to the concept of net assimilation rate. Ann Bot 10:41–72

PGPR: Current Vogue in Sustainable Crop Production

21

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Abstract

Nowadays a large scale of crop produce are pesticide ridden. Heavy application of these hazardous pesticides is not only very costly which leaves financial burden to the farmers but is also harmful to our biodiversity leading to loss of various endangered living species. However, growers are being trained worldwide, and they are progressively switching over their agriculture from chemical or conventional agriculture to organic or sustainable agriculture. Sustainable agriculture reveals crop cultivation with "no chemicals." But organically cultivated produce are mirage due to their exorbitant prices, at least for the urban dwellers. To resolve this conundrum, the role of plant growth-promoting rhizobacteria (PGPR) has been discussed in the process of plant growth promotion, with their mechanisms and their importance in crop production on sustainable basis. The application of PGPR strain is conducive and creates thrust toward organic farming at every level of farmers, whether it be large landowner or small-scale farmers. However, PGPR strain performance varies from lab to field and even from field to field due to host specificity. Besides, some strains of PGPR have the potential to promote growth of a particular plant, while in another plant they do not respond. There are various ways that promote plant growth such as N_2 fixation, P solubilization, siderophore production, phytohormone production, and also the control of phytoparasitic pathogens. In addition to the beneficial role, some important aspects of negativity inducted by the PGPR have also been discussed. Sustainable agriculture, if done in the light of PGPR module, will not only remove the financial burden of the farmers but also prove to be conducive, congenial, and putative. Further studies to commercialize the potent strain of PGPR are stridently needed which will unravel certain yet to be explored mechanisms.

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21.1 Introduction

The total world population is expected to increase by 8.5 billion by 2030 (Anonymous 2015). This significant population increase is surmised due to unchecked and consistent increase in the population of developing or underdeveloped countries. This significant increase brings about the poverty and hunger. India has been home to 194.6 million undernourished people, the highest in the world (Anonymous 2015). To obviate this issue, sustainable crop production is the best weapon known so far against poverty and hunger especially powerful in underdeveloped countries. Microorganisms are the best living entities providing the best ecological services in the sustenance of ecological balance. Thus, a group of bacteria that help in plant growth promotion by exhibiting beneficial inputs on crop plant are known as plant growth-promoting rhizobacteria (PGPR) (Zhou et al. 2016). They do have some advanced diagnostic features such as colonization of the host's rhizosphere, rhizoplane, and the interior region of the root system. Besides, some bacteroides make the way to enter inside the root building up endophytic population which ultimately benefits the crop plants (Compant et al. 2005). Similarly, some bacterial species are able to enhance the root surface area providing essential nutrients that reach to the plant, thereby inducing plant productivity (Adesemoye and Kloepper 2009). The thread line toward the role of biofertilizers in nutrient uptake and environment stress management has provided a relaxation to the researchers up to some extent but not fully. Hence, we are in urgent need to manage these stresses through eco-friendly ways. Many countries are utilizing PGPR as biofertilizers in sustainable agriculture and also forcing nearby nations to use them in a proper way (Singh et al. 2011). However, there are some issues/factors to use PGPR, such as performance of strains under field conditions, because it has been seen that bacterial strains having the same biological potential do not respond under the field conditions that may be due to failure in the host's root colonization. To eliminate the food issues for the crowded population, natural biofertilizers in sustainable module are being used. It has been a well-established fact that application of suitable PGPR strain enhances the productivity under favorable climate conditions (Okon and Labandera-González 1994; Singh et al. 2011). A large number of genera of PGPR have been applied worldwide to check the potentiality in plant growth promotion and found to possess great potential in sustainable crop production such as silviculture, horticulture, and environmental remediation (Jeffries et al. 2003; Reed and Glick 2005; Fravel 2005; Aeron et al. 2011; Karličić et al. 2016). The role of different organic molecules released by PGPR like indoleacetic acid (Park et al. 2005; Shao et al. 2015), gibberellic acid (Mahmoud et al. 1984; Ortega-Baes and Rojas-Aréchiga 2007; Castillo et al. 2015), and cytokinins (Amara et al. 2015) is appreciable to various extents in agriculture. In addition, plant hormones such as IAA and cytokinin-producing PGPR are found to be conducive growth promoters of various horticultural crops, *Sesamum indicum*, *Trifolium repens*, *Arachis hypogaea*, *Cajanus cajan*, *Trigonella foenum*, *Mucuna pruriens*, *Pinus roxburghii*, and *Mimosa pudica* (Noel et al. 1996; Hirsch et al. 1997; Kumar et al. 2005). Growth stimulation in plant through PGPR has been observed through various mechanisms such as colonization of plant root,

plant growth stimulation, and reduction in plant disease (Kloepper and Schroth 1978). To unravel the complex mechanisms involved in rhizobial interactions is a very important issue in the determination of the sustainability; however, some abiotic factors such as temperature, soil nature, smog, etc. can't be avoided. Because varying temperatures have good binding with aeration, pH gradient promotes the microbial growths (Shen et al. 2015).

Application of PGPR into soil must be evaluated meticulously. However, the indigenous strain may trigger defense mechanism induction which helps in the reduction of the pathogen potential by releasing root flavonoids (Parmar and Dufresne 2011). Therefore, during the microorganism selection, extreme care through rigorous filed studies to fully understand interactive traits is needed which may ease the expected turmoil. Besides, PGPR provide the potential role in the biotic and abiotic stress reduction, also help in the elimination of pesticides' residual effects, and thereby help in the plant and microflora development through sustainable ways (Khan 2005; Kang et al. 2010; Xun et al. 2015).

Moreover, for successful colonization and proliferation of PGPR, interaction among the microorganisms is necessary especially between the local strains. The bacterial population around the rhizosphere remains always higher than the population existing through the soil (Lynch and Leij 1990). These aspects have made a clear note that the higher amount of nutrient remains available around the root region. Conjoint application of compatible traits accelerates symbiotic activities which help in the enhanced nutrient acquisition by switching on some gene that allows recognition and release of root exudates (Verma and Yadav 2012).

21.2 The Rhizosphere: Dwelling Point for PGPR

The rhizosphere is considered to be the most important portion of the ecological habitat in soil where PGPR along with other microorganisms remain in close contact with the roots of the plant (Brink 2016). PGPR may have some specific alliances with plants which may have provided the role in growth enhancement. Production of some biomolecules for plant growth promotion such as phytohormones, metabolites, etc. may modify the rhizosphere microbiota and environment affecting microbial diversity associated with the rhizosphere (Frankenberger and Arshad 1995; Davison 1988). Different types of close association in bacteria with roots may be formed such as on root surface (rhizoplane) and soil just after the root (rhizosphere) (Brink 2016). PGPR respond to various processes like exchange of signal molecules and nutrients and colonize the root tissue creating a protection layer of root tissues. In addition, mucigel consists of plant mucilage, bacterial exopolymers, and soil particles of the immediate layer of rhizobacteria. It has been reported that plant roots covered by mucigel have higher water content than noncovered ones; hence, mucigel plays a crucial role in the root protection and protects from dehydration (Miller and Wood 1996). In addition, contents of root exudates help in the enrichment and selection of bacteria and ultimately help in the healthy rhizosphere formation. Plant root exudates act as source of carbon for microbial

growth. Besides, there are certain organic molecules which perform chemotaxis of microbes within the rhizosphere. In addition, root exudates are much helpful in the maintenance of steady concentration of some flavonoids and mineral nutrients, flocked after decomposition of organics and other recycled wastes (Dakora and Phillips 2002). Thus depending upon the nature and types of organics, released flavonoids and other molecules, specific PGPR diversity develops into the rhizosphere. Several PGPR have the ability to attach with roots and extract the nutrients from soil making them available to the plants. More specifically, some strains of PGPR have been found to penetrate the root tissue and make direct communication with the organic nutrients present in the apoplast (Gupta et al. 2017).

21.3 Mechanisms of Actions

Generally there are two types of mechanisms involved in the plant growth promotion, i.e., (1) direct and (2) indirect.

21.3.1 Direct Mechanism

21.3.1.1 Nitrogen Fixation

Nitrogen (N) is an important element for growth and development; hence, it is surmised to be very essential. However, 78% N_2 present in the atmosphere is not available to the growing plants. Generally, N_2 is converted into a useable form through nitrogen fixation process where nitrogen changes to ammonia through nitrogenase enzyme (Kim and Rees 1994). Biological nitrogen fixers are ubiquitous in nature, and available around the world, they function at mild temperature (Raymond et al. 2004). They are economically sound, beneficial, eco-friendly, and alternative to hazardous pesticides. Around two-thirds of global nitrogen is fixed through biological nitrogen fixation process (Rubio and Ludden 2008).

Generally, two categories of nitrogen-fixing organisms are found: (1) symbiotic nitrogen-fixing bacteria (rhizobia) which includes members of the family *Rhizobiaceae* forming symbiosis with leguminous plants (Ahemad and Khan 2010; Zahran 2001) and nonleguminous plants (*Frankia*) and (2) nonsymbiotic nitrogen-fixing bacteria such as cyanobacteria, *Azotobacter*, *Azospirillum*, *Azoarcus*, *Gluconacetobacter diazotrophicus*, etc. (Bhattacharyya and Jha 2012). Although nitrogen-fixing bacteria make available only a short amount of the fixed nitrogen to the plants (Glick 2012), interestingly, some other type of symbiotic nitrogen-fixing bacteria infects the root and establishes symbiosis with the roots of crop plants.

In the establishment of the symbiotic relationship, dinitrogenase reductase containing iron protein and dinitrogenase having metal cofactors are involved (Minamisawa et al. 2016). Dinitrogenase reductase gives electrons with high reducing energy, while dinitrogenase forming metal cofactor uses these electrons to reduce N_2 to NH_3. There are three nitrogen-fixing cofactors such as (1) Mo-nitrogenase, (2) V-nitrogenase, and (3) Fe-nitrogenase. Structure wise, nitrogen-fixing living

system varies from genus to genus; mostly nitrogen fixation process is completed by the activity of the molybdenum-nitrogenase (Bishop and Jorerger 1990). The nitrogen fixation process is carried out by an enzyme known as nitrogenase complex (Kim and Rees 1994).

21.3.1.2 Phosphate Solubilization

The second important plant growth-limiting nutrient is phosphorus (P) after nitrogen; this is available in plenty in both organic and inorganic forms (Khan et al. 2009). Despite having a large reservoir of P in the soil, the sufficient amount of P to the plant is not reachable due to availability of P into H_2PO_4 forms which are inaccessible to the plants (Bhattacharyya and Jha 2012). The insoluble P is available in the soil and remains in an inactive state as inorganic mineral forms like apatite or as organic forms such as inositol phosphate, phosphotriesterase, and phosphomonoesters (Glick 2012). To obviate the P deficiency in soils, farmers have started to apply phosphatic fertilizers in agricultural lands. Plants obtain fewer amounts of applied fertilizers, and the rest is rapidly converted into insoluble forms of P in the soil which are reserved again and reach beyond the catch limits of the plants (Mckenzie and Roberts 1990). Importantly, continuous application of P is not a solution because regular application of these P fertilizers is not only very costly to the farmers but is also an unsafe means to the environments. Moreover, organisms having phosphate-solubilizing activity, known as phosphate-solubilizing microorganisms (PSM), help in the availability of P to the plants (Khan et al. 2006). PSB are considered to be a supplier of P in P-limited soil and replenish the P through various means (Zaidi et al. 2009). Some bacteria such as *Serratia*, *Microbacterium*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Erwinia*, *Flavobacterium*, *Pseudomonas*, *Enterobacter*, *Rhizobium*, and *Beijerinckia* are known to be the important and ecologically sound rhizobacteria (Bhattacharyya and Jha 2012). Solubilization of inorganic phosphorus is carried out by the action of organic acids (low molecular weights) which have been synthesized by various PGPR groups (Zaidi et al. 2009). A large number of phosphatase enzymes catalyzing the hydrolysis phosphoric esters are involved in the mineralization (Glick 2012). Moreover, phosphate solubilization and mineralization may occur in the same bacterial species simultaneously (Tao et al. 2008).

21.3.1.3 Siderophore Production

Iron is a key element for all microorganisms to thrive well; however, certain lactobacilli, are an exception (Neilands 1995). In some environments, iron does not occur in the accessible form, but they are available in plenty as an inaccessible form (Rajkumar et al. 2010). Generally, bacteria catch iron atoms through organic molecules which act as an iron chelator, siderophores having high iron-binding affinities. Generally, water-soluble siderophores are common, and they are categorized into extracellular and intracellular siderophores (Khan et al. 2009). In gram-positive and gram-negative bacteria, Fe^{+3} in Fe^{3+}-siderophore complex on the membrane of bacteria is reduced to Fe^{2+} which is accessible to bacterial membrane, further released into the cell through gating mechanisms of inner and outer membrane of bacteria (Ansari et al. 2016). However, there may

be loss of some amount of siderophores (Rajkumar et al. 2010; Neilands 1995). Hence, it may be concluded that siderophore acts as iron solubilizers under an iron-limited environment (Indiragandhi et al. 2008). Besides iron, some other heavy metals like Al, Cd, Cu, Ga, In, Pb, and Zn are being chelated by siderophores (Neubauer et al. 2000). In addition, siderophore complex enhances the solubility of metal concentration (Rajkumar et al. 2010). Therefore, bacterial released chelating molecules assist well in the alleviation of stress imposed on plant by heavy metals (Schmidt 1999). Plenty of research have advocated well for plant growth promotion as a result of siderophore releasing bacterial applications (Rajkumar et al. 2010; Ansari et al. 2016). Crowley and Kraemer (2007) reported that siderophores released by bacteria help iron to be made available to the oat, and the plant has mechanisms for utilization of complex under iron-deprived environment. Moreover, *Pseudomonas fluorescens* C7 enhanced the iron content significantly in plant tissue and improved plant yield (Vansuyt et al. 2007). Inoculation of *Pseudomonas* strain GRP3 on iron nutrition of *Vigna radiata* resulted in a decline in chlorotic injuries and enhanced plant growth (Sharma et al. 2003).

21.3.1.4 Phytohormone Production

Most of PGPR isolated from the soil especially rhizosphere have the ability to synthesize and release phytohormones like IAA as secondary molecules (Patten and Glick 1996). Generally, IAA released by PGPR may alter the growth and development of the plant because endogenous pool of plant IAA may be deviated by the enhanced acquisition of IAA (Glick 2012; Spaepen et al. 2007). Moreover, IAA also plays a crucial role in plant defense mechanisms against a wide range of phytopathogenic bacteria (Spaepen and Vanderleyden 2011). Thus, IAA released by PGPR is recognized as effective molecules and plays a role in pathogenesis and phytostimulation (Spaepen and Vanderleyden 2011). It has been reported that IAA is a significant factor in various cellular processes, such as cell division, differentiation, and vascular bundle formation, and also surmised that auxins play a role in the nodule formation (Glick 2012; Spaepen et al. 2007). It is reported that application of *Rhizobium leguminosarum* bv. *viciae* enhanced 60-fold more root nodules than uninoculated ones (Camerini et al. 2008). In spite of these, certain environmental factor regulates the IAA biosynthesis in different genera of PGPR (Spaepen et al. 2007).

21.3.1.5 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase

Ethylene is an essential hormone for carrying out normal plant growth and development (Khalid et al. 2006). This phytohormone is produced by almost all plants and plays an important role in the reduction of multifarious physiological changes in plants. In addition, ethylene is also considered to be a stress hormone (Saleem et al. 2007). It has been reported that under deprived conditions due to various environmental factors such as extreme drought, water logging, heavy metals, and pathogenicity, the ethylene reaches to its elevated level and affects negatively the plant, thereby reducing the crop growth and development (Saleem et al. 2007; Bhattacharyya

and Jha 2012). PGPR possess enzymes, e.g., 1-aminocyclopropane-1-carboxylate (ACC) deaminase, to help in plant biomass enhancement by reducing the ethylene level (Nadeem et al. 2007; Zahir et al. 2008). Some bacterial strains possessing ACC deaminase activity have been documented such as *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; Zahir et al. 2009; Kang et al. 2010). These bacterial genera have the ability to convert ACC to 2-oxobutanoate and NH_3 (Arshad et al. 2007). Various types of biotic and abiotic stress have been relaxed by ACC deaminase producers (Glick 2012; Lugtenberg and Kamilova 2009). Besides, these PGPR help in the root elongations, seed formation, and enhancement in root nodule formation (Nadeem et al. 2007; Shaharoona et al. 2008; Nadeem et al. 2009; Glick 2012).

21.3.2 Indirect Mechanism

Management of plant disease through the application of bioagents is an eco-friendly and novel approach (Lugtenberg and Kamilova 2009; Rizvi et al. 2016; Ansari et al. 2016). Significant indirect mechanisms of plant growth promotion in PGPR through biocontrol agents have been discussed (Glick 2012). Generally, food competitions, niche exclusions, induction of systemic resistance, and anti-fungal metabolite production are the main mode of biological control of PGPR. A large number of PGPR have been reported to produce antifungal metabolites such as HCN, phenazines, pyrrolnitrin, 2,4-diacetylphloroglucinol, pyoluteorin, viscosinamide, and tensin (Bhattacharyya and Jha 2012). In addition, proper synchronization of PGPR with root leads to development of plant resistance against some pathogenic bacteria, fungi, and viruses (Rizvi et al. 2016). This process is known as induced systemic resistance (ISR) (Lugtenberg and Kamilova 2009).

Under natural environment having stress, mechanisms used by the PGPR for plant growth promotion are common. However, under stress conditions some strains of PGPR fail to survive because of inability to tolerate the stress. But the significant increase in plant growth takes place by various mechanisms, for example, reduction in stress-induced ethylene level, production of exopolysaccharides, induced systemic resistance, etc. (Glick et al. 2007; Saharan and Nehra 2011; Sandhya et al. 2009; Saravanakumar et al. 2007; Upadhyay et al. 2011). As far as stress management is concerned, plant growth is affected by nutritional perturbations such as elevation in Na^+ which causes iron toxicity and disrupts the usual uptake of various essential ions. Some strains of PGPR protect crop plants from excessive Na^+ concentration by producing exopolysaccharides and through biofilm transformations which ultimately reduce Na^+ uptake (Geddie and Sutherland 1993; Khodair et al. 2008; Qurashi and Sabri 2012). In addition, PGPR protect plants from phytopathogens through various mechanisms such as antibiosis, competition, and parasitism (Beneduzi et al. 2012; Cassells and Rafferty-McArdle 2012;

Deshwal et al. 2003; Gula et al. 2013; Heydari and Pessarakli 2010; Khokhar et al. 2012; Perneel et al. 2008; Ping and Boland 2004). PGPR adopt one or more mechanisms for crop protection. PGPR check the phytopathogens' growth by antibiosis mechanisms where antimicrobial compounds inhibit pathogen's growth released by bacteria (Glick 1995). Similarly, PGPR have also been reported to check availability of iron required for pathogens which is necessary for plant growth. (Subba Rao 1993).

It is enough to conclude that PGPR accelerate plant growth by deploying some mechanisms and help in the crop protection from various deleterious plant pathogens which directly or indirectly affect the plant growth. In addition, there may be some specificity in the bacterial genera, i.e., some mechanisms may be present in one particular strain while absent in another.

21.4 Commercialization of PGPR

Different strains of bacteria have responded to various extents under different climatic environment. This may be due to different climatic factors and edaphic factor which are considered to affect the performance of beneficial PGPR (Zaidi et al. 2009). The importance of PGPR has generated an impetus to commercialize the PGPR in the industrial level so that potential strains of PGPR may be exploited from the soil and transferred to the farmers' even low scale of land (Table 21.1).

Table 21.1 Various strains of plant growth-promoting rhizobacteria (PGPR) exerting beneficial impact on plant health

S. no.	PGPR	Role	Reference
1	*Bacillus megaterium*, *Arthrobacter chlorophenolicus*, and *Enterobacter* sp.	Enhanced plant growth and yield attributed by solubilization of phosphorus; nitrogen fixation; production of phytohormones such as auxins, cytokinins and gibberellins; sequestering of iron by production of siderophores; and lowering of ethylene concentration	Idris et al. (2004)
2	*Azotobacter*, *Bacillus*, *Enterobacter*, and *Xanthobacter*	Significantly enhanced nitrogen accumulation, growth and grain yield of rice plants	Mirzai et al. (2010), Bal et al. (2013), Khalid et al. (2009), and Singh et al. (2011).
3	*Bacillus lentimorbus*	Enhanced plant growth as well as antioxidant capacity of the edible parts of spinach, carrots and lettuce, under salinity and drought stress	Nautiyal et al. (2008), Ahmad et al. (2013), and Naveed et al. (2014)

S. no.	PGPR	Role	Reference
4	*Pseudomonas aeruginosa*	Improved the growth of *Vigna radiata* (mung beans) plants under drought conditions. PGPR-inoculated plants tend to improve the water-use efficiency of plants. Hence, these bacteria can be beneficial to the environment in terms of reducing excessive usage of water	Sarma and Saikia (2014), Ahmad et al. (2013), and Naveed et al. (2014)
5	*Bacillus megaterium* and *Pantoea agglomerans*	Inoculation of these bacteria into maize roots increased the ability of the root to absorb water under the salinity conditions. Here, bacteria that can grow under hypersaline conditions were better able to colonize the root rhizospheres and external spaces of roots that are themselves exposed to high-salinity conditions	Marulanda et al. (2010) and Gond et al. (2015)
6	*Azospirillum brasilense*	Improved salt tolerance of the jojoba plant during in vitro rooting	Gonzalez et al. (2015)
7	*Azospirillum*	Inoculation of lettuce plants with *Azospirillum* sp. not only improved lettuce quality but also extended the storage life of a lettuce grown under salt stress	Gabriela et al. (2015)
8	*Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Corynebacterium*, *Pseudomonas*, *Rhizobium*, and *Serratia*	Beneficial to the whole rhizosphere microbiota through the highly nutritive and energetic rhizodepositions and, in turn, improved plant growth	Rawat and Mushtaq (2015)
9	*Pseudomonas fluorescens* and *Bacillus subtilis*	Soil application of both *P. fluorescens* and *B. subtilis* alone or in combination was able to reduce the nematode population and improve the onion growth	Munshid et al. (2013)
10	*Azotobacter* sp., *Bacillus cereus, B. megaterium, B. subtilis*	Individual and/or mixed treatment of PGPR when used as a soil drench treatment resulted in reduced root rot/wilt incidence and severity on some evergreen fruit transplants under greenhouse conditions compared with control. The mixed treatment of PGPR gave the highest protection against root rot/wilt diseases compared with the use of individual treatment. Also, all treatments significantly increased plant growth when compared with control treatment	Abdel-Monaim et al. (2014)
11	*Pseudomonas fluorescens*	Singly and in various combinations with botanical enhanced growth and productivity parameters of fenugreek (*Trigonella* sp.)	Rizvi et al. (2013)

(continued)

Table 21.1 (continued)

S. no.	PGPR	Role	Reference
12	*Azospirillum brasilense strain Cd*	Improved plant growth and nutrition as well as reduced root-knot nematode in roots in micropropagated banana	Rodrigues et al. (2008)
13	*Azospirillum brasilense* and *Rhizobium leguminosarum bv. ciceri*	Improved the nodulation of chickpea; the effect of this interaction was further enhanced by organic matter present in the growth medium	Fabbri and Del Gallo (1995)
14	*Azotobacter chroococcum*	Production of growth substances (auxin, gibberellin, etc.) and in turn enhanced crop production	Wani et al. (2013)
15	*Bacillus* spp.	Elicit induced systemic resistance (ISR) and also elicit plant growth promotion	Kloepper et al. (2004)
16	*Bacillus subtilis, Pseudomonas fluorescens*, and *Aspergillus awamori*	Significantly increased plant growth and chlorophyll contents of pathogen-inoculated tomato plants	Singh and Siddiqui (2015)

21.5 Pros and Cons of PGPR Applications

A critical observation pertaining to the administration of any foreign strain of PGPR is done in order to know up to what extent they have adaptability to the native strain. If they are incompatible with each other, there may be some perturbation regarding the performance of the bacteria. Besides, native strain initiates the development of resistance in the plant against deleterious phytopathogens by releasing flavonoids and phytoalexins (Parmar and Dufresne 2011). In order to understand the interactive character of the microorganisms and their utilization as a potential application, rigorous studies on field experiment are required. The rhizosphere is an ideal place for the proliferation of these microorganisms influenced by the various environmental factors like physical, chemical, and biological processes of the root (Sørensen 1997). These microorganisms nurture well in and around the root area of plants which may be due to root exudates which are then used by the microbial growth (Doornbos et al. 2012; Phillips et al. 2011).

21.5.1 Beneficial Aspects of PGPR

PGPR present in the soil environment can cause beneficial alterations in plant health either through the production of plant growth regulators or ameliorating the plant nutrition by enhancing nutrient uptake from the soil (Zahir et al. 2004). Besides, a large number of these rhizobacteria help the plant to overcome several biotic as well as abiotic stresses such as drought, salinity, flooding, and heavy metal toxicity, and hence they capacitate the plant to sustain under adverse environmental situations (Tiwari et al. 2016). Different free-living soil bacterial strains

of a particular genus consist of similar metabolic potential of enhancing plant growth (Gamalero et al. 2009; Belimov et al. 2005; Ma et al. 2011; Nadeem et al. 2007; Sandhya et al. 2009; Zahir et al. 2008). PGPR minimize the detrimental effects of the plant pathogens through several mechanisms that in turn lead to healthy and disease-free plants, thereby improving the plant growth indirectly (Glick and Bashan 1997). This task of PGPR may be fulfilled either by the release of anti-pathogenic substances or by making the plant more resistant against attacking pathogen through activation of induced systemic resistance (Persello-Cartieaux et al. 2003). As far as direct growth promotion is concerned, PGPR adopt different pathways such as providing the host plant a useful compound or facilitating the plant to use the beneficial compounds already present in the soil (Kloepper et al. 1991). They can also help the plant by fixing atmospheric nitrogen and producing siderophores that chelate iron that gets available to the plants. PGPR have also been reported to produce phytohormones and solubilizing nutrients such as phosphorus, thus making it available to the plants (Patten and Glick 2002). The efficiency of these rhizobacteria is also determined by the host plants as well as the soil characteristics (Gamalero et al. 2009). Overall, PGPR enable them to promote plant growth and development by various ways. Some strains utilize more than one mechanism to go through normal as well as stressed environmental conditions. In addition to rhizobacterial strain, plant growth and development also rely on the types of interaction with the host plant and also with the soil environment.

21.5.2 Harmful Aspects of PGPR

PGPR play a valuable role in the sustenance of soil health and enhancement of plant growth and developments; they are also reported to show pernicious effects on plant growth and developmental process (Saharan and Nehra 2011). For instance, *Pseudomonas* species produce cyanide that is implicated to have both advantageous and detrimental effects. Cyanide-producing PGPR not only inhibit the growth of certain pathogens but also cause injurious impact on plant growth (Martínez-Viveros et al. 2010). Moreover, the auxin production by PGPR, depending on its concentration, may be beneficial or detrimental for plant health (Vacheron et al. 2013). A low concentration of auxin promotes plant growth, while at elevated level root retardation has been noticed (Patten and Glick 2002). Another compound rhizobitoxine released by *Bradyrhizobium elkanii* acts in both manners. Being an inhibitor of ethylene synthesis, it can mitigate the adverse effects of stress-induced ethylene on the formation of nodule (Vijayan et al. 2013). But in some cases such as foliar chlorosis in soya bean, it has also been reported to act as a toxin (Xiong and Fuhrmann 1996). Enormous varieties of biosurfactant produced by the microorganisms are being considered as an interesting group of materials for application in various areas of agriculture such as food, health care, biotechnology, and biomedical approaches (Banat et al. 2010). It has also been observed that simultaneous application of PGPR and fungi accelerates to be pathogenic, while PGPR individually remain nonpathogenic

(Banat et al. 2010). The above discussion flashes the light on the negative impacts of PGPR in addition to its positive role. However, these detrimental impacts may take place under certain specific conditions and that too by some distinct strains.

21.6 Conclusion and Future Prospects

High level of hazardous pesticide application is very costly and leaves financial burden to the farmers. Their application also leaves a mark of loss of red data list species. No doubt, different governments have initiated various steps to train the local farmers to cultivate their land in organic ways. Application of PGPR is one of the cost-effective and conducive ways. It is concluded that application of PGPR helps to enhance plant growth through various mechanisms like induction of IAA, P solubilization, and siderophore production. Sometimes it has been seen that consortia of different strains are much more effective than their sole application. More studies on PGPR will increase our knowledge of rhizosphere biology and will provide the new avenues to open for new door for the sustainable agriculture. Application of consortia of different strains of PGPR will help in the nutrient management.

References

Abdel-Monaim MF, EL-Morsi MEA, Hassan MAE (2014) Control of root rot and wilt disease complex of some evergreen fruit transplants by using plant growth promoting rhizobacteria in the New Valley Governorate. Egypt J Phytopathol Pest Manag 1(3):23–33

Adesemoye AO, Kloepper JW (2009) Plant–microbes interactions in enhanced fertilizer-use efficiency. Appl Microbiol Biotechnol 85:1–12

Aeron A, Kumar S, Pandey P, Maheshwari DK (2011) Emerging role of plant growth promoting rhizobacteria in agrobiology. In: Bacteria in agrobiology: crop ecosystems. Springer, Heidelberg, pp 1–36

Ahemad M, Khan MS (2010) Phosphate-solubilizing and plantgrowth- promoting *Pseudomonas aeruginosa* PS1 improves greengram performance in quizalafop-p-ethyl and clodinafop amended soil. Arch Environ Contam Toxicol 58:361–372

Ahmad M, Zahir ZA, Khalid M (2013) Efficacy of Rhizobium and Pseudomonas strains to improve physiology, ionic balance and quality of mung bean under salt-affected conditions on farmer's fields. Plant Physiol Biochem 63:170–176

Amara U, Khalid R, Hayat R (2015) Soil bacteria and phytohormones for sustainable crop production. In: Bacterial metabolites in sustainable agroecosystem. Springer, pp 87–103

Anonymous (2015) India tops world hunger list with 194 million people. The Hindu. http://www.thehindu.com/todays-paper/tp-national/india-tops-world-hunger-list-with-194-million-people/article7257822.ece. Accessed 22 Oct 2016

Ansari RA, Rizvi R, Mahmood I, Safiuddin, Sumbul A (2016) Siderophore: augmentation in plant and soil health. In: Kumar V et al (eds) Probiotics and agroecosystem. Springer, Singapore. ISBN 978-981-10-4058-0 (in press)

Arshad M, Saleem M, Hussain S (2007) Perspectives of bacterial ACC deaminase in phytoremediation. Trends Biotechnol 25:356–362

Bal H, Nayak B, Das L, Subhasis A, Tapan K (2013) Isolation of ACCdeaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. Plant Soil 366:93–105

Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG, Fracchia L, Smyth TJ, Marchant R (2010) Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotehnol 87:427–444

Belimov AA, Hontzeas N, Safronova VI, Demchinskaya SV, Piluzza G, Bullitta S, Glick BR (2005) Cadmium-tolerant plant growth promoting rhizobacteria associated with the roots of Indian mustard (*Brassica juncea* L. Czern.) Soil Biol Biochem 37:241–250

Beneduzi A, Ambrosini A, Passaglia LMP (2012) Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol 35:1044–1051

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Bishop PE, Jorerger RD (1990) Genetics and molecular biology of an alternative nitrogen fixation system. Plant Mol Biol 41:109–125

Brink SC (2016) Unlocking the secrets of the rhizosphere. Trends Plant Sci 21(3):169–170

Camerini S, Senatore B, Lonardo E, Imperlini E, Bianco C, Moschetti G, Rotino GL, Campion B, Defez R (2008) Introduction of a novel pathway for IAA biosynthesis to rhizobia alters vetch root nodule development. Arch Microbiol 190:67–77

Cassells AC, Rafferty-McArdle SM (2012) Priming of plant defenses by PGPR against fungal and bacterial plant foliar pathogens. In: Maheshwari DK (ed) Bacterai in agrobiology: stress management. Springer, Berlin, pp 1–26

Castillo P, Molina R, Andrade A, Vigliocco A, Alemano S, Cassán FD (2015) Phytohormones and other plant growth regulators produced by PGPR: the genus Azospirillum. In: Handbook for *Azospirillum*. Springer, pp 115–138

Compant SB, Reiter A, Sessitsch J, Nowak C, Clement E, Barka A (2005) Endophytic colonization of *Vitis vinifera* L. by plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. App Environ Microbiol 71:1685–1693

Crowley DE, Kraemer SM (2007) Function of siderophores in the plant rhizosphere. In: Pinton R et al (eds) The rhizosphere, biochemistry and organic substances at the soil-plant interface. CRC Press, pp 73–109

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245:35–47

Dashti N, Zhang F, Hynes R, Smith DL (1997) Application of plant growth-promoting rhizobacteria to soybean [Glycine max (L.) Merrill] increases protein and dry matter yield under short-season conditions. Plant Soil 188:33–41

Davison J (1988) Plant beneficial bacteria. Nat Biotechnol 6:282–286

Deshwal VK, Dubey RC, Maheshwari DK (2003) Isolation of plant growth-promoting strains of Bradyrhizobium (Arachis) sp. with biocontrol potential against Macrophomina phaseolina causing charcoal rot of peanut. Curr Sci 84:443–448

Doornbos RF, van Loon LC, Bakker PA (2012) Impact of root exudates and plant defense signaling on bacterial communities in the rhizosphere. A review. Agron Sustain Dev 32:227–243

Fabbri P, Del Gallo M (1995) Specific interaction between chickpea (Cicer arietinum) and three chickpea-Rhizobium strains inoculated singularly and in combination with Azospirillumbrasilense Cd. In: Fendrik I, Del Gallo M, Vanderleyden J, de Zamaroczy M (eds) Azospirillum VI and related microorganisms, genetics – physiology -ecology, vol G37, NATO ASI Series, Series G: Ecological sciences. Springer, Berlin, pp 207–212

Frankenberger Jr WT, Arshad M (1995) Phytohormones in soils: microbial production and function. Marcel Dekker Inc

Fravel DR (2005) Commercialization and implementation of biocontrol 1. Annu Rev Phytopathol 43:337–359

Gabriela F, Casanovas EM, Quillehauquy V, Yommi AK, Goni MG, Roura SI, Barassi CA (2015) Azospirillum inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress. Sci Hortic 195:154–162

Gamalero E, Berta G, Glick BR (2009) The use of microorganisms to facilitate the growth of plants in saline soils. In: Khan MS, Zaidi A, Musarrat J (eds) Microbial strategies for crop improvement. Springer, Berlin

García de Salamone IE, Hynes RK, Nelson LM (2001) Cytokinin production by plant growth promoting rhizobacteria and selected mutants. Can J Microbiol 47(5):404–411
Geddie JL, Sutherland IW (1993) Uptake of metals by bacterial polysaccharides. J Appl Bacteriol 74:467–472
Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117
Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Hindawi Publishing Corporation, Scientifica
Glick BR, Bashan Y (1997) Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. Biotechnol Adv 15:353–378
Glick BR, Cheng Z, Czarny J, Cheng Z, Duan J (2007) Promotion of plant growth by ACC deaminase-producing soil bacteria. Eur J Plant Pathol 119:329–339
Gond SK, Bergen MS, Torres MS, White JF, Kharwar RN (2015) Effect of bacterial endophyte on expression of defense genes in Indian popcorn against *Fusarium moniliforme*. Symbiobosis
Gonzalez AJ, Larraburu EE, Llorente BE (2015) Azospirillum brasilense increased salt tolerance of jojoba during in vitro rooting. Ind Crop Prod 76:41–48
Gula A, Ozaktanb H, Kıdogluc F, Tuzela Y (2013) Rhizobacteria promoted yield of cucumber plants grown in perlite under Fusarium wilt stress. Sci Hortic 153:22–25
Gupta A, Gupta R, Singh RL (2017) Microbes and environment. In: Principles and applications of environmental biotechnology for a sustainable future. Springer, Singapore, pp 43–84
Haghighi BJ, Alizadeh O, Firoozabadi AH (2011) The role of plant growth promoting rhizobacteria (PGPR) in sustainable agriculture. Adv Environ Biol 5:3079–3083
Heydari A, Pessarakli M (2010) A review on biological control of fungal plant pathogens using microbial antagonists. J Biol Sci 10:273–290
Hirsch AM, Fang Y, Asad S, Kapulnik Y (1997) The role of phytohormones in plant-microbe symbioses. Plant Soil 194:171–184
Idris EES, Bochow H, Ross H, Boriss F (2004) Use of Bacillus subtilis as biocontrol agent. 6. Phytohormone action of culture filtrate prepared from plant growth promoting Bacillus amyloliquefaciens FZB24, FZB42, FZB45 and *Bacillus subtilis* FZB37. J Plant Dis Prot 111:583–597
Indiragandhi P, Anandham R, Madhaiyan M, Sa TM (2008) Characterization of plant growth-promoting traits of bacteria isolated from larval guts of diamondback moth *Plutella xylostella* (Lepidoptera: Plutellidae). Curr Microbiol 56:327–333
Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea J (2003) The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37:1–16
Kang BG, Kim WT, Yun HS, Chang SC (2010) Use of plant growth-promoting rhizobacteria to control stress responses of plant roots. Plant Biotechnol Rep 4:179–183
Karličić V, Ćurguz VG, Raičević V (2016) The alleviation of reforestation challenges by beneficial soil microorganisms. Reforesta 1(1):238–260
Khalid A, Akhtar MJ, Mahmoo MH, Arshad M (2006) Effect of substrate-dependent microbial ethylene production on plant growth. Microbiology 75:231–236
Khalid A, Arshad M, Shaharoona B, Mahmood T (2009) Plant growth promoting rhizobacteria and sustainable agriculture. In: Khan MS et al (eds) Microbial strategies for crop improvement. Springer, Berlin, p 133
Khan AG (2005) Role of soil microbes in the rhizospheres of plants growing on trace metal contaminated soils in phytoremediation. J Trace Elem Med Biol 18(4):355–364
Khan MS, Zaidi A, Wani PA (2006) Role of phosphatesolubilizing microorganisms in sustainable agriculture – a review. Agron Sustain Dev 27:29–43
Khan MS, Zaidi A, Wani PA, Oves M (2009) Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. Environ Chem Lett 7:1–19
Khodair TA, Galal GF, El-Tayeb TS (2008) Effect of inoculating wheat seedlings with exopolysaccharide-producing bacteria in saline soil. J Appl Sci Res 4:2065–2070
Khokhar MK, Gupta R, Sharma R (2012) Biological control of plant pathogens using biotechnological aspects: a review, 1: 277. http://dx.doi.org/10.4172/scientificreports.277

Kim J, Rees DC (1994) Nitrogenase and biological nitrogen fixation. Biochemistry 33:389–397
Kloepper JW, Schroth MN (1978) Plant growth-promoting rhizobacteria on radishes. In: Proceedings of the 4th international conference plant pathogenic bacteria. Angers, France
Kloepper JW, Zablotowick RM, Tipping EM, Lifshitz R (1991) Plant growth promotion mediated by bacterial rhizosphere colonizers. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Kluwer Academic Publishers, Dordrecht, pp 315–326
Kloepper JW, Ryu CM, Zhang S (2004) Induced systemic resistance and promotion of plant growth by Bacillus spp. Phytopathology 94:1259–1266
Kumar K, Gupta SC, Baidoo SK, Chander Y, Rosen CJ (2005) Antibiotic uptake by plants from soil fertilized with animal manure. J Environ Qual 34(6):2082–2085
Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556
Lynch JM, Leij F (1990) Rhizosphere. Wiley
Ma Y, Rajkumar M, Luo Y, Freitas H (2011) Inoculation of endophytic bacteria on host and non-host plants-effects on plant growth and Ni uptake. J Hazard Mater 195:230–237
Mahmoud A, El-Sheikh AM, Baset AS (1984) Germination ecology of *Rhazya stricta* Decne, vol 15. J Coll Sci, KSU, UK, pp 5–25
Manivannan M (2011) Effect of PGPR as biofertilizer on growth and yield of paddy. Int J Pharm Biol Arch 2:6–10
Martínez-Viveros O, Jorquera MA, Crowley DE, Gajardo G, Mora ML (2010) Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. J Soil Sci Plant Nutr 10:293–319
Marulanda A, Azcón R, Chaumont F, Ruiz-Lozano JM, Aroca R (2010) Regulation of plasma membrane aquaporins by inoculation with a *Bacillus megaterium* strain in maize (*Zea mays* L.) plants under unstressed and salt-stressed conditions. Planta 232:533–543
McKenzie RH, Roberts TL (1990) Soil and fertilizers phosphorus update. In: Proceedings of Alberta Soil Science Workshop proceedings, 20–22 February, Edmonton, pp 84–104
Miller KJ, Wood JM (1996) Osmoadaptation by rhizosphere bacteria. Annu Rev Microbiol 50(1):101–136
Minamisawa K, Imaizumi-Anraku H, Bao Z, Shinoda R, Okubo T, Ikeda S (2016) Are symbiotic methanotrophs key microbes for N acquisition in paddy rice root? Microbes Environm 31(1):4
Mirzai A, Vazan S, Naseri R (2010) Response of yield and yield components of safflower (*Carthamus tinctorius* L.) to seed inoculation with *Azotobacter* and *Azospirillum* and different nitrogen levels under dry land condition. World Appl Sci J 11(10):1287–1291
Munshid H, Simon S, Lal AA (2013) Antagonistic potential of bacillus subtilis and *Pseudomonas fluorescens* on *Meloidogyne incognita* of green onion (*allium fistulosum*). Int J Bot Res 3(3):15–22
Nadeem SM, Zahir ZA, Naveed M, Arshad M (2007) Preliminary investigations on inducing salt tolerance in maize through inoculation with rhizobacteria containing ACC deaminase activity. Can J Microbiol 53:1141–1149
Nadeem SM, Zahir ZA, Naveed M, Arshad M (2009) Rhizobacteria containing ACC-deaminase confer salt tolerance in maize grown on salt-affected fields. Can J Microbiol 55:1302–1309
Nadeem SM, Naveed M, Zahir ZA, Asghar HN (2013) Plant-microbe interactions for sustainable agriculture: fundamentals and recent advances. In: Arora NK (ed) Plant microbe symbiosis: fundamentals and advances. Springer, India, pp 51–103
Nautiyal CS, Govindarajan R, Lavania M, Pushpangadan P (2008) Novel mechanisms of modulating natural antioxidants in functional foods: involvement of plant growth promoting rhizobacteria NRRL B-30488. J Agric Food Chem 56:4474–4481
Naveed M, Hussain MB, Zahir ZA, Mitter B, Sessitsch A (2014) Drought stress amelioration in wheat through inoculation with *Burkholderia phytofirmans* strain PsJN. Plant Growth Regul 73:121–131
Neilands JB (1995) Siderophores: structure and function of microbial iron transport compounds. J Biol Chem 270:26723–26726

Neubauer U, Furrer G, Kayser A, Schulin R (2000) Siderophores, NTA, and citrate: potential soil amendments to enhance heavy metal mobility in phytoremediation. Int J Phytoremediation 2:353–368

Noel TC, Sheng C, Yost CK, Pharis RP, Hynes MF (1996) *Rhizobium leguminosarum* as a plant growth-promoting rhizobacterium: direct growth promotion of canola and lettuce. Can J Microbiol 42(3):279–283

Okon Y, Labandera-González C (1994) Agronomic applications of *Azospirillum*: an evaluation of 20 years of worldwide field inoculation. Soil Biol Biochem 26:1591–1601

Ortega-Baes P, Rojas-Aréchiga M (2007) Seed germination of Trichocereus terscheckii (Cactaceae): light, temperature and gibberellic acid effects. J Arid Environ 69(1):169–176

Park M, Kim C, Yang J, Lee H, Shin W, Kim S, Sa T (2005) Isolation and characterization of diazotrophic growth promoting bacteria from rhizosphere of agricultural crops of Korea. Microbiol Res 160(2):127–133

Parmar N, Dufresne J (2011) Beneficial interactions of plant growth promoting rhizosphere microorganisms. In: Singh A et al (eds) Bioaugmentation, biostimulation and biocontrol, soil biology 28. Springer, Berlin, pp 27–42. doi:10.1007/978-3-642-19769-7_2

Patten CL, Glick BR (1996) Bacterial biosynthesis of indole-3-acetic acid. Can J Microbiol 42:207–220

Patten CL, Glick BR (2002) Role of *Pseudomonas putida* indoleacetic acid in development of the host plant root system. Appl Environ Microbiol 68:3795–3801. doi:10.1128/AEM.68.8.3795-3801.2002

Perneel M, D'Hondt L, De Maeyer K, Adiobo A, Rabaey K, Hofte M (2008) Phenazines and biosurfactants interact in the biological control of soil-borne diseases caused by *Pythium* spp. Environ Microbiol 10:778–788

Persello-Cartieaux F, Nussaume L, Robaglia C (2003) Tales from the underground: molecular plant-rhizobacteria interactions. Plant Cell Environ 26:189–199. doi:10.1046/j.1365-3040.2003.00956.x

Phillips RP, Finzi AC, Bernhardt ES (2011) Enhanced root exudation induces microbial feedbacks to N cycling in a pine forest under long-term CO_2 fumigation. Ecol Lett 14(2):187–194

Ping L, Boland W (2004) Signals from the underground, bacterial volatiles promote growth Arabidopsis. Trends Plant Sci 9:263–266

Qurashi AW, Sabri AN (2012) Bacterial exopolysaccharide and biofilm formation stimulate chickpea growth and soil aggregation under salt stress. Braz J Microbiol:1183–1191

Rajkumar M, Ae N, Prasad MNV, Freitas H (2010) Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol 28:142–149

Rawat S, Mushtaq A (2015) Plant growth promoting rhizobacteria, a formula for sustainable agriculture: a review. Asian J Plant Sci Res 5:43–46

Raymond J, Siefert JL, Staples CR, Blankenship RE (2004) The natural history of nitrogen fixation. Mol Biol Evol 21:541–554

Reed MLE, Glick BR (2005) Growth of canola (*Brassica napus*) in the presence of plant growth promoting bacteria and either copper or polycyclic aromatic hydrocarbons. Can J Microbiol 51:1061–1069

Rizvi R, Mahmood I, Tiyagi S (2013) Potential role of organic matters and phosphate solubilizing bacteria (PSB) on the growth and productivity of fenugreek. J Agric Sci Technol 15:639–647

Rizvi R, Mahmood I, Ansari S (2016) Interaction between plant symbionts, bio-organic waste and antagonistic fungi in the management of *Meloidogyne incognita* infecting chickpea. J Saudi Soc Agric Sci

Rodrigues EP, Rodrigues LS, de Oliveira ALM, Baldani VLD, Teixeira KRS, Urquiaga S, Reis VM (2008) Azospirillum amazonense inoculation: effects on growth, yield and N_2 fixation of rice (Oryza sativa L.) Plant Soil 302:249–261

Rubio LM, Ludden PW (2008) Biosynthesis of the iron-molybdenum cofactor of nitrogenase. Annu Rev Microbiol 62:93–111

Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res LSMR, p 21

Saleem M, Arshad M, Hussain S, Bhatti AS (2007) Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. J Indian Microbiol Biotechnol 34:635–648

Sandhya V, Ali SKZ, Grover M, Reddy G, Venkateswarlu B (2009) Alleviation of drought stress effects in sunflower seedlings by exopolysaccharides producing *Pseudomonas putida* strain P45. Biol Fertil Soils 46:17–26

Saravanakumar D, Harish S, Loganathan M, Vivekananthan R, Rajendran L, Raguchander T et al (2007) Rhizobacterial bioformulation for the effective management of Macrophomina root rot in mung bean. Arch Phytopathol Plant Prot 40:323–337

Sarma RK, Saikia RR (2014) Alleviation of drought stress in mung bean by strain Pseudomonas aeruginosa GGRK21. Plant Soils 377:111–126

Schmidt W (1999) Mechanisms and regulation of reduction-based iron uptake in plants. New Phytol 141:1–26

Shaharoona B, Arshad M, Khalid A (2007a) Differential response of etiolated pea seedlings to inoculation with rhizobacteria capable of utilizing 1-aminocyclopropane-1-carboxylate or L-methionine. J Microbiol 45:15–20

Shaharoona B, Jamro GM, Zahir ZA, Arshad M, Memon KS (2007b) Effectiveness of various Pseudomonas spp. and *Burkholderia caryophylli* containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) J Microbiol Biotechnol 17:1300–1307

Shaharoona B, Naveed M, Arshad M, Zahir ZA (2008) Fertilizer-dependent efficiency of Pseudomonads for improving growth, yield, and nutrient use efficiency of wheat (Triticum aestivum L.) Appl Microbiol Biotechnol 79:147–155

Shao J, Xu Z, Zhang N, Shen Q, Zhang R (2015) Contribution of indole-3-acetic acid in the plant growth promotion by the rhizospheric strain Bacillus amyloliquefaciens SQR9. Biol Ferti Soils 51:321–330

Sharma A, Johri BN, Sharma AK, Glick BR (2003) Plant growth-promoting bacterium Pseudomonas sp. strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck). Soil Biol Biochem 35:887–894

Shen C, Ni Y, Liang W, Wang J, Chu H (2015) Distinct soil bacterial communities along a small-scale elevational gradient in alpine tundra. Front Microbiol 6

Singh N, Siddiqui ZA (2015) Effects of Bacillus subtilis, Pseudomonas fluorescens and Aspergillus awamori on the wilt-leaf spot disease complex of tomato. Phytoparasitica 43:61–75

Singh JS, Pandey VC, Singh DP (2011) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. Agric Ecosyst Environ 140:339–353

Sørensen J (1997) The rhizosphere as a habitat for soil microorganisms. In: van Elsas JD, Trevors JT, Wellington EMH (eds) Modern soil microbiology. Marcel Dekker, New York, pp 21–45

Spaepen S, Vanderleyden J (2011) Auxin and plant-microbe interactions. Cold Spring Harb Perspect Biol. doi:10.1101/cshperspect.a001438

Spaepen S, Vanderleyden J, Remans R (2007) Indole-3-acetic acid in microbial and microorganism-plant signalling. FEMS Microbiol Rev 31:425–448

Subba Rao NS (1993) Biofertilizer in agriculture and forestry, 3rd edn. Oxford and IBH, New Delhi

Tao GC, Tian SJ, Cai MY, Xie GH (2008) Phosphate solubilizing and -mineralizing abilities of bacteria isolated from. Pedosphere 18:515–523

Tewari S, Arora NK (2013) Transactions among microorganisms and plant in the composite rhizosphere. In: Arora NK (ed) Plant microbe symbiosis: fundamentals and advances. Springer, pp 1–50

Tiwari S, Lata C, Chauhan PS, Nautiyal CS (2016) *Pseudomonas putida* attunes morphophysiological, biochemical and molecular responses in *Cicer arietinum* L. during drought stress and recovery. Plant Physiol Biochem 99:108–117

Upadhyay SK, Singh JS, Singh DP (2011) Exopolysaccharide-producing plant growth-promoting rhizobacteria under salinity condition. Pedosphere 21:214–222

Vacheron J, Desbrosses G, Bouffaud M, Touraine B, Moenne-Loccoz Y, Muller D (2013) Plant growth-promoting rhizobacteria and root system functioning. Front Plant Sci 4:1–19

Vansuyt G, Robin A, Briat JF, Curie C, Lemanceau P (2007) Iron acquisition from Fe-pyoverdine by Arabidopsis thaliana. Mol Plant Microbe Interact 20:441–447

Verma JP, Yadav J (2012) Evaluation of plant growth promoting rhizobacteria and their effect on plant growth and grain yield of chickpea (Cicer arietinum L.) under sustainable agriculture Production. Agri Sci Engg (ICASE), 127

Vijayan R, Palaniappan P, Tongmin SA, Elavarasi P, Manoharan N (2013) Rhizobitoxine enhances nodulation by inhibiting ethylene synthesis of *Bradyrhizobium elkanii* from Lespedeza species: validation by homology modelingand molecular docking study. World. J Pharm Pharm Sci 2:4079–4094

Wani SA, Chand S, Ali T (2013) Potential use of Azotobacter chroococcum in crop production: an overview. Curr Agric Res 1:35–38. doi:10.12944/CARJ.1.1.04

Xiong K, Fuhrmann JJ (1996) Comparison of rhizobitoxine-induced inhibition of betacystathionase from different bradyrhizobia and soybean genotypes. Plant Soil 186:53–61

Xun F, Xie B, Liu S, Guo C (2015) Effect of plant growth-promoting bacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) inoculation on oats in saline-alkali soil contaminated by petroleum to enhance phytoremediation. Environ Sci Pollut Res 22:598–608

Zahir AA, Arshad M, Frankenberger WT (2004) Plant growth promoting rhizobacteria: applications and perspectives in agriculture. Adv Agron 81:97–168

Zahir ZA, Munir A, Asghar HN, Shaharoona B, Arshad M (2008) Effectiveness of rhizobacteria containing ACC-deaminase for growth promotion of pea (*Pisum sativum*) under drought conditions. J Microbiol Biotechnol 18:958–963

Zahir ZA, Ghani U, Naveed M, Nadeem SM, Asghar HN (2009) Comparative effectiveness of Pseudomonas and Serratia sp. containing ACC-deaminase for improving growth and yield of wheat (*Triticum aestivum* L.) under salt-stressed conditions. Arch Microbiol 191:415–424

Zahran HH (2001) Rhizobia from wild legumes: diversity, taxonomy, ecology, nitrogen fixation and biotechnology. J Biotechnol 91:143–153

Zaidi A, Khan MS, Ahemad M, Oves M (2009) Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiol Immunol Hung 56:263–284

Zhou D, Huang XF, Chaparro JM, Badri DV, Manter DK, Vivanco JM, Guo J (2016) Root and bacterial secretions regulate the interaction between plants and PGPR leading to distinct plant growth promotion effects. Plant Soil 401(1–2):259–272

Application of Bioinoculants for Sustainable Agriculture

22

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Abstract

Agriculture provides the principle means of livelihood for the majority of the Indian population. Hence, there is a need for sustainable agriculture which can be achieved by engineering/manipulating the rhizospheric microflora. The use of biofertilizers (plant growth-promoting rhizobacteria and fungus) is cost-effective and eco-friendly which helps in mobilization of soil nutrients, increasing drought resistance and biocontrol over conventional fertilizers. Application of bioinoculants to the host plants serves as a biofertilizer (P solubilization), a biostimulator (phytohormone production), a stress regulator (drought and salinity), and a biocontrol agent (against phytopathogens). Further research on the exploitation of bioinoculants can be used as an innovative technology in organic farming for better crop productivity.

22.1 Introduction

Agriculture provides principal means of livelihood for majority of Indian population. Agriculture plays an imperative role and is considered as backbone of India's economy. Over 58% of the rural households depend on agriculture for their livelihood. Agriculture, fisheries, and forestry are the major contributors to the gross domestic product. India has attained self-sufficiency in food staples, but the

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productivity is below that of other nations. India is one of the largest exporters of food and agricultural products. A large proportion of India's export trade is based on the agricultural products. A number of industries are based on agricultural produce. Agriculture also provides employment opportunities to very large percentage of population in addition to food and raw material. Increasing population leads to a greater demand of production; hence, the increase in agricultural productivity is the main target to meet the human needs. A remarkable enhancement in the agricultural outputs can be achieved by introducing various improved agricultural methods.

The modern agricultural practices are heavily dependent on the use of chemical pesticides, inorganic fertilizers, and growth regulators which have not only raised the agriculture production but have also resulted in depletion of natural resource, environmental deterioration, and loss of crop diversity. Inorganic fertilizers are already considered as one of the important agents for causing soil pollution. Application of chemical fertilizers even at balanced level affects the soil fertility and productivity under continuous cropping and is unsustainable burden on agricultural system. Modern agriculture is not sustainable in the long run; hence, the concept of sustainable agriculture has emerged in recent years which emphasizes more on the conservation of natural resources and environment. A need for sustainable agriculture can be achieved by engineering the rhizospheric microflora. Sustainable agriculture is very much essential in today's world as it offers the potential to meet the future agricultural needs which are not provided by the conventional agriculture. Recently, the emphasis has shifted to eco-friendly and sustainable agriculture.

Sustainable agriculture is the amalgamation of three main objectives: (1) economic profitability, (2) environmental health, and (3) social and economic equity. Sustainable agricultural practices incorporate variety of approaches. Some of the benefits of sustainable farming include (1) preservation of environment, (2) economic profitability, (3) efficient use of nonrenewable resources, (4) public health protection, and (5) social and economic equity. Some of the specific strategies which have to be considered in sustainable agriculture are topography, soil characteristics, climate, and pests. Soil is believed to be the most vulnerable and delicate living medium which has to be protected and taken care of to safeguard the long-term productivity and stability.

Soil is a dynamic ecosystem which provides a support/medium to the plant's life. It consists of organic matter, minerals, and various organisms. Numerous microorganisms dwell luxuriously in the soil. Rhizosphere is the region of the soil surrounding the plant roots wherein most intensive interactions between the plants and bacterial or fungal partners take place. Microorganisms inhabiting the rhizosphere region of soil play a cardinal role in agriculture by promoting the exchange of plant nutrients and reducing the application of chemical fertilizers to a large extent. There are several mechanisms by which rhizospheric microorganisms stimulate the plant growth (Kiely et al. 2006). Soil microflora mostly consists of free-living microorganisms such as fungi, actinomycetes, PGPR, PSB, and AM fungi. All these organisms contribute to the growth and development of various plants. The organic compounds released from the plant roots serve as nutrients to the microbial community present in the rhizospheric soil. The root exudates serve the dual purpose of increasing microbial population along with augmenting the soil structure. The

microorganism inhabiting the rhizosphere engineers the rooting pattern, activates the plant defense mechanism, and improves nutrient uptake in plants (Cruz et al. 2002; Barea et al. 2005).

In addition to the normal microflora, application of beneficial microorganisms to various horticultural crops helps in better yield and productivity. The sustainable agriculture can be achieved by engineering the rhizospheric microflora. Exploitation of specific microorganisms in the rhizosphere leads to higher microbial diversity in the soil which in turn helps in playing a significant role in maintaining the soil health (Mishra and Sundari 2015; Rodriguez et al. 2007). There are several mechanisms by which rhizospheric microorganisms stimulate the plant growth. Several scientific articles endorse the affirmative effects of microbial inoculation on promotion of plant growth.

22.2 Bioinoculants

Bioinoculants/microbial inoculants are the formulation of advantageous microorganisms that when added to the soil directly or indirectly improves the nutrient availability to the host plants and promotes plant growth. Currently, developments in sustainability involve the exploitation of beneficial microorganisms and use of less available sources of plant nutrients. Organic farming has emerged as an important area and offers long-term sustainability. Bioinoculants when applied as seed treatment or seedling root dip or as a soil application lead to the multiplication of microbes rapidly, thereby helping in developing the thick population in the rhizosphere. Various bacteria, endophytic fungi, arbuscular mycorrhizal fungi, and blue-green algae are found helpful in promoting as well as enhancing the plant growth and yield, respectively.

22.2.1 Arbuscular Mycorrhizal Fungi as a Bioinoculant

Arbuscular mycorrhizal fungi (AM fungi) are a group of fungi that have the ability to colonize the plant roots and form a symbiotic association with the plants (Schulz and Boyle 2005). Arbuscular mycorrhizal fungi are aseptate in nature and are characterized by the formation of typical structures like arbuscles and vesicles found in the root cortex. Arbuscles are the main site of communication between the host and the fungus. The vesicles are hyphal swellings present in the root cortex that acts as a storage organ to AM fungi. These structures may be intra- and intercellular and develop into thick walls in older roots. These thick-walled structures may function as propagules (Biermann and Linderman 1983). The hyphae of AM fungi form a bridge connecting the plant root with large areas of the soil and serve as a channel to direct the nutrients to the plants. They help in increasing the contact between the plant roots and greater soil area.

Symbiotic association of AM fungi with plants also plays a major role in alleviating the plant salt stress. Arbuscular mycorrhizal fungi improve the soil quality and health. Mycorrhizal plants notably absorb more phosphorus (Parniske 2008) from the soil than nonmycorrhizal plants under stressful conditions. Recently, co-inoculation of AM fungi (*Rhizophagus intraradices* and *Rhizophagus fasciculatus*)

along with *Acinetobacter junii* supplemented with varied levels of rock phosphate has led to the better growth and enhanced crop yield in both *Solanum lycopersicum* and *Capsicum annuum* L. (Tallapragada et al. 2015a).

Symbiotic association of host plants with AM fungi effectively helps plants to overcome the drought stress. The hyphae of AM fungi penetrate deep into the soil and help in effectual absorption of water and nutrients from the soil. The extraradical hyphae of arbuscular mycorrhiza produce certain hydrolytic enzymes responsible for the hydrolysis of macromolecules such as chitin, lignin, nucleic acid, and protein into simple monomers. AM fungi thus help in the uptake of the converted monomers more efficiently. AM fungi are distinguished in improving the various physiological processes such as assimilation of water as well as nutrients. They are also efficient in maintaining the osmotic balance in plants (Ruiz-Lozano 2003). Several reports suggest that the mycorrhiza-colonized plants depict much higher relative water content compared to nonmycorrhizal plants under salt stress (Al-Khaliel 2010; Jahromi et al. 2008). Similarly, stomatal conductance is also higher in mycorrhizal plants than nonmycorrhizal plants resulting in an increased demand for transpiration. The water status in mycorrhizal plants is mainly improved by high turgor pressure. Inoculation of AM fungi greatly influences the photosynthetic pigments, proline accumulation, nutrient uptake, and antioxidant enzymes in the host plants. Recently, Hashem et al. (2015) have reported that the AM fungi enhance salt tolerance in *Panicum turgidum* Forssk by changing photosynthetic and antioxidant pathways. Also, reports on the enhanced chlorophyll contents in AM fungi colonized *Solanum lycopersicum* L. (Hajiboland et al. 2010) and lettuce plants (Aroca et al. 2013) under salt stress are available in the literature.

Application of *Glomus mosseae* helped the *Erythrina variegata* Linn. to overcome drought stress at four different levels. Colonization of arbuscular mycorrhizal fungi in *Triticum aestivum* L. enhanced the growth and grain yield and helped the plants in mitigating the water stress (Al-Karaki et al. 2004). According to Porcel et al. (2006), aquaporins might be playing a cardinal role in ameliorating the water and salt stress in AM fungus-colonized plants.

22.2.2 Plant Growth-Promoting Rhizobacteria as a Bioinoculant

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring, free-living, soilborne bacteria, which colonize the rhizosphere region of soil that helps in the plant growth promotion through direct and indirect mechanism. Nitrogen fixation, phosphate solubilization, HCN production, and phytohormone production are some of the mechanisms that are involved in PGPR-mediated plant growth promotion. Several reports on the enhancement of growth and yield of various plants upon PGPR inoculation are available in the literature (Kanchana et al. 2013). Application of certain strains of PGPR at an early stage of development to various crop plants improves the biomass production through direct effects on root and shoot growth. Fluorescent *Pseudomonas*, *Bacillus* spp., *Azotobacter* spp., and *Azospirillum* spp. are some of the PGPR which are being widely exploited for the plant growth

promotion. PGPR play an imperative role in agricultural systems, especially as a biofertilizer and biocontrol agent.

PGPR also modify root functioning, improve plant nutrition, and influence the physiology of the whole plant. Plant growth-promoting rhizobacteria ameliorate the salt stress by exhibiting some of the important traits. Under salinity, plants tend to produce ethylene; higher concentration of which results in the inhibition of root growth and finally affects the overall growth of plants. The ethylene level is regulated by a key enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase. Plant growth-promoting rhizobacteria produce ACC deaminase which helps in mitigating the inhibitory effects of salinity by lowering the ethylene level, thereby helping prolific root growth which in turn is beneficial for the nutrient uptake and maintenance of plant growth. The possible role of *Bacillus pumilus* and *Pseudomonas pseudoalcaligenes* in alleviating the salt stress of paddy under greenhouse conditions was investigated by Jha and Subramanian (2013). According to Yildirim et al. (2008), PGPR offer an economical and simple treatment to salt-sensitive radish plants. Saber et al. (2013) have established the positive effects of *Azotobacter chroococcum*, *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Azospirillum lipoferum* on the rapeseed cultivars under saline conditions. Increase in chlorophyll a and b at different levels of salinity was observed with ACC deaminase-producing rhizobacterial inoculated maize plants (Nadeem et al. 2010). Co-inoculation of *Azospirillum* and AM fungi enhanced the growth of rice plants under water deficit and well-watered conditions (Sanchez et al. 2010).

PGPR are reported to influence the growth, yield, and nutrient uptake of plants by various mechanisms. They are involved in (1) nitrogen fixation, (2) increasing supply of other nutrients, (3) phytohormone production, and (4) controlling fungal and bacterial pathogens. There has been a lot of scope in PGPR studies, and an increasing number of PGPR are being commercialized for various crops (Saharan and Nehra 2011).

22.2.3 *Piriformospora indica* as a Bioinoculant

Piriformospora indica (*P. indica*) is a novel root fungus which was first discovered by Varma et al. (1999) from the rhizospheric soils of *Ziziphus nummularia* and *Prosopis juliflora* grown in Indian Thar Desert. It is a multifaceted fungus which exerts numerous functions on the host plant (Fig. 22.1). They can colonize both in mono- and dicotyledonous plants including *Arabidopsis thaliana*, barley, wheat, and tobacco (Varma et al. 1999; Waller et al. 2005; Serfling et al. 2007). *P. indica* enhances growth and yield of host plant and protects them against biotic stress (resistance to diseases) or abiotic stress (salt stress) (Rai et al. 2001; Barazani et al. 2005; Waller et al. 2008). *P. indica* is strictly limited to the root cortex. It develops into intracellular coils which are totally different from that of the arbuscules of AM fungi (Varma et al. 1999).

Kumar et al. (2009) have reported the antioxidant enzyme activities in maize plants colonized with *P. indica*. According to Waller et al. (2005), the endophytic

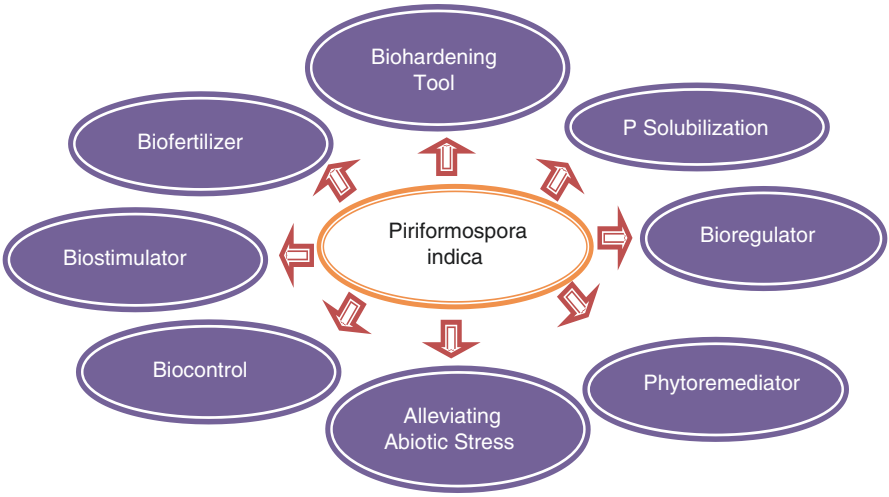

Fig. 22.1 Functions of multifaceted fungus *Piriformospora indica*

fungus *P. indica* reprograms barley to salt stress tolerance and disease resistance and also increases the yield of barley. Association of *P. indica* with *Arabidopsis thaliana* roots represents a novel system and increases the growth and yield of *Arabidopsis thaliana* (Peskan-Berghofera et al. 2004). The cell wall extract of *P. indica* promotes growth of *Arabidopsis* seedlings and induces intracellular calcium elevation in roots (Vadassery et al. 2009). *P. indica* also promotes the growth of *Adhatoda vasica* Nees, as well as *Bacopa monnieri* L. which are used in the preparation of Ayurvedic medicine (Rai and Varma 2005; Prasad et al. 2008). A phosphate transporter from *P. indica* plays an important role in phosphate transport to the host plant (Yadav et al. 2010). An increase in yield of tomato was achieved upon interaction of *P. indica* with fungal and viral pathogens. The interactive effect of three advantageous microorganisms (*P. indica* and *Pseudomonas* strains R62 and R81) on the growth of *Vigna mungo* through their inorganic carrier-based formulations has been reported (Kumar et al. 2012). *P. indica* also affects the plant growth by auxin production (Sirrenberg et al. 2007). According to Druege et al. (2007), *P. indica* promotes the adventitious root formation in cuttings. Rai (2010) has reported the strategies involving *P. indica* to conserve endangered medicinal plants. *P. indica* acts as a potential biocontrol agent against *Trichoderma* species which causes disease in wheat (Ghahfarokhi and Goltapeh 2010). Two varieties of *Glycine max* Linn. were found to be resistant to water stress upon *P. indica* inoculation (Rathod et al. 2011). Biotization with *P. indica* and *Pseudomonas fluorescens* improves survival rate, nutrient acquisition, field performance, and saponin content of micropropagated *Chlorophytum* spp. (Gosal et al. 2010). Interaction of *P. indica* with strawberry plants helps it to modify for better adaptability to adverse climate (Husaini et al. (2012)). Culture filtrate of *P. indica* influences the growth and

yield of oil seed in *Helianthus annuus* (Bagde et al. 2011). Co-inoculation of *P. indica* and *Azospirillum* strains has played an imperative role in ameliorating the salt stress in wheat plants (Zarea et al. 2012).

22.3 Consortium of Advantageous Microorganisms

A conglomeration of advantageous microorganisms enhances the efficiency of the bioinoculant in an effective way than single inoculum by making them more consistent in their performance. In recent times, the emphasis is on microbial consortia studies and their effect upon plant growth (Nain et al. 2009; Meena et al. 2009; Ögüt et al. 2005; Valverde et al. 2006). Application of microbial consortium covers almost all the aspects of plant growth promotional traits thereby assisting in enhanced growth of plants. Few scientists have reported considerable impact of consortia probably due to collective mutualistic effects of consortia inoculation over single inoculation (Meena et al. 2009; Ögüt et al. 2005), while others have reported vice versa (Sattar et al. 2008). Several studies are available in the literature which describes the ambiguous and contradictory impact of consortia application under greenhouse and field conditions (Table 22.1).

Table 22.1 Effect of bioinoculants on various crops grown under greenhouse conditions over the last few years

Sl. No.	Bioinoculants	Experimental system/host	Effects	Reference
1.	Endomycorrhizal fungi and *Pseudomonas fluorescens* supplemented with varied phosphorus levels	*Capsicum annuum* L.	Enhanced growth and yield	Tanwar et al. (2013)
2.	*Glomus fasciculatum*, *Bacillus megaterium*, and *Pseudomonas fluorescens*	*Ocimum basilicum* L.	Enhanced biomass	Hemavathi et al. (2006)
3.	*Glomus intraradices* and *Rhizobium tropici* CIAT899	*Phaseolus vulgaris* L.	Significant increase in crop yield (increase in P uptake and N fixation)	Tajini et al. (2011)
4.	*Pseudomonas striata* and *Piriformospora indica*	*Cicer arietinum* L.	Synergistic effect and enhanced crop yield	Meena et al. (2009)
5.	*Piriformospora indica* and *fluorescent Pseudomonas* R62 and R81	*Vigna mungo* L.	Growth promotion	Kumar et al. (2012)
6.	*Piriformospora indica*, *Glomus mosseae*, and *Rhizobium*	*Vigna mungo* L.	Found incompatible	Ray and Valsalakumar (2010)

22.4 Role of Bioinoculants on Phosphate Solubilization

Phosphorus (P) is one of the major essential macronutrients for biological growth and development (Fernandez et al. 2007). It also offers resistance to plants against various diseases and promotes crop maturity. Most agricultural soils contain large reserves of total P; a part of the accumulated P depends on regular application of chemical fertilizers or sludge from wastewater treatment (De-Bashan and Bashan 2004). Both P fixation and precipitation occur in soil because of the large reactivity of phosphate ions with numerous soil constituents (Fernandez et al. 2007). Plants obtain phosphorous from the soil. Soils invariably do not contain requisite amount of available phosphorus. Only a limited portion of phosphorous in the soil is taken up by plants, while the remaining portion is in the immobilized form. The insoluble form of phosphate is converted to its soluble form by a group of microorganisms known as phosphate-solubilizing microorganisms present in the soil. Microbial biodiversity in soil plays a vital role in the metabolism of complex molecules. Soil microorganisms contribute extensively in solubilizing insoluble form of phosphates and help plants to acquire soluble form of phosphate. Several reports on solubilization of different inorganic phosphates by various bacteria and fungi are available in the literature. Many insoluble forms of calcium, iron, and aluminum phosphate occur in soil. Rock phosphate and single superphosphates are being chiefly employed to sustain soil phosphorus level in the available form for plants. Also, zinc is one of the limiting factors in crop production. Ivanova et al. (2006) have reported the solubilization of Tunisian phosphorite (P_2O_5) by *Erwinia* sp. and *Azotobacter* sp. isolated from the agricultural soil. Compared to *Erwinia* sp., the solubilization of Tunisian phosphorite was rapid with that of *Azotobacter* sp. Efficient solubilization of tricalcium phosphate by *Penicillium* sp. (endophytic fungi) isolated from tea (*Camellia sinensis* L.) has been investigated (Nath et al. 2012). Among all the bacteria tested, three strains of *Pseudomonas fluorescens* (CB501, CD511, and CE509) were selected based on their ability to solubilize three types of phosphates, tricalcium phosphate ($Ca_3(PO_4)_2$), aluminum phosphate ($AlPO_4 \cdot H_2O$), and iron phosphate ($FePO_4 \cdot 2H_2O$), present in liquid medium. Park et al. (2009) have reported *Pseudomonas fluorescens* RAF15 as a potential candidate for the development of biofertilizer. The probable mechanism involved would be the combined effect of decrease in pH as well as the synthesized carboxylic acids (citric acid, malic acid, tartaric acid, and gluconic acid). *Aspergillus* sp. PS 104 was found to be an excellent rock phosphate solubilizer under in vitro conditions (Kang et al. 2008).

22.4.1 Mechanism of Phosphate Solubilization by Microorganisms (PSMs)

The probable mechanism involved in solubilization of phosphate might be due to the organic acid production and proton extrusion as depicted in Fig. 22.2 (Jones 1998; Reyes et al. 1999). Acid phosphatases and phytases secreted by certain

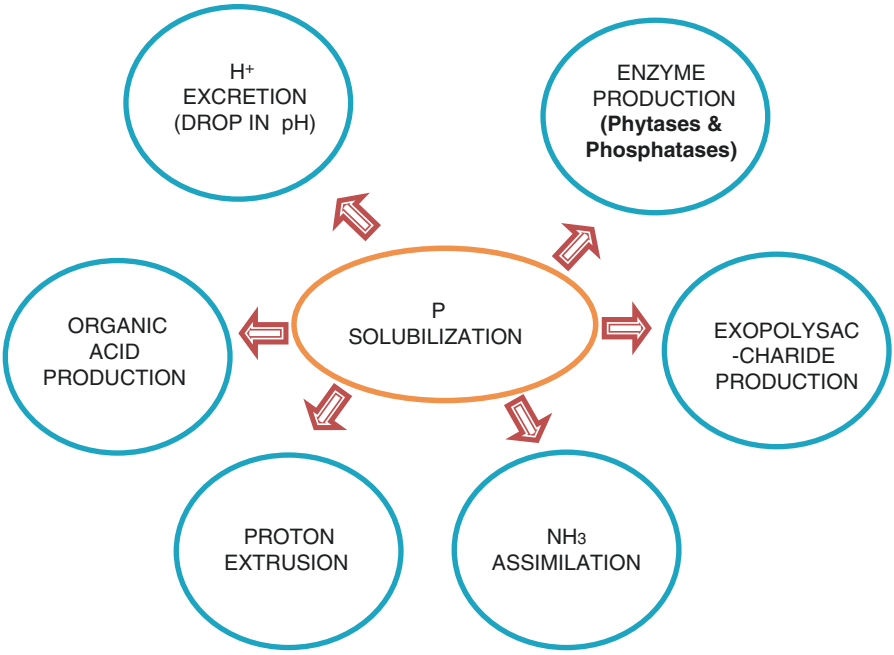

Fig. 22.2 Schematic representation of mechanism involved in phosphate solubilization

rhizospheric microorganisms play a cardinal role in the solubilization of phosphate (Aseri et al. 2009). Solubilization of organic phosphates takes place mainly by acid phosphatase enzyme. Arbuscular mycorrhizal fungi (AM fungi) are capable of solubilizing organic phosphates as they possess acid phosphatase enzyme in their hyphal tips (Balaz and Vosatka 1997). The organic acids produced by the PSMs help in solubilizing the insoluble phosphates to their soluble form by lowering the pH, chelating the cations, and also competing with phosphate for adsorption sites in the soil. Inorganic acids such as hydrochloric acid can also solubilize phosphate but are less effective compared to organic acids at the same pH (Kim et al. 1997). Various organic acids produced by the PSMs are more likely involved in the dissociation of tricalcium phosphate (Deubel and Merbach 2005). Solubilization of Fe and Al occurs via proton release by PSMs. Organic phosphates are catalyzed through the hydrolysis of C-O-P ester bond by phosphatase and phytase released by PSMs (Yadav and Tarafdar 2011).

22.5 Effect of Phosphate-Solubilizing Microorganisms on Crop Production

Mitigation of phosphate deficiency by the application of chemical fertilizer generates serious issues about the continued viability of current agricultural practice. This has led to the investigation of more eco-friendly and economically feasible

strategies to improve crop production in low-phosphorous soils. Efficient utilization of phosphate-solubilizing microorganisms is one such ideal strategy to improve crop production. Microorganisms having the potential to solubilize phosphate increase the availability of soluble phosphate and aid in the enhancement of plant growth by improving biological nitrogen fixation (Ponmurugan and Gopi 2006). Several reports suggest that dual inoculation of AM fungi and phosphate-solubilizing bacteria (PSB) helps in efficient mobilization of phosphates in plants (Tallapragada et al. 2015b). Although application of phosphate-solubilizing bacteria increased the biological yield, the maximum grain weight was achieved when the same bacteria were applied with arbuscular mycorrhizae (Mehrvarz et al. 2008). According to Afzal and Bano (2008), application of phosphate-solubilizing bacteria and AM fungi (dual inoculation) along with P fertilizer was found to be better in improving grain yield of wheat by 30–40% than P fertilizer alone. Increase in the chlorophyll content was observed in barley upon the application of both mycorrhiza and *Pseudomonas putida* (Mehrvarz et al. 2008). Microorganisms inhabiting the rhizospheric region of soil interact with the plant roots and help in promoting the plant growth via increase in nutrient uptake. Application of beneficial microorganisms such as *Bradyrhizobium*, *Glomus fasciculatum*, and *Bacillus subtilis* demonstrated 24% of enhancement in the yield of green gram (Zaidi and Khan 2006). Tallapragada et al. (2015a, b) have reported the effect of co-inoculation of *Glomus mosseae* and *Acinetobacter junii* on the growth and yield of *Lycopersicon esculentum* supplemented with varied concentrations of rock phosphates.

P. indica plays an important role in the phosphate solubilization. It is known to solubilize different sources of organic phosphates as well as polyphosphates with the help of an enzyme, acid phosphatase (66 kDa), present in its hyphal tips (Malla et al. 2004). Yadav et al. (2010) have reported that *P. indica* plays an important role in phosphate transport to the host plant. Plants inoculated with wild-type *P. indica* illustrated higher amounts of phosphate compared to the plants that were inoculated with PiPT gene-silenced *P. indica* suggesting the role of PiPT gene in phosphate transportation. Colonization of barley plants with *P. indica* helps in increasing grain yield by accelerating early development of barley plants under low-phosphorous condition (Achatz et al. 2010).

The organic acids and phosphatase enzyme produced by certain soil microorganisms help in the conversion of Fe and Al oxide forms of phosphate, thereby making it available for plants. Phosphate-solubilizing microorganisms are very effective in increasing the available P in soil for the enhanced plant growth and crop yield. Hence, application of phosphate-solubilizing bacteria as a biofertilizer has enormous potential for making use of the fixed P in the soil.

22.6 Role of Phytohormones Produced by Bioinoculants on the Stimulation of Plant Growth

Phytohormones are organic compounds which are essential for plant growth at very low concentrations. Plant growth-promoting rhizobacteria and certain endophytic fungi produce phytohormones such as gibberellic acids (GA) and indole-3-acetic

acid (IAA) that are believed to be related to their ability in stimulating plant growth. **Gibberellins** are associated with seed germination, stem elongation, flowering, and fruit development (Boğa et al. 2009). Gibberellic acid stimulates the plant growth by increasing cell elongation in some plant species and also by increasing both cell elongation and cell division in others (Jupe et al. 1988). Indole-3-acetic acid and GA are nothing but the secondary metabolites produced by fungi during the stationary phase. Several microorganisms, such as *Gibberella fujikuroi*, *Aspergillus niger*, *Fusarium fujikuroi* SG2, *Rhizobium* sp., *Azospirillum brasilense*, and *Pseudomonas* sp., are known to produce gibberellic acid (Pandya and Desai 2014; Bilkay et al. 2010; Uthandi et al. 2010).

Auxins (indole-3-acetic acid) were the ones to be first isolated and characterized as a plant hormone. IAA is considered as the most important native auxin and is known to be involved in increasing the root growth and root length resulting in greater root surface area which enables the plant to access more nutrients from soil (Vessey 2003). It also functions as an important signal molecule in regulating various plant development processes such as cell differentiation and gene regulation (Ryu and Patten 2008). Regulation of these processes by auxin is believed to be involved in auxin-induced changes in gene expression. However, the complete description of the mechanism by which auxin regulates cell growth is not clear. The immediate effect of exposure of plant tissues to auxin is proton excretion, occurring within minutes.

Several bacteria such as *Bacillus cereus*, *Rhizobium japonicum*, *Bradyrhizobium japonicum*, *Klebsiella pneumoniae*, *Azospirillum* spp., *Acetobacter diazotrophicus* L1, *Azotobacter chroococcum*, *Azotobacter beijerinckii*, *Azotobacter vinelandii*, *Pseudomonas fluorescens*, etc. and fungi such as *Aspergillus*, *Penicillium*, and *Trichoderma* have already been reported advantageous for IAA biosynthesis (Khin et al. 2012; Boiero et al. 2007; Datta and Basu 2000; Dhara et al. 2009; Lenin and Jayanthi 2012; Ona et al. 2005; Resende et al. 2014; Bilkay et al. 2010).

Extensive studies on the IAA production by different microorganisms and its exogenous application on various crops have been reported by many scientists. Dhara et al. (2009) have reported on the isolation of IAA-producing *Klebsiella pneumonia* strains from the rhizospheric region of *Triticum aestivum*, and they have studied its effect on the same plant. Culture filtrate of *Pseudomonas putida* UB1 was found to have profound impact on the increase in lateral root formation of maize seedlings (Bharucha et al. 2013). The enhancement of lateral roots by IAA-producing bacteria has been reported by Lwin et al. (2012). Exogenous application of L-tryptophan and IAA showed a positive response on the yield of soya bean plants (Sudadi 2012). *Pseudomonas fluorescens* strain Psd isolated from the rhizospheric region of *Vigna mungo* exhibited multiple plant growth-promoting traits of which synthesis of phytohormones was found to be one of the important traits (Upadhyay and Srivastava 2010). Increase in germination rate, roots, shoots, and plant growth was observed with the application of IAA and plant growth-promoting rhizobacteria (Fatima et al. 2009). According to Tsavkelova et al. (2006), *Bacillus* sp. modulated the plant development through the production of phytohormones. High proportion of rhizospheric microorganisms is able to produce plant growth hormones (IAA), which aid in stimulating the plant growth and offer more

branching and larger surface area. The production of tryptophan-dependent indole-3-acetic acid by *Bacillus amyloliquefaciens* FZB42 and its effect on plant growth promotion have been extensively studied by Idris et al. (2007). Recently Tallapragada et al. (2015a, b) have reported the isolation and optimization of IAA-producing *Burkholderia seminalis* and its effect on the seedlings of tomato.

Few reports on the production of auxins by *Piriformospora indica* (*P. indica*) are also available in the literature. *P. indica* produces phytohormones such as indole-3-acetic acid and indole lactic acid through an intermediate indole pyruvic acid, upon L-tryptophan (TRP) feeding. A positive effect on the micropropagation of *Thymus vulgaris* in the presence of different auxin densities supplemented with *P. indica* has been demonstrated by Hossein et al. (2012). According to Hilbert et al. (2012), the auxin produced by *P. indica* is not involved in the stimulation of barley biomass, but Sirrenberg et al. (2007) have reported that *P. indica* induced increase in root branching in *Arabidopsis thaliana* through IAA production. Recently Hilbert et al. (2013) have reported that exogenous auxin affects the oxidative burst in barley roots colonized by *P. indica*. Schäfer et al. (2009) have demonstrated that plant hormone signaling is obviously recruited by *P. indica* in order to manipulate plant defense and most probably plant metabolism. Plant hormones might further be a key to explain the broad host spectrum of *P. indica*. They have also concluded that phytohormonal state and signaling are very important aspects during plant colonization by *P. indica*.

22.7 Role of Bioinoculants in the Mitigation of Abiotic Stress

Plant species need to surmount a wide variety of environmental stresses that have adverse effect on their growth, production, and development. Drought and salinity are the two important abiotic stresses that will affect crop development and yield. The basic physiology of both drought and salinity overlaps with each other. They mainly target plant's water balance and/or inducing photoinhibition and photooxidative stress (Polanco et al. 2014). Typically plants try to reduce their water loss by closing the stomata (Schachtman and Goodger 2008) and by adjusting their root hydraulic conductance (Aroca et al. 2013).

22.7.1 Drought

Drought is one such stress which affects plant productivity worldwide and is expected to increase with climatic changes. Recurrent dry periods and scattered rainfall affect the crop production in generally well-rain-fed areas. Plants can counter drought stress at various levels such as at morphological, metabolic, and cellular levels with modifications that allow the plants to avoid the stress or to increase their tolerance. Application of bioinoculants helps the plants to alleviate drought and salinity stress in addition to their own intrinsic mechanism (Fig. 22.3). Various plant growth-promoting rhizobacteria, endophytic fungi, and arbuscular mycorrhizal fungi are known to confer resistance to drought stress in various crops.

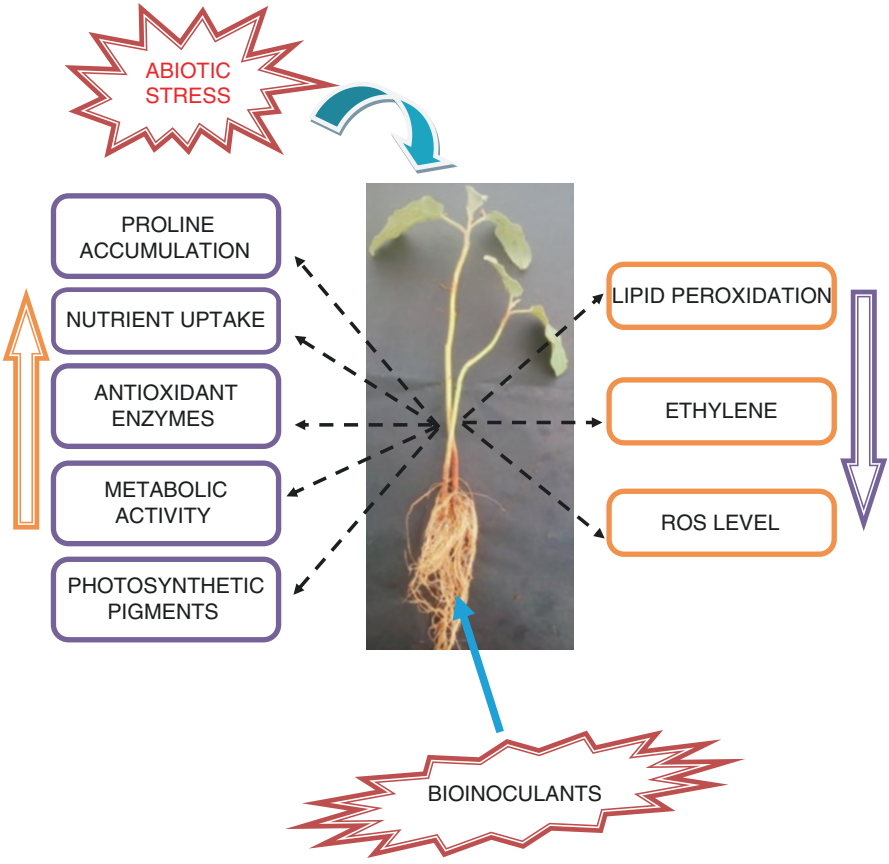

Fig. 22.3 Schematic representation of the mechanism involved in bioinoculant-mediated abiotic stress tolerance

22.7.2 Arbuscular Mycorrhizal Fungi in Mitigating Water Stress

Symbiosis of AM fungi with plants offers protection to its host plants against the harmful/ill effects of water stress via drought avoidance mechanism (Sanchez et al. 2010). Maintenance of adequate hydration level (relative water content) in the host plants is one of the important strategies offered by AM fungi to avoid water stress (Augé and Moore 2005). The effectual absorption of water and nutrients from the soil is one of the proposed actions rendered by AM fungi. Colonization of AM fungi helps in maintaining the hydration level as well as turgor pressure (osmotic adjustment) in the leaves thereby helping them to sustain even during water deficit conditions. Under water deficit conditions, colonization of AM fungi triggers the accumulation of proline (an osmoregulator) (Yooyongwech et al. 2013). Proline also acts as an osmoprotectant which in turn helps the plants to overcome the stress. Conversely, in several studies, a lower accumulation of proline has also been

observed in mycorrhizal plants relative to nonmycorrhizal counterparts (Doubková et al. 2013), suggesting that AM symbiosis enhanced host plant resistance to drought. In fact, proline could also be considered as a marker of the potential injury caused by water deficit, indicating lower stress in the mycorrhiza-colonized plants compared to the nonmycorrhizal plants.

Extensive studies on the application of PGPR in mitigating the water stress have been reported. Among them, utilization of rhizobacteria under water stress has improved the antioxidant and photosynthetic pigments in basil plants as reported by Heidari and Golpayegani (2012). In addition to an increase in the general plant growth promotion, some PGPR promote root development (Vacheron et al. 2013) and alter root morphology with the production of phytohormones such as indole-3-acetic acid (IAA) resulting in the increased root surface area and number of root tips. Root tips and root surfaces are the important sites of nutrient uptake which are likely to be involved in the mechanism by which PGPR lead to increased nutrient uptake via stimulation of root development. Increase in the leaf area, stalk length, and shoot dry biomass of Brassica oilseed rape was found to be noteworthy upon dual inoculation of *Bacillus* spp. and *Pantoea* sp. in *Zea mays* L. seedlings under drought conditions. Significant increase in the yield of Brassica oilseed rape upon inoculation with *Pseudomonas fluorescens* or *Pseudomonas putida* under water deficit has been investigated (Arvin et al. 2012).

Very few reports with respect to application of *P. indica* to different crops under drought studies have been reported in the literature. The dual inoculation of *Glomus mosseae* and *P. indica* has led to the enhancement of the antioxidant activity in wheat and thereby improving drought resistance. *P. indica* confers resistance to drought as well as salinity by activating the antioxidant enzyme activities and also by upregulating mRNA levels (Nomura et al. 2008).

22.7.3 Salinity

Soil salinity is one of the major threats to agriculturists and has received much attention worldwide. It has ill effects on plant growth and yield. Majority of the earth's surface is affected by salinity (Arzani 2008). Five percent of the total cultivable land available is being affected by salinity particularly arid and semi-arid areas (Evelin et al. 2009). The electrical conductivity of saline soils is usually high with a low pH (8.5) and less sodium absorption (<13). Reduction in the plant growth as well as lower yield is due to the higher concentration of Na^+ and Cl^- ions (Parida and Das 2005). Although salinity affects the plant growth, the tolerance level to salinity varies from one plant to another. Reduction in leaf surface area followed by termination of leaf expansion is one of the earlier responses of the plants to salinity. Decrease in the plant water uptake due to the low osmotic potential affects various physiological processes of plants. According to Tejera et al. (2006), under high salinity, the plant biomass reduced considerably. Various microorganisms such as AM fungi, PGPR, and endophytic fungi are known to alleviate salt stress. Among AM fungi, *Glomus*, *Gigaspora*,

Scutellospora, *Acaulospora*, and *Entrophospora* are some of the commonly occurring genera. Several studies have revealed that AM fungi do exist in the saline soil (Evelin et al. 2009; Porcel et al. 2012). Although the exact mechanism of AM fungi in mitigating salt stress is not apparent, most of the studies illustrate that AM fungi improve the plant growth and yield by enhancing nutrient uptake. AM fungi are known to engineer the physiological processes of colonized plants like water assimilation capacity of plants by adjusting the osmotic balance as well as increasing the hydraulic conductivity (Ruiz-Lozano 2003). The mycorrhiza-colonized peanut plants illustrated considerable amount of relative water content in their leaves under salt stress (Al-Khaliel 2010). Similarly, *Glomus intraradices*-colonized lettuce plants were able to sustain/uphold higher water content than noncolonized plants in spite of salt level (Jahromi et al. 2008). Increase in the stomatal conductivity was illustrated by the plants upon AM fungi colonization leading to increase in transpiration rate (Jahromi et al. 2008).

PGPR that elicited plant tolerance against salt stress have been extensively studied. Inoculation of *Achromobacter piechaudii* to tomato seedlings exposed to high salt reduced the ethylene content indicating that bacterial ACC deaminase was functional (Mayak et al. 2004). Induced systemic tolerance to salt stress was also investigated in a new study with *Arabidopsis* using *Bacillus subtilis* GB03. Jha and Subramanian (2013) have reported the possible role of *Bacillus pumilus* and *Pseudomonas pseudoalcaligenes* in alleviating salt stress and enhancing growth of paddy plants GJ-17. Significant variation in the antioxidant levels and growth physiology were also observed. *Staphylococcus kloosii* EY37 and *Kocuria erythromyxa* EY43 were found to be effective in mitigating the harmful effects of salt stress in *Raphanus sativus* L. plants (Yildirim et al. 2008).

Piriformospora indica not only exhibits growth-promotional activity but also offers resistance to plants with respect to biotic and abiotic stresses. The rice plants inoculated with *P. indica* showed higher biomass, antioxidant activity, free proline content, and relative water content with lesser lipid peroxidation compared to uninoculated plants under salt stress (Bagheri et al. 2013). The obtained results of this research indicated the effective role of this fungus in improving the growth of rice plants under salt stress conditions (Bagheri et al. 2013). According to Jogawat et al. (2013), the rice plants were able to tolerate 200 and 300 mM of salt stress in the presence of *P. indica*.

22.8 Role of Bioinoculants in Ameliorating Biotic Stress

Plants have the ability to defend themselves against most microbial pathogens with a complex array of physical barriers and antimicrobial compounds, which are either preformed or induced. Most of the plants studied in natural ecosystems are infested by fungi which do not exhibit any disease symptoms. It is well known that biological control is an eco-friendly strategy to reduce crop damage caused by plant pathogens (Murali et al. 2012). Rhizosphere-resident antagonistic

microorganisms are ideal biocontrol agents, as the rhizosphere provides frontline defense for roots against infection by the pathogens (Murali et al. 2012). Biocontrol research has gained considerable attention and showed potential as a viable alternative to chemical control strategies. Certain plant growth-promoting fungus (PGPF) along with AM fungi is reported to suppress the disease effectively and induce systemic resistance in cucumber (Chandanie et al. 2006). PGPF isolates are known to effectively control soilborne diseases such as damping-off caused by species of *Fusarium*, *Rhizoctonia*, and sclerotium (Waqas et al. 2015). The isolates afforded better protection when they were challenged of inoculation. Waqas et al. (2015) have reported the positive effect of *Penicillium citrinum* and *Aspergillus terreus* on the enhanced growth as well as disease suppression in sunflower plant infected with *Sclerotium rolfsii*. *Trichoderma harzianum* was found to be a potential biocontrol agent against *Alternaria alternata* on tobacco plants. According to the literature, *Trichoderma* isolates were found capable of inducing systemic resistance and act directly on the pathogens (Murali et al. 2012). *Penicillium chrysogenum* induces significant resistance against *Fusarium oxysporum* f. sp. *vasinfectum* and *Verticillium dahliae* in potted cotton plants under glasshouse conditions (Dong and Cohen 2002). The potential of *Pseudomonas* sp. and *Bacillus* sp. isolates against the wilt of *Solanum melongena* L. plants caused by *Fusarium oxysporum* was investigated by Altinok et al. (2013). *Pseudomonas fluorescens* and *Bacillus subtilis* depicted a great potential for plant growth and biocontrol of *Fusarium oxysporum* in tomato plants. Sudharani et al. (2014) have reported the beneficial role of consortia of biocontrol agents and PGPR in the production of cabbage under nursery condition. The exact mechanism involved in the biological control is not yet clear; however, the plausible reasons may be due to the collective effect of (1) production of siderophores and antibiotics, (2) competition for available nutrients at the root surface (Kamilova et al. 2005), (3) synthesis of hydrolytic enzymes that help in breaking the cell wall of pathogens (Neeraja et al. 2010), and (4) regulation of ethylene levels in plants via ACC deaminase enzyme (Van Loon 2007).

Colonization of *Piriformospora indica* has led to the reduction in the disease symptoms of wheat caused by the stem base pathogen *Pseudocercosporella herpotrichoides* (Serfling et al. 2007). Disease severity of *Verticillium dahlia* on tomato plants was reduced with the colonization of *P. indica* (Fakhro et al. 2010). *P. indica*-colonized plants were more resistant against *Blumeria graminis* infection in shoots and *Fusarium culmorum* in roots (Waller et al. 2005). In barley, a number of defense-related genes were strongly upregulated in fungus-colonized plants compared to the untreated control by leaf pathogens inducing powdery mildew infections (Molitor et al. 2011). *P. indica* and *Sebacina vermifera* were found to be most potent biocontrol agents against the root pathogen *Gaeumannomyces graminis* var. *tritici* (Ghahfarokhi and Goltapeh 2010). The production of reactive oxygen species and synthesis of antioxidants were also observed during abiotic stress in barley, wheat, and maize (Waller et al. 2005; Serfling et al. 2007; Kumar et al. 2009). The mechanisms of *P. indica*-induced resistance were similar to that of the growth-promoting rhizobacteria.

Conclusion

Owing to its exceptional ability to efficiently promote plant growth by effective P solubilization, stimulation via phytohormone production, protection against plant pathogens, and alleviation to abiotic stress by adapting to various mechanisms, bioinoculants have received much attention over the last few decades. They efficiently engineer the root architecture upon colonization in the host plants. Improved resistance offered by bioinoculants in plants and the molecular mechanisms governing the amelioration of stress need to be addressed. Deeper understanding of the role of bioinoculant in providing resistance against the detrimental effects of climate may provide new perspectives with respect to the stress adaptation mechanisms in host plant, thereby assisting in designing a better approach to tackle the abiotic stresses. Extensive studies on molecular mechanisms involved in mitigation of various abiotic and biotic stresses may open up new avenues which can be further explored to enhance the crop production. They can be further explored and used as an innovative technology in organic farming for better crop productivity.

References

Achatz B, Kogel K-H, Franken P, Waller F (2010) *Piriformospora indica* mycorrhization increases grain yield by accelerating early development of barley plants. Plant Signal Behav 5(12):1685–1687

Afzal A, Bano A (2008) Rhizobium and phosphate solubilizing bacteria improve the yield and phosphorous uptake in Wheat (*Triticum aestivum*). Int J Agric Biol 10(1):85–88

Al-Karaki G, McMichael B, Zak J (2004) Field response of wheat to arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14:263–269

Al-Khaliel AS (2010) Effect of salinity stress on mycorrhizal association and growth response of peanut infected by *Glomus mosseae*. Plant Soil Environ 56:318–324

Altinok HH, Dikilitas M, Yildiz HN (2013) Potential of Pseudomonas and Bacillus isolates as biocontrol agents against fusarium wilt of eggplant. Biotechnol Biotechnol Equip 27(4):3952–3958

Aroca R, Ruiz-Lozano JM, Zamarreño AM, Paz JA, García-Mina JM, Pozo MJ, López-Ráez JA (2013) Arbuscular mycorrhizal symbiosis influences strigolactone production under salinity and alleviates salt stress in lettuce plants. J Plant Physiol 170(1):47–55

Arvin P, Vafabakhsh J, Mazaheri D, Noormohamadi G, Azizi M (2012) Study of drought stress and plant growth promoting rhizobacteria (PGPR) on yield, yield components and seed oil content of different cultivars and species of Brassica oilseed rape. Ann Biol Res 3(9):4444–4451

Arzani A (2008) Improving salinity tolerance in crop plants: a biotechnological view. In Vitro Cell Dev Biol Plant 44(5):373–383

Aseri GK, Jain N, Tarafdar JC (2009) Hydrolysis of organic phosphate forms by phosphatases and phytase producing fungi of arid and semi-arid soils of India. Am-Eurasian J Agric Environ Sci 5:564–570

Augé RM, Moore JL (2005) Arbuscular mycorrhizal symbiosis and plant drought resistance In: Mehrotra VS (ed) Mycorrhiza: role and applications. Allied Publishers Limited, New Delhi, pp 136–157

Bagde US, Prasad R, Varma A (2011) Influence of culture filtrate of *Piriformospora indica* on growth and yield of seed oil in *Helianthus annuus*. Symbiosis 53(2):83–88

Bagheri AA, Saadatmand S, Niknam V, Nejadsatari T, Babaeizad V (2013) Effect of endophytic fungus, *Piriformospora indica* on growth and activity of antioxidant enzymes of rice (*Oryzae sativa* L) under salinity stress. Int J Biomed Adv Res 11:1337–1350

Balaz M, Vosatka M (1997) Effect of Mycorrhiza and different forms of phosphorous on the growth of *Calamagrostis villosa*. Biol Plantarum 39:281–288

Barazani O, Benderoth M, Groten K, Kuhlemeier C, Baldwin IT (2005) *Piriformospora indica* and *Sebacina vermifera* increase growth performance at the expense of herbivore resistance in *Nicotiana attenuata*. Oecologia 146:234–243

Barea JM, Pozo MJ, Azcon R, Azcon-Aguilar C (2005) Microbial co-operation in the rhizosphere. J Exp Bot 56:1761–1778

Bharucha U, Patel K, Trivedi UB (2013) Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on mustard (*Brassica nigra*). Agric Res 2:215–221

Biermann B, Linderman RG (1983) Increased geranium growth using pre-transplant inoculation with a mycorrhizal fungus. J Am Soc Hortic Sci 108:972–976

Bilkay IS, Şafak K, Nilüfer A (2010) Indole-3-acetic acid and gibberellic acid production in *Aspergillus niger*. Turk J Biol 34:313–318

Boğa A, Binokay S, Sertdemir Y (2009) The toxicity and teratogenicity of gibberellic acid (GA3) based on the frog embryo teratogenesis assay-Xenopus (FETAX). Turk J Biol 33:181–188

Boiero L, Perrig D, Masciarelli O, Penna C, Cassán F, Luna V (2007) Phytohormone production by three strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. Appl Microbiol Biotechnol 74:874–880

Chandanie W, Kubota M, Hyakumachi M (2006) Interactions between plant growth promoting fungi and arbuscular mycorrhizal fungus *Glomus mosseae* and induction of systemic resistance to anthracnose disease in cucumber. Plant and Soil 286:209–217

Cruz AF, Ishii T, Matsumoto I, Kadoya K (2002) Network establishment of vesicular arbuscular mycorrhizal hyphae in the rhizosphere between trifoliate orange and some plants. J Jpn Soc Hortic Sci 71:19–25

Datta C, Basu P (2000) Indole acetic acid production by a Rhizobium species from root nodules of a leguminous shrub *Cajanus cajan*. Microbiol Res 155:123–127

De-Bashan LE, Bashan Y (2004) Recent advances in removing phosphorus from wastewater and its future use as fertilizer. Water Res 38:4222–4246

Deubel A, Merbach W (2005) Influence of microorganisms on phosphorus bioavailability in soils. In: Buscot F, Varma A (eds) Microorganisms in soils: roles in genesis and functions. Springer, Berlin, p 62

Dhara PS, Hemangi GC, Vijay MK, Dilip DD, Balu AC (2009) Isolation and characterization of Indole acetic acid producing *Klebsiella pneumonia* strain from rhizosphere of wheat (*Triticum aestivum*) and their effect on plant growth. Indian J Exp Boil 47:993–1000

Dong HZ, Cohen Y (2002) Dry mycelium of Penicillium chrysogenum induces resistance against Verticillium wilt and enhances growth of cotton plants. Phytoparasitica 30:147–157

Doubková P, Vlasáková E, Sudová R (2013) Arbuscular mycorrhizal symbiosis alleviates drought stress imposed on *Knautia arvensis* plants in serpentine soil. Plant Soil 370:149–161

Druege U, Baltruschat H, Franken P (2007) *Piriformospora indica* promotes adventitious root formation in cuttings. Sci Hortic 112:422–426

Evelin H, Kapoor R, Giri B (2009) Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. Ann Bot 104(7):1263–1280

Fakhro A, Andrade-Linares DR, von Bargen S, Bandte M, Büttner C, Grosch R, Schwarz D, Franken P (2010) Impact of *Piriformospora indica* on tomato growth and on interaction with fungal and viral pathogens. Mycorrhiza 20(3):191–200

Fatima Z, Saleemi M, Zia M, Sultan T, Aslam M, Riaz-Ur-Rehman, Chaudhary MF (2009) Antifungal activity of plant growth-promoting rhizobacteria isolates against *Rhizoctonia solani* in wheat. Afr J Biotechnol 8(2):219–225

Fernandez LA, Zalba P, Gomez MA, Sagardoy MA (2007) Phosphate solubilization activity of bacterial strains in soil and their effect on soybean growth under greenhouse conditions. Biol Fertil Soils 43:805–809

Ghahfarokhi RM, Goltapeh ME (2010) Potential of the root endophytic fungus *Piriformospora indica; Sebacina vermifera* and *Trichoderma* species in biocontrol of take-all disease of wheat *Gaeumannomyces graminis* var *tritici in vitro*. J Agric Technol 6(1):11–18

Gosal SK, Karlupia A, Gosal SS, Chhibba IM, Varma A (2010) Biotization with *Piriformospora indica* and *P fluorescens* improves survival rate, nutrient acquisition, field performance and saponin content of micropropagated chlorophytum Sp. Indian J Biotechnol 9:289–297

Hajiboland R, Aliasgharzadeh A, Laiegh SF, Poschenrieder C (2010) Colonization with arbuscular mycorrhizal fungi improves salinity tolerance of tomato (*Solanum lycopersicum* L) plants. Plant Soil 331:313–327

Hashem A, AbdAllah EF, Alqarawi AA, Aldubise A, Egamberdieva D (2015) Arbuscular mycorrhizal fungi enhances salinity tolerance of Panicum turgidum Forssk by altering photosynthetic and antioxidant pathways. J Plant Interact 10(1):230–242

Heidari M, Golpayegani A (2012) Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L). J Saudi Soc Agric Sci 11(1):57–61

Hemavathi VN, Sivakumr BS, Suresh CK, Earanna N (2006) Effect of Glomus fasciculatum and plant growth promoting rhizobacteria on growth and yield of *Ocimum basilicum*. Karnataka J Agric Sci 19:17–20

Hilbert M, Lars M, Yi D, Jorg H, Monica S, Zuccaroa A (2012) Indole derivative production by the root endophyte *Piriformospora indica* is not required for growth promotion but for biotrophic colonization of barley roots. New Phytol 196:520–534

Hilbert M, Nostadta R, Zuccaroa A (2013) Exogenous auxin affects the oxidative burst in barley roots colonized by *Piriformospora indica*. Plant Signal Behav 8(4):e235721–e235721

Hossein KD, Ebrahim MG, Ahmad M, Varma A (2012) Evaluation of different densities of auxin and endophytic fungi (*Piriformospora indica* and *Sebacina vermifera*) on *Mentha piperita* and *Thymus vulgaris* growth. Afr J Biotechnol 11(7):1644–1650

Husaini AM, Abdin MZ, Khan SY, Xu W, Aquil S, Anis M (2012) Modifying strawberry for better adaptability to adverse impact of climate change. Curr Sci 102(12):1660–1673

Idris EES, Iglesias DJ, Talon M, Borriss R (2007) Tryptophan-dependent production of indole-3-acetic acid (IAA) affects level of plant growth promotion by Bacillus amyloliquefaciens FZB42. Mol Plant Microbe Interact 20:619–626

Ivanova I, Bojinova D, Nedialkova K (2006) Rock phosphate solubilization by soil bacteria. J Univ Chem Technol Metallurgy 41(3):297–302

Jahromi F, Aroca R, Porcel R, Ruiz-Lozano JM (2008) Influence of salinity on the in vitro development of *Glomus intraradices* and on the in vivo physiological and molecular responses of mycorrhizal lettuce plants. Microb Ecol 55:45–53

Jha Y, Subramanian RB (2013) Paddy plants inoculated with PGPR show better growth physiology and nutrient content under saline conditions. Chil J Agric Res 73(3):213–219

Jogawat A, Shreya S, Madhunita B, Vikram D, Kumar M, Meenakshi D, Varma A, Oelmüller R, Narendra T, Kumar AJ (2013) *Piriformospora indica* rescues growth diminution of rice seedlings during high salt stress. Plant Signal Behav 8(10):e26891–e26896

Jones DL (1998) Organic acids in the rhizosphere – a critical review. Plant Soil 205(1):25–44

Jupe SC, Causton DR, Scott IM (1988) Cellular basis of the effects of gibberellin and the pro gene on stem growth in tomato. Planta 174:106–111

Kamilova F, Validov S, Azarova T, Mulders I, Lugtenberg B (2005) Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. Environ Microbiol 7(11):1809–1817

Kanchana D, Jayanthi M, Kanchana D, Saranraj P, Sujitha D (2013) Evaluation of plant growth promoting substance production by Azospirillum sp isolated from rhizosphere of Chilli (*Capsicum annuum* L). Int J Microbiol Res 4(3):300–304

Kang SC, Pandey P, Khillon R, Maheshwari DK (2008) Process of rock phosphate solubilization by Aspergillus sp PS 104 in soil amended medium. J Environ Biol 29(5):743–746

Khin ML, Moe MM, Tar T, Aung WZM (2012) Isolation of plant hormone (indole-3-acetic acid – IAA) producing rhizobacteria and study on their effects on maize seedling. Eng J 16(5):137–144

Kiely PD, Haynes JM, Higgins CH, Franks A, Mark GL, Morrissey JP, O'Gara F (2006) Exploiting new systems-based strategies to elucidate plant-bacterial interactions in the rhizosphere. Microb Ecol 51:257–266

Kim KY, Jordan DD, McDonald GA (1997) Solubilization of hydroxyapatite by Khan et al: phosphorus solubilizing bacteria for crop production 56 *Enterobacter agglomerans* and cloned *Escherichia coli* in culture medium. Biol Fertil Soils 24:347–352

Kumar M, Yadav V, Tuteja N, Johri AK (2009) Antioxidant enzyme activities in maize plants colonized with *Piriformospora indica*. Microbiology 155:780–790

Kumar V, Sahai V, Bisaria VS (2012) Production of amylase and Chlamydospores by *Piriformospora indica*, a root endophytic fungus. Biocatal Agric Biotechnol 1:124–128

Lenin G, Jayanthi M (2012) Indole acetic acid, gibberellic acid and siderophore production by PGPR isolates from rhizospheric soils of *Catharanthus roseus*. Int J Pharm Biol Sci Arch 3(4):933–938

Lwin KM, Myint MM, Tar T, Aung WZM (2012) Isolation of plant hormone (indole-3-acetic acid – IAA) producing rhizobacteria and study on their effects on maize seedling. Eng J 16(5):137–144

Malla R, Prasad R, Kumari R, Giang PH, Pokharel U, Oelmüller R, Varma A (2004) Phosphorus solubilizing symbiotic fungus: *Piriformospora indica* endocytobiosis. Cell Res 15(2):579–600

Mayak S, Tirosh T, Glick BR (2004) Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. Plant Physiol Biochem 42:565–572

Meena KK, Mesapogu S, Kumar M, Yandigeri MS, Singh G et al (2009) Coinoculation of the endophytic fungus *Piriformospora indica* with the phosphate solubilising bacterium *Pseudomonas striata* affects population dynamics and plant growth in chickpea. Biol Fertil Soils 46:169–174

Mehrvarz S, Chaichi MR, Alikhani HA (2008) Effects of phosphate solubilizing microorganisms and phosphorus chemical fertilizer on yield and yield components of barley (*Hordeum vulgare* L). Am-Eurasian J Agric Environ Sci 3(6):822–828

Mishra N, Sundari KS (2015) Native PGPM consortium: a beneficial solution to support plant growth in the presence of phytopathogens and residual organophosphate pesticides. J Bioproces Biotech 5(2):1–8

Molitor A, Zajic D, Voll LM, Pons-Kühnemann J, Samans B, Kogel K-H, Waller F (2011) Barley leaf transcriptome and metabolite analysis reveals new aspects of compatibility and *Piriformospora indica*–mediated systemic induced resistance to powdery mildew. Mol Plant Microbe Interact 24(12):1427–1439

Murali M, Amruthesh KN, Sudisha J, Niranjana SR, Shetty HS (2012) Screening for plant growth promoting fungi and their ability for growth promotion and induction of resistance in pearl millet against downy mildew disease. J Phytol 4(5):30–36

Nadeem S, Zahir Z, Naveed M, Arshad M (2010) Rhizobacteria capable of producing ACC-deaminase may mitigate salt stress in wheat. Soil Sci Am J 74(2):533–542

Nain L, Rana A, Joshi M, Jadhav SD, Kumar D et al (2009) Evaluation of synergistic effects of bacterial and cyanobacterial strains as biofertilizers for wheat. Plant Soil 331:217–230

Nath R, Sharma GD, Barooah M (2012) Efficiency of tricalcium phosphate solubilization by two different endophytic *Penicillium* sp isolated from tea (*Camellia sinensis* L). Eur J Exp Biol 2(4):1354–1358

Neeraja C, Anil K, Purushotham P, Suma K, Sarma P, Moerschbacher BM, Podile AR (2010) Biotechnological approaches to develop bacterial chitinases as a bioshield against fungal diseases of plants. Crit Rev Biotechnol 30:231–241

Nomura H, Komori T, Kobori M, Nakahira Y, Shiina T (2008) Evidence for chloroplast control of external Ca^{2+-} induced cytosolic Ca^{2+} transients and stomatal closure. Plant J 53:988–998

Ögüt M, Akdag C, Düzdemir O, Sakin MA (2005) Single and double inoculation with Azospirillum/Trichoderma: the effects on dry bean and wheat. Biol Fertil Soils 41:262–272

Ona O, Van Impe J, Prinsen E, Vanderleyden J (2005) Growth and indole-3-acetic acid biosynthesis of *Azospirillum brasilense* Sp245 is environmentally controlled. FEMS Microbiol Lett 246:125–132

Pandya ND, Desai PV (2014) Screening and characterization of GA3 producing *Pseudomonas monteilii* and its impact on plant growth promotion. Int J Curr Microbiol App Sci 3(5):110–115

Parida AK, Das AB (2005) Salt tolerance and salinity effect on plants: a review. Ecotoxicol Environ Saf 60:324–349

Park KH, Lee CY, Son HJ (2009) Mechanism of insoluble phosphate solubilization by Pseudomonas fluorescens RAF15 isolated from ginseng rhizosphere and its plant growth-promoting activities. Lett Appl Microbiol 49:222–228

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763–775

Peskan-Berghofera T, Shahollari B, Giong PH, Hehl S, Markerta C, Blanked V, Kost G, Varma A, Oelmullera R (2004) Association of *Piriformospora indica* with *Arabidopsis thaliana* roots represents a novel system to study beneficial plant–microbe interactions and involves early plant protein modifications in the endoplasmic reticulum and at the plasma membrane. Physiol Plant 122:465–477

Polanco LR, Rodrigues FA, Nascimento KJT, Cruz MFA, Curvelo CRS, DaMatta FM, Vale FXR (2014) Photosynthetic gas exchange and antioxidative system in common bean plants infected by *Colletotrichum lindemuthianum* and supplied with silicon. Trop Plant Pathol 39(1):035–042

Ponmurugan P, Gopi C (2006) Distribution pattern and screening of phosphate solubilizing bacteria isolated from different food and forage crops. J Agron 5:600–604

Porcel R, Aroca R, Azcón R, Ruiz-Lozano JM (2006) PIP aquaporin gene expression in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants in relation to drought stress tolerance. Plant Mol Biol 60:389–404

Porcel R, Aroca R, Ruíz-Lozano JM (2012) Salinity stress alleviation using arbuscular mycorrhizal fungi: a review. Agron Sustain Dev 32:181–200

Prasad R, Bagde US, Puspangadan P, Varma A (2008) *Bacopa monnieri* L: pharmacological aspects and case study involving *Piriformospora indica*. Int J Integr Biol 3(2):100–110

Rai MK (2010) Review: biotechnological strategies for conservation of rare and endangered medicinal plants. Biodiversitas 11(3):157–166

Rai M, Varma A (2005) Arbuscular mycorrhiza-like biotechnological potential of *Piriformospora indica,* which promotes the growth of *Adhatoda vasica* Nees. Electron J Biotechnol 8(1):107–112

Rai M, Acharya D, Singh A, Varma A (2001) Positive growth responses of the medicinal plants *Spilanthes calva* and *Withania somnifera* to inoculation by *Piriformospora indica* in a field trial. Mycorrhiza 11:123–128

Rathod DP, Brestic M, Shao HB (2011) Chlorophyll *a* fluorescence determines the drought resistance capabilities in two varieties of mycorrhized and non-mycorrhized *Glycine max* Linn. Afr J Microbiol Res 5(24):4197–4206

Ray JG, Valsalakumar N (2010) Arbuscular mycorrhizal fungi and *Piriformospora indica* individually and in combination with *Rhizobium* on green gram. J Plant Nutr 33:285–298

Resende MP, Jakoby ICMC, dos Santos LCR, Soares MA, Pereira FD, Souchie EL et al (2014) Phosphate solubilization and phytohormone production by endophytic and rhizosphere *Trichoderma* isolates of guanandi (*Calophyllum brasiliense* Cambess). Afr J Microbiol Res 8:2616–2623

Reyes I, Bernier L, Simard RR, Antoun H (1999) Effect of nitrogen source on the solubilization of different inorganic phosphates by an isolate of *Penicillium rugulosum* and two UV-induced mutants. FEMS Microbiol Ecol 28:281–290

Rodriguez H, Fraga R, Gonzalez T, Bashan Y (2007) Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. Dev Plant Soil Sci 102:15–21

Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress new perspectives for molecular studies. Mycorrhiza 13:309–317

Ryu R, Patten CL (2008) Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by 4 TyrR in *Enterobacter cloacae* UW5. Am Soc Microbiol 190(21):1–35

Saber Z, Pirdashti H, Heidarzade A (2013) Plant growth promoting rhizobacteria effects on yield and yield components of four rapeseed (*Brassica napus* L) cultivars under salt condition. Int J Agric Crop Sci 5(17):1869–1873

Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res 21:1–30

Sanchez MR, Aroca R, Munoz Y, Polon R, Lozano JMR (2010) The arbuscular mycorrhizal symbiosis enhances the photosynthetic efficiency and the antioxidative response of rice plants subjected to drought stress. J Plant Physiol 167:862–869

Sattar MA, Rahman MF, Das DK, Choudhury TMAA (2008) Prospects of using *Azotobacter, Azospirillum* and *Cyanobacteria* as supplements of urea nitrogen for rice production in Bangladesh. In: Proceedings of Australia Centre for International Agricultural Research, no 130, pp 59–66

Schachtman DP, Goodger JQD (2008) Chemical root to shoot signalling under drought. Trends Plant Sci 13:281–287

Schäfer P, Pfiffi S, Voll LM, Zajic D, Chandler PM, Waller F, Scholz U, Pons-Kuhnemann J, Sonnewald S, Sonnewald U, Kogel K-H (2009) 'Manipulation of plant innate immunity and gibberellin as factor of compatibility in the mutualistic association of barley roots with Piriformospora indica'. The Plant Journal, 59: 461–474.

Schulz B, Boyle C (2005) The endophytic continuum. Mycol Res 109(6):661–666

Serfling A, Wirsel SGR, Lind V, Deising H (2007) Performance of the biocontrol fungus *Piriformospora indica* on wheat under greenhouse and field condition. Phytopathology 97:523–531

Sirrenberg A, Gobel C, Gron S, Czempinski N, Ratzinger A, Karlovsky P, Santos P, Feussnera I, Pawlowskia K (2007) *Piriformospora indica* affects plant growth by auxin production. Physiol Plant 131:581–589

Sudadi S (2012) Exogenous application of tryptophan and indole acetic acid (IAA) to induce root nodule formation and increase yield of soybean. Agric Sci Res J 2(4):134–139

Sudharani M, Shivaprakash MK, Prabhavathi MK (2014) Role of consortia of biocontrol agents and PGPR s in the production of cabbage under nursery condition. Int J Curr Microbiol App Sci 3(6):1055–1064

Tajini F, Trabelsi M, Drevon JJ (2011) Co-inoculation with *Glomus intraradices* and *Rhizobium tropici* CIAT899 increases P use efficiency for N_2 fixation in common bean under P deficiency in hydroaeroponic culture. Symbiosis 53:123–129

Tallapragada P, Dikshit R, Seshagiri S (2015a) Isolation and optimization of IAA producing *Burkholderia seminalis* and its effect on seedlings of tomato Songklanakarin. J Sci Technol 37(5):553–559

Tallapragada P, Dikshit R, Seshagiri S (2015b) Effect of *Rhizophagus spp* and plant growth-promoting *Acinetobacter junii* on *Solanum lycopersicum* and *Capsicum annuum*. Braz J Bot 38(2):273–280

Tanwar A, Aggarwal A, Kadian N, Gupta A (2013) Arbuscular mycorrhizal inoculation and super phosphate application influence plant growth and yield of *Capsicum annuum* L. J Soil Sci Plant Nutr 13(1):55–66

Tejera NA, Soussi M, Lluch C (2006) Physiological and nutritional indicators of tolerance to salinity in chickpea plants growing under symbiotic condition. Environ Exp Bot 58:17–24

Tsavkelova EA, Klimova SY, Cherdyntseva TA, Netrusov AI (2006) Microbial producers of plant growth stimulators and their practical use: a review. Appl Biochem Microbiol 42:117–126

Upadhyay A, Srivastava S (2010) Evaluation of multiple plant growth promoting traits of an isolate of Pseudomonas fluorescens strain Psd. Indian J Exp Biol 48(6):601–609

Uthandi S, Karthikeyan S, Sabarinathan KG (2010) Gibberellic acid production by *Fusarium fujikuroi* SG2. J Sci Ind Res 69:211–214

Vacheron J, Desbrosses G, Bouffaud M-L, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Dyé FW, Combaret CP (2013) Plant growth-promoting rhizobacteria and root system functioning. Front Plant Sci 4(356):1–19

Vadassery J, Ranf S, Drzewiecki C, Mithofer A, Mazars C, Scheel D, Lee J, Oelmuller R (2009) A cell wall extract from the endophytic fungus *Piriformospora indica* promotes growth of Arabidopsis seedlings and induces intracellular calcium elevation in roots. Plant J 59:193–206

Valverde A, Burgos A, Fiscella T, Rivas R, Velazquez E et al (2006) Differential effects of coinoculations with *Pseudomonas jessenii* PS06 (a phosphate solubilizing bacterium) and

Mesorhizobium ciceri C-2/2 strains on the growth and seed yield of chickpea under greenhouse and field conditions. Plant Soil 287:43–50

Van Loon LC (2007) Plant responses to plant growth-promoting rhizobacteria. Eur J Plant Pathol 119:243–254

Varma A, Savita V, Sudha Sahay N, Butehorn B, Franken P (1999) *Piriformospora indica*, a cultivable plant–growth–promoting root endophyte. Appl Environ Microbiol 65(6):2741–2744

Vessey JK (2003) Plant growth promoting rhizobacteria as biofertilizers. Plant Soil 255:571–586

Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, Heier T, Huckelhoven R, Neumann C, von Wettstein D, Franken P, Kogel K-H (2005) The endophytic fungus *Piriformospora indica* reprograms barley to salt–stress tolerance, disease resistance, and higher yield. Proc Natl Acad Soc U S A 102(38):13386–13391

Waller F, Mukherjee K, Deshmukh SD, Achatz B, Sharma M, Schäfer P, Kogel KH (2008) Systemic and local modulation of plant responses by *Piriformospora indica* and related *Sebacinales* species. J Plant Physiol 165:60–70

Waqas M, Khana AL, Hamayuna M, Shahzada R, Kanga S-M, Kime J-G, Leea I-J (2015) Endophytic fungi promote plant growth and mitigate the adverse effects of stem rot: an example of *Penicillium citrinum* and *Aspergillus terreus*. J Plant Interact 10(1):280–287

Yadav BK, Tarafdar JC (2011) *Penicillium Purpurogenum*, unique P mobilizers in arid agroecosystems. Arid Land Res Manag 25(1):87–99

Yadav V, Kumar M, Deep DK, Kumar H, Sharma R, Tripathi T, Tuteja N, Saxena AK, Johri AK (2010) A phosphate transporter from the root endophytic fungus *Piriformospora indica* plays a role in the phosphate transport to the host plant. J Biol Chem 285(34):26532–26544

Yildirim E, Turan M, Donmez MF (2008) Mitigation of salt stress in radish (*Raphanus sativus* L) by plant growth promoting rhizobacteria. Rom Biotechnol Lett 13(5):3933–3943

Yooyongwech S, Phaukinsang N, Cha-Um S, Supaibulwatana K (2013) Arbuscular mycorrhiza improved growth performance in *Macadamia tetraphylla* L grown under water deficit stress involves soluble sugar and proline accumulation. Plant Growth Regul 69:285–293

Zaidi A, Khan MS (2006) Co-inoculation effects of phosphate solubilizing microorganisms and *Glomus fasciculatum* on green gram – Bradyrhizobium symbiosis. Turk J Agric 30:223–230

Zarea MJ, Hajinia S, Karimi N, Goltapeh EM, Rejali F, Varma A (2012) Effect of *Piriformospora indica* and *Azospirillum* strains from saline or non-saline soil on mitigation of the effects of NaCl. Soil Biol Biochem 45:139–146

Role of *Trichoderma* spp. as a Biocontrol Agent of Fungal Plant Pathogens

23

Manish Kumar and Shabbir Ashraf

Abstract

In developing countries, there is rampant use of chemical pesticides to control plant diseases by agriculturist. Pesticides not only pollute the soil but also cause environmental pollution and human hazard. Among the fungal biocontrol agents, *Trichoderma* spp. are being used most abundantly against plant pathogens. Several species of *Trichoderma* which produce volatile and non-volatile antibiotics and enzymes are antagonistic to phytopathogenic fungi and nematodes. *Trichoderma* spp. are free-living and abundantly present in the soil and rhizosphere region and are mycoparasitic of several soilborne plant pathogens. It has also been exploited successfully as a biocontrol agent for controlling the foliar diseases of economically important plants. The fungus is effective against pathogens causing various diseases of the root region of plants, viz. collar rot, foot rot, damping off, etc. In the rhizosphere region, some strains of *Trichoderma* spp. release metabolites which improve the growth of seedling, and it also causes resistance against abiotic stress. *Trichoderma* spp. have great potential against soilborne pathogens, and it may be able to replace chemical pesticides in the near future.

23.1 Introduction

Chemical pesticides are used abundantly by farmers in the developing countries polluting soil and water leading to health problem in human beings and animals. Among the Asian countries, the largest producer of pesticides is India. It ranks 12th

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in the use of pesticides in the world. The export of agricultural commodities like vegetables and fruits has been banned or restricted from developed countries due to pesticide residue. In the last few years, integrated pest management strategies and avoidance or regulation of pesticides by using more fungal biocontrol agents especially *Trichoderma* spp. reduced the use of pesticides against economically important crops. *Trichoderma* spp. are the most widely used fungal biocontrol agents against fungal diseases of pulses, grapes, cotton, onion, carrot, peas, plums, maize, apple, etc. *Trichoderma* spp. grow very fast and can produce polysaccharide-degrading enzymes, so it can be grown on a large number of substrates. They can also tolerate different kinds of environmental condition (Papavizas 1985; Elad et al. 1993).

The fungal genus *Trichoderma* includes important species for production of antibiotics and enzymes (Howell 2003; Viesturs et al. 1996) and biocontrol activity against fungi and nematodes (Brunner et al. 2005; Sahebani and Hadavi 2008). It also helps in induction of systemic acquired resistance in plants by endophytism (Brunner et al. 2005; Hanson and Howell 2004; Kubicek et al. 2001). *Trichoderma* species can also enhance plant growth and development (Chang et al. 1986; De Souza et al. 2008; Gravel et al. 2007). Insertion or resident living organisms allude to purposeful utilisation of biological control other than disease-resistant host plants to suppress the activities of plant pathogens (Pal and Gardener 2006).

23.2 Mass Multiplication of *Trichoderma* spp. and Their Commercialisation

Grains are the best source of nutritive media; Jowar (*Sorghum bicolor*) and Bajra (*Pennisetum typhoides*) are soaked in water for 10 h. The soaked grains filled in polypropylene bags followed by autoclaving for 30 min at 15 psi pressure. The bags are left for cooling overnight followed by autoclaving. Five ml of stock solution (10^6–10^8 CFU/ml) of starter culture should be inoculated and incubated at 25 ± 2 °C for 15 days in a temperature-controlled room. After visual checking of bags for 15 days to estimate contamination, the bags dry overnight at 35 °C in hot air oven. The obtained formulation is mixed with presterilised talc 1:9 (*Trichoderma* spore/talc) ratio. After testing and using a standardised method, products are ready for packaging as well as transportation for purposeful utilisation (Srivastava et al. 2015). A mixture of peat and wheat bran (1:1, v/v) has been also used widely. pH should remain constant below 5.5 during growth period which prevents from bacterial contamination. After 1–2 years, the shelf life of fungus and the number of colony forming units (CFUs) decrease and have been experimented in the greenhouse and field which has been successfully proven (Chet 1987; Elad and Chet 1995; Sivan and Chet 1992) (Table 23.1).

Table 23.1 Fungal bioagents (*Trichoderma* spp.) with their manufacturers and trade names

Trade name	Bioagents	Manufacturers
Biofungus	*Trichoderma* spp.	Grondortsmettingen De Cuester n. V.Belgium
Bas-derma	*Trichoderma viride*	Basarass Biocontrol Res. Lab., India
Binab T	*Trichoderma harzianum* (ATCC 20476) and *Trichoderma polysporum* (ATCC 20475)	Bio-Innovation AB, UK
Trichopel	*Trichoderma harzianum* and *Trichoderma viride*	Agrimm Technologies Ltd., New Zealand
T-22 G, T-22 HB	*Trichoderma harzianum* strain KRL-AG2	THT Inc., USA
Bioderma	*Trichoderma harzianum/Trichoderma viride*	Biotech International Ltd., India
TY	*Trichoderma* spp.	Mycocontrol, Israel
Trieco	*Trichoderma viride*	Ecosense Labs Pvt. Ltd., Mumbai, India
Tri-control	*Trichoderma* spp.	Jeypee Biotechs, India

Source: Ashraf and Zuhaib (2013)

23.3 Mechanism

Plant diseases as a result of interaction among various components consist of host pathogens and environment, i.e. disease triangle. Bioagents are the organisms which manage the diseases by interaction components of disease triangle. Interaction of pathogens and bioagents concedes to handle the soil environment to create a conducive condition for successful biocontrol strategies against plant disease (Chet 1987). Biocontrol agents involve several types of mechanisms in achieving disease control. However, the conclusive evidences for the involvement of a particular factor in biological control are determined by the strict correlation between the appearance of factor and the biological control (Handelsman and Parke 1989).

23.3.1 Mycoparasitism

Hyperparasitism is one of the main mechanisms that involve *Trichoderma* spp. which is considered a direct form of antagonism (Pal and Gardener 2006). It acts as a biocontrol agent against target pathogens, dissolution and coiling of target pathogens and their activity as enzymes. Weindling in 1932 was the first scientist to report that *Trichoderma lignorum* are antagonistic to *Rhizoctonia solani.* The term mycoparasitism is used for an antagonist, a parasitic fungus whose host is another fungus (Hawksworth 1983). Mycoparasitism is a mechanism in which generally there is lytic enzyme. Chet et al. (1981) described that generally mycoparasitism occurs in four

steps: chemotropism and recognition, attachment and coiling, cell wall penetration and digestion of host cell. Harman (2000) reported that chitinase and β-1,3-glucanase have a role in biological control by *Trichoderma* spp. The mycelium and resting spores of several phytopathogenic fungi present in soil are invaded and parasitised (mycoparasitism) by several fungi. Among the most common mycoparasitic fungi of *Trichoderma* species is *T. harzianum*.

23.3.2 Competition

Microorganisms most commonly die of starvation as nutrients are in limited quantity in their immediate environment around plant surfaces and soil. Thus, to establish themselves well in the environment, both pathogen and biocontrol agents give each other a tough competition for food and space. Biocontrol agents mainly compete for vital micronutrients such as iron in oxidised and aerated soils. *Trichoderma* spp. synthesise iron-chelating siderophores to cope up with this problem of micronutrient scarcity from other pathogenic fungi (Benítez et al. 2004), as biocontrol agents are highly specialised in substance uptaking system as compared to the pathogens (Nelson 1990). Fe (II) ions are chelated by siderophores to form siderophore-Fe complex which is specifically recognised by the membrane-bound protein receptors of biocontrol agents (Mukhopadhyay and Mukherjee 1998), thus making iron available in lower quantity for the pathogen. Competition for space by the biocontrol agents causes delayed root colonisation by the pathogen, making it weak to establish and cause the disease.

23.3.3 Induction of Plant Defence

Trichoderma spp. are not only fast growers that produces large spores but are also opportunistic invaders that stimulates hypersensitivity and induces systemic acquired resistance (SAR) and induced systematic resistance (ISR) in host plants (Vinale et al. 2008). *Trichoderma hamatum* causes physiological changes in tomato plants by colonising the roots and inducing systemic changes to prevent disease damage (Alfano et al. 2007). A study conducted on cucumber plant revealed that *Trichoderma asperellum* possess two different genes encoding phenylalanine and hydroperoxide lyase that induce systematic resistance and phytoalexin accumulation to provide resistance against *P. syringae* pv. *lachrymans* (Yedidia et al. 2003). Induction of resistance (localised or systemic) is an essential component of *Trichoderma* spp. for inhibiting plant diseases (Harman et al. 2004).

23.3.4 Plant Growth Enhancer

Apart from controlling the plant pathogens, *Trichoderma* spp. also intensify the plant growth, act as biofertilizer and stimulate the defence mechanism in plant (Harman et al. 2004). Improved growth of lettuce, pepper plants and tomato has

been observed with the use of *Trichoderma* (Vinale et al. 2006). Treatment of maize plants with *Trichoderma harzianum* strain T-22 showed two lines of increase in root length in comparison to the controlled plants (Harman et al. 2004). Secondary metabolites produced by *Trichoderma* spp. such as koningin A (*Trichoderma koningii*) and 6-pentyl-alpha-pyrone (*T. harzianum*) act as growth regulator in plants (Cutler et al. 1986). Gluconic acid and citric acid production and reduction in soil pH increased solubilisation of phosphate minerals (such as Fe, Mg and Mn), and micronutrients are some other important activities exhibited by *Trichoderma* spp. (Benítez et al. 2004; Harman et al. 2004; Vinale et al. 2008).

23.3.5 Plant Root Colonisation

Plant root colonisation with *Trichoderma* spp. enhances defence reaction in plants by inducing the production of β-1,3-glucanase, peroxidases, phenylalanine, chitinases and hydroperoxides activating biosynthetic pathogens and causing phytoalexin accumulation (Yedidia et al. 2003; Harman et al. 2004). Electron microscopy for the physical interaction between *Trichoderma harzianum* and cucumber plant showed fungal penetration of the root in epidermal and outer cortex region. This interaction was symbiotic in which *Trichoderma* stimulate the increased activity of peroxidase and chitinase, thus protecting the plant from disease and in return the plant providing nutritional niche to the fungus.

23.4 *Trichoderma* Act as Bioagents Against Soilborne Pathogen

Initiation of chemotrophic reaction due to interaction between *Trichoderma* and its host is expressed by direct mycoparasitic hyphal growth towards the host plant (Chet et al. 1981). Fungal hyphae first coil around the host plant which is followed by contact (Benhamou and Chet 1993) and penetration due to release of lytic enzymes by the *Trichoderma* spp., partially degrading the host cell wall (Elad et al. 1982). Antagonistic interaction of *Trichoderma* has been shown by several possible mechanisms:

(a) Volatile and non-volatile antibiotic synthesis by the fungus (Baker and Griffin 1995)
(b) Limiting factors (space or nutrient uptake) competing with host (Sivan and Chet 1989)
(c) Direct mycoparasitism by host cell wall degradation due to lytic enzyme production by *Trichoderma* (Chet et al. 1998)

Biocontrol of soilborne diseases is the dynamic environment that makes an interesting setting by the interactions which lead to disease reduction against pathogen (Rovira 1991; Hawes 1991; Waisel et al. 1991). The effect of *Trichoderma*

Table 23.2 Use of *Trichoderma* spp. against different plant pathogens of the soil

Host plant	Trichoderma spp.	Causative agent	References
Gossypium hirsutum (Cotton)	*Trichoderma viride*, *Trichoderma harzianum*	*Rhizoctonia solani*, *Sclerotium rolfsii*, *Pythium aphanidermatum*	Gaur and Sharma (2005)
Brassica oleracea (Cauliflower)	*Trichoderma viride*, *Trichoderma harzianum*	*Rhizoctonia solani*, *Pythium aphanidermatum*	Sharma et al. (2004) and Ahuja et al. (2012)
Citrus	*Trichoderma viride*, *Trichoderma harzianum*	*Fusarium solani*	Kalita et al. (1996)
Vigna unguiculata (Cowpea)	*Trichoderma harzianum*	*Rhizoctonia solani*	Pan and Das (2011)
Capsicum annuum L. (Chilli)	*Trichoderma viride*, *Trichoderma harzianum*	*Sclerotium rolfsii*, *Fusarium oxysporum*, *Pythium* spp.	Rini and Sulochana (2006)
Cicer arietinum (Chickpea)	*Trichoderma harzianum*, *Trichoderma viride*	*Fusarium oxysporum*, *R. solani*, *A. niger*, *Chaetomium* spp., *S. rolfsii*, *Penicillium* spp., *M. phaseolina*	Mukherjee and Raghu (1997), Pandey et al. (2003), and Poddar et al. (2004)
Vigna mungo (Black gram)	*Trichoderma viride*, *Trichoderma harzianum*	*Macrophomina phaseolina*, *Alternaria alternata*	Mishra et al. (2011)

harzianum on plant-parasitic nematodes in the greenhouse has been also investigated. *Trichoderma harzianum* parasitism on *Meloidogyne javanica.* Two isolates of *Trichoderma lignorum* and the T-203 have been evaluated for their nematicidal activity (Spiegel and Chet 1998). Economically important soilborne pathogens cause plant disease like damping off and root rot resulting in rapid collapse of plant seedlings.

Therefore, biocontrol agents are efficient first and foremost against soilborne pathogens which protect young seedlings against detrimental attack by infective inoculum (Table 23.2).

23.5 Gene for Biocontrol in *Trichoderma* spp.

A number of soilborne pathogens are effectively managed by the wide use of *Trichoderma* spp. (Table 23.3) as they can efficiently synthesise cell wall-degrading enzymes. Isolation and cloning of many commercially important biocontrol genes of *Trichoderma* spp. for massive commercial production have been successfully proven by many researchers (Massart and Jijakli 2007).

Table 23.3 Some biocontrol genes of *Trichoderma* and their function

Gene	Source	Activity
Tvsp1	*Trichoderma virens*	Serine protease-encoding gene successfully managed *Rhizoctonia solani* affecting cotton seedling
TgaA and *TgaB*	*Trichoderma virens*	Antagonistic role against *Rhizoctonia solani* and *Sclerotium rolfsii*
egl1.	*Trichoderma longibrachiatum*	Biocontrol of cucumber damping off by *Pythium ultimum*
Th-Chit	*Trichoderma harzianum*	This gene is responsible for the antifungal activity in transgenic tobacco plant
ThPG1	*Trichoderma harzianum*	Endopolygalacturonase-encoding genes that act against cell wall degradation of pathogens such as *Rhizoctonia solani* and *Pythium ultimum*
Taabc2	*Trichoderma atroviride*	This gene has a significant role in ATP-binding cassette (ABC) transporter in cell membrane pump that helps in the mycoparasitism activity by playing significant role in ATP- binding cassette transporting in cell membrane
tac1	*Trichoderma virens*	Antagonistic role in mycoparasitic activity against *Rhizoctonia solani* and *Pythium ultimum*

Source: Srivastava et al. (2014a, b)

Phytopathogenic activity of *Trichoderma* spp. is attributed to mycoparasitism, competition and antibiosis (Janisiewicz and Korsten 2002). Among which, *Trichoderma harzianum* is isolated to be the most potent strain (Guo et al. 2002). Kuć (2001) proved *Trichoderma* possess genes that impart resistance against biotic and abiotic stress (Table 23.3).

Conclusion

Fungicides which are chemically based can manage or control effectively, but they pollute soil and water causing harm to human health. Nature has been gifted with biocontrol agents like *Trichoderma* spp. which are antagonistic to most fungal pathogens of plants. It is found mostly in soil and rhizosphere region. Weindling (1932) is the pioneer scientist who discovered mycoparasitism against pathogenic fungi. Soilborne fungal pathogens of plant can be controlled by species and races of *Trichoderma*. There are genes in *Trichoderma* which helps the host plants in resistance against pathogenic fungi. Integration of genetic engineering and molecular biology will open new vistas in developing fungal bioagents for the benefit of plants and safety of environment as well as mankind. The biocontrol commercial products having *Trichoderma* should have a long shelf life and can be stored at room temperature. They are safe, environment friendly and can be very easily used by farmers. However, it needs more work to be done to develop stable, cost-effective and easy to apply formulations, which can control more than one pathogen.

References

Ahuja DB, Ahuja UR, Srinivas P, Singh RV, Malik M, Sharma P, Bamawale OM (2012) Development of farmer-led integrated management of major pests of cauliflower cultivated in rainy season in India. J Agric Sci 4:79–83

Alfano G, Ivey ML, Cakir C, Bos JIB, Miller SA, Madden LV, Hoitink HAJ (2007) Systemic modulation of gene expression in tomato by *Trichoderma hamatum* 382. Phytopathology 97:429–437

Ashraf S, Zuhaib M (2013) Fungal biodiversity: a potential tool in plant disease management. In: Malik A, Grohmann E, Alves M (eds) Management of microbial resources in the environment. Springer, Dordrecht, pp 69–90

Baker R, Griffin GJ (1995) Molecular strategies for biological control of fungal plant pathogens. In: Reuveni R (ed) Novel approaches to integrated pest management. Lewis, Boca Raton, pp 153–182

Benhamou N, Chet I (1993) Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani*: ultrastructure and gold cytochemistry of the mycoparasitic process. Phytopathology 83:1062

Benítez T, Rincón AM, Limón MC, Codón AC (2004) Mecanismos de biocontrol de cepas de Trichoderma. Int Microbiol 7:249–260

Brunner K, Zeilinger S, Ciliento R, Woo SL, Lorito M, Kubicek CP, Mach RL (2005) Improvement of the fungal biocontrol agent *Trichoderma atroviride* to enhance both antagonism and induction of plant systemic disease resistance. Appl Environ Microbiol 71:3959–3965

Chang YC, Chang YC, Baker R, Kleifeld O, Chet I (1986) Increased growth of plants in the presence of the biological control agent *Trichoderma harzianum*. Plant Dis 70:145–148

Chet I (1987) Innovative approaches to plant disease control. Wiley, New York

Chet I, Harman GE, Baker R (1981) Trichoderma hamatum: its hyphal interactions with *Rhizoctonia solani* and *Pythium* spp. Microb Ecol 7:29–38

Chet I, Benhamou N, Haran S (1998) Mycoparasitism and lytic enzymes. In: Harman GE, Kubicek CP (ed) Trichoderma and Gliocladium. Vol. 2: enzymes, biological control and commercial application. London, Taylor and Francis, pp 153–172

Cutler HG, Cox RH, Crumley FG, Cole PD (1986) 6-Pentyl-α-pyrone from *Trichoderma harzianum*: its plant growth inhibitory and antimicrobial properties. Agric Biol Chem 50:2943–2945

De Souza JT, Bailey BA, Pomella AWV, Erbe EF, Murphy CA, Bae H, Hebbar PK (2008) Colonization of cacao seedlings by *Trichoderma stromaticum*, a mycoparasite of the witches' broom pathogen, and its influence on plant growth and resistance. Biol Control 46:36–45

Elad Y, Chet I (1995) Practical approaches for biocontrol implementation. In: Reuvuni R (ed) Novel approaches to integrated pest management. Lewis, Boca Raton, pp 323–338

Elad Y, Chet I, Henis Y (1982) Degradation of plant pathogenic fungi by *Trichoderma harzianum*. Can J Microbiol 28:719–725

Elad Y, Zimand G, Zaqs Y, Zuriel S, Chet I (1993) Use of *Trichoderma harzianum* in combination or alternation with fungicides to control cucumber grey mould (Botrytis cinerea) under commercial greenhouse conditions. Plant Pathol 42:324–332

Gaur RB, Sharma RN, Sharma RR, Vinod Singh Gautam (2005) Efficacy of *Trichoderma* for *Rhizoctonia* root rot control in chickpea. J Mycol Plant Pathol 35:144–150

Gravel V, Antoun H, Tweddell RJ (2007) Growth stimulation and fruit yield improvement of greenhouse tomato plants by inoculation with *Pseudomonas putida* or *Trichoderma atroviride*: possible role of indole acetic acid (IAA). Soil Biol Biochem 39:1968–1977

Guo R, Liu X, Gao K, Gao B, Shi B, Zhen Z (2002) Progress in biocontrol research with Trichoderma. Chinese J Biol Control 18:180–184

Handelsman J, Parke JL (1989) Mechanisms of biocontrol of soil borne plant pathogens. Plant-microbe interactions (USA). In: Kosuge T, Nester EW (eds) Plant-microbe interactions, molecular and genetic perspectives, vol 3. McGraw-Hill, New York, pp 27–61

Hanson LE, Howell CR (2004) Elicitors of plant defense responses from biocontrol strains of Trichoderma viren. Phytopathology 94:171–176

Harman GE (2000) Myths and dogmas of biocontrol changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Dis 84:377–393

Harman GE, Lorito M, Lynch JM (2004) Uses of *Trichoderma* spp. to alleviate or remediate soil and water pollution. Adv Appl Microbiol 56:313–330

Hawes MC (1991) Living plant cells released from the root cap: a regulator of microbial populations in the rhizosphere. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Springer, Dordrecht, pp 51–59

Hawksworth DL (1983) A key to the lichen-forming, parasitic, parasymbiotic and saprophytic fungi occurring on lichens in the British Isles. Lichenologist 15(01):1–44

Howell CR (2003) Mechanisms employed by Trichoderma species in the biological control of plant diseases: the history and evolution of current concepts. Plant Dis 87:4–10

Janisiewicz WJ, Korsten L (2002) Biological control of postharvest diseases of fruits. Annu Rev Phytopathol 40:411–441

Kalita P, Bora LC, Bhagabati KN (1996) Phylloplane microflora of citrus and their role in management of citrus canker. Indian Phytopathol 49:234–237

Kubicek CP, Mach RL, Peterbauer CK, Lorito M (2001) *Trichoderma*: from genes to biocontrol. J Plant Pathol:11–23

Kuć J (2001) Concepts and direction of induced systemic resistance in plants and its application. Eur J Plant Pathol 107:7–12

Massart S, Jijakli HM (2007) Use of molecular techniques to elucidate the mechanisms of action of fungal biocontrol agents: a review. J Microbiol Methods 69:229–241

Mishra BK, Mishra RK, Mishra RC, Tiwari AK, Yadav RS, Dikshit A (2011) Biocontrol efficacy of *Trichoderma viride* isolates against fungal plant pathogens causing disease in Vigna radiata L. Arch Appl Sci Res 3:361–369

Mukherjee PK, Raghu K (1997) Effect of temperature on antagonistic and biocontrol potential of shape Trichoderma sp. on *Sclerotium rolfsii*. Mycopathologia 139:151–155

Mukhopadhyay ASN, Mukherjee PK (1998) Biological control of plant diseases: status in India. In: Singh SP, Husain SS (eds) Biological suppression of plant diseases: phytopathogens, nematodes and weeds, vol 7. Project Directorate of Biological Control, Bangalore, pp 1–20

Nelson EB (1990) Exudate molecules initiating fungal responses to seed seeds and roots. Plant Soil 129:61–73

Pal KK, Gardener BM (2006) Biological control of plant pathogens. Plant Health Instructor 2:1117–1142

Pan S, Das A (2011) Control of cowpea (*Vigna sinensis*) root and collar rot (*Rhizoctonia solani*) with some organic formulations of *Trichoderma harzianum* under field condition. J Plant Prot Sci 3:20–25

Pandey G, Pandey RK, Pant H (2003) Efficacy of different levels of *Trichoderma viride* against root-knot nematode in chickpea (*Cicer arietinum* L.) Ann Plant Prot Sci 11:101–103

Papavizas GC (1985) *Trichoderma* and *Gliocladium*: biology, ecology, and potential for biocontrol. Annu Rev Phytopathol 23:23–54

Poddar RK, Singh DV, Dubey SC (2004) Integrated application of *Trichoderma harzianum* mutants and carbendazim to manage chickpea wilt (*Fusarium oxysporum* f. sp. *ciceris*). Indian J Agric Sci 74:346–348

Rini CR, Sulochana KK (2006) Management of seedling rot of chilli (*Capsicum annuum* L.) using *Trichoderma* spp. and fluorescent pseudomonads (*Pseudomonas fluorescens*). J Trop Agric 44:79–82

Rovira AD (1991) Rhizosphere research-85 years of progress and frustration. In: Keister DL, Cregan PB (eds) The rhizosphere and plant growth. Springer, Dordrecht, pp 3–13

Sahebani N, Hadavi N (2008) Biological control of the root-knot nematode *Meloidogyne javanica* by *Trichoderma harzianum*. Soil Biol Biochem 40:2016–2020

Sharma SK, Kalra K, Kocher GS (2004) Fermentation of enzymatic hydrolysate of sunflower hulls for ethanol production and its scale-up. Biomass Bioenergy 27:399–402

Sivan A, Chet I (1989) The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. Phytopathology 79:198–203

Sivan A, Chet I (1992) Microbial control of plant diseases. In Mitchel R (ed) Environmental microbiology. Wiley-Liss, New York, pp 335–354

Spiegel Y, Chet I (1998) Evaluation of *Trichoderma* spp. as a biocontrol agent against soilborne fungi and plant-parasitic nematodes in Israel. Integr Pest Manag Rev 3:169–175

Srivastava M, Shahid M, Singh A, Kumar V, Pandey S, Srivastava YK (2014a) To develop strain specific molecular marker for easy and proper identification of fungal species based on molecular characters: a review. J Mool Bimark Diagn 5:172

Srivastava M, Shahid M, Pandey S, Singh A, Kumar V, Gupta S, Maurya M (2014b) *Trichoderma* genome to genomics: a review. J Data Mining Genomics Proteomics 5(3):1000172

Srivastava M, Sonika P, Shahid M, Kumar V, Singh A, Trivedi S, Srivastava YK (2015) *Trichoderma*: a magical weapon against soil borne pathogens. Afr J Agric Res 10:4591–4598

Viesturs U, Leite M, Treimanis A, Eremeeva T, Apsite A, Eisimonte M, Jansons P (1996) Production of cellulases and xylanases by *Trichoderma viride* and biological processing of lignocellulose and recycled paper fibers. Appl Biochem Biotechnol 57:349–360

Vinale F, Marra R, Scala F, Ghisalberti EL, Lorito M, Sivasithamparam K (2006) Major secondary metabolites produced by two commercial *Trichoderma* strains active against different phytopathogens. Lett Appl Microbiol 43:143–148

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Woo SL, Lorito M (2008) *Trichoderma*–plant–pathogen interactions. Soil Biol Biochem 40:1–10

Waisel Y, Eshel A, Kafkafi U (1991) Plant roots: the hidden half. Marcel Dekker, Inc

Weindling R (1932) *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology 22:837–845

Yedidia I, Shoresh M, Kerem Z, Benhamou N, Kapulnik Y, Chet I (2003) Concomitant induction of systemic resistance to Pseudomonas syringae pv. lachrymans in cucumber by *Trichoderma asperellum* (T-203) and accumulation of phytoalexins. Appl Environ Microbiol 69:7343–7353

Microbes as Biocontrol Agents

24

Babbal, Adivitiya, and Yogender Pal Khasa

Abstract

Phytopathogens pose a major threat to ecosystem stability and food production, indicating the need for developing methods to control the severe losses caused by these pathogens. To control these pathogens, the use of various chemical pesticides is majorly practiced. These pesticides are associated with environmental and health hazards and also pose a risk of resistance development in phytopathogens against them forcing the researcher towards the development of alternative and innovative methods by which sustainable management of plant diseases can be achieved. To control plant diseases and have pesticide-free food worldwide, the use of natural antagonistic microorganisms known as biocontrol agents or biological control agents (BCA) is employed. BCA can act on these pathogens through a number of mechanisms such as antibiosis, hyperparasitism, enzyme production, induction of plant resistance mechanisms and competition for essential nutrients and space and through plant growth promotion. Apart from controlling phytopathogens, these microbial agents also promote plant growth and stress tolerance. BCA can be used as bioinsecticides, bionematicides and biopesticides. They are also used for the management of post-harvest diseases. Recently, recombinant microbes have been developed with enhanced biocontrol capabilities. Several commercially available BCA are currently being used for the efficient control of plant disease with improved productivity of many crops. These majorly include GB34, Kodiak, Serenade and Companion containing *Bacillus* as the active ingredient, Biosave 10LP and Bio-jet containing *Pseudomonas* as the active ingredient and Soilguard, Trichodex and Trichojet containing *Trichoderma* as the active ingredient. Thus, use of microbes such as fungi, bacteria, yeast and viruses

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holds an enormous potential as biocontrol agents to replace conventional chemical-based pesticides and provide food security in a safe and eco-friendly manner.

24.1 Introduction

Plant diseases are one of the major concerns to achieve the goal of food security for the growing population worldwide. The crop yields are severely affected by the loss incurred due to widespread pathogens in cereals and fruits. A number of methods, such as crop rotation, use of resistant plant varieties, etc., have been adopted to control the plant diseases. However, the use of chemical pesticides is still a prevalent approach to contain many plant pathogens. The excessive usage of chemical pesticides poses a major health and environmental hazard; therefore, other eco-friendly alternatives must be explored to control this damage. Biocontrol using natural antagonistic microorganisms, i.e. biocontrol agents (BCA), is an environmentally safe method and in some cases is the only option available to prevent plant diseases (Cook 1993). Furthermore, besides preventing plant diseases, BCA also promote the growth of the plants. They also enhance stress tolerance, aid in nutrient acquisition and induce disease resistance in plants. Based on the mechanisms and effects, the products from BCA can be used as biofertilizers, as plant strengtheners and as biopesticides. According to a BCC research, the global market for biopesticides will increase up to USD 83.7 billion by 2019 (Wu et al. 2015).

Biological control can be defined as the use of non-pathogenic antagonistic microorganisms by humans to suppress the disease-causing pathogens in an environmental-friendly manner (Cook 1993). According to the US National Research Council, biocontrol agents should be defined as the use of naturally or genetically modified organisms and the use of their genes or gene products in order to decrease the harmful effects of pathogens.

There are two main principles of biological control:

1. The use of biological control agents relies on the phenomenon of ‘natural control’ to suppress the population of the destructive plant pathogens.
2. Biological control does not cause the elimination of pests. It only results in the reduction of pest species, such that both the pest and the natural enemy are maintained at lower densities in the agroecosystem.

BCA are being used in greenhouses and field crops towards diminishing the disease and/or suppression of pathogen on various legumes, cereals, flowers, fruits and ornamental plants.

In a broad way, biological control can also be defined as the destruction of harmful pathogens by using the activities of natural enemies (Cook 1993). Most narrowly, biological control is the use of a single antagonist against a single pathogen in a single-cropping system (Cook 1993).

In 1874, William Roberts reported the antagonistic activity of microorganisms and for the first time coined the term ‘antagonism’. The term biological control was defined by C.F. Von in 1914. Sanford, in 1926, reported the antagonistic activity of microorganisms present in green manure against potato scab disease caused by *Streptomyces scabies*. Weindling, in 1932, reported the antagonistic activity of

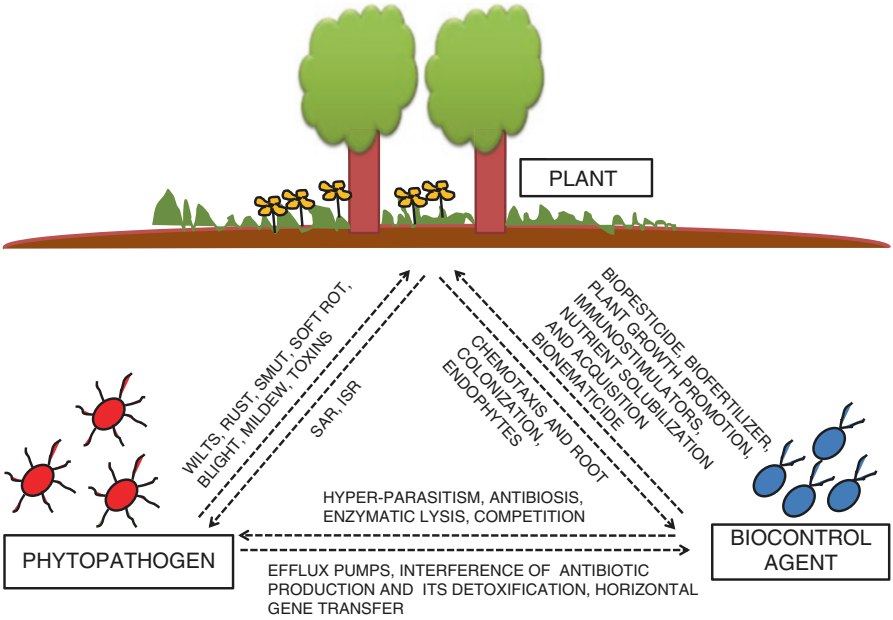

Fig. 24.1 Plant-microbe interactions and role of biocontrol agents in disease management

Trichoderma lignorum against *Rhizoctonia solani* which causes a variety of soil-borne diseases. Kloepper, in 1980, described the role of siderophores produced by antagonistic microbes to suppress the growth of *Erwinia carotovora*. Howell, in 1993, discovered P and Q strains of *Trichoderma virens*. The strains belonging to P group cannot act as biocontrol agents of seedling disease in cotton and do not induce resistance in cotton and can cause disease in susceptible seeds, whereas strains belonging to the Q group can efficiently act as biocontrol agents of seedling disease in cotton and can induce a high-level expression of phytoalexins (Junaid et al. 2013).

Thus, biological control of plant diseases is an effective and eco-friendly method to control phytopathogens, to preserve our nature and to prevent various health hazards associated with the continuous use of chemical pesticides. In the current chapter, various mechanisms used by biocontrol agents against the phytopathogens, potential of different microbes like fungi, bacteria and yeast to suppress dreadful plant pathogens and potential of plant growth-promoting bacteria to be used as BCA, current status of the BCA, their formulations, mass production and delivery methods have been discussed. Furthermore, various mechanisms used by phytopathogens to overcome the BCA are also described (Fig. 24.1).

24.2 General Mechanism of Action

Plant diseases result due to the interactions among the components of the disease triangle, i.e. pathogen, host and environment. A biocontrol agent interacts with these components and prevents the occurrence of disease (Junaid et al. 2013). In order to successfully control the plant diseases, it is important to understand the

mechanism of action of biocontrol agents, which results in disease suppression. The mechanism of action of BCA can be broadly classified as direct antagonism and indirect antagonism which shall be covered in the following sections. Understanding of these mechanisms and their conducive environmental conditions can help in the development of a highly efficient biocontrol agent. This can be achieved by improvement in the environmental conditions in which these agents act and developing a strain which employs multiple mechanisms to control plant diseases.

24.2.1 Direct Antagonism

It is the consequence of a direct physical interaction and/or high degree of selectivity for the pathogen by the biocontrol-active microorganisms. The direct antagonism mechanisms include hyperparasitism, antibiosis and enzyme production.

24.2.1.1 Hyperparasitism

The most direct type of antagonism is hyperparasitism of a plant pathogen by an obligate parasite (Harman et al. 2004). In hyperparasitism, the specific BCA directly attacks the pathogen or its propagules. This is one of the pre-eminent mechanisms employed by microbes acting as BCA. Fungal antagonists that attack pathogens having biotrophic nature act by antibiosis and mycoparasitism only. Generally, mycoparasitism involves four steps:

(a) Chemotropism where a chemical stimulus from the pathogen attracts the biocontrol fungi. For example, production of water-soluble or volatile substances by pathogens that act as chemoattractant for their parasite.
(b) Recognition in which a specific antagonistic microbe attacks only certain pathogenic fungi. Lectins produced by pathogen and carbohydrate-surface receptors of biocontrol agent play a critical role in their specific interaction.
(c) Attachment and cell wall degradation where the antagonistic fungal hyphae can either grow alongside the host hyphae or coil around it and produce cell wall-degrading enzymes such as chitinases and β-1,3-glucanase.
(d) Penetration in which the biocontrol agents produce structures like appressoria to penetrate the fungal cell wall.

There are four major groups of hyperparasites, namely, hypovirus, facultative parasites, predators and obligate bacterial pathogens. One of the examples of hypoparasites is the hypovirus that infects the fungus *Cryphonectria parasitica*, the causal agent of chestnut blight. The hypovirus causes hypovirulence, i.e. decrease in the pathogenicity of the pathogen. This phenomenon has successfully resulted in the control of chestnut blight at many places (Milgroom and Cortesi 2004). In some cases it is possible that multiple hyperparasites attack a single fungal pathogen. For example, *Acremonium alternatum*, *Cladosporium oxysporum*, *Acrodontium crateriforme* and *Ampelomyces quisqualis* have the capability to parasitize the powdery mildew fungi (Milgroom and Cortesi 2004). One of the best-known examples of fungal antagonism

is the mycoparasitism of powdery mildew fungi by *Ampelomyces* spp. The molecular level of mycoparasitism was first stated in 1994, in which the role of the endochitinase-encoding gene (*ech42*) was elucidated. Vinale et al. (2008) demonstrated that in order to induce mycoparasitism and cause degradation of chitin from the fungal cell wall, the expression of the endochitinase *ech42* or exochitinase *nagI* gene is required.

Other examples of mycoparasitism include the mycoparasitism of rust fungi, i.e. *Puccinia* and *Uromyces* by *Sphaerellopsis filum. Trichoderma lignorum* can parasitize *Rhizoctonia solani* and thus can prevent damping-off of the citrus seedlings. Certain species of *Trichoderma* can successfully parasitize *Rhizoctonia bataticola* and *Armillaria mellea* which cause dry root rot of chickpea and Armillaria root rot, respectively. *Pythium oligandrum* is a mycoparasite of various *Pythium* spp., while several sclerotia-forming fungi can be parasitized by *Sporidesmium sclerotivorum* (Baker and Cook 1974; Sundheim and Tronsmo 1988).

24.2.1.2 Antibiosis

Antibiotics are secondary metabolites produced by microbes that can directly retard the growth of other organisms. They are low molecular weight organic molecules, effective at lower concentrations. Production of antibiotics is an important trait of biocontrol agents. This has been shown by genetic manipulation of the genes involved in antibiotic production. Thomashow and Weller (1988) demonstrated that mutant strains of *Pseudomonas* which were unable to produce phenazines or phloroglucinols cannot cause effective destruction of pathogens as compared to wild-type strains. Biocontrol agents are known to produce majorly three types of antibiotics, namely, polar/non-volatile, non-polar/volatile and water soluble. Volatile antibiotics are more effective as they can act on sites other than the site of their production. It was found that several trace metals like zinc and various carbon sources affect the genetic stability of bacteria, thus upsetting their capability to produce antibiotics. Biocontrol of crown gall caused by *Agrobacterium tumefaciens* by agrocin 84 produced by *Agrobacterium radiobacter* is one of the best-known examples of antibiosis. The ability of some biocontrol agents to produce multiple antibiotics is an effective strategy to control different pathogens. For example, *Bacillus cereus* strain UW85 produces both kanosamine and zwittermicin, and these are used to control damping-off caused by *Phytophthora medicaginis* and *Phytophthora aphanidermatum* (Smith et al. 1993). Strains that produce multiple antibiotics of different classes are the most effective BCA. Strains of *Pseudomonas putida* WCS358r, which were genetically engineered to produce the antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG) and phenazine, cause effective suppression of pathogens (Glandorf et al. 2001). Thomashow and Weller (1988) demonstrated that mutant strains which were unable to produce phenazines or phloroglucinols cannot cause effective destruction of pathogens as compared to wild-type strains. *Pseudomonas fluorescens* strain 2-79 can control the economically prevalent take-all disease of wheat caused by *Gaeumannomyces graminis* var. *tritici* by producing the antibiotic phenazine. *P. fluorescens* CHA0 produces several antibiotic products like 2,4-diacetylphloroglucinol and pyoluteorin which are important for the suppression of soilborne fungal pathogens causing root diseases.

24.2.1.3 Enzymes

Many microbial antagonists act by the mechanism of production of various lytic enzymes. These enzymes efficiently cause the suppression of the pathogen's activity. For example, *Serratia marcescens* acts against *Sclerotium rolfsii*, the causal agent of southern blight, by chitinase production (Ordentlich et al. 1988). *Lysobacter enzymogenes* strain C3 depends on the β-1,3-glucanase for its biocontrol activity. These enzymes cause the degradation of complex cell wall components of the pathogen to obtain nutrition thereby suppressing the pathogen's activity. *Myxobacteria* and *Lysobacter* are known to produce ample amounts of lytic enzymes. The role of enzymes in disease suppression was shown by creating mutants deficient in genes coding for specific enzymes. For example, mutant strains of *Serratia marcescens* deficient in the gene coding for chitinase (ChiA) were less efficient in controlling *Fusarium* wilt in greenhouse conditions. When the *ChiA* gene was introduced in other microbes, it resulted in an increase in their biocontrol efficiency along with imparting biocontrol activity to the nonbiocontrol microbes. The *Escherichia coli* strain, having the *ChiA* gene inserted in its genome, caused significant reduction in the disease incidence of southern blight of bean caused by *Sclerotium rolfsii.* Harman and Hayes (1994) developed transgenic plants having increased resistance to pathogenic fungi by transforming plants with the endochitinase gene from *T. harzianum.* Various enzymes produced by *Paenibacillus* spp., such as chitinase, protease, cellulase and amylase, have an important role in the fungal disease suppression. Studies have shown that the products of lytic enzyme activity can have an indirect effect on the pathogen. For example, the oligosaccharides released upon fungal cell wall lysis can induce resistance in plants (Howell 1998). Other by-products such as hydrogen cyanide and volatile compounds like ammonia also play a role in disease suppression. Hydrogen cyanide (HCN) production by *P. fluorescens* CHA0 is mainly responsible for preventing black rot of tobacco caused by *Thielaviopsis basicola*. HCN inhibits the cytochrome oxidase pathway and thus can effectively cause pathogen destruction even at picomolar concentrations. Howell et al. (1980) described the role of ammonia produced by *Enterobacter cloacae* in suppression of damping-off of cotton caused by *Pythium. E. cloacae* produces ammonia having antifungal activity via deaminating amino acids under low concentration of sugars.

24.2.2 Indirect Antagonism

It does not involve any direct physical interactions between the pathogen and the BCA. Indirect antagonism includes induction of systemic resistance in plant, competition for nutrient and space and plant growth promotion.

24.2.2.1 Induction of Systemic Resistance

The most indirect form of antagonism is the suppression of disease by induction of plant defence mechanisms by the non-pathogenic microorganisms (Kloepper et al. 1980; Maurhofer et al. 1994; Lafontaine and Benhamou 1996; Silva et al. 2004).

The induced resistance can be systemic or local depending on its source, type and amount of stimuli. Nonexpressor of pathogenesis-related genes 1, i.e. NPR1 and salicylic acid, are the major elicitors of systemic acquired resistance (SAR). The SAR is triggered by the local infection and remains systemic in the plant body thus providing a long-term resistance to subsequent pathogen attacks. Another resistance mechanism known as induced systemic resistance is mediated by ethylene and jasmonic acid. Various compounds are known to induce resistance in plants. For example, proteins with enzymatic activity like xylanases, cellulases and endochitinase can induce plant defence-related proteins. *T. virens* produces hydrophobin-like protein SM1 which can induce the synthesis of phytoalexins that acts as a toxin against the attacking pathogen. Another group of proteins which can induce resistance in plants are the products of avirulence (Avr) genes. They result in activation of the hypersensitive response and other defence-related responses in plants having specific R (resistance) gene for a particular Avr gene. Many microbial products such as lipopolysaccharide (LPS) and flagellin from gram-negative bacteria, cold-shock proteins of various bacteria, invertase from yeast, chitin and ergosterol from fungi and transglutaminase and alpha-glucanase from oomycetes can elicit defence responses in plants. Root colonization by biocontrol agents also results in the induction of resistance mechanisms in plants. The induction of SAR defence response usually results in deposition of callose, thickening of cell wall by lignification, synthesis of lytic enzymes like chitinases, and glucanases, synthesis of peroxidases and pathogenesis-related (PR) proteins, as well as synthesis of low molecular weight antimicrobial compounds like phytoalexins. This mechanism of SAR is generally employed by various plant growth-promoting rhizobacteria (PGPR). Poromarto et al. (1988) reported that binucleate *Rhizoctonia* (BNR) AG-K suppresses the growth of *Rhizoctonia solani* on soybean via inducing resistance. Maurhofer et al. (1994) revealed that *P. fluorescens* strain CHA96 induces the production of PR proteins like β-1,3-glucanases and endochitinases in plants, thereby causing resistance against the black root rot of tobacco. Van Peer et al. (1991) found that plants treated with *P. fluorescens* strain WCS417r possessed high levels of phytoalexins compared to non-treated plants. Thus, induction of systemic resistance by beneficial microbes is an effective method to control various phytopathogens under field conditions.

24.2.2.2 Competition

It is an indirect method of antagonism used by biocontrol agents. It does not involve the direct interaction between the pathogen and the antagonistic microbe. The pathogen and the biocontrol agents can compete with each other for nutrients, space, essential micronutrients like iron and manganese, specific growth substances or stimulants for germination. Production of high-affinity iron chelators, i.e. siderophores, by BCA starves the pathogenic microbes for the essential element iron. Kloepper et al. (1980) were the first to describe the importance of siderophore production as the mechanism of suppression of *Erwinia carotovora* by *P. fluorescens* strains A1, TL3B1, BK1 and BK10. Some more studies have demonstrated the role of siderophores in disease suppression by *P. fluorescens* (Loper 1988). Schippers et al. (1986) described the role of pyoverdine (siderophore) in the biocontrol of

pathogens by generating mutants using transposon insertion to inactivate the gene coding for pyoverdine (Pvd). The Pvd⁻ *P. fluorescens* 3551 was unable to control the damping-off of cotton caused by *Pythium* species. Competition for specific substances required for germination, like fatty acids that stimulate germination of *Pythium* spp., can cause disease suppression by E6 *Enterobacter cloacae*. Thus, competition for growth stimulants, like fatty acids and their peroxidation products, volatile compounds such as acetaldehyde and ethanol are effective methods to control disease. Elad and Chet (1987) demonstrated that some bacterial strains can inhibit the oospore germination of *Pythium* spp. by utilizing essential exudate component. In addition to this, the competition for physical occupation of site reduces the root colonization by pathogen.

24.2.2.3 Plant Growth Promotion

Biocontrol agents have been shown to increase the solubilization and uptake of various nutrients. Thus, apart from causing the suppression of disease-causing pathogens, biocontrol agents can successfully enhance plant growth. *Aspergillus niger* strain AN-27 produces 2-methylene-3-hexyl-butanedioic acid and 2-carboxy-methyl-3-hexyl-maleic anhydride which causes increased root and shoot length in crop plants (Selvakumar and Srivastava 2000). Woo et al. (2006) reported that seed treatment with *T. viride* resulted in increased fresh and dry weight of root, shoot and nodules of broad beans. The bacterial species associated with plants can affect the hormonal balance. Several bacteria can reduce the level of ethylene hormone by producing ACC (1-aminocyclopropane-1-carboxylate) deaminase which degrades the ACC, a precursor of ethylene synthesis. A number of PGPR (plant growth-promoting rhizobacteria) like *Azospirillum*, *Erwinia*, *Pseudomonas*, *Bacillus*, *Serratia* and *Rhizobium* can solubilize phosphate. These bacteria can convert the non-soluble phosphate to soluble form either by acidification of phosphate salts or via enzymatic action. The PGPR acidify the soil rhizosphere via production of organic acids resulting in improved solubilization of nutrients such as Ca, K, Fe, Cu, Zn and Mn (Berg 2009).

24.3 Types of Interactions Contributing to Biological Control

In order to understand the various mechanisms used by biocontrol agents to suppress the activity of pathogens, it is very important to understand the interactions contributing to their biocontrol activity. These interactions include mutualism, commensalism, competition, parasitism and predation. Several complex interactions between the pathogen-biocontrol agent and biocontrol agent-plant and environmental factors play a significant role in disease control (Harman et al. 2004; Hoitink et al. 2006; Alfano et al. 2007).

Plants and microbes have established diverse forms of mutualistic interactions during co-evolution (Germida and Siciliano 2001; Cardinale et al. 2015). Mutualism can be defined as the association between two or more species in which both the species get benefited from the interaction. The mutualistic association of *Rhizobium* with the roots of leguminous plants is a classical example of mutualism where the host plant provides nutrient to the bacteria and the bacteria in turn provide NH_3 to the plant for

amino acid synthesis. Most of the microbes acting as biocontrol agents can be considered as facultative mutualists. Sometimes, mutualism involves long-term physical and biochemical interactions between the interacting partners, e.g. the interaction between the plants and mycorrhizal fungi. The mycorrhizal interaction prevents the root infection by stimulating host defences. It also provides stress tolerance to plants. Such a mycorrhizal colonization has been used to reduce the damage caused by *Pseudomonas syringae* on tomato plants (Garcia-Garrido and Ocampo 1989).

Another form of interaction, commensalism, is the symbiotic relationship between organisms in which one organism gets benefitted and the other organism remains unaffected (Fitter and Garbaye 1994). Many of the plant-associated microbes are considered as commensals (Katska 1994; Chisholm et al. 2006). However, their presence may pose a challenge to phytopathogens (Cook 1993). Overall, an absence of significant disease suppression or pathogen infection in the presence of non-pathogenic microbes can be considered as commensalism. The competition for nutrients, space and growth stimulators between the BCA and pathogens results in disease suppression.

Parasitism is described as the interaction between organisms where generally the smaller one, i.e. the parasite, gets benefited and the host or pathogen gets harmed. Hyperparasitism, in which one organism parasitizes the other organism, particularly the pathogen, can result in biocontrol such as the parasitism of pathogen *Rhizoctonia solani* by BCA *T. viride*. In this type of interaction, the parasite, i.e. the antagonistic microbe, harms the host, i.e. the phytopathogen. In contrast to parasitism, predation refers to the killing of one organism by another for sustenance, e.g. the fungus-feeding nematode which consumes the pathogen biomass for sustenance (Cook 1993).

24.4 Various Categories of Biological Control Agents

Several microorganisms like bacteria, fungi (especially *Trichoderma*) and yeast are capable of acting as BCA. Fungal BCA such as *Scutellospora* sp., *Glomus*, *Gigaspora margarita* and particularly *Trichoderma* act against a wide range of pathogens, thus indicating their significance as biofungicides. A variety of yeast species like *S. cerevisiae*, *Wickerhamomyces anomalus*, *Candida* and *Pichia* have been shown to successfully inhibit the growth of various pathogens. Similarly, various bacterial species such as *Pseudomonas*, *Erwinia*, *Ralstonia*, *Clavibacter*, *Bacillus*, *Enterobacter*, lactic acid bacteria and several others have a high biocontrol efficiency against the phytopathogens. The details about various categories of BCA are discussed in the following sections.

24.4.1 Fungi as Biocontrol Agents

Fungi constitute 10% of all BCA species. *Glomus versiforme* was found to be an effective biocontrol against the bacterial wilt-causing pathogen *Ralstonia solanacearum.* It causes induced systemic resistance (ISR) in the plant. *Pythium oligandrum* is also effective against bacterial wilt. The leachate of Shiitake mycelia

contains an antibiotic ingredient which inhibits the phytopathogen *R. solanacearum.* Even endomycorrhizal fungi, i.e. *Scutellospora* sp., *Glomus mosseae* and *Gigaspora margarita*, have antagonistic activity against bacterial wilt pathogen *R. solanacearum*. Some fungi also have the ability to degrade various pesticides. For example, *Trichoderma harzianum* can degrade organochlorine pesticides, mostly endosulfan and polycyclic aromatic hydrocarbons, including anthracene.

Among the various fungal BCA, one of the most promising fungal antagonists is *Trichoderma* which has been extensively used for disease suppression. It has the ability to hyperparasitize other fungi, due to the production of cell wall lytic enzymes and antibiosis. Moreover, it also produces various secondary metabolites and can induce resistance in plants. These features make it the most favourable biocontrol agent for plant protection (Woo et al. 2014). Weindling (1932, 1934) was the first to report the biocontrol capability of *Trichoderma.* He demonstrated the biocontrol activity of *Trichoderma lignorum* against the fungal pathogen *Rhizoctonia solani.* Later it was found that the same species is effective against other common fungal pathogens such as *Pythium*, *Sclerotium rolfsii*, *Phytophthora* and *Rhizopus.* In addition, *T. harzianum* and *T. virens* have been shown as efficient inhibitors of *Ganoderma* growth. *Trichoderma* effectively controls the phytopathogens such as *Sclerotium rolfsii* which causes damping-off, seed rot and root rot of mung bean and sunflower. *Trichoderma* spp. not only inhibits the growth of pathogens, but it also benefits its host plant by stimulating the colonization of the rhizosphere, promoting plant and root growth and enhancing the defence mechanisms of the plant (Vinale et al. 2008; Harman et al. 2004). More than 60% of the registered biofungicides worldwide are ruled by various *Trichoderma* strains. In India, around 250 products are accessible for field applications. Currently, various commercial products of *Trichoderma* are available in the market as biopesticides, as plant growth promoters and as a soil amendment. They can act on fungal pathogens through multiple modes of action.

It has been shown that a high percentage of specific *Trichoderma* strains and various antagonists are associated with the endemic plants which protect them from various pathogens. Due to the advancement in technology and higher resolution of sequence data, a deeper understanding regarding the function and structure of microbial communities is possible (Berg 2015). Berg (2015) showed that endemic plants harbour a unique subset of *Trichoderma* species as part of their microbiome and these unique specific microbiomes accomplish essential functions for their host and can influence its growth, germination and health.

The most direct mechanism used by *Trichoderma* against fungal pathogens is mycoparasitism. As discussed earlier, mycoparasitism is a complex process involving majorly four steps: chemotrophic growth and recognition, attachment and coiling around fungal hyphae, penetration of cell wall and degradation by lytic enzymes (Chet et al. 1998). Various cell wall lytic enzymes such as chitinases, proteases and glucanases play a major role in mycoparasitism. The ability of *Trichoderma* to mycoparasitize pathogenic fungi is the major reason for their success as biofungicides. It has been shown that they also have nematicidal activity and thus can also act as bionematicides. Several factors involved in their

mycoparasitism have been identified. The role of G-protein-coupled receptor Gpr1 in mycoparasitism was elucidated using gene silencing experiments (Omann et al. 2012). Similarly, the deletion of gene coding for Tga3 Gα protein revealed the role of G-protein-coupled receptor-mediated signalling in mycoparasitism (Zeilinger et al. 2005). Mitogen-activated protein kinase (MAPK) pathway may also have a role in mycoparasitism and biocontrol (Kumar et al. 2010). Deletion of the TmKA/TvK1 MAPK gene affects the biocontrol efficiency of *T. virens*. Mukherjee et al. (2012) found that mutants having deletion in this MAPK gene were less effective in parasitizing the sclerotia of *S. rolfsii* and *R. solani*, whereas Mendoza-Mendoza et al. (2003) found improvement in the biocontrol activity of mutant strain of *T. virens* against *R. solani* and *P. ultimum*. Druzhinina et al. (2012) demonstrated the role of hydrophobins in the attachment of mycoparasitic *Trichoderma* to the host fungi.

Trichoderma is a rapidly colonizing fungus, which can utilize various substrates and competes very well for nutrient and space. Furthermore, it produces high-affinity iron chelators, i.e. siderophores, and thus starves the pathogenic fungi for iron. It can also modify the rhizosphere so that it is not suitable for pathogen growth and can inhibit their spore germination and kill the cells. Further, *Trichoderma* spp. can effectively enhance the root development and plant growth and induce plant defence mechanisms (Harman et al. 2004). Some strains can colonize the root surface throughout their lifetime. In a study, it was demonstrated that maize plant treated with *T. harzianum* strain T-22 leads to a twofold increase in root development in comparison with untreated plants (Harman et al. 2004). Cutler et al. (1986, 1989) described the role of secondary metabolites koninginin A and 6-pentyl-alpha-pyrone produced by *T. koningii and T. harzianum*, respectively, as plant growth regulators. *Trichoderma* spp. produces citric and gluconic acids, which decrease the pH of the soil and increase the solubilization of micronutrients, phosphates and mineral components such as magnesium, iron and manganese (Benitez et al. 2004; Harman et al. 2004; Vinale et al. 2008).

Trichoderma stimulates various defence mechanisms in plants such as the hypersensitive response, induced systemic resistance (ISR) and systemic acquired resistance (SAR). For example, *T. asperellum* induced the expression of phenylalanine and hydroperoxidase lyase and caused the accumulation of phytoalexin in cucumber plants against *Pseudomonas syringae* pv. *lachrymans* (Yedidia et al. 2003). Plants react to *Trichoderma* invasion by rapid ion fluxes, oxidative burst, deposition of callose and synthesis of polyphenols followed by activation of ethylene and salicylate signalling which results in the elicitation of the induced systemic resistance in plants (ISR) (Shoresh et al. 2010). Various elicitors of the plant immune system produced by *Trichoderma* have been recognized, such as xylanase, alamethicin and trichovirin II (peptaibol) (Mukherjee et al. 2012). The best-characterized elicitor from *Trichoderma* is glycosylated Sm1/Ep11, a hydrophobin-like cysteine-rich protein of the cerato-platanin family (Djonovic et al. 2006; Seidl et al. 2006). It has been seen that the deletion of the gene coding for the elicitor Sm1 impairs the elicitation of ISR in maize (Djonovic et al. 2007).

Root colonization by *Trichoderma* results in the induction of various enzymes such as β-1,3 glucanases, chitinases, peroxidases, phenylalanine and accumulation of phytoalexins. Once the colonization occurs, they penetrate the root and grow intercellularly in the epidermis and outer cortex. The release of various chemicals from both the plant and *Trichoderma* favours the colonization of roots by the fungus both internally and externally. *Trichoderma* secretes a hormonal signal which facilitates root colonization (Contreras-Cornejo et al. 2009). The role of auxin in root colonization was demonstrated by knocking out the gene *accd* coding for ACC deaminase. Besides this, *Trichoderma* also secretes cysteine-rich hydrophobins, e.g. Qid74 (from *T. harzianum*) and TasHyd1 (from *T. asperellum*) which help in root attachment (Viterbo and Chet 2006; Samolski et al. 2012). They also secrete endopolygalacturonase and expansin-like proteins having cellulose-binding modules which help in root penetration (Brotman et al. 2008).

Secondary metabolites and enzymes have a major role in the activity of *Trichoderma* against phytopathogens. *Trichoderma* produces various antibiotics having antifungal and antibacterial properties. *T. harzianum* produces a pyrone-like antibiotic which suppresses the growth of *Gaeumannomyces graminis.* According to Sivasithamparam and Ghisalberti (1998), secondary metabolites produced by *Trichoderma* spp. assemble into three types: (1) water-soluble compounds like heptelidic acid, (2) volatile compounds like 6-pentyl-alpha-pyrone and (3) peptaibol compounds. Deletion of the gene coding for β-1,6-glucanase, i.e. *tvbgn3*, results in significant reduction in the mycoparasitic and biocontrol activity of *T. virens* against *Pythium ultimum* (Djonovic et al. 2006). Proteases like Prb1/Sp1 also have a role in mycoparasitism. Secretome analysis revealed that among various fungi, *Trichoderma* has one of the largest sets of proteases. Druzhinina et al. (2012) demonstrated the role of various subtilisin-like proteases of the S8 family, dipeptidyl and tripeptidyl peptidases. Beside all these enzymes, laccases have role in the colonization of sclerotial structures by *T. virens*. Secondary metabolites like peptaibols also play a role in fungus-fungus interaction. For example, peptaibol trichokonin VI of *T. pseudokoningii* induces programmed cell death in *Fusarium oxysporum* (Shi et al. 2012). Even volatile compounds like 6-pentyl-2H-pyran-2-one (6-PP) produced by *T. atroviride* have an important role in *Trichoderma*-plant and *Trichoderma*-fungal pathogen interactions (El-Hasan et al. 2008; Vinale et al. 2009).

Several endophytic species of *Trichoderma* such as *T. taxi*, *T. martiale*, *T. amazonicum*, *T. evansii*, *T. stromaticum* and *T. theobromicola* have been discovered. These endophytic species have biocontrol ability and can protect plants from phytopathogens and abiotic stress factors by inducing various changes at the transcriptome level (Bailey et al. 2006; Bae et al. 2009; Druzhinina et al. 2011).

The *Trichoderma*-based biocontrol agents have been given in Table 24.1. However, all these products are not registered as biocontrol agents, but they are marketed as either soil conditioners or plant growth promoters.

Certain entomopathogenic fungi have shown to control even the insect population, indicating the potential of antagonistic microbes as bioinsecticides. Zamani et al. (2013) reported the activity of fungi *Beauveria bassiana* (Whiteguard) against the red flour beetle *Tribolium castaneum*, an insect of barley, *Hordeum vulgare*,

Table 24.1 Commercially available bacterial, yeast and fungal biocontrol agents

Biocontrol agent	Target pathogen/crop	Commercialized product	Company
Bacterial biocontrol agents			
Bacillus			
B. subtilis strain GB34	*Rhizoctonia*, *Fusarium*	GB34	Gustafson (USA)
B. subtilis strain GB03	*Rhizoctonia*, *Aspergillus*	Kodiak, Companion	Growth products (USA)
B. subtilis FZB24	Potatoes, vegetables, ornamentals, strawberries, bulbs, turf and woods	FZB24 li, TB, WG RhizoPlus	AbiTep
B. subtilis QST716	Tobacco, tomato, lettuce, spinach	Serenade	AgraQuest
B. subtilis GB03, other *B. subtilis*, *B. licheniformis* and *B. megaterium*	*Rhizoctonia*, *Pythium*, *Fusarium* and *Phytophthora*	Companion	Growth Products
Pseudomonas			
P. aureofaciens strain TX-1	*Pythium*, *Rhizoctonia solani*	Bio-jet, spot less	Eco Soil System
P. fluorescens strain A506	Fire blight, bunch rot	Frostban	Plant Health Technologies
P. chlororaphis	Leaf stripe, net blotch, *Fusarium* sp., sot blotch, leaf spot, etc. on barley and oats	Cedomon	BioAgri AB
P. trivialis 3Re27	Lettuce	Salavida	Sourcon Padena
Pseudomonas spp.	*Rhizoctonia Solani*	Proradix	Sourcon Padena
P. syringae ESC10 and ESC11	*Penicillium* spp., *Mucor piriformis*, *Geotrichum candidum*	Bio-Save 10LP, 110	EcoScience Corp. (Longwood, FL)
P. chlororaphis 6328	*Pythium* spp., *Rhizoctonia solani, Fusarium oxysporum*	AtEze	Eco Soil Systems Inc. (San Diego, CA)
P. chlororaphis strain	Leaf stripe, net blotch, *Fusarium* spp., spot blotch, leaf spot	Cedemon	BioAgri (Uppsala, Sweden)
Agrobacterium			
A. radiobacter strain 84	*A. tumefaciens*	Galtrol	AgBioChem (USA)
A. radiobacter strain K 1026	*A. tumefaciens*	Nagol	Biocare

(continued)

Table 24.1 (continued)

Biocontrol agent	Target pathogen/crop	Commercialized product	Company
Other bacteria			
Burkholderia cepacia	Control of *Rhizoctonia, Fusarium* and *Pythium* spp.	Deny	Stine Microbial Products (USA)
Streptomyces griseoviridis K61	Soilborne pathogens like *Phomopsis* spp., *Botrytis* spp., *Pythium* spp., *Phythophthora* spp.	Mycostop	Kemira Oyj (Finland)
Azospirillum spp.	Paddy, millets, oilseeds, fruits, vegetables, sugarcane, banana	Biopromoter	Manidharma Biotech
Bradyrhizobium japonicum	Soybean	Soil implant	Nitragin
Serratia plymuthica HROC48	Strawberries, oilseed rape	Rhizostar	Prophyta Biologischer Pflanzenschutz
Yeast biocontrol agents			
Metschnikowia fructicola	*P. digitatum*, *P. italicum*, *P. expansum*, *B. cinerea*, *Rhizopus stolonifer*, *Aspergillus niger*, *Fusarium* and *Sclerotinia sclerotium*	Shemer	Lesaffre-Bionext (France/Europe)
Candida oleophila	*Botrytis* spp., *Penicillium* spp.	Aspire	Ecogen, Inc. (USA)
Fungal biocontrol agents			
Trichoderma			
T. harzianum T-22	*Pythium* spp., *Rhizoctonia solani*, *Fusarium* spp.	Root shield, plant shield T22, Planter box	BioWorks (USA)
T. harzianum T-39	*Botrytis cinerea*	Trichodex	BioWorks (USA)
T. harzianum	*Rhizoctonia solani*, *Sclerotium rolfsii*, *Pythium* and other fungal diseases	Trichoderma 2000, Trichopel	Mycontrol (EfA1) Ltd. (Israel) and Agrimm Technologies Ltd. (New Zealand)
T. harzianum, *T. polysporum, T. viride*	Wilt-causing fungi, soil and foliar pathogens	Binab T, Trichodowels, Trichoject, Trichoseal and others	Wood Bio-Innovation (Sweden), Agrimms Biologicals (New Zealand)

Table 24.1 (continued)

Biocontrol agent	Target pathogen/crop	Commercialized product	Company
Others			
Cryptococcus albidus	*Botrytis* spp., *P. expansum*	Yield plus	Anchor Yeast (South Africa) belonging to Lallemand group (South Africa)
Pythium oligandrum	*Pythium ultimum*	Polyversum, Polygandron	Plant Protection Institute (Slovak Republic)
Fusarium oxysporum	*Fusarium oxysporum*	Fusaclean	Natural Plant Protection (France)
Glomus intraradices	Increases cotton growth	Ascend/BuRIZE	Bioscientific Inc. (USA)
Aureobasidium pullulans	*B. cinerea*	Boni Protect	BioProtect GmbH belonging to BioFirm from BIOMIN (Austria)
Gliocladium catenulatum strain JI446	Soilborne pathogens	Prima stop soil guard	Kemira Agro Oy (Finland)
Aspergillus flavus AF36	*Aspergillus flavus*	Alfa guard	Circle One Global (USA)
Ampelomyces quisqualis isolate M-10	Powdery mildew	AQ10	Ecogen (USA)

Burges (1998), Butt et al. (1999, 2001), Junaid et al. (2013), Mark et al. (2006), Berg (2009), and Haissam (2011)

which is one of the important crops economically. It can also act on wide range of insects such as caterpillars, beetles, aphids, etc. Also, Greenguard based on *Metarhizium* has potential to be used as bioinsecticide. It has been used widely against the African desert locust (*Schistocerca gregaria* Forscal) and other grasshoppers.

24.4.2 Yeast Species as Biocontrol Agents

A variety of yeast species also have the potential to act as biocontrol agents. Their ability to survive in an acidic environment, production of killer toxin, competition for nutrients and production of various volatile compounds makes them a favourable candidate for biocontrol of various fungal-borne plant diseases.

The biological control using antagonistic yeasts has been described and is considered as a substitute to synthetic fungicide (Droby et al. 2002; Zhang et al. 2009; Geng et al. 2011). These antagonistic yeasts rapidly colonize the leaf and fruit surfaces because of the high-sugar content present on these surfaces. Thus, phylloplane is the primitive source for the isolation of yeasts. In many of the studies, it was

found that very few antagonistic yeasts are present in the soil (approximately 9.5%), while approximately 90.5% of yeasts were isolated from the phylloplane (Chanchaichaovivat et al. 2007; Wang et al. 2009). Yeasts also use multiple modes of action against the pathogen. For example, Bruce et al. (2003) described the suppression of growth of wood-rot fungi, *Sclerophoma pithyophila*, by *S. cerevisiae* by approximately 75% via the production of volatile compounds. Similarly, Fialho et al. (2010) reported that production of volatile compounds by *S. cerevisiae* inhibited the growth of the fungus *Phyllosticta citricarpa*, a causal agent of citrus black spot by up to 83%. Parafati et al. (2015) verified that *Wickerhamomyces anomalus* strains inhibited the mycelial growth of *Botrytis cinerea* via the production of volatile organic compounds on PDA at pH 4.5. Certain yeast species can also produce killer toxins that are lethal to filamentous fungi. For example, *Pichia membranifaciens* inhibits the growth of *Botrytis cinerea* via the action of a killer toxin (Santos et al. 2004). Ferraz et al. (2016) demonstrated that for the biological control of the fungus *Penicillium expansum* in apples, killer toxin producing antagonistic yeasts like *Candida guilliermondii* and *Pichia ohmeri* could be used. Saravanakumar et al. (2009) described that the yeast *Metschnikowia pulcherrima* can effectively control several fungal pathogens on harvested apples. This yeast effectively outcompetes for iron thus inhibiting the growth of the fungal pathogens.

24.4.3 Bacterial Species as Biocontrol Agents

There are several bacterial endophytic species which hold the ability to be used as biocontrol agents. Endophytes are microorganisms that are associated with plant tissues and cause no apparent symptoms or infection. Endophytic microbes greatly influence the physiology of their host plant. In this context, various endophytic microbes have a potential to be used as biocontrol agents because of their ability to carry out nutrient assimilation and produce a variety of secondary metabolites, particularly antibiotics. Bacterial endophytes have the ability to colonize the same ecological niche as that of the plant pathogens, particularly the pathogens causing vascular wilt diseases, which point out their significance as biopesticides against wilt diseases. Bacterial vascular wilts are mainly caused by *Pseudomonas*, *Ralstonia*, *Erwinia* and *Clavibacter.* Vascular wilts can affect both the woody perennials and annual crops thus causing major food losses (Yadeta and Thomma 2013). These vascular wilt pathogens invade the xylem vascular tissues and thus obstruct the transportation of water and minerals. Two major genera of fungi that cause vascular wilts are *Fusarium* and *Verticillium. Verticillium* infects a variety of plant species such as cotton and tomatoes (Sharma and Nowak 1998; Bolek et al. 2005).

The bacterial endophytes colonize the apoplastic intercellular spaces of plants (Rosenblueth and Martínez-Romero 2006; Weyens et al. 2009). The most commonly isolated endophytic bacteria are *Enterobacter*, *Bacillus*, *Pseudomonas* and *Agrobacterium* (Hallmann et al. 1997). These endophytes have the ability to inhibit the harmful effects of pathogenic organisms. Most of the endophytes use more than one mode of action to inhibit the pathogen growth. They act on the pathogens by the

mechanism of inducing host resistance, antibiosis, growth promotion, competition, parasitism and signal interference (quorum sensing) (Amer and Utkhede 2000; Collins and Jacobsen 2003; Jataraf et al. 2005; Jorjani et al. 2011; Mansoori et al. 2013).

Mercado-Blanco et al. (2004) demonstrated that *Pseudomonas fluorescens* PICF7 suppressed the growth of *Verticillium* wilt on olive trees. It enters the plant through root hairs and hinders the colonization of the pathogens. The treatment of olive tree root in greenhouse condition with *Pseudomonas fluorescens* PICF7 reduced the incidence and severity of the disease by 82% and 96%, respectively. The bacterium acts on *Verticillium* by the induction of systemic resistance in roots as well as other distant tissues. Tjamos et al. (2004) described the efficiency of *Paenibacillus* K165 strain against the soilborne pathogen *V. dahliae.* This biocontrol strain lessens the severity and disease symptoms in eggplant and potato in glasshouse and field experiments where its biocontrol activity was mediated via ISR induction in plants.

Erdogan and Benlioglu (2010) isolated four different strains of *Pseudomonas*, namely, FP22, FP23, FP30 and FP35, from the roots of different cotton plants and studied their effect on the verticillium wilt-causing pathogen. They demonstrated that treatment of cotton seeds with these *Pseudomonas* strains significantly caused the reduction of disease severity and also resulted in the higher growth of cotton plants compared to untreated plants. The bacterial endophytes that can successfully be used as biocontrol of bacterial wilt diseases include *Bacillus amyloliquefaciens* Bg-C31 and *Streptomyces virginiae* which control the disease caused by *Ralstonia solanacearum* on capsicum and tomato plant, respectively (Hu et al. 2010).

Matsukuma et al. (1994) and Okazaki et al. (1995) described the colonization of various actinomycetes on plants either as parasites or symbionts. Actinomycetes isolated from the rhizospheric soil, particularly *Streptomyces* spp., are recognized to be an excellent biocontrol agent. Kunoh (2002) isolated *Streptomyces galbus* from rhododendron plants and examined its efficacy to protect the host plant from two major pathogens *Phytophthora cinnamomi* and *Pestalotiopsis sydowiana* and demonstrated that the pretreatment of plant seedlings with *Streptomyces galbus* effectively controls the *P. sydowiana* growth. *S. galbus* act on the root rot-causing pathogens by more than one mechanism. It produces polyene macrolide antibiotics, such as actinomycin X_2 and fungichromin, and volatile compounds like monoterpenes such as linalool and linalool 3,7-oxide. Linalools can suppress the spore germination of numerous phytopathogenic fungi. They also induce systemic acquired resistance in the plant. Molecular analysis of this resistance revealed that treatment of *Arabidopsis* seedlings with *S. galbus* resulted in high-level expression of defence-related genes such as *PDF1.2* (plant defensin1.2) gene, whereas slight expression of *PR-1* and *PAL* (phenylalanine ammonia lyase) genes was also observed. The *PDF1.2* gene is involved in jasmonic acid pathway which results in induction of ISR, whereas *PR-1* and *PAL* genes are involved in SAR. Moreover, the production of camalexin, a phytoalexin, was also significantly enhanced. Furthermore, *S. galbus*-treated plant seedlings are resistant to drought conditions and can also be used as part of an integrated system because of its resistance to various pesticides.

Most of the bacterial strains used as biopesticides belong to the genera *Bacillus*, *Pseudomonas* and *Agrobacterium*. Various strains of *Bacillus* have an enormous potential to be used as biofertilizers and biocontrol agents due to their ability to colonize rhizosphere, induce plant resistance, compete for nutrients with phytopathogens, produce endospores, antibiotics and promote plant growth. They can colonize diverse habitats and synthesize many substances that have successfully been used in agriculture. *Bacillus* strains produce a wide variety of antimicrobial compounds such as ribosomally synthesized bacteriocins and nonribosomally synthesized lipopeptides and polyketides. The nonribosomally produced lipopeptides such as iturins (iturin A, bacillomycin and mycosubtilin), surfactin and fengycin by *Bacillus* strains are gaining enormous attention due to their ability to effectively cause disease suppression. These compounds contain a lipid tail attached to a short cyclic oligopeptide. These lipopeptides act in a synergistic manner. Both the surfactin and iturin display an antibacterial activity.

Bacillus strains have also been used to control the crown gall disease caused by *Agrobacterium tumefaciens* strains. Many studies have described the antibacterial activity of bacteriocins against the *Agrobacterium* strains. Frikha-Gargouri et al. (2017) recently discovered a novel strain of *Bacillus*, i.e. *Bacillus methylotrophicus* 39b, which can effectively control the crown gall pathogen. This was the first report describing the antagonistic activity of *Bacillus methylotrophicus* 39b against *Agrobacterium tumefaciens* strains C58 and B6. The antibacterial compounds produced by *Bacillus methylotrophicus* 39b were found to be stable in a pH range from 2 to 8 with 100% activity at 70 °C and 90% activity at 100 °C. The antibacterial compounds were resistant to proteolytic enzymes and were extracted with methanol indicating the presence of a hydrophobic moiety. Using PCR (for the presence of the gene coding for lipopeptides) and LC-MS analysis of the methanol extracts, it was confirmed that the nature of antibiotic substance produced by strain 39b was lipopeptide surfactin. *Bacillus methylotrophicus* 39b can be used for long-term protection of plants against *Agrobacterium tumefaciens* strains C58 and B6 as this strain is endophytic in nature and can persist at high density for more than 45 days. Ben Abdallah et al. (2015) also showed the antibacterial activity of lipopeptides produced by *B. amyloliquefaciens* strain 32a against *A. tumefaciens*. Certain bacteria like *Bacillus* can be used to control *Phytophthora infestans*, the causal agent of late blight of potato. The mycelial growth of *P. infestans* was controlled by microbial preparations such as Serenade (*Bacillus subtilis* QST 713) and Sonata (*Bacillus pumilus* QST 2808).

The genetic basis of various mechanisms employed by *Bacillus* and the potential of lactic acid bacteria as biocontrol agent is discussed in the following section:

(a) *Molecular insights into various mechanisms employed by Bacillus*: In order to enhance the biocontrol efficiency of various *Bacillus*-based formulations, it is important to study the mechanisms involved at the genetic level. *B. amyloliquefaciens* FZB42 strain was used as a model to find the genetic features linked with their biocontrol ability (Wu et al. 2015). Computational genome analysis

of this model organism revealed the presence of ten giant gene clusters (corresponding to 10% of the whole genome) associated with the synthesis of many compounds responsible for its biocontrol activity. The genes responsible for the synthesis of ribosomal and nonribosomal associated secondary metabolites having antibacterial properties were identified. Various genes whose products inhibit the pathogens actions were recognized using chemical mass spectroscopy and by creating knockouts. Using this approach, a total of ten gene clusters were found which are involved in Sfp (4′-phosphopantetheine transferase)-dependent nonribosomal synthesis of polyketides and cyclic lipopeptides (cLPs), Sfp-independent nonribosomal synthesis of bacilysin and ribosomal synthesis of the bacteriocins plantazolicin and amylocyclicin which are highly modified (Chowdhury et al. 2015). The ability of Sfp-dependent nonribosomal cyclic lipopeptides and bacilysin (dipeptide) to inhibit the growth of *Erwinia amylovora*, a causative agent of fire blight, was demonstrated by the construction of a double mutant (RS06 *Δsfp Δbac*), which cannot synthesize bacilysin (*Δbac*) and nonribosomal peptides (*Δsfp*), and it was found that the double mutant was incapable of inhibiting the growth of *E. amylovora.* Using a similar approach of creating mutant strains of FZB42, the antagonistic role of difficidin and bacilysin was demonstrated against *Xanthomonas oryzae*, the causative agent of bacterial blight of rice. Genes involved in aromatic acid synthesis (*aro* genes) and bacilysin synthesis (*bacB* gene) are responsible for the inhibition of *Microcystis aeruginosa*, the causative agent of algal blooms, by *B. amyloliquefaciens* FZB42 (Wu et al. 2014). It has also been found that surfactins, volatile organic compounds (VOCs) such as 2,3-butanediol and acetoin, act as an elicitor of plant defence mechanisms (Ryu et al. 2004). The antifungal activity of *B. amyloliquefaciens NJN-6* against *Fusarium oxysporum* f. sp. *cubense* depends on its ability to produce volatile organic compounds. The overexpression of genes involved in secondary metabolite synthesis (antifungal lipopeptide bacillomycin D and antibacterial bacilysin) such as *degU* significantly enhances the biocontrol efficiency of FZB42 against *Fusarium* wilt. The global transcriptional regulator gene (DegU) controls the nonribosomal synthesis of secondary metabolites such as bacillomycin D (Koumoutsi et al. 2007). Using promoter exchange method, the ability of *Bacillus* strains to produce cyclic lipopeptides such as mycosubtilin and iturin A and surfactin can be significantly enhanced (Wu et al. 2015). In order to enhance the production of the biosurfactant surfactin, inducible promoter P_{spac} was used in *B. subtilis* (Sun et al. 2009). Similarly, increased production of 2,3-butanediol (plant defence elicitor) was achieved by engineering genes coding for acetolactate synthase (*alsS*), acetolactate decarboxylase (*alsD*) and butanediol dehydrogenase (*bdhA*) under IPTG-inducible P_{spac} promoter (de Oliveira and Nicholson 2016). Several factors important for bacilysin production such as DegU, nitrogen sources and scandium in the growth medium were identified by optimizing culture conditions (Mariappan et al. 2012; Inaoka and Ochi 2011). Genetic engineering using the Cre/Lox site-specific recombination along with PCR for replacement of the native promoter

with the constitutive promoter P_{repB} and P_{spac} was used to overproduce bacilysin (Wu et al. 2015).

(b) *Use of lactic acid bacteria to control the late blight of potato caused by Phytophthora infestans*: Lactic acid bacteria can produce various active metabolites such as 3-hydroxy fatty acids, organic acids, hydrogen peroxide, carbon dioxide, cyclic dipeptides and proteinaceous compounds having antifungal and antibacterial properties (Axel et al. 2012). Due to the diverse antifungal compounds produced by the LAB, they can also be tested for their potential against *P. infestans* and a large number of other fungal pathogens such as *Botrytis*, *Alternaria*, *Candida*, *Aspergillus*, *Endomyces*, *Penicillium*, *Fusarium*, *Sclerotium Monilinia*, *Microsporum*, *Rhizopus*, *Sclerotium* and *Trichophyton* (Axel et al. 2012). Wang et al. (2010, 2012) described the antagonistic nature of *Lactobacillus plantarum* IMAU10014 and *Lactobacillus plantarum* Bx62 against the *Phytophthora drechsleri* Tucker, a causal agent of root rot of cucumber and pistachio trees. There are two commercial LAB-based products available in the market, namely, AgroMos™ and EM5. However, the effective control of late blight by these products has not yet been reported. One of the products, EM5, was used for the biocontrol of *P. infestans* which shows up to 30% of pathogen inhibition. Dorn et al. (2007) showed that AgroMos™ which is based on *L. plantarum* does not possess anti-oomycete activity against *P. infestans*. Thus, there is a need to discover other novel strains of LAB having high capability to inhibit *P. infestans* and other pathogens and to continue research on the various potential antifungal compounds produced by different species of LAB.

24.5 Potential of Plant Growth-Promoting Microorganisms to be Used as Biocontrol Agents

Plant growth-promoting bacteria (PGPB) are microorganisms inhabiting the rhizosphere that benefit the associated host plant by more than one mechanism. Plant growth-promoting bacteria (PGPB) are potential agents for the biological control of plant pathogens. They have tremendous ability to be used as biocontrol agents particularly as biofertilizers because of their significant impact on the plant health, suppression of disease-causing microbes and ability to facilitate nutrient assimilation. PGPB belong to the genera *Acinetobacter*, *Arthrobacter*, *Acetobacter*, *Alcaligenes*, *Azoarcus*, *Azotobacter*, *Azospirillum*, *Beijerinckia*, *Burkholderia*, *Bacillus*, *Klebsiella*, *Enterobacter*, *Derxia*, *Gluconacetobacter*, *Rhodococcus*, *Serratia* and *Stenotrophomonas* (Berg 2009). PGPB act by the production of various toxic compounds such as phenazines, hydrogen cyanide (HCN) and pyrrolnitrin as well as other enzymes, antibiotics and metabolites. They aid in the plant growth by the production of siderophores, organic acids, phytohormones (indole acetic acid) and enzymes (phosphatase, nitrogenase, dehydrogenase, etc.) and by carrying out nitrogen fixation and phosphate solubilization and induction of systemic resistance. Because of their ability to fix atmospheric nitrogen via a symbiotic or

non-symbiotic association, their importance for use in organic farming as biofertilizers is increasing.

24.5.1 Factors Involved in Root Colonization by PGPB

PGPB inhabit the rhizosphere and multiply in the presence of native microflora. In order to increase the efficacy of PGPB as biocontrol agent, it becomes necessary to understand the genetic and environmental factors affecting the interaction between the root and the microbes. The competition between the phytopathogens and the PGPB to colonize the nutrient-rich rhizosphere zone is the mechanism by which PGPB protect the plants from phytopathogens. Rhizosphere is a significant carbon sink and is rich in nutrients containing almost 40% photosynthate material (Degenhardt et al. 2003). PGPB are attracted towards the plant roots by a chemotactic response. The chemical attractants present in the root exudates include amino acids, organic acids and specific sugars (Welbaum et al. 2004). In some of the bacterial strains, lipopolysaccharide (LPS) particularly the O-antigen plays a role in the root colonization abilities of these bacteria (Dekkers et al. 1998a). For example, the O-antigen chain of *P. fluorescens* PCL1205 plays a significant role in the colonization of tomato roots. Other factors reported to be involved in root colonization by PGPB include the synthesis of vitamins (e.g. vitamin B1) and enzymes (NADH dehydrogenases) (Dekkers et al. 1998a, b; Simons et al. 1996) and the presence of type IV pili. Root colonization by endophytic bacteria *Azoarcus* sp. depends on the type IV pili (Steenhoudt and Vanderleyden 2000). The ability of *P. fluorescens* to colonize root surface depends on the production of site-specific recombinase (Dekkers et al. 1998b). Root exudates and root mucilage also have roles in root colonization by PGPB.

24.5.2 Mechanisms Used by PGPB Against Phytopathogens

(a) *Production of siderophores*: Siderophores are low molecular weight compounds produced by PGPB under iron-limiting conditions (Whipps 2001). The siderophores produced by PGPB deprive the fungal pathogen of the essential element iron due to their higher affinity over the siderophores produced by the fungal pathogen (O'Sullivan and O'Gara 1992; Loper and Henkels 1999).
(b) *Antibiosis*: Production of various antibiotics against pathogens is one of the mechanisms used by PGPB. Various compounds like 2,4-diacetylphloroglucinol (2,4-DAPG), phenazine, pyrrolnitrin, pyoluteorin, hydrogen cyanide, oomycin A and cyclic lipopeptides are produced by *Pseudomonads*, and zwittermicin A, kanosamine and oligomycin A are produced by *Streptomyces*, *Bacillus* and *Stenotrophomonas* spp., respectively (Compant et al. 2005).
(c) *Enzyme production*: Production of lytic enzymes is an important factor to control phytopathogens. *Serratia plymuthica* C48 produced chitinase which inhibits the spore germination and germ tube elongation in *Botrytis cinerea*

(Frankowski et al. 2001). *Serratia marcescens* acts as an antagonist of *Sclerotium rolfsii* by production of extracellular cellulases (Ordentlich et al. 1988). Proteases produced by *S. plymuthica* IC14 are considered important factors in suppressing the pathogens *B. cinerea* and *S. sclerotiorum*, the causal agents of grey-mould rot and white rot, respectively (Kamensky et al. 2003). *Streptomyces* sp. strain 385 and *Paenibacillus* sp. strain 300 produce β-1,3-glucanase in order to lyse the fungal cell wall of *F. oxysporum* f. sp. *cucumerinum* (Singh et al. 1999).

(d) *Degradation and detoxification of virulence factors*: Detoxification of virulence factors of pathogens can be considered as a significant mechanism employed by antagonistic microbes against phytopathogens. The mechanism of detoxification involves the reversible binding of specific protein to the toxin as well as detoxification of toxins by several esterases produced by biocontrol agents such as *Pantoea dispersa* (Zhang and Birch 1997). *Pantoea dispersa* SB1403 produces albicidin protein AlbD (albicidin hydrolase) which causes detoxification of albicidin toxin which is crucial for *Xanthomonas albilineans* to cause sugar cane leaf scald disease (Compant et al. 2005). Certain biocontrol agents like strains of *Ralstonia solanacearum* and *B. cepacia* can hydrolyse fusaric acid which is a phytotoxin produced by *Fusarium* species (Toyoda and Utsumi 1991).

Another mechanism used by PGPB is the degradation of autoinducer signals used in quorum sensing to activate virulence gene expression. This mechanism is very effective as it can be used to effectively control the pathogens that activate their virulence genes through a quorum-sensing mechanism. This method can be used to alleviate disease even post infection (Compant et al. 2005).

(e) *Induction of systemic resistance*: ISR is an effective mechanism used by PGPB against phytopathogens. Both the free-living rhizobacterial strains and the endophytic microorganisms can activate ISR. *P. fluorescens* EP1 activates the ISR in sugar cane against the red rot pathogen *Colletotrichum falcatum* (Viswanathan and Samiyappan 1999). *Burkholderia phytofirmans* PsJN activates ISR against *Verticillium dahliae* infection on tomato and *Botrytis cinerea* in grapevine (Barka et al. 2000, 2002), *Bacillus pumilus* SE34 activates ISR in pea roots against *F. oxysporum* f. sp. *pisi* and *F. oxysporum* f. sp. *vasinfectum* on cotton roots (Conn et al. 1997), and *P. denitrificans* 1-15 and *P. putida* 5-48 induce ISR in oak plants against *Ceratocystis fagacearum*. PGPR which elicit ISR in one plant may not be able to do so in others, indicating the specificity of the interaction. Other substances involved in ISR include antibiotics, *N*-acyl-homoserine lactones, volatile organic compounds (e.g. 2,3-butanediol) and siderophores (Compant et al. 2005).

(f) *Plant growth promotion*: Plant-associated microorganisms can provide certain macronutrients and micronutrients to plants. The nitrogen fixation by *Rhizobium* is the biological process by which N_2 is converted to NH_3. In this mutualistic association, the bacteria metabolize root exudates and in turn provide a nitrogen source to the host plant for amino acid synthesis. Certain free-living bacteria such as *Burkholderia*, *Azospirillum* and *Stenotrophomonas* can also carry out nitrogen fixation (Dobbelare et al. 2003). PGPR can indirectly promote plant

growth by liberating phosphorus from organic substrates like phytates. *Azospirillum* enhances the nutrient uptake in plants by promoting root growth. In addition, PGPR can also provide sulphate to plants via oxidation of sulphur compounds (Banerjee and Yesmin 2002).

24.6 Variability in Sensitivity of Plant Pathogens to Biocontrol Agents

Different isolates of the same pathogen can show variability in their sensitivity to the biocontrol agents. Mazzola et al. (1995) showed that different isolates of *Gaeumannomyces graminis* var. *tritici* (the causal agent of take-all disease of wheat) have different sensitivity to the antibiotics 2,4-diacetylphloroglucinol (2,4-DAPG) and phenazine-1-acide carboxylique (PCA) produced by strains of fluorescent *Pseudomonas* spp. Similarly, among 117 isolates of *Fusarium oxysporum*, approximately 17% isolates were shown to be naturally tolerant to 2,4-DAPG (Schouten et al. 2004). No correlation was observed between the geographical position of the isolates and their sensitivity or tolerance to 2,4-DAPG suggesting the widespread nature of the isolates tolerant to these antibiotics. A wide range of variations in sensitivity was observed among the isolates of *Botrytis cinerea* to pyrrolnitrin, produced by several biocontrol agents such as *Pseudomonads* and *Serratia plymuthica* (Ajouz et al. 2011).

The success of biocontrol agents under field conditions can be affected by the different species of pathogens occurring in the same place. Various species of *Pythium* show variability in their sensitivity towards different antibiotics produced by biocontrol agents. *P. ultimum* var. *sporangiferum* was found to have the minimum sensitivity to phenazine-1-acide carboxylique (PCA) (Gurusiddaiah et al. 1986), whereas *P. deliense* was found to be least sensitive and *P. volutum* most sensitive to 2,4-DAPG (De Souza et al. 2003). Further, *P. medicaginis* shows the minimum sensitivity to kanosamine produced by *Bacillus cereus* (Milner et al. 1996). Mazzola et al. (2007) found that amidst the eight *Pythium* species studied *P. ultimum* var. *ultimum*, *P. rostratum* and *P. heterothallicum* have the least sensitivity to cyclic lipopeptide massetolide. These examples illustrate why certain biocontrol agents do not have high success under field conditions.

The variability in sensitivity levels could be because of the following reasons:

24.6.1 Variability in Sensitivity to Biocontrol Agents Having a Single Mode of Action

Variation was observed among the crown gall pathogen *Agrobacterium tumefaciens*, towards the biocontrol agent *A. rhizogenes* strain K84, acting through the production of the antibiotic agrocin 84 (Moore and Warren 1979). Among the various strains analysed for their sensitivity towards agrocin 84, all those belonging to biotype 3 were found to be resistant, and most strains belonging to biotype 1 and 2 were found to be susceptible (Van Zyl et al. 1986). In a recent study, it was

demonstrated that there is a high level of diversity both in the pathogen and biocontrol agents (Otto-Hanson et al. 2013). The researchers used 15 isolates of *Streptomyces scabies* (the causal agent of potato scab) and 19 strains of antagonistic *Streptomyces* sp. and found that, among the 19 isolates of biocontrol agent, only one was able to inhibit all the 15 isolates of *S. scabies*. Among the 15 isolates of *S. scabies*, one was found to be least susceptible and was inhibited by only four isolates of antagonists (Otto-Hanson et al. 2013). These results demonstrate the difficulty associated with the successful use of biocontrol agents which are based on single species.

24.6.2 Variability of Phytopathogens to Hyperparasites

The emergence of resistant varieties of *Cryphonectria parasitica* has been reported by various researchers. This fungal pathogen causes chestnut blight disease. It is effectively controlled by the use of the hypovirus *Cryphonectria* hypovirus 1 (CHV1) which hyperparasitizes the fungus and inhibits its sexual reproduction. Chestnut blight has been successfully controlled using CHV1 in Europe and in some parts of the USA except Eastern North America. However, the development of resistance in certain isolates of *C. parasitica* should be carefully monitored. Similarly, different bacteriophages have been tested against the fire blight pathogen *Erwinia amylovora.* A total of 5 phages were tested against 52 strains of *E. amylovora.* Among the 52 strains, only 22 were sensitive to all the phages and 23 were sensitive to more than one phage (Schnabel and Jones 2001). Four strains of the plant pathogen *Helminthosporium solani* also showed diversity in their sensitivity to the mycoparasite *Acremonium strictum*, with *in vitro* inhibition of sporulation by 35–65%, mycelial growth by 32–40% and spore germination by 43–53% (RiveraVaras et al. 2007).

24.6.3 Variability in Sensitivity of Plant Pathogens to Biocontrol Agents Acting Through More Than One Mode of Action

It was observed that certain isolates of *B. cinerea* were found to be resistant to the yeast *Rhodotorula glutinis* PM4, which inhibits the *B. cinerea* by producing rhodotorulic acid, a toxic compound and by competition for nutrients (Sansone et al. 2005). In a study, it was found that among 29 isolates of *B. cinerea*, some were completely resistant to *R. glutinis* PM4 (Buck and Jeffers 2004). Certain isolates of *B. cinerea* were also found to be resistant to *Bacillus subtilis* QST713, which act through multiple mechanisms such as hyperparasitism, antibiosis, competition and induction of resistance (Paulitz and Belanger 2001; Lahlali et al. 2013). Bardin et al. (2013) reported that the strain L13 of *Fusarium* sp. can effectively control the 41 isolates of *B. cinerea* when used at the recommended dose of 10^7 spores/ml. However, isolates of *B. cinerea* displayed diversity of sensitivity towards the *Fusarium* sp., when the biocontrol strain was applied at a tenfold reduced dose.

24.7 Mass Production, Formulation, Additives and Delivery

In order to control plant diseases in a sustainable manner, it is important to produce the BCA or the effective form of antagonistic microbes at a large-scale cost-effectively. Further, the development of effective formulations with suitable additives to increase the shelf life and to develop a suitable, potent delivery method is important for the successful management of a specific disease.

24.7.1 Mass Production

Mass production of the antagonistic microbes is a major concern due to the various difficulties associated with large-scale production, determining the nutritional and environmental conditions suitable for the biomass production. Mass production is achieved through solid state or liquid-state fermentation. Liquid-state fermentation has been used for the mass production of fungal biocontrol agents. The medium used for multiplication should be nutrient rich, inexpensive and readily available. Molasses-yeast medium, wheat bran and potato dextrose are commonly used for the large-scale production of *Trichoderma* spp. (Prasad and Rangeshwaran 1998, 2000; Prasad et al. 2002). Solid-state fermentation is an appropriate method for the production of fungal biopesticides as it provides high conidia content to micropropagules. Various cheap substrates like millets, ragi and sorghum are used in this method (Lewis 1991; Jeyarajan 2006). These grains are moistened, sterilized and then inoculated with the BCA, e.g. *Trichoderma*, for 10–15 days. The BCA-coated grains can then be used for seed treatment in the powdered form.

24.7.2 Formulations

An ideal formulation is one which is not toxic to the host plant, is easy to handle, has a long shelf life, is compatible with other agrochemicals, is cost-effective and is stable over a temperature range from −5 to 35 °C. The formulations should work under different environmental conditions, to provide reliable control of plant diseases (Kumar et al. 2014):

(a) *Talc-based formulations*: In this method, the biomass is first grown in the liquid medium, and then it is mixed with talc powder in the ratio of 1:2 and dried under shade (up to 8% moisture content).
(b) *Vermiculite-wheat bran-based formulation*: In this method, the molasses-yeast medium is used for the growth of antagonistic microbes such as *Trichoderma* for almost 10 days. 33 g wheat bran and 100 g vermiculite are sterilized in an oven at 70 °C for 3 days. To this, 20 g of fungal biomass and 0.05 N of growth medium are added, mixed and then dried in shade (Lewis 1991).
(c) *Pesta granule-based formulations*: To 100 g of wheat flour, 52 ml of fermenter biomass was added and mixed properly to form a consistent dough. Then this dough was pressed and folded several times, and 1 mm thick pesta (sheets) was

made and air-dried. Subsequently, pesta granules were made by passing the pesta sheets through a mesh. The granular-based formulations are more successful because they are applied as several millimetre particles which can be completely colonized by the biocontrol agent and thus have high inoculum size (Connick et al. 1991).

(d) *Coffee husk-based formulation*: This product is made using the waste product of the coffee-curing industry, i.e. coffee husk. This product based on *Trichoderma* can effectively suppress foot rot of black pepper caused by *Phytophthora* (Sawant and Sawant 1996).

(e) *Oil-based formulations*: Oil-based formulations generally have a long shelf life and can be used under adverse environmental conditions such as dry weather. *Trichoderma* containing oil-based formulations are now being used as foliar sprays. They are prepared by mixing the vegetable/mineral oils with the conidia harvested from the liquid-/solid-state fermentation, in stable emulsion formulations. The oil used must not be toxic to fungal spores, humans, plants and animals.

(f) *Encapsulation method/alginate prill-based formulation*: A large number of encapsulation methods are available for the biocontrol agents. Encapsulation-based formulations have several advantages like increased shelf life, improved handling, reduced application and co-encapsulation with small amounts of pesticides or nutrients (Vemmer and Patel 2013).

The sodium alginate-based formulation is the most popular method for encapsulation of microbial biopesticides. Calcium alginate-based beads have been successfully used for the delivery of *Trichoderma*, *Fusarium*, *Gliocladium*, *Alternaria*, *Penicillium*, *Pseudomonas* and *Bacillus* spp. (Connick et al. 1991). In this method small spherical beads containing the immobilized cells are made using calcium chloride and sodium alginate. First, sodium alginate is dissolved in one portion, and the food base and distilled water are dissolved in another, and both portions are autoclaved and then mixed with the biomass. This mixture is added dropwise into the calcium chloride solution to form spherical beads (Connick et al. 1991).

24.7.3 Methods of Application

(a) *Application of BCA directly to the infection site*: The direct application of biocontrol agents at high population densities at the infection site and seed coating are the most successful methods for the effective control of several pathogens.

(b) *One place application*: In this strategy, the antagonistic microbes are applied at one place (during each crop year) at lower concentrations. These microbes then multiply and spread to other parts of the plants and to other nearby crops providing augmentative control against pathogens. For example, when non-toxigenic strains of *Aspergillus flavus* were applied on wheat seeds to outcompete the toxigenic strains of *A. flavus*, then these non-toxigenic strains

spread to the cotton flowers, thus providing protection to both wheat plant and cotton flowers against toxigenic *A. flavus* (Islam et al. 2005; Kloepper et al. 2004).

(c) *Occasional application*: In this method, an occasional application of the BCA maintains the population level of the pathogens below the threshold level. The occasional application of hypovirulent strains or attenuated strains of pathogens is done to protect the plant from the virulent strains of pathogens (Milgroom and Cortesi 2004).

24.7.4 Additives Used to Enhance the Efficacy of Biocontrol Agents

One of the major problems associated with the use of BCA is that they rapidly lose their effectiveness below 85–90% relative humidity. It has been reported that the use of additives can enhance the efficacy of biocontrol agents and overcome the humidity requirements.

Treatment of seeds with *Trichoderma* strains along with 10% pelgel enhanced the efficacy of *Trichoderma* against the *Pythium* sp. on various crops (Lo et al. 1997). It was reported that the use of BCA along with surfactants can result in control of plant diseases at the same level as that obtained by the use of chemical pesticides. Detergents like Triton X-100 are very efficient in increasing the efficacy of biocontrol agents. They can reduce the growth of pathogens and can increase the adhesion of spores to the infection site. The biofungicide AQ-10 containing conidia of *Ampelomyces quisqualis* which is reported against powdery mildew fungi when used with the wetting agent, i.e. AddQ, provides better control of the powdery mildew disease since the activity of the biocontrol agent *Ampelomyces* highly depends on humidity. Other agents used are 2% paraffin oil and Tween 20 (Kiss 2003).

24.7.5 Delivery

In order to successfully control the disease, the delivery of the biocontrol agent at the site of action is very essential. Therefore, the following strategies are employed:

(a) *Seed treatment*: It is one of the easiest and effective methods to control the seed-/soilborne plant diseases. In this method, the seed is coated with the antagonistic microbe which then colonizes the roots of the germinating seedlings and rhizosphere. Generally, the dry powder of the antagonist is used at 3–10 g/kg seed for commercial use. *T. viride*, *T. virens* and *T. harzianum* are effective seed protectants against *R. solani* and *Pythium* spp. (Mukherjee and Mukhopadhyay 1995).

(b) *Seed biopriming*: Biopriming is the method in which the seed is coated with the biocontrol agent and incubated under warm conditions. This method results in

the rapid and uniform emergence of the seedling. Seed biopriming is successfully used in chickpea, tomato, soybean and brinjal (Mishra et al. 2001). The bioprimed seeds of the chickpea and rajma coated with *Trichoderma asperellum* T42, *Rhizobium* sp. RH4 and *P. fluorescens* OKC showed higher germination of seedlings as well as better plant growth as compared to non-bioprimed control seedlings. It was also concluded that the use of multiple biocontrol agents resulted in better growth and development than the use of individual species (Yadav et al. 2013).

(c) *Soil treatment*: Various soilborne diseases can be effectively controlled by the treatment of soil with biocontrol agents, either before or at the time of plantation. Several reports stated that a wide range of fungal pathogens can be efficiently controlled by the soil treatment method. Seedling blight, root rot and stem rot of jute can be commendably controlled by the application of *T. viride* to the soil (Srivastava et al. 2010). Various strains of *Trichoderma* can also control the seed-borne pathogenic fungi such as *F. oxysporum*, *F. moniliforme*, *R. solani* and *A. alternata* (Mustafa et al. 2009). *Trichoderma* is also capable of colonizing farmyard manure (FYM), and therefore, treatment of soil with FYM colonized by *Trichoderma* is the most effective and beneficial method for the management of soilborne diseases.

(d) *Root treatment*: In this method the root is dipped in the spore or cell suspension containing the antagonistic microbes before transplanting. This method not only results in disease suppression but also results in increased seedling growth in the case of rice, brinjal, chilli, capsicum and tomato (Singh and Zaidi 2002).

(e) *Foliar spraying/wound dressings*: The ability of biocontrol agents to control foliar diseases is largely affected by the fluctuation in environmental conditions. Various foliar diseases can be effectively controlled by the spray application of bacterial and fungal antagonists. Smith et al. (1993) reported the use of a foliar spray of *Bacillus cereus* against the cotton leak of cucumber.

(f) *Multiple delivery systems*: The use of multiple delivery systems results in a significant increase in the population of the biocontrol agent. For example, seed treatment and foliar application of *T. viride* on linseed reduced the incidence of *Alternaria* blight. Gaur et al. (2010) demonstrated that *Sclerotinia* rot of mustard can be managed by foliar spray and seed treatments with mixed formulations of *T. viride* (Tv-1) and *T. hamatum* (HP-20). Several species of *Trichoderma* were used to develop these formulations. Among them, *T. harzianum*-based Pusa Biopellet for soil application and Pusa 5SD for seed treatment were best in terms of their shelf life and efficacy (Dubey et al. 2009). These formulations were found to be highly effective in controlling diseases of pulse crops, namely, dry root rot of chickpea and mung bean, wet root rot and wilt of chickpea.

24.8 Strategies Employed by Pathogens to Overcome the Effect of Biocontrol Agents

There are very few studies regarding the development of resistance against biocontrol agents. However, various mechanisms involved in the resistance to toxic substances produced by microorganisms have been extensively studied. In the following section, few of these mechanisms are discussed:

(a) *Active efflux*: The microbial cells contain ABC (ATP-binding cassette) and MSF (major facilitator superfamily) transporters for effluxing several toxic compounds outside the cell. The resistance of *B. cinerea* to the antibiotic compounds produced by *Pseudomonas* is due to the presence of these efflux pumps (Schoonbeek et al. 2002). Several antibiotics have been shown to induce the gene coding for ABC transporter, providing the description of the involvement of ABC transporters in protection of plant pathogens against the antibiotics produced by these beneficial microbes (De Waard et al. 2006). In addition, ABC transporters are also responsible for development of multidrug resistance among several pathogenic fungi against the chemical fungicides belonging to different families (Kretschmer et al. 2009). Burse et al. (2004) demonstrated that the membrane transporter NorM is responsible for the development of resistance in *E. amylovora* to various toxins produced by *P. fluorescens* and *Pantoea agglomerans.* Thus, these efflux pumps have a major role in the development of resistance against various antibiotics and toxic metabolites produced by biocontrol agents.

(b) *Metabolization*: Antibiotics and other metabolites produced by biocontrol agents can induce the expression of enzymes such as catalases, laccases, peroxidases and superoxide dismutase in pathogenic fungi. These compounds cause the degradation of antibiotics and other metabolites thus providing resistance to the disease-causing pathogen. *Rhizoctonia solani* produces laccase in response to the metabolites produced by *P. fluorescens*. These laccases reduce the permeability of the fungal cell wall against toxic compounds and detoxify the antifungal compounds (Crowe and Olsson 2001).

(c) *Interference in the biosynthesis of various metabolites*: Phytopathogens can interfere in the synthesis of antibiotics. For example, fusaric acid produced by *F. oxysporum* inhibits the synthesis of the antibiotic 2,4-DAPG produced by *P. fluorescens* CHA0 (Notz et al. 2002). Besides this, plant pathogens can also alter their surrounding environment and reduce the effectiveness of BCA. For example, *Gaeumannomyces graminis* var. *tritici* acidifies the surrounding environment and renders the biocontrol agent *P. fluorescens* ineffective (Ownley et al. 1992).

(d) *Transfer of resistance genes*: The transfer of plasmids carrying an antibiotic resistance gene is another mechanism employed by phytopathogens to confer resistance to biocontrol agents. For example, the crown gall pathogen *A. tumefaciens* became resistant to *A. radiobacter* K84 upon acquiring the plasmid pAgK84. The plasmid present in the biocontrol agent *A. radiobacter* K84 con-

tains the gene which codes for the antibiotic agrocin 84, as well as the gene coding for the resistance to this antibiotic. In order to minimize the risk associated with the transfer of plasmid pAgK84, a new strain of K84 known as K1026 was constructed which carries the gene coding for the antibiotic but lacks the region required for the transfer of the plasmid (Stockwell et al. 1996).

(e) *Resistance to cell wall-degrading enzymes produced by biocontrol agents*: Plant pathogens use several strategies to overcome the effect of biocontrol agents. Most of the biocontrol agents which act through hyperparasitism produce enzymes such as chitinases and glucanases. In order to protect themselves from such lytic enzymes, plant pathogens produce melanin polymers which protect pathogens from harsh environmental conditions and also help in host invasion (Bell and wheeler 1986). Additionally, they can also inhibit the synthesis of such enzymes. For example, *Fusarium graminearum* and *Fusarium culmorum* produce the mycotoxin DON (deoxynivalenol) which can effectively inhibit the expression of the gene coding for chitinase in *Trichoderma atroviride* (Lutz et al. 2003).

(f) *Resistance to defence compounds produced by plants*: Pathogens can use a combination of strategies to overcome the plant defence mechanisms induced by the biocontrol agents. This includes detoxification of host defence molecules and interference with the signalling mechanism (Morrissey and Osbourn 1999). Phytopathogens can use ABC transporters to evacuate various metabolites out of the cell. They are the major components responsible for the virulence and aggressiveness of pathogens. *Grosmannia clavigera*, a fungal pathogen of pine trees, can cope up with monoterpenes because of such ABC transporters. Similarly ABC transporters are responsible for the resistance of phytopathogens to certain phytoalexins produced by plants, e.g. *B. cinerea* to resveratrol (Schoonbeek et al. 2001) and *Nectria haematococca* to pisatin. Many plant pathogens can prevent themselves from the oxidative damage caused by the hypersensitive response, and certain pathogens like *Pythium ultimum* can decrease the population density of biocontrol agents either by decreasing the expression of genes involved in antimicrobial activity or by exploiting the nutrients faster than the biocontrol agents (Fedi et al. 1997; Duffy et al. 2003).

24.9 Commercialized Biocontrol Agents

The first commercialized product of biocontrol agent was registered in 1979 by the US Environmental Protection Agency (EPA) for the control of crown gall disease. It contained the bacterium *Agrobacterium radiobacter* strain K84. Ten years later in 1989, the first fungus *Trichoderma harzianum* ATCC 20476 was registered for the control of plant diseases. **AQ 10** biofungicide containing a conidial suspension of *Ampelomyces* was developed by Ecogen, Inc., USA, against powdery mildew fungi. Other products used to control powdery mildew disease include **Sporodex**, which contains *Pseudozyma flocculosa* formulated as a liquid suspension (Kiss 2003). **Vertelac** consisting of *Verticillium lecanii* is used for the control of whiteflies in

greenhouse system, but it also controls powdery mildew (Kiss 2003). Various other commercialized products based on yeast, bacteria and fungi are given in Table 24.1.

24.10 Current Status

Recently, biocontrol agents have been used as a strategy to reduce the impact of various mycotoxins in Argentina and USA. Mycotoxins are secondary metabolites produced by filamentous fungi.

Peanut (*Arachis hypogaea*) is an economically important crop in Argentina, the number one exporter of peanut in the world. It exports 400,000 tonnes of edible peanuts worldwide. However, aflatoxins as contaminants of peanut crops present a great economic loss. Several control methods have been applied to limit aflatoxin both at preharvest and postharvest level such as the use of drought-resistant cultivars, good cultural practices, irrigation and postharvest sorting by blanching and electronic devices (Dorner 2008; Torres et al. 2014).

In order to prevent the pre-harvest contamination of aflatoxin in peanut crops, competitive non-toxigenic strains of *Aspergillus flavus* and *Aspergillus parasiticus* were applied as biological control to the soil of developing crops. This method is based on the fact that competitive non-toxigenic strains will compete with the pathogen for nutrients, space and site of infection for growth on peanut crops and will produce inhibitory metabolites. Furthermore, the non-toxigenic strains do not recombine with the toxigenic strain thereby preventing the reoccurrence of aflatoxigenicity (Abbas et al. 2011; Ehrlich 2014). It has been shown previously that preharvest treatment of peanuts with biocontrol agents also prevents the postharvest infection during storage (Dorner and Cole 2002). While selecting for atoxigenic strains, it is a prerequisite to consider the phenotype and genotype of strains and select those strains that have completely or partially lost the aflatoxin synthesis gene cluster (Barros et al. 2007). This method of using competitive non-aflatoxigenic strains for the competitive elimination of toxigenic strains has been demonstrated under field conditions in peanuts (Dorner et al. 2003; Dorner and Lamb 2006; Pitt and Hocking 2006; Alaniz Zanon et al. 2013), maize (Abbas et al. 2011; Atehnkeng et al. 2008, 2014) and cotton (Cotty 1994).

A. flavus AFCHG2 strain was used to reduce the production of aflatoxin in peanuts. Long-grain rice was chosen as the substrate to prepare the inoculum for field trials. This substrate gave a dried and granulated product, which assisted in the suitable distribution of the biopesticide. Using this methodology of competitive exclusion, a significant decline in the toxigenic isolates of *A. flavus/A. parasiticus* occurred in the soil and peanuts during 2009–2010. A major decrease in the aflatoxin was detected in the treated crops averaging 71%. Thus, this study revealed the significance of microbes as biocontrol agents in controlling various crop-related problems in a substantial manner (Alaniz Zanon et al. 2013). In the USA, an overall mean reduction in aflatoxin of 85.2% was obtained in Georgia and Alabama during 2004 after treatment with non-toxigenic strain of *A. flavus* (Dorner 2004).

Other mycotoxins which cause contamination of economically important crops include fumonisins and deoxynivalenol (in cereals) and ochratoxin A (in grapes). Wheat, which is one of the most essential cereal crops, is often infected by the *Fusarium graminearum* which causes *Fusarium* head blight disease resulting in an extensive loss of quality and yield. The major problem associated with this disease is the contamination of wheat grains by the mycotoxin and deoxynivalenol (DON) (McMullen et al. 2012). Similarly, grapes and grape-derived products are commonly contaminated with ochratoxin A (OTA). This toxin is mainly produced by fungal species like *Penicillium verrucosum*, *Aspergillus* section *nigri*, *Aspergillus ochraceus* and *Penicillium nordicum* that predominantly colonize cocoa, grapes, cereals and coffee.

Ochratoxin A (OTA) is one of the most important mycotoxins of concern for human and animal health. The IARC (International Agency for Research on Cancer) has classified OTA as a possible human carcinogen, group 2B (IARC 1993). Based on the available scientific toxicological and exposure data, the European Union established 2 μg/kg as the maximum permitted level for OTA in wines (European Commission 2006). Thus preclusion of the fungi-producing OTA is the most promising strategy for preventing the entry of this toxin in food chains. Yeast like *Kluyveromyces thermotolerans*, *Metschnikowia fructicola* and *Aureobasidium pullulans* showed promising results to neutralize this toxin in grapes.

Recently, a new yeast, *Candida azyma*, has been discovered for the biological control of *Geotrichum citri-aurantii* in citrus fruits. Citrus fruits are the most commonly produced fruits in the world. However, due to their acidic nature, they often get infected with fungi post-harvesting. The most common disease that occurs in post-harvest citrus fruits is sour rot caused by *Geotrichum citri-aurantii.* Ferraz et al. (2016) demonstrated the use of yeast species for the biological control of sour rot in 'Pera oranges'. They had successfully isolated the three most potential yeast isolates from the citrus fruit, namely, *Rhodotorula minuta* (ACBL-23), *Candida azyma* (ACBL-44) and *Aureobasidium pullulans* (ACBL-77), which controlled sour rot effectively. Abd-Alla et al. (2007) found that *Candida* sp. and *Saccharomyces* sp. isolates can inhibit *G. citri-aurantii* up to 51.1%; however, *Cryptococcus* sp. causes inhibition up to 31.5%. Maldonado et al. (2010) found that the antagonist *Streptomyces* inhibited *G. citri-aurantii* by 29%. Hernandez-Montiel et al. (2010) described that when *Citrus aurantifolia* fruits were treated with the yeast *Debaryomyces hansenii*, a significant reduction in the size of the lesion and in the incidence of infection caused by *G. citri-aurantii* was observed. Ren et al. (2012) demonstrated that the recombinant isolate of *Pichia pastoris* GS115 expressing the cecropin A, an antifungal peptide, diminished the lesions caused by *G. citri-aurantii* in citrus fruits. In this study it was found that ACBL-77 results in inhibition of up to 91% in the incidence of disease, while ACBL-44 and ACBL-23 resulted in 95% inhibition of lesion development. This study proves that production of killer toxin by antagonist yeast is the main mode of action for the control of *G. citri-aurantii*.

Recently, it has been found that enzymes from endophytic fungi can be used as biopesticides for the control of various insects in an eco-friendly manner. Thus endophytic fungi can be used as biocontrol agents for regulating several important

plant diseases. For example, alpha-glucosidase inhibitors from the endophytic *Cladosporium* sp. have the potential to be used as biocontrol agents. Endophytes are microorganisms that grow either inter- or intracellularly in the tissues of host plants and provide them protection and benefits by producing excess of useful substances (Strobel 2003; Tan and Zou 2001). Use of entomopathogenic microorganisms is an alternative to reduce or abolish the use of chemical pesticides for the control of pests and plant diseases. Endophytic fungi have been shown to produce toxins with the ability to repel pests, induce weight loss, reduce growth and development and increase the death rate of pests. In recent studies, the use of inhibitors of digestive enzymes that affect the growth and development of pest species has been described. The inhibitors of digestive enzymes like α-glucosidase and α-amylase can prevent the cleavage of the oligosaccharides to monosaccharides thereby slowing the digestion process. In insects, α-glycosidases are generally present in the salivary secretions, hemolymph and alimentary canal. In plants, α-glycosidase inhibitors (AGI) are present as part of the natural defence mechanism, and they are abundantly present in cereal crops (Franco et al. 2000; Carlini and Grossi-de-Sá 2002; Nair et al. 2013). Singh et al. (2015) carried out a study on the economical production of fungal AGI as bioinsecticides. In this study, endophytes were isolated from *T. cordifolia*, and their potential to act as bioinsecticide against the pest *Spodoptera litura* was examined. *Spodoptera litura* is a polyphagous pest which causes economic losses of many commercial crops. It is also resistant to a variety of chemical pesticides thus highlighting the necessity of using bioinsecticides. There are some fungi like *Aspergillus aculeatus* (Ingavat et al. 2009), *Aspergillus terreus* (Dewi et al. 2007), *Colletotrichum* sp. TSC13 (Artanti et al. 2012) and *Cladosporium herbarum* (Saito 1982) which produce α-glucosidase inhibitors having good inhibitory activity. However, endophytic *Cladosporium* sp. has been recently discovered as a producer of AGI. The results of this study indicated that AGI production by endophytic *Cladosporium* sp. adversely affects the survival of the insect *S. litura.* The LC_{50} (lethal concentration) value of AGI was found to be 22.12 µl/ml. A substantial amount of decline was observed in the activity of α-amylase and α-glucosidase in the gut of the larvae. The larvae of pests which were fed with the fungal extract showed significant reduction in the activity of α-amylase by approximately 20.97–64.40%. Similarly, AGI inhibited the glucosidase activity by 6.11–24.93%. Recently, Singh et al. (2012) reported the antagonistic effect of *Alternaria* sp. on *S. litura.* Endophytic *Nigrospora* sp. has been demonstrated to affect the reproductive potential of *S. litura* (Thakur et al. 2012). Thus it was concluded that the inhibitors of digestive enzymes from endophytic microorganisms hold the potential as an effective bioinsecticides.

A new strain, *Paenibacillus polymyxa* APEC128, has been recently discovered to control the postharvest disease anthracnose on apple caused by *Colletotrichum gloeosporioides* and *C. acutatum*, thus increasing the shelf life of apples and reducing the use of fungicides. This BCA strain produces the enzyme amylase and protease in copious amounts and cellulase and chitinase at moderate levels contributing to their biocontrol activity. It was observed that the postharvest treatment of apples with *Paenibacillus polymyxa* APEC128 at a concentration of 1×10^7 cfu/ml causes

a significant suppression of *C. gloeosporioides* by 83.6% and *C. acutatum* by 79%. *Paenibacillus* spp. use multiple mechanisms to cause disease suppression, like competition, induced resistance, antibiosis and production of cell wall-degrading enzymes chitinases and β-1,3-glucanase. In vitro, the strain APEC128 causes inhibition of fungal pathogens, and this was attributed to their potential to produce antifungal compounds. This study indicates the potential use of *Paenibacillus polymyxa* APEC128 as a biocontrol against anthracnose pathogens, both in the field and postharvesting (Kim and Jeon 2016).

Recently, Raza et al. (2016) described the role of various biocontrol agents against the *Fusarium* wilt-causing pathogen. *Fusarium* wilt is one of the major diseases occurring worldwide. *Fusarium oxysporum* was ranked fifth among the top 10 fungal pathogens. These are saprophytic fungi, having the capability to colonize the rhizosphere for long periods of time. The control of *Fusarium* wilt includes the use of non-pathogenic strains of *Fusarium*, use of antagonistic microbes and use of resistant plant varieties. Cucumber (*Cucumis sativus L.*), banana (*Musa* spp.) and tomato (*Solanum lycopersicum*) are the major crops affected by *Fusarium* wilts. *F. oxysporum* f. sp. *cucumerinum*, *F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *cubense* are the casual agents of *Fusarium* wilt of cucumber, tomato and banana, respectively. Among the various strains examined to have biocontrol efficiency against *Fusarium* wilt, *Bacillus*, *Trichoderma*, *Pseudomonas*, *Chaetomium*, *Serratia*, *Streptomyces* and the non-pathogenic *Fusarium* spp. showed significant capability to inhibit the phytopathogens. In tomato and banana, *Bacillus* strains showed maximum biocontrol efficiency (BCE) of 60% and 65%, respectively. However, in cucumber, *Chaetomium* spp. showed the maximum biocontrol efficiency of 82% among the various strains examined. *Bacillus*, *Trichoderma*, *Pseudomonas* (in banana, cucumber and tomato), *Chaetomium* and *Serratia* (in cucumber) and *Streptomycetes* (in tomato) were found to be the most effective biocontrol agents against *Fusarium* wilt. It was found that the use of biocontrol strains along with various organic and inorganic amendments considerably increased the BCE in banana, cucumber and tomato by 21%, 24% and 4% respectively.

24.11 Future Prospects

Biocontrol agents can be established as part of an integrated pest management (IPM) system to diminish the effect of chemical fungicides on the environment. More investment is needed in the field of research and development of the biocontrol agents. The registration procedure should be fast, and trials must be done to make it more reliable. Additional studies need to be done on dose, formulation, the effect of various environmental conditions on the efficacy of BCA and the effect of BCA on the native microflora of plants. It is essential to completely understand the mechanism of action of biocontrol agents so that more effective antagonists can be developed. There is a need to improve the formulation methods so that formulations having improved shelf life and delivery systems can be made. One of the promising methods of effective formulation is encapsulation of microbial cells. However, even

till now alginate-based beads are the most commonly used encapsulation method. So, there is a need to make efforts to construct new methods and to improve the already existing methods for formulations and delivery. It is also important to gain knowledge about the interaction of the capsule matrix with the physico-chemistry of the soil or agroecosystem. The environmental conditions play a major role in determining the efficacy of biocontrol agents. Thus, it is necessary to study the environmental conditions in which the biocontrol agents work optimally. Also it is important to determine the factors contributing to the colonization of rhizosphere and the effect of using BCA on the native population of soil. Further, there exists a need to discover the novel strains or previously uncharacterized strains and to develop genetically engineered microbes and plants in order to increase the effectiveness of BCA. With the use of metagenomic approaches, several non-culturable microorganisms can be identified, and their potential as biocontrol agents can be determined using various molecular methods. It is also important to determine the genes, gene products and other signalling molecules responsible for the antagonistic activity of microbes. There is also a need to develop effective methods for the mass production of BCA and to solve technical problems associated with their large-scale production and to improve formulation methods so that even gram-negative bacteria can be effectively used. A major difficulty in the development of BCA is the time and cost associated with the registration process (Berg 2009). It is very important to know that these antagonistic microbes are safe to use and there is no risk associated with them, i.e. they are not human pathogens.

Conclusions

Biocontrol agents hold a potential to replace the chemical pesticides. They can be used as biofertilizers, biopesticides and plant growth stimulators. BCA use different mechanisms of action to control the activity of plant pathogens which involves either the direct interaction between the BCA and the pathogen such as hyperparasitism, production of lytic enzymes and antibiotics or the indirect interaction between BCA and pathogen such as competition for nutrients and space and induction of resistance in plants. Various types of interactions contribute to the biological control, e.g. commensalism, mutualistic relationship between plant and BCA, competition between antagonistic microbes and phytopathogens, predation and parasitism. An understanding of these interactions is very important. Various categories of microbes like fungi, bacteria and yeast can act as biocontrol agents. These microbes have successfully contributed to the sustainable management of phytopathogens. Even PGPB also hold the potential to be used as BCA due to their ability to produce antibiotics and enzymes, in detoxification of virulence factors, in quorum quenching, in induction of plant defence mechanisms and in plant growth promotion. However, plant pathogens also use certain strategies to overcome the effect of BCA such as the use of efflux pumps, metabolization of secondary metabolites and resistance to enzymes and defence compounds produced by plants. So, it is important to understand the response of pathogens to BCA so that recombinant strains or multiple BCA effective against a pathogen can be developed and well screened for its biocontrol efficiency.

Further, it is important to determine the variability of different strains of pathogens occurring at the same place to the BCA used to suppress them. Moreover, mass production of BCA in a cost-effective manner, development of formulations containing suitable additives with increased shelf life and an effective delivery system are important parameters for the popularization and effective commercialization of BCA.

References

Abbas HK, Zablotowicz RM, Horn BW, Phillips NA, Johnson BJ, Jin X, Abel CA (2011) Comparison of major biocontrol strains of nonaflatoxigenic *Aspergillus flavus* for the reduction of aflatoxins and cyclopiazonic acid in maize. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 28:198–208

Abd-Alla MA, El-Mohamedy RSR, El-Mougy NS (2007) Control of sour rot disease of lime fruits using saprophytic isolates of yeast. Egypt J Phytopathol 35:39–51

Ajouz S, Walker AS, Fabre F, Leroux P, Nicot PC, Bardin M (2011) Variability of *Botrytis cinerea* sensitivity to pyrrolnitrin, an antibiotic produced by biological control agents. BioControl 56:353–363

Alaniz Zanon MS, Chiotta ML, Giaj-Merlera G, Barros G, Chulze S (2013) Evaluation of potential biocontrol agent for aflatoxin in Argentinean peanuts. Int J Food Microbiol 162:220–225

Alfano G, Ivey ML, Cakir C, Bos JI, Miller SA, Madden LV, Kamoun S, Hoitink HA (2007) Systemic modulation of gene expression in tomato by *Trichoderma hamatum* 382. Biol Control 97:429–437

Amer GA, Utkhede RS (2000) Development of formulation of biological agents for the management of root rot lettuce and cucumber. Can J Microbiol 46:809–816

Artanti N, Tachibana S, Kardono LBS, Sukiman H (2012) Isolation of [alpha]-glucosidase inhibitors produced by an endophytic fungus, *Colletotrichum* sp. TSC13 from Taxus sumatrana. Pak J Biol Sci 15(14):673

Atehnkeng J, Ojiambo PS, Ikotun T, Sikora RA, Cotty PJ, Bandyopadhyay R (2008) Evaluation of atoxigenic isolates of *Aspergillus flavus* as potential biocontrol agents for aflatoxin in maize. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 25:1264–1271

Atehnkeng J, Ojiambo PS, Cotty PJ, Bandyopadhyay R (2014) Field efficacy of a mixture of atoxigenic *Aspergillus flavus* Link: Fr vegetative compatibility groups in preventing aflatoxin contamination in maize (*Zea mays* L.) Biol Control 72:62–70

Axel C, Zannini E, Coffey A, Guo J, Waters DM, Arendt EK (2012) Eco-friendly control of potato late blight causative agent and the potential role of lactic acid bacteria: a review. Appl Microbiol Biotechnol 96:37–48

Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL, Bailey BA (2009) The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. J Exp Bot 60:3279–3295

Bailey BA, Bae H, Strem MD, Roberts DP, Thomas SE, Crozier J, Holmes KA (2006) Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* species. Planta 224:1449–1464

Baker KF, Cook RJ (1974) Biological control of plant pathogens. WH Freeman and Company

Banerjee M, Yesmin L (2002) Sulfur-oxidizing plant growth promoting Rhizobacteria for enhanced canola performance. US Patent

Bardin M, Comby M, Troulet C, Nicot PC (2013) Relationship between the aggressiveness of *Botrytis cinerea* on tomato and the efficacy of biocontrol. IOBCWPRS Bull 86:163–168

Barka EA, Belarbi A, Hachet C, Nowak J, Audran JC (2000) Enhancement of in vitro growth and resistance to gray mould of Vitis vinifera cocultured with plant growth-promoting rhizobacteria. FEMS Microbiol Lett 186:91–95

Barka EA, Gognies S, Nowak J, Audran JC, Belarbi A (2002) Inhibitory effect of endophyte bacteria on *Botrytis cinerea* and its influence to promote the grapevine growth. Biol Control 24:135–142

Barros G, Chiotta M, Reynoso M, Torres A, Chulze S (2007) Molecular characterization of *Aspergillus* section *flavi* isolates collected from peanut fields in Argentina using AFLPs. J Appl Microbiol 103:900–909

Bell JA, Wheeler MH (1986) Biosynthesis and functions of fungal melanins. Annu Rev Phytopathol 24:411–451

Ben Abdallah D, Frikha-Gargouri O, Tounsi S (2015) *Bacillus amyloliquefaciens* strain 32a as a source of lipopeptides for biocontrol of *Agrobacterium tumefaciens* strains. J Appl Microbiol 119:196–207

Benitez T, Rincon AM, Limon MC, Codon AC (2004) Biocontrol mechanism of *Trichoderma* strains. Int Microbiol 7:249–260

Berg G (2009) Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Berg G (2015) Beyond borders: investigating microbiome interactivity and diversity for advanced biocontrol technologies. Microb Biotechnol 8:5–7

Bolek Y, Bell AA, El-Zik KM, Thaxton PM, Magill CW (2005) Reaction of cotton cultivars and F2 population to stem inoculation with isolates *Verticillium dahliae*. J Phytopathol 153:269–273

Brotman Y, Briff E, Viterbo A, Chet I (2008) Role of swollenin, an expansin-like protein from *Trichoderma*, in plant root colonization. Plant Physiol 147:779–789

Bruce A, Douglas S, Susan V, Ron EW (2003) Effect of volatiles from bacteria and yeast on the growth and pigmentation of sapstain fungi. Int Biodeterior Biodegradation 51:101–108

Buck JW, Jeffers SN (2004) Effect of pathogen aggressiveness and vinclozolin on efficacy of *Rhodotorula glutinis* PM4 against *Botrytis cinerea* on geranium leaf disks and seedlings. Plant Dis 88:1262–1268

Burges HD (1998) Formulation of microbial pesticides. Kluwer Academic Publishers, Boston

Burse A, Weingart H, Ullrich MS (2004) NorM, an *Erwinia amylovora* multidrug efflux pump involved in in vitro competition with other epiphytic bacteria. Appl Environ Microbiol 70:693–703

Butt TM, Goettel MS, Papierok B (1999) Directory of specialists involved in the development of fungi as biocontrol agents. Colin Butt Design & Print, Warley

Butt TM, Jackson CW, Magan N (2001) Fungal biological control agents: progress, problems and potential. CABI International, Wallingford

Cardinale M, Grube M, Erlacher A, Quehenberger J, Berg G (2015) Bacterial networks and co-occurrence relationships in the lettuce root microbiota. Environ Microbiol 17:239–252

Carlini CR, Grossi-de-Sá MF (2002) Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. Toxicon 40:1515–1539

Chanchaichaovivat A, Ruenwongsa P, Panijpan B (2007) Screening and identification of yeast strains from fruits and vegetables: potential for biological control of postharvest chilli anthracnose (*Colletotrichum capsici*). Biol Control 42:326–335

Chet I, Benhamou N, Harman S (1998) Mycoparasitism and lytic enzymes. In: Harman GE, Kubicek CP (eds) *Trichoderma* and *Gliocladium*, vol 2. Taylor and Francis Ltd., London, pp 153–172

Chisholm ST, Coaker G, Day B, Staskawicz BJ (2006) Host-microbe interactions: shaping the evolution of the plant immune response. Cell 124:803–814

Chowdhury SP, Hartmann A, Gao XW, Borriss R (2015) Biocontrol mechanisms by root-associated *Bacillus amyloliquefaciens* FZB42 a review. Front Microbiol 6:780

Collins DP, Jacobsen B (2003) Optimizing a *Bacillus subtilis* isolate for biological control of sugar beet Cercospora leaf spot. Biol Control J 26:153–161

Compant S, Duffy B, Nowak J, Clément C, Barka EA (2005) Use of plant growth-promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. Appl Environ Microbiol 71:4951–4959

Conn KL, Nowak J, Lazarovits G (1997) A gnotobiotic bioassay for studying interactions between potato and plant growth-promoting rhizobacteria. Can J Microbiol 43:801–808

Connick W, Daigle D, Quimby P (1991) An improved invert emulsion with high water retention for mycoherbicide delivery. Weed Technol 5:442–444

Contreras-Cornejo HA, MacíasRodríguez L, CortésPenagos C, LópezBucio J (2009) *Trichoderma virens,* a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin dependent mechanism in *Arabidopsis*. Plant Physiol 149:1579–1592

Cook RJ (1993) Making greater use of introduced microorganisms for biological control of plant pathogens. Annu Rev Phytopathol 31:53–80

Cotty PJ (1994) Influence of field application of an atoxigenic strain *of Aspergillus flavus* on the populations of *A. flavus* infection cotton bolls and on aflatoxin content of cottonseed. Phytopathology 84:1270–1277

Crowe JD, Olsson S (2001) Induction of laccase activity in *Rhizoctonia solani* by antagonistic *Pseudomonas fluorescens* strains and a range of chemical treatments. Appl Environ Microbiol 67:2088–2094

Cutler HG, Cox RH, Crumley FG, Cole PD (1986) 6-Pentyl-alpha pyrone from *Trichoderma harzianum*: its plant growth inhibitory and antimicrobial properties. Agric Biol Chem 50:2943–2945

Cutler HG, Himmetsbach DS, Arrendale RF, Cole PD, Cox RH (1989) Koninginin A: a novel plant regulator from *Trichoderma koningii*. Agric Biol Chem 53:2605–2611

de Oliveira RR, Nicholson WL (2016) Synthetic operon for (R,R)-2,3-butanediol production in *Bacillus subtilis* and *Escherichia coli*. Appl Microbiol Biotechnol 100:719–728

De Souza JT, Arnould C, Deulvot C, Lemanceau P, Gianinazzi-Pearson V, Raaijmakers JM (2003) Effect of 2,4-diacetylphloroglucinol on *Pythium*: cellular responses and variation in sensitivity among propagules and species. Phytopathology 93:966–975

De Waard MA, Andrade AC, Hayashi K, Schoonbeek HJ, Stergiopoulos I, Zwiers LH (2006) Impact of fungal drug transporters on fungicide sensitivity, multidrug resistance and virulence. Pest Manag Sci 62:195–207

Degenhardt J, Gershenzon J, Baldwin IT, Kessler A (2003) Attracting friends to feast on foes: engineering terpene emission to make crop plants more attractive to herbivore enemies. Curr Opin Biotechnol 14:169–176

Dekkers LC, van der Bij AJ, Mulders IHM, Phoelich CC, Wentwoord RAR, Glandorf DCM, Wijffelman CA, Lugtenberg BJJ (1998a) Role of the O-antigen of lipopolysaccharide, and possible roles of growth rate and of NADH:ubiquinone oxidoreductase (nuo) in competitive tomato root-tip colonization by *Pseudomonas fluorescens* WCS365. Mol Plant Microbe Interact 11:763–771

Dekkers LC, Phoelich CC, van der Fits L, Lugtenberg BJJ (1998b) A site-specific recombinase is required for competitive root colonization by *Pseudomonas* fluorescens WCS365. Proc Natl Acad Sci U S A 95:7051–7056

Dewi RT, Iskandar YM, Hanafi M, Karnado LBS, Angelina M, Dewijanti ID, Banjajarnmohar SDS (2007) Pakistan. J Biochem 6:465–471

Djonovic S, Pozo MJ, Dangott LJ, Howell CR, Kenerley CM (2006) Sm1, a proteinaceous elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and systemic resistance. Mol Plant Microbe Interact 19:838–853

Djonovic S, Vargas WA, Kolomiets MV, Horndeski M, Wiest A, Kenerley CM (2007) A proteinaceous elicitor Sm1 from the beneficial fungus *Trichoderma virens* is required for induced systemic resistance in maize. Plant Physiol 145:875–889

Dobbelare S, Vanderleydern J, Okon Y (2003) Plant growth promoting effects of diazotrophs in the rhizosphere. Crit Rev Plant Sci 22:107–149

Dorn B, Musa T, Krebs H, Fried PM, Forrer HR (2007) Control of late blight in organic potato production: evaluation of copper free preparations under field, growth chamber and laboratory conditions. Eur J Plant Pathol 119(2):217–240

Dorner JW (2004) Biological control of aflatoxin contamination of crops. J Toxicol Toxin Rev 23:425–450

Dorner JW (2008) Management and prevention of mycotoxins in peanuts. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 25:203–208
Dorner JW, Cole RJ (2002) Effect of application of nontoxigenic strains of *Aspergillus flavus* and *A. parasiticus* on subsequent aflatoxin contamination of peanuts in storage. J Stored Prod Res 38:329–339
Dorner JW, Lamb MC (2006) Development and commercial use of afla-guard, an aflatoxin biocontrol agent. Mycot Res 22:33–38
Dorner JW, Cole RJ, Connick WJ, Daigle DJ, McGuire MR, Shasha BS (2003) Evaluation of biological control formulations to reduce aflatoxin contamination in peanuts. Biol Control 26:318–324
Droby S, Vinokur V, Weiss B, Cohen L, Daus A, Goldschmidt EE, Porat R (2002) Induction of resistance to *Penicillium digitatum* in grapefruit by the yeast biocontrol agent *Candida oleophila*. Phytopathology 92:393–399
Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Mukherjee PK, Zeilinger S, Grigoriev IV, Kubicek CP (2011) *Trichoderma*-the genomics of opportunistic success. Nat Rev Microbiol 9:749–759
Druzhinina IS, Shelest E, Kubicek CP (2012) Novel traits of *Trichoderma* predicted through the analysis of its secretome. FEMS Microbiol Lett 337:1–9
Dubey SC, Bhavani R, Singh B (2009) Development of Pusa 5SD for seed dressing and Pusa Biopellet 10G for soil application formulations of *Trichoderma harzianum* and their evaluation for integrated management of dry root rot of mungbean (*Vigna radiata*). Biol Control 50:231–242
Duffy B, Schouten A, Raaijmakers JM (2003) Pathogen self-defense: mechanisms to counteract microbial antagonism. Annu Rev Phytopathol 41:501–538
Ehrlich KC (2014) Nonaflatoxigenic *Aspergillus flavus* to prevent aflatoxin contamination in crops: advantages and limitations. Front Microbiol 5:50
Elad Y, Chet I (1987) Possible role of competition for nutrients in biocontrol of *Pythium* damping-off by bacteria. Phytopathology 77:190–195
El-Hasan A, Walker F, Buchenauer H (2008) *Trichoderma harzianum* and its metabolite 6-pentyl-alpha pyrone suppress fusaric acid produced by *Fusarium moniliforme*. J Phytopathol 156:79–87
Erdogan O, Benlioglu K (2010) Biological control of *Verticillium* wilt on cotton by the use of fluorescent *Pseudomonas* spp. under field conditions. Biol Control 53:39–45
Fedi S, Tola E, Moenne-Loccoz Y, Dowling DN, Smith LM, O'Gara F (1997) Evidence for signalling between the phytopathogenic fungus *Pythium ultimum* and *Pseudomonas fluorescens* F113: *P. ultimum* represses the expression of genes in *P. fluorescens* F113, resulting in altered ecological fitness. Appl Environ Microbiol 63:4261–4266
Ferraz LP, da Cunha T, da Silva AC, Kupper KC (2016) Biocontrol ability and putative mode of action of yeasts against *Geotrichum citri-aurantii* in citrus fruit. Microbiol Res 188:72–79
Fialho MB, Toffano L, Pedroso MP, Augusto F, Pascholati SF (2010) Volatile organic compounds produced by *Saccharomyces cerevisiae* inhibit the in vitro development of *Guignardia citricarpa*, the causal agent of citrus black spot. World J Microbiol Biotechnol 26:925–932
Fitter AH, Garbaye J (1994) Interactions between mycorrhizal fungi and other soil microorganisms. Plant Soil 159:123132
Franco OL, Rigden DJ, R Melo F, Bloch C, Silva CP, Grossi de Sá MF (2000) Activity of wheat α-amylase inhibitors towards bruchid α-amylases and structural explanation of observed specificities. Eur J Biochem 267:2166–2173
Frankowski J, Lorito M, Scala F, Schmidt R, Berg G, Bahl H (2001) Purification and properties of two chitinolytic enzymes of *Serratia plymuthica* HRO-C48. Arch Microbiol 176:421–426
Frikha-Gargouri O, Ben Abdallah D, Ghorbel I, Charfeddine I, Jlaiel L, Triki MA, Tounsi S (2017) Lipopeptides from a novel *Bacillus methylotrophicus* 39b strain suppress *Agrobacterium* crown gall tumours on tomato plants. Pest Manag Sci 73:568–574
Garcia-Garrido JM, Ocampo JA (1989) Effect of VA mycorrhizal infection of tomato on damage caused by *Pseudomonas syringae*. Soil Biol Biochem 21:65–167

Gaur RB, Sharma RN, Gautam VS, Dangi RP (2010) Management of *Sclerotinia* rot of mustard through bioagents. Indian Phytopathol 63:392–397

Geng P, Chen S, Hu M, Rizwan-ul-Haq M, Lai K, Qu F, Zhang Y (2011) Combination of *Kluyveromyces marxianus* and sodium bicarbonate for controlling green mold of citrus fruit. Int J Food Microbiol 151:190–194

Germida JJ, Siciliano SD (2001) Taxonomic diversity of bacteria associated with the roots of modern, recent and ancient wheat cultivars. Biol Fertil Soils 33:410–415

Glandorf DC, Verheggen P, Jansen T, Jorritsma JW, Smit E, Leefang P, Wernars K, Thomashow LS, Laureijs E, Thomas-Oates JE, Bakker PA, Van Loon LC (2001) Effect of genetically modified *Pseudomonas putida* WCS358r on the fungal rhizosphere microflora of fieldgrown wheat. Appl Environ Microbiol 67:33713378

Gurusiddaiah S, Weller DM, Sarkar A, Cook RJ (1986) Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens* inhibitory to *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp. 8. Antimicrob Agents Chemother 29:488–495

Haissam JM (2011) *Pichia anomala* in biocontrol for apples: 20 years of fundamental research and practical applications. Antonie Van Leeuwenhoek 99:93–105

Hallmann J, Quadt-Hallmann A, Mahaffee WF, Kloepper JW (1997) Bacterial endophytes in agricultural crops. Can J Microbiol 43:895–914

Harman GE, Hayes CK (1994) Biologically based technologies for pest control: pathogens that are pests of agriculture. A Report to the Office of Technology Assessment US Congress 75 pp

Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species-opportunistic, avirulent plant symbionts. Nat Rev Microbiol 2:43–56

Hernandez-Montiel LG, Ochoa JL, Troyo-Diéguez E, Larralde-Corona CP (2010) Biocontrol of postharvest blue mold (*Penicillium italicum* Wehmer) on Mexican lime by marine and citrus *Debaryomyces hansenii* isolates. Postharvest Biol Technol 56:181–187

Hoitink HAJ, Madden LV, Dorrance AE (2006) Systemic resistance induced by *Trichoderma* spp.: Interactions between the host, the pathogen, the biocontrol agent, and soil organic matter quality. Phytopathology 96:186–118

Howell CR (1998) The role of antibiosis in biocontrol. In: *Trichoderma* and *Gliocladium*, vol 2: Enzymes, biological control and commercial applications. Taylor & Francis, London, pp 173–184

Howell CR, Beier RC, Stipanovi RD (1980) Production of ammonia by *Enterobacter cloacae* and its possible role in the biological control of pythium pre-emergence damping off by the bacterium. Phytopathology 78:105–1078

Hu HQ, Li XS, He H (2010) Characterization of an antimicrobial material from a newly isolated *Bacillus amyloliquefaciens* from mangrove for biocontrol of *Capsicum* bacterial wilt. Biol Control 54:359–365

Inaoka T, Ochi K (2011) Scandium stimulates the production of amylase and bacilysin in *Bacillus subtilis*. Appl Environ Microbiol 77:8181–8183

Ingavat N, Dobereiner J, Wiyakrutta S, Mahidol C, Ruchirawat S, Kittakoop P (2009) Aspergillusol A, an α-glucosidase inhibitor from the marine-derived fungus *Aspergillus aculeatus*. J Nat Prod 72:2049–2052

Islam MT, Hashidoko Y, Deora A, Ito T, Tahara S (2005) Suppression of damping-off disease in host plants by the rhizoplane bacterium *Lysobacter* sp. strain SB-K88 is-linked to plant colonization and antibiosis against soilborne peronosporomycetes. Appl Environ Microbiol 71:3786–3796

Jataraf J, Radhakrim NV, Hannk P, Sakoof R (2005) Biocontrol of tomato damping-off caused by *Pythium aphanidermatum*. BioControl 15:55–65

Jeyarajan R (2006) Prospects of indigenous mass production and formulation of *Trichoderma.* In: Current status of biological control of plant diseases using antagonistic organisms in India. Project Directorate of Biological Control Bangalore, pp 74–80

Jorjani M, Heydari A, Zamanizadeh HR, Rezaee S, Naraghi L (2011) Controlling sugarbeet mortality disease by application of new bioformulations. J Plant Prot Res 52:303–307

Junaid JM, Dar NA, Bhat TA, Bhat AH, Bhat MA (2013) Commercial biocontrol agents and their mechanism of action in the management of plant pathogens. Int J Mod Plant Anim Sci 1:39–57

Kamensky M, Ovadis M, Chet I, Chernin L (2003) Soil-borne strain IC14 of *Serratia plymuthica* with multiple mechanisms of antifungal activity provides biocontrol of *Botrytis cinerea* and *Sclerotinia sclerotiorum* diseases. Soil Biol Biochem 35:323–331

Katska V (1994) Inter-relationship between vesicular-arbuscular mycorrhiza and rhizosphere microflora in apple replant disease. Biol Plant 36:99–104

Kim YS, Jeon Y (2016) Biological control of Apple Anthracnose by *Paenibacillus polymyxa* APEC128, an Antagonistic Rhizobacterium. Plant Pathol J 32:251–259

Kiss L (2003) A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. Pest Manag Sci 59:475–483

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) *Pseudomonas* siderophores: a mechanism explaining disease suppression in soils. Curr Microbiol 4:317–320

Kloepper JW, Ryu CM, Zhang S (2004) Induce systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology 94:1259–1266

Koumoutsi A, Chen XH, Vater J, Borriss R (2007) DegU and YczE positively regulate the synthesis of bacillomycin D by *Bacillus amyloliquefaciens* strain FZB42. Appl Environ Microbiol 73:6953–6964

Kretschmer M, Leroch M, Mosbach A, Walker AS, Fillinger S, Mernke D, Hahn M (2009) Fungicide-driven evolution and molecular basis of multidrug resistance in field populations of the grey mould fungus *Botrytis cinerea*. PLoS Pathog 5(12):e1000696

Kumar A, Scher K, Mukherjee M, PardovitzKedmi E, Sible GV, Singh US, Kale SP, Mukherjee PK, Horwitz BA (2010) Overlapping and distinct functions of two *Trichoderma virens* MAP kinases in cell-wall integrity, antagonistic properties and repression of conidiation. Biochem Biophys Res Commun 398:765–770

Kumar S, Thakur M, Rani A (2014) *Trichoderma*: mass production, formulation, quality control, delivery and its scope in commercialization in India for the management of plant diseases. Afr J Agric Res 9:3838–3852

Kunoh H (2002) Endophytic actinomycetes: attractive biocontrol agents. J Gen Plant Pathol 68:249–252

Lafontaine PJ, Benhamou N (1996) Chitosan treatment: an emerging strategy for enhancing resistance of greenhouse tomato plants to infection by *Fusarium oxysporum* f.sp. *radicis-lycopersici*. Biocontrol Sci Technol 6:111–124

Lahlali R, Peng G, Gossen BD, McGregor L, FQ Y, Hynes RK et al (2013) Evidence that the bio-fungicide Serenade (*Bacillus subtilis*) suppresses club-root on canola via antibiosis and induced host resistance. Phytopathology 103:245–254

Lewis JA (1991) Formulation and delivery system of bio-control agents with emphasis on fungi. In The rhizosphere and plant growth Springer, Dordrecht, pp 279–287

Lo CT, Nelson EB, Harman GE (1997) Biological control of *Pythium, Rhizoctonia* and *Sclerotinia* infected diseases of turfgrass with *Trichoderma harzianum*. Phytopathology 84:1372–1379

Loper JE (1988) Role of fluorescent siderophore production in biological control of *Pythium ultimum* by *Pseudomonas fluorescens* strain. Phytopathology 78:166–172

Loper JE, Henkels MD (1999) Utilization of heterologous siderophores enhances levels of iron available to *Pseudomonas putida* in the rhizosphere. Appl Environ Microbiol 65:5357–5363

Lutz MP, Feichtinger G, Defago G, Duffy B (2003) Mycotoxigenic *Fusarium* and deoxynivalenol production repress chitinase gene expression in the biocontrol agent *Trichoderma atroviride* P1. Appl Environ Microbiol 69:3077–3084

Maldonado MC, Orosco CE, Gordillo MA, Navarro AR (2010) In vivo and in vitro antagonism of *Streptomyces* sp RO3 against *Penicillium digitatum* and *Geotrichum candidum*. Afr J Microbiol Res 4:2451–2456

Mansoori M, Heydari A, Hassanzadeh N, Rezaee S, Naraghi L (2013) Evaluation of *Pseudomonas* and *Bacillus* bacterial antagonists for biological control of cotton *Verticillium* Wilt disease. J Plant Prot Res 53:11–14

Mariappan A, Makarewicz O, Chen XH, Borriss R (2012) Two-component response regulator DegU controls the expression of bacilysin in plant growth promoting bacterium *Bacillus amyloliquefaciens* FZB42. J Mol Microbiol Biotechnol 22:114–125

Mark GL, Morrissey JP, Higgins P, O'Gara F (2006) Molecular-based strategies to exploit *Pseudomonas* biocontrol strains for environmental biotechnology applications. FEMS Microbiol Ecol 56:167–177

Matsukuma S, Okuda T, Watanabe J (1994) Isolation of actinomycetes from pine litter layers. Actinomycetology 8:57–65

Maurhofer M, Hase C, Meuwly P, Metraux JP, Defago G (1994) Induction of systemic resistance to tobacco necrosis virus. Phytopathology 84:139–146

Mazzola M, Fujimoto DK, Thomashow LS, Cook RJ (1995) Variation in sensitivity of *Gaeumannomyces graminis* to antibiotics produced by fluorescent *Pseudomonas* spp. and effect on biological control of take all of wheat. Appl Environ Microbiol 61:2554–2559

Mazzola M, Zhao X, Cohen MF, Raaijmakers JM (2007) Wheat cultivar specific selection of 2,4-diacetylphloroglucinol producing fluorescent *Pseudomonas* species from resident soil populations. Phytopathology 97:1348–1355

McMullen M, Bergstrom G, De Wolf E, DillMacky R, Hershman D, Shaner G, Van Sanford D (2012) A unified effort to fight an enemy of wheat and barley: *Fusarium* head blight. Plant Dis 96:1712–1728

Mendoza-Mendoza A, Pozo MJ, Grzegorski D, Martínez P, García JM, Olmedo-Monfil V, Cortés C, Kenerley C, Herrera-Estrella A (2003) Enhanced biocontrol activity of *Trichoderma* through inactivation of a mitogen-activated protein kinase. Proc Natl Acad Sci U S A 100:15965–15970

Mercado-Blanco J, Rodríguez-Jurado D, Hervás A, Jiménez-Diaz RM (2004) Suppression of *Verticillium* wilt in olive planting stocks by root-associated fluorescent *Pseudomonas* spp. Biol Control 30:474–486

Milgroom MG, Cortesi P (2004) Biological control of chestnut blight with hypo-virulence: a critical analysis. Annu Rev Phytopathol 42:311–338

Milner JL, Silosuh L, Lee JC, He HY, Clardy J, Handelsman J (1996) Production of kanosamine by *Bacillus cereus* UW85. Appl Environ Microbiol 62:3061–3065

Mishra DS, Singh US, Dwivedi TS (2001) Comparative efficacy of normal seed treatment and seed biopriming with commercial formulations of *Trichoderma* spp. In: 53rd Annual meeting of Indian Phytopathological Society and national symposium on ecofriendly approaches for *Trichoderma*, Chennai, pp 21–23

Moore LW, Warren G (1979) *Agrobacterium radiobacter* strain 84 and biological control of crown gall. Annu Rev Phytopathol 17:163–179

Morrissey JP, Osbourn AE (1999) Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Microbiol Mol Biol Rev 63:708–724

Mukherjee PK, Mukhopadhyay AN (1995) In situ, mycoparasitism of *Gliocladium virens* on *Rhizoctonia solani*. Indian Phytopathol 48:101–102

Mukherjee PK, Buensanteai N, MoranDiez ME, Druzhinina IS, Kenerley CM (2012) Functional analysis of nonribosomal peptide synthetases (NRPSs) in *Trichoderma virens* reveals a polyketide synthase (PKS)/NRPS hybrid enzyme involved in induced systemic resistance response in maize. Microbiology 158:155–165

Mustafa A, Khan MA, Inam-ul-Haq M, Khan SH, Pervez MA (2009) Mass multiplication of *Trichoderma* spp on organic substrate and their effect in management of seed borne fungi. Pak. J Phytopathol 21:108–114

Nair SS, Kavrekar V, Mishra A (2013) In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. Eur J Exp Biol 3:128–132

Notz R, Maurhofer M, Dubach H, Haas D, Defago G (2002) Fusaric acid producing strains of *Fusarium oxysporum* alter 2,4-diacetylphloroglucinol biosynthetic gene expression in *Pseudomonas fluorescens* CHA0 in vitro and in the rhizosphere of wheat. Appl Environ Microbiol 68:2229–2235

Okazaki T, Takahashi K, Kizuka M, Enokita R (1995) Studies on actinomycetes isolated from plant leaves. Annu Rep Sankyo Res Lab 47:97–106

Omann MR, Lehner S, Escobar Rodriguez C, Brunner K, Zeilinger S (2012) The seven transmembrane receptor Gpr1 governs processes relevant for the antagonistic interaction of *Trichoderma atroviride* with its host.

Ordentlich A, Elad Y, Chet I (1988) The role of chitinase of *Serratia marcescens* in biocontrol of *Sclerotium rolfsii*. Phytopathology 78:84–88

O'Sullivan DJ, O'Gara F (1992) Traits of fluorescent *Pseudomonas* spp. involved in suppression of plant root pathogens. Microbiol Rev 56:662–676

Otto-Hanson LK, Grabau Z, Rosen C, Salomon CE, Kinkel LL (2013) Pathogen variation and urea influence selection and success of *Streptomyces* mixtures in biological control. Phytopathology 103:34–42

Ownley BH, Weller DM, Thomashow LS (1992) Influence of in situ and in vitro pH on suppression of *Gaeumannomyces graminis* var *tritici* by *Pseudomonas fluorescens* 279. Phytopathology 82:178–184

Parafati L, Vitale A, Restuccia C, Cirvilleri G (2015) Biocontrol ability and action mechanism of food-isolated yeast strains against *Botrytis cinerea* causing post-harvest bunch rot of table grape. Food Microbiol 47:85–92

Paulitz TC, Belanger RR (2001) Biological control in greenhouse systems. Annu Rev Phytopathol 39:103–133

Pitt JI, Hocking AD (2006) Mycotoxins in Australia: biocontrol of aflatoxin in peanuts. Mycopathologia 162:233–243

Poromarto SH, Nelson BD, Freeman TP (1988) Association of binucleate Rhizoctonia with soybean and mechanism of biocontrol of *Rhizoctonia solani*. Phytopathology 88:1056–1067

Prasad RD, Rangeshwaran R (1998) A modified liquid medium for mass production of *Trichoderma* by fermentation process. In: Abstracts of national symposium on eco friendly approaches in the management of plant diseases, Shimoga

Prasad RD, Rangeshwaran R (2000) Shelf life and bioefficacy of *Trichoderma harzianum* formulated in various carrier materials. Plant Dis Res 15:38–42

Prasad RD, Rangeshwaran R, Anuroop CP, Phanikumar PR (2002) Bioefficacy and shelf life of conidial and chlamydospore formulation of *Trichoderma harzianum*. J Biol Control 16:145–148

Raza W, Ling N, Zhang R, Huang Q, Xu Y, Shen Q (2016) Success evaluation of the biological control of Fusarium wilts of cucumber, banana, and tomato since 2000 and future research strategies. Crit Rev Biotechnol:1–11

Ren X, Kong Q, Wang H, Yu T, Zhou W, Zheng X (2012) Biocontrol of fungal decay of citrus fruit by *Pichia pastoris* recombinant strains expressing cecropin A. Food Chem 131:796–801

RiveraVaras VV, Freeman TA, Gudmestad NC, Secor GA (2007) Mycoparasitism of *Helminthosporium solani* by *Acremonium strictum*. Phytopathology 97:1331–1337

Rosenblueth M, Martínez-Romero E (2006) Bacterial endophytes and their interactions with hosts. Mol Plant Microbe Interact 19:827–837

Ryu CM, Farag MA, Hu CH, Reddy MS, Kloepper JW, Paré PW (2004) Bacterial volatiles induce systemic resistance in *Arabidopsis*. Plant Physiol 134:1017–1026

Saito N (1982) alpha-Amylase inhibitor from fungus *Cladosporium herbarum*. J Biol Chem 257:3120–3125

Samolski I, Rincon AM, Pinzon LM, Viterbo A, Monte E (2012) The qid74 gene from *Trichoderma harzianum* has a role in root architecture and plant biofertilization. Microbiology 158:129–138

Sansone G, Rezza I, Calvente V, Benuzzi D, Sanz De Tosetti MI (2005) Control of *Botrytis cinerea* strains resistant to iprodione in apple with rhodotorulic acid and yeasts. Postharvest Biol Technol 35:245–251

Santos A, Sanchez A, Marquina D (2004) Yeasts as biological agents to control *Botrytis cinerea*. Microbiol Res 159:331–338

Saravanakumar D, Spadaro D, Garibaldi A, Gullino ML (2009) Detection of enzymatic activity and partial sequence of a chitinase gene in *Metschnikowia pulcherrima* strain MACH1 used as post-harvest biocontrol agent. Eur J Plant Pathol 123:183–193

Sawant IS, Sawant SD (1996) A simple method for achieving high cfu of *Trichoderma harzianum* on organic wastes for field applications. Indian Phytopathol 9:185–187

Schippers B, Geels EP, Bakker PAHM, Bakker AW, Weisbeek PJ, Lugtenberg B (1986) Methods of studying growth stimulating *Pseudomonads*: problems and progress. In: Swinburne TR (ed) Iron siderophores and plant disease

Schnabel EL, Jones AL (2001) Isolation and characterization of Five bacteriophages and assessment of phage resistance in strains of *Erwinia amylovora*. Appl Environ Microbiol 67:59–64

Schoonbeek H, Del Sorbo G, De Waard MA (2001) The ABC transporter BcatrB affects the sensitivity of *Botrytis cinerea* to the phytoalexin resveratrol and the fungicide fenpiclonil. Mol Plant Microbe Interact 14:562–571

Schoonbeek H, Raaijmakers JM, De Waard MA (2002) Fungal ABC transporters and microbial interactions in natural environments. Mol Plant Microbe Interact 15:1165–1172

Schouten A, Van Den Berg G, EdelHermann V, Steinberg C, Gautheron N, Alabouvette C et al (2004) Defence responses of *Fusarium oxysporum* to 2,4-diacetylphloroglucinol, a broad spectrum antibiotic produced by *Pseudomonas fluorescens*. Mol Plant Microbe Interact 17:1201–1211

Seidl V, Marchetti M, Schandl R, Allmaier G, Kubicek CP (2006) EPL1, the major secreted protein of *Hypocrea atroviridis* on glucose, is a member of a strongly conserved protein family comprising plant defense response elicitors. FEBS J 273:4346–4359

Selvakumar R, Srivastava KD (2000) Management of spot blotch of wheat using bio control agent. Im: National conference on Swadeshi Vighan, New Delhi, 26–28 December

Sharma VK, Nowak J (1998) Enhancement of *Verticillium* wilt resistance in tomato transplants by in vitro co-culture of seedlings with a plant growth-promoting rhizobacterium (*Pseudomonas* sp. strain PsJN). Can J Microbiol 44:528–536

Shi M, Chen L, Wang XW, Zhang T, Zhao PB, Song XY, Sun CY, Chen XL, Zhou BC, Zhang YZ (2012) Antimicrobial peptaibols from *Trichoderma pseudokoningii* induce programmed cell death in plant fungal pathogens. Microbiology 158:166–175

Shoresh M, Harman GE, Mastouri F (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. Annu Rev Phytopathol 48:21–43

Silva HSA, Romeiro RDS, Macagnan D, Halfeld-Vieira BDA, Pereira MCB, Mounteer A (2004) Rhizobacterial induction of systemic resistance in tomato plants: non-specific protection and increase in enzyme activities. Biol Control 29:288–295

Simons M, vander Bij AJ, de Weger LA, Wijffelman CA, Lugtenberg BJ (1996) Gnotobiotic system for studying rhizosphere colonization by plant growth-promoting *Pseudomonas* bacteria. Mol Plant Microbe Interact 9:600–607

Singh US, Zaidi NW (2002) Current status of formulation and delivery of fungal and bacterial antagonists for disease management in India. In: Rabindra RJ, Hussaini SS, Ramanujam B (eds) Microbial biopesticide formulations and application. Project Directorate of Biological Control, Bangalore, p 269

Singh PP, Shin YC, Park CS, Chung YR (1999) Biological control of Fusarium wilt of cucumber by chitinolytic bacteria. Phytopathology 89:92–99

Singh B, Thakur A, Kaur S, Chadha BS, Kaur A (2012) Acetyl-cholinesterase inhibitory potential and insecticidal activity of an endophytic *Alternaria* sp. from *Ricinus communis*. Appl Biochem Biotechnol 168:991–1002

Singh B, Kaur T, Kaur S, Manhas RK, Kaur A (2015) An alpha-glucosidase inhibitor from an endophytic *Cladosporium* sp. with potential as a biocontrol agent. Appl Biochem Biotechnol 175:2020–2034

Sivasithamparam K, Ghisalberti EL (1998) Secondary metabolism in *Trichoderma* and *Gliocladium*. *Trichoderma* and *Gliocladium* basic biology taxonomy and. Genetics 1:139–191

Smith KP, Havey MJ, Handelsman J (1993) Suppression of cottony leak of cucumber with *Bacillus cereus* strain UW85. Plant Dis 77:139–142

Srivastava RK, Singh RK, Kumar N, Singh S (2010) Management of Macrophomina disease complex in jute (*Corchorus olitorius*) by *Trichoderma viride*. J Biol Control 24:77–79

Steenhoudt O, Vanderleyden J (2000) *Azospirillum*, a free-living nitrogen-fixing bacterium closely associated with grasses: genetic, biochemical and ecological aspects. FEMS Microbiol Rev 24:487–506

Stockwell VO, Kawalek MD, Moore LW, Loper JE (1996) Transfer of pAgK84 from the biocontrol agent *Agrobacterium radiobacter* K84 to under field conditions. Phytopathology 86:31–37

Strobel GA (2003) Endophytes as sources of bioactive products. Microbes Infect 5:535–544

Sun HBX, Lu F, Lu Y, Wu Y, Lu Z (2009) Enhancement of surfactin production of *Bacillus subtilis* fmbR by replacement of the native promoter with the Pspac promoter. Can J Microbiol 55:1003–1006

Sundheim L, Tronsmo A (1988) Hyperparasites in biological control. In: Biocontrol of plant diseases, pp 53–69

Tan RX, Zou WX (2001) Endophytes: a rich source of functional metabolites. Nat Prod Rep 18:448–459

Thakur A, Kaur S, Kaur A, Singh V (2012) Detrimental effects of endophytic fungus *Nigrospora* sp. on survival and development of *Spodoptera litura*. Biocontrol Sci Technol 22:151–161

Thomashow LS, Weller DM (1988) Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. J Bacteriol 170:3499–3508

Tjamos EC, Tsitsigiannis DI, Tjamos SE, Antoniou PP, Katinakis P (2004) Selection and screening of endorhizosphere bacteria from solarized soils as biocontrol agents against *Verticillium dahliae* of solanaceous hosts. Eur J Plant Pathol 110:35–44

Torres AM, Barros GG, Palacios SA, Chulze SN, Battilani P (2014) Review on pre- and post-harvest management of peanuts to minimize aflatoxin contamination. Food Res Int 62:11–19

Toyoda H, R Utsumi (1991) Method for the prevention of *Fusarium* diseases and microorganisms used for the same. US Patent 4,988,586

van Peer RG, Niemann GJ, Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of *Fusarium* wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathology 81:728–734

van Zyl FGH, Strijdom BW, Staphorst JL (1986) Susceptibility of *Agrobacterium tumefaciens* strains to two agrocin-producing *Agrobacterium* strains. Appl Environ Microbiol 52:234–238

Vemmer M, Patel AV (2013) Review of encapsulation methods suitable for microbial biological control agents. Biol Control 67:380–389

Vinale F, Sivasithamparam K, Ghisalberti LE, Marra R, Woo LS, Lorito M (2008) *Trichoderma*-plant-pathogen interactions. Soil Biol Biochem 40:1–10

Vinale F, Ghisalberti EL, Sivasithamparam K, Marra R, Ritieni A, Ferracane R, Woo S, Lorito M (2009) Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. Lett Appl Microbiol 48:705–711

Viswanathan R, Samiyappan R (1999) Induction of systemic resistance by plant growth-promoting rhizobacteria against red rot disease caused by *Colletotrichum falcatum* went in sugarcane. In: Proceedings of the Sugar Technology Association of India Sugar Technology Association, New Delhi, 61, pp 24–39

Viterbo A, Chet I (2006) TasHyd1, a new hydrophobin gene from the biocontrol agent *Trichoderma asperellum*, is involved in plant root colonization. Mol Plant Pathol 7:249–258

Wang X, Li G, Jiang D, Huang HC (2009) Screening of plant epiphytic yeasts for biocontrol of bacterial fruit blotch (*Acidovorax avenae* subsp *citrulli*) of hami melon. Biol Control 50:164–171

Wang HK, Shi YC, Zhang HP, Qi W (2010) Study on the inhibition of *Phytophthora drechsleri* Tucker by *Lactobacillus plantarum* Bx62 isolated from koumiss. J Tianjin Univ Sci Technol:1

Wang H, Yan Y, Wang J, Zhang H, Qi W (2012) Production and characterization of antifungal compounds produced by *Lactobacillus plantarum* IMAU10014. PLoS One 7(1):e29452

Weindling R (1932) *Trichoderma lignorum* as a parasite of other soil fungi. Phytopathology 22:837–845

Weindling R (1934) Studies on lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopathology 24:1153–1179

Welbaum G, Sturz AV, Dong Z, Nowak J (2004) Fertilizing soil microorganisms to improve productivity of agroecosystems. Crit Rev Plant Sci 23:175–193

Weyens N, Van der Lelie D, Taghavi S, Vangronsveld J (2009) Phytoremediation: plant-endophyte partnerships take the challenge. Curr Opin Biotechnol 20:248–254

Whipps JM (2001) Microbial interactions and biocontrol in the rhizosphere. J Exp Bot 52:487–511

Woo SL, Scala F, Ruocco M, Lorito M (2006) The molecular biology of the interaction between *Trichoderma* spp phytopathogenic fungi and plants. Phytopathology 6:181–185

Woo SL, Ruocco M, Vinale F, Nigro M, Marra R, Lombardi N, Pascale A, Lanzuise S, Manganiello G, Lorito M (2014) *Trichoderma*-based products and their widespread use in agriculture. Open Mycol J 8:71–126

Wu L, Wu H, Chen L, Xie S, Zang H, Borriss R et al (2014) Bacilysin from *Bacillus amyloliquefaciens* FZB42 has specific bactericidal activity against harmful algal bloom species. Appl Environ Microbiol 80:7512–7520

Wu L, Wu HJ, Qiao J, Gao X, Borriss R (2015) Novel routes for improving biocontrol activity of *Bacillus* based bioinoculants. Front Microbiol 6

Yadav SK, Dave A, Sarkar A, Singh HB, Sharma BK (2013) Co-inoculated biopriming with *Trichoderma, Pseudomonas* and *Rhizobium* improves crop growth in *Cicer arietinum* and *Phaseolus vulgari*. Int J Agric Biol 6:255–259

Yadeta KA, Thomma J (2013) The xylem as battleground for plant hosts and vascular wilt pathogens. Front Plant Sci 4:1–12

Yedidia I, Shoresh M, Kerem K, Benhamou N, Kapulnik Y, Chet I (2003) Concomitant induction of systemic resistance to *Pseudomonas syringae* pv. *lachrymans* in cucumber by *Trichoderma asperellum* (T-203) and the accumulation of phytoalexins. Appl Environ Microbiol 69:7343–7353

Zamani Z, Aminaee MM, Khaniki GB (2013) Introduction of *Beauveria bassiana* as a biological control agent for *Tribolium castaneum* in Kerman province. Arch Phytopathol Plant Prot 46:2235–2243

Zeilinger S, Reithner B, Scala V, Peiss I, Lorito M, Mach RL (2005) Signal transduction by Tga3, a novel G protein alpha subunit of *Trichoderma atroviride*. Appl Environ Microbiol 71:1591–1597

Zhang L, Birch RG (1997) The gene for albicidin detoxification from *Pantoea dispersa* encodes an esterase and attenuates pathogenicity of *Xanthomonas albilineans* to sugarcane. Proc Natl Acad Sci U S A 94:9984–9989

Zhang HY, Wang L, Ma LC, Dong Y, Jiang S, Xu B, Zheng XD (2009) Biocontrol of major postharvest pathogens on apple using *Rhodotorula glutinis* and its effects on postharvest quality parameters. Biol Control 48:79–83

Arbuscular Mycorrhizal Fungi for Sustainable Agriculture

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Abstract

Arbuscular mycorrhizal fungi (AMF) have obligatory symbiotic relationship with more than 80% of terrestrial plant species. AMF symbiosis acclimatizes plants for their better survival, enhanced growth and development in biotic as well abiotic environment, thereby promoting sustainable growth and development of plants. Being highly competitive and better suited, plants with AMF association with ease tolerate environmental stress to face plethora of various biotic as well as abiotic changes. These fungal symbionts offer an eco-friendly biological sound substitute to chemical fertilizers and pesticides for managing both plant quality and quantity in agriculture, horticulture and forestry. AMF are now regarded as the cornerstone of sustainable agriculture; as such, there is a necessity to accelerate their integration in agricultural production systems. It becomes important now that soil scientists and agriculturalists pay due attention to the management of AMF in the formal way to increase, restore or maintain soil fertility which indirectly influences the growth and development of plant. Present review emphasizes the mycorrhizal symbiosis as a keystone to plant productivity and diversity because of their influence on almost all metabolic processes of plants and maintains and, in many cases, stimulates plant growth and development due to their diverse functionality/benefits to host plant, consolidated here.

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25.1 Introduction

Mycorrhiza is an obligatory symbiotic relationship between soilborne fungi with the roots of higher plants (Sieverding 1991). German forest pathologist Frank (1885) coined the term mycorrhiza. Mycorrhiza word is derived from two words, one Greek mykes (mushroom/fungus) and other Latin rhiza (roots) literally meaning fungus roots (Allen 1991). Kamienski (1881) reported for the first time that root fungi play important role in growth of plants. In the mycorrhizal literature, the term symbiosis is used to describe a highly interdependent mycorrhizal obligatory symbiosis; the host plant receives mineral, while the fungus obtains carbon atoms from the photosynthetic plant (Harley and Smith 1983; Allen 1991). Mycorrhizal associations involve three-way communication between mutualistic fungi, host plants and soil factors, and recently arbuscular or ectomycorrhizal helping bacteria strains are reported to promote this association (Garbaye 1994; Barea et al. 2002; Johansson et al. 2004; Artursson et al. 2006; Duponnois 2006; Frey-Klett et al. 2007; Rigamonte et al. 2010). Mycorrhiza is considered as fundamental part of the plant as 95% of all the plant families are predominantly mycorrhizal (Remy et al. 1994). Except for some angiospermic families, viz. Proteaceae (Nicolson 1967; Brundrett et al. 1996) Zygophyllaceae, Cruciferae (Verma 1998), Betulaceae, Dipterocarpaceae, Myrtaceae, Fagaceae (Nicolson 1967) and Cactaceae, Chenopodiaceae, Cyperaceae, Amaranthaceae and Juncaceae (Hirrel et al. 1978), all other show mycorrhizal association. The mycorrhizal symbiosis is now essential for productivity and diversity of plants and also played significant role in ecological presence. As a consequence, plant health for productivity is directly depending on this relationship, and any loss or change to this relationship can produce serious consequence for plant growth and development. Available fossil and molecular evidences support the concept that this symbiosis is of ancient origin around 450 million years, implying a co-evolution of plants and fungi forming a possible integral part in the establishment of a land flora (Remy et al. 1994).

Mycorrhizal symbiosis has been studied as a relationship between soilborne fungi with underground root of the host plant, and now it has been reported that besides roots, many underground stem modifications and other associated structures show association with these fungi. For the first time Taber and Trappe (1982) reported AMF in the vascular system of rhizomatous tissues and scales like leaves of *Zingiber officinale* L. Arbuscular mycorrhizal fungi have been reported in garlic bulbs (Kunwar et al. 1999), tubers of *Colocasia esculenta* and *Gloriosa superba* L. (Bhat and Kaveriappa 1997; Khade and Rodrigues 2003) and scales of corm of *Crocus sativus* (Lone et al. 2016).

Present review emphasizes the mycorrhizal symbiosis as important to plant productivity and diversity because of their influence on almost all metabolic processes of plant. AMF symbiosis hence has an important role in growth and development of plants.

25.2 Genesis of Arbuscular Mycorrhizal Fungi

The AMF symbiosis as per fossil records dates back around 460 years ago of Ordovician age (Redecker 2000). The arbuscules were discovered in *Aglaophyton major* which provides unambiguous indication that mycorrhizae were established

more than 400 million years ago (Remy et al. 1994; Simon et al. 1993; Taylor et al. 1993). These arbuscules are supposed to have been reasons in the colonization of land by plants during that time (David-Schwart et al. 2003; Remy et al. 1994). AMF are common in ecosystems and very ancient, as fossil and molecular phylogeny evidence suggests (Simon et al. 1993; Remy et al. 1994; Redecker 2000).

During earlier period the co-evolution of plants and AMF may have been main factor in the evolution of first rootless plant to establish on land (Pirozynski and Mulloch 1975; Schubler 2002; Simon et al. 1993). A large body of data suggest that extant hornworts and liverworts had features of earlier plants that occupied land (Edwards et al. 1995; Qui and Lee 2000; Wellman et al. 2003) which often form symbiotic association with fungi (Read et al. 2000).

25.3 Development of Functional Arbuscular Mycorrhizal Fungi

Arbuscular mycorrhizal fungi during mycorrhizal formation undergo many developmental stages. AMF spores after germination show limited hyphal development during asymbiotic stage and later switch to the presymbiotic stage to form extensive hyphal branching in the presence of root exudates (Buee et al. 2000). The fungal hyphae after branching and just before its penetration into the root epidermis makes contact with the root surface through the formation of appressorium which is followed by symbiotic colonization of the cortex tissue of roots, involving formation of either intracellular arbuscules or hyphal coils and along with sporulative extraradical mycelium production (Smith and Read 1997a, b). Later on after the formation of appressorium, root colonization can take place *via* two different modes (Smith and Read 1997a, b) either by the *Arum* type or through *Paris* type of colonization. The *Arum* type of colonization is characterized by spreading of the hyphae intercellularly till they're spread to the inner cortex, where the fungus makes penetration into the cell wall of host plant and ramifies extensively to form arbuscules, while in *Paris* type of colonization, development of fungi is intracellular and hyphal coils give rise to arbuscules. In *Arum*-type arbuscules, numerous modifications have been observed in host cells during the development (Timonen and Peterson 2002). Increased activity of ATPase enzyme can be detected in the periarbuscular membrane, which is probably responsible for the nutrient uptake from the matrix between the host plants and the fungus (Smith et al. 1993).

For production of AMF inoculum, various techniques are available, viz. through nutrient film technique, aeroponic culture system and root organ culture (Raja and Mahadevan 1991). For large-scale production of inoculum, the traditional pot culture technique employing trap plants is a widely available and practised method (Chellappan et al. 2002). Potty (1985) reported in cassava (*Manihot esculenta*) tuber peel that AMF *Glomus mosseae* multiplied and increases number of spores. Further it was proposed that for mass production of AMF, peel system could be used as inoculum. Ganesan and Mahadevan (1998) reported that arbuscules, vesicles and hyphae of *Glomus aggregatum* colonize on cassava tuber surface and that may be further used as inoculum.

To increase the production of AMF spores, sucrose-agar globule with root exudates as a source of inoculum was introduced by Selvaraj and Kim (2004). In chickpea it is reported that AMF colonization fostered the growth up to 43% of total dry matter (Farzaneh et al. 2009). In our laboratory, an in vitro hyphal inoculation has been induced in the isolated cucumber roots by providing *Glomus* species. Spores under petri plate condition to be used later as inoculums on other plants in fields (Agarwal et al. 2013).

AMF after entering the host plant undergoes many morphological and physiological changes before development into spore reproductive bodies. Techniques like nutrient film technique, root organ culture, aeroponic culture system, pot culture and petri plate culture are readily employed for large-scale production of AMF.

25.4 Arbuscular Mycorrhizal Fungi and Soil Formation

Arbuscular mycorrhizal fungal hyphae have important role in maintaining soil aggregates by increasing the soil nutrient uptake by plants (Tesdall and Oades 1979; Miller and Jastrow 1990; Hodge 2000; Hodge et al. 2001; Wilson et al. 2009). The exudation of extracellular polysaccharides and glomalin helps in entangling soil particles within the hyphae network (Treseder and Turner 2007; Rillig et al. 2014). Glomalin a polysaccharide has ability to carbon, which aides in formation of organic matter, attachment it to silt, clay particles and sand which are described as an important factor in the formation of soil (Miller and Jastrow 1990; Bossuyt et al. 2001). The AMF association is also reported to change soil characteristics and transplant shock (Bagyaraj and Varma 1995), restore degraded land and reclaim and enrich soil fertility (Charles et al. 2006).

AMF have important role in growth and survival of plant species and influence plant succession and structure of community in tropical regions (Janos 1996). AMF benefits include better access to soil nutrients and enhancement of soil aggregation, stability and protection against phytopathogens (Newsham et al. 1995b; Rillig and Mummey 2006). Their benefits also influence sustainability and biodiversity in terrestrial ecosystems (Van der Heijden et al. 1998). Arbuscular mycorrhizal fungi increase the nutrient uptake by plants. Also these stabilize soil aggregates, prevent soil erosion and enhance soil nutrient value and fertility which are prime facts for secondary succession and plant community structure.

25.5 Arbuscular Mycorrhizal Fungi and Soil Nutrient Uptake

The mutualistic association of roots and AMF provides the fungus direct access to carbohydrates fractions produced by the plant during photosynthesis and then translocation of these solutes to the roots (Harrison 2005). In return, the plant utilizes the large surface area of mycelium for water absorption (Bohra et al. 2007) and, thereby, dissolved mineral nutrients from the soil (Jeffries et al. 2003; Bohra et al. 2007). This additively improves the absorption of mineral capabilities of the plant roots

(Selosse et al. 2006). Mycorrhizal research has shown the increased nutrient uptake mainly phosphorus in mycorrhizal plants compared to control ones with no mycorrhiza inoculation (Akthar and Siddiqui 2008; Tong-jian et al. 2010; Hart and Forsythe 2012; Lone et al. 2015a, 2016). In addition to the phosphorus, the hypha also helps in other element uptake to the host such as calcium, aluminium, cadmium, arsenic, selenium (Khan et al. 2000; Al-Agel et al. 2005), sulphur and zinc (Khaliq and Sanders 2000) and potassium and copper (Nasim 2005). The land plants not only acquire soil nutrients such as phosphorus and nitrogen through the root surface area but also through AMF (Kobae et al. 2010). It has been reported that higher phosphorous and potassium uptake were assimilated in AMF-inoculated *Chlorophytum borivilianum* plants (Solanki et al. 2011).

The nutrient uptake in selected medicinal plants had significantly higher nutrient contents in plants colonized with mycorrhiza than controls having no AMF inoculation (Guissou 2009; Goussous and Mohammad 2009; Karagiannidis et al. 2012; Lone et al. 2015b, 2016). Mycorrhizal small hyphal diameter than smaller roots of plants exhibits large volume of soil, providing large surface area for water and mineral absorption (Sharma et al. 1991; Okon et al. 1996). AMF are among the most important plant root symbiosis fungi enhancing plant phosphorous uptake (Pasqualini et al. 2007; Farahani et al. 2009). AMF and plant roots improve nutrient and water uptake like nitrogen, phosphorus and micronutrients and thus increase plant growth (Smith and Read 2008; Goussous and Mohammad 2009; Elahi et al. 2010). AMF facilitate plant soil nutrient uptake (especially phosphorus) *via* extraradical hyphae that translocate soil nutrient from some distance away of the root-depleted rhizosphere to the root cortex alleviating plant nutrition (Smith and Read 2008). While AMF obtain 100 percent of their carbon from the host plants (Harrison 2005). The growth of the host plant is promoted by AMF, due to better nutrient uptake with particular emphasis on phosphorus nutrition (Smith and Read 2008; Farzaneh et al. 2011; Smith and Smith 2011, 2012).

AMF affect nutrient uptake in onion and saffron (Lone et al. 2015a, 2016). Farzaneh et al. (2009) observed no effect of soil sterilization on the growth enhancement due to AMF inoculation, but other studies showed that AMF inoculation increased dry matter content accumulation and uptake of nutrients compared with native AMF communities (Biro et al. 2000). The inoculation of AMF significantly increases leaf phosphorous, potassium, calcium, magnesium, manganese, zinc, copper, boron and molybdenum concentration, but leaf sodium is significantly decreased (Cartmill et al. 2008). AMF inoculation affected the nutrient uptake of key elements such as nitrogen, phosphorous, sulphur, zinc and manganese in *Solanum lycopersicum* (Nzanza et al. 2011). Mycorrhizal *Glomus fasciculatum* species inoculation in *Withania somnifera* increases the vegetative growth by obtaining different elements particularly phosphorus and nitrogen from the soil (Halder and Ray 2006). Mycorrhiza besides nitrogen content increase also showed higher phosphorous, potassium and calcium, iron, cobalt and molybdenum levels in roots and shoots of *Glomus* species-inoculated plants than those inoculated only with bacteria *Rhizobium* (Ferrera-Cerrato and Villerias 1985). Plant roots are not capable alone of taking up immobilized phosphate ions in soils with a base pH. The

rhizosphere pH is lowered by mycorrhiza due to release of H^+ ions and selective uptake of NH_4^+ (ammonium-ions) and increasing the solubility of phosphorus precipitates. The AMF benefit the host plant by improving uptake of nutrients like phosphorus, nitrogen and micronutrients (Ward et al. 2001; Javaid 2007, 2009; Javaid and Riaz 2009).

It has been reported that shoot and root dry matter and phosphorus in wheat (*Triticum aestivum*) were higher in AMF-colonized plants than control (Al-Karaki et al. 2004). Mycorrhizae substantially increase zinc and copper contents of the shoot at low phosphorus levels in soils. However, in the case of soybean (*Glycine max*), it was found grown in high-phosphorus level soils, and the mycorrhizae lower zinc and copper contents of colonized plants (Lambert and Weidensaul 1991). Visible symptoms of phosphorus and zinc deficiency occur in peanut (*Arachis hypogaea*) plants, when grown in sterilized soils without AMF inoculation (Mathur and Vyas 2000).

Due to phosphorus starvation in plants, inhibition of photosynthesis and respiration, cell division restriction and expansion and impairment of nutrient uptake and transport of nutrient occur (Baas and Kuiper 1989). These starvation effects result in substantial reductions in yield in potato (Harris 1978; Pursglove and Sanders 1981; MacKay et al. 1988). AMF inoculation in *Pogostemon patchouli* pellet seedlings showed increase in growth, nutritional value status and total chlorophyll in leaves compared to non-inoculated seedlings grown without AMF; extent of increase varies with AMF species present (Selvaraj et al. 2009). The plant combinations and all AMF are not always compatible, as some fungi are more beneficial to the host plant and have more adaptability to edaphoclimatic conditions showing marked functional and structural differences among species and even among morphotypes of the same fungal species (Linderman and Davis 2004). It is necessary to know the compatibility between a determined host and the AMF for making a satisfactory inoculum for the specific plant cultivar (Rodríguez et al. 2004). AMF facilitate and increase the plant nutrient uptake as their extensive hyphal network provides a large surface area for water absorption, thereby improving the mineral absorption by the host plant roots. These act as barriers and save plants from cell division restriction and expansion, inhibition of respiration and photosynthesis and also the impairment of root nutrient uptake.

25.6 Arbuscular Mycorrhizal Fungi Affect Secondary Compound Production

The process of AMF colonization involves a series of different steps each characterized by specific structures and morphological and physiological changes in the host plant (Bonfante and Perotto 1995; Smith 1995; Gianinazzi-Pearson 1996; Harrier 2001). The production of secondary plant compounds has been shown to be altered once AMF colonize the host plant, the products of defence-related chemicals even phytoalexins (VanEtten et al. 1994; Scharrff et al. 1997). It has been observed that the first step of arbuscular mycorrhizal colonization can induce

the production of those compounds in the host plant. However, this response is usually small in association between highly compatible host plant and AMF (Spanu et al. 1989; Harrison and Dixon 1993; Vierheiling et al. 1994; Volpin et al. 1994). Overall, the mechanism associated with plant defence plays a key role in arbuscular mycorrhizal colonization and compatibility with the host plants (Garcia-Garrido and Ocampo 2002). Phenolic content significantly increased due to mycorrhizal colonization (Devi and Reddy 2002). Silva et al. (2008) reported that oleoresin production can be increased in *Zingiber officinale* by mycorrhizal inoculation and also increases the value of ginger rhizome. Mycorrhiza-colonized seedlings showed distinct variations than the non-mycorrhizal ones for most of the growth parameters. *Wedilla chinensis* showed different responses with pre-inoculated different AMF species, and also phytochemical constituents showed significant variation in AMF-inoculated ones than the control having no inoculation (Nisha and Rajeshkumar 2010). Essential oil production in selected medicinal plants was higher in mycorrhizal plants compared to control having no inoculation (Karagiannidis et al. 2012). Khaosaad et al. (2006) reported that higher phosphorus uptake due to mycorrhizal association is not responsible for increasing the essential oils, but it is directly dependent on association between AMF and oregano plant. Secondary metabolites in leaves of *Pogostemon patchouli* pellet seedlings showed increase, when the seedlings were grown in the presence of AMF than control. However, the extent of increase varied with different AMF species (Selvaraj et al. 2009). In onion bulb AMF induce phytochemical changes; compounds like sitosterol, stigmosterol and amyrins are produced in arbuscular mycorrhizal ones than non-arbuscular mycorrhizal ones (Lone et al. 2015b). Lone et al. (2015b) reported that AMF inoculation in onion not only induces changes in phytochemical constituents but also increases the indigestible oligosaccharides like fructofuranosylnystose, nystose, kestose and other carbohydrate factions in onion bulb.

Secondary metabolite production shows synergism in AMF-colonized plants. This symbiotic association increased phytoalexins, pathogen-related proteins, active principle compounds, phenolic content and also the phytochemical constituents of plants, thereby increasing plant capability to face a plethora of various biotic and abiotic changes.

25.7 Arbuscular Mycorrhizal Fungi and Plant Diseases

Mycorrhiza-inoculated plants have tolerance against diseases (Bagyaraj 1984; Graham 2001), especially to those caused by soilborne microbial pathogens and more so pathogens (Reddy et al. 2006). The tolerance in plants against diseases is thought to be achieved by changing physiology in host plant due to improved nutritional status, production of phenolic compounds and anatomical changes (Morandi et al. 1984; Morandi and Gianinazzi-Pearson 1986; Zhang et al. 2013). Morandi et al. (1984) observed AMF-associated plants produced phytoalexins and isoflavonoid compounds which provide protection against pathogens. Production of plant

growth regulators like cytokinins, indoleacetic acid and gibberellin-like substances gets boosted by AMF association (Smith and Read 1997a, b). Mycorrhizal fungi provides an eco-friendly biological substitute for maintaining plant growth and development, quality and yield in agriculture, forestry and horticulture conversely to the use of chemical fertilizers and pesticides (Wood 1992). Phenolic compounds play a key role in plant growth and defence by acting as signalling molecules in the initiation of legume-rhizobia symbiosis, help in establishment of arbuscular mycorrhizal symbiosis and can act as agents in plant defence (Santi et al. 2010). Mycorrhiza competes with pathogens for acquiring colonization sites, improves the nutrient pool for the plant and thereby increases the tolerance of the plants against any attack by pathogens. Mycorrhizal association in plants may have also enhanced resistance against the appearance of disease symptoms (Smith and Read 1997a, b).

AMF are obligate mutualistic symbionts to most of plant species and are common across most terrestrial biomes (Smith and Read 2008). Besides the foremost functions of the symbiosis, experimental studies have well established that arbuscular mycorrhizal plants get protection from the pathogens in contrast to their non-mycorrhizal counterparts (Newsham et al. 1995a; Filion et al. 1999, 2003; Borowicz 2001; Vazquez-Prieto and Miatello 2010). Till now, different mechanisms pertaining to explain how AMF-associated plants get tolerance against pathogens have been proposed (Azcon-Aguilar and Barea 1996; Whipps 2004; Dalpe 2005). Several studies suggest that each and every AMF species varies both in the expression of traits associated with mechanisms related to plant resistance against pathogens (Hart and Reader 2002; Pozo and Azcon-Aguilar 2007) and in their capacity for protecting the host plants against soilborne pathogens (Newsham et al. 1995b; Maherali and Klironomos 2007; Sikes et al. 2009). Therefore, probability arises that a consortium of AMF obtained from different AMF species may exhibit greater potential in protecting the host plants against pathogens as compared to a single AMF species; this concept could probably provide a boom for the beneficial relationships observed between the richness of AMF inoculum and the growth of individual associated plants and ortho-diversity and productivity of plant communities associating with this combined inoculum (Van der Heijden et al. 1998; Maherali and Klironomos 2007). Most of the research that is mainly confined to the mechanisms involved in AMF-mediated pathogen protection fails to understand the richness of AMF communities in natural systems (Azcon-Aguilar and Barea 1996; Borowicz 2001; Whipps 2004; Dalpe 2005). Wehner et al. (2009) proposed that the mechanisms involved in AMF-mediated pathogen protection must be known, and the ways through which results of these interactions get influenced due to AMF diversity must be known.

These mycorrhizal symbionts offer and enhance host plant resistance to diseases caused by root pathogens and soilborne pathogenic microbes. These, therefore, provide a substitute of chemical fertilizers and pesticides by offering an eco-friendly, biologically sound biofertilizer that will greatly help in maintaining plant growth, quality as well as quantity in agriculture, horticulture and forestry.

25.8 Arbuscular Mycorrhizal Fungi Effects on Plant Morphology and Physiology

AMF colonization affects plant biomass allocation and results in changes in biomass allocation to different plant parts (Garrido et al. 2010; Shuab et al. 2014, 2016; Lone et al. 2015b;). AMF could increase plant growth by tapering the leaf area per unit of plant biomass (Kavanova et al. 2006). Sharma and Adholeya (2000) and Shuab et al. (2014) showed that the onion plants on AMF inoculation may significantly increase bulb diameter and enhance bulb yield, shoot dry and fresh weights and shoot phosphorous content. Nair (1998) reported that increased level of AMF inoculation was helpful for plant growth and development of cowpea (*Vigna unguiculata*) under field conditions. Jackson (1984) noticed that in groundnut, there is a positive relationship between phosphorous availability, AMF colonization and pod yield (*Arachis hypogaea*). AMF inoculation significantly increased shoot and root growth at transplanting stage (Sohn et al. 2003). Halder and Ray (2006) showed that mycorrhiza-treated *Withania somnifera* plants showed better vegetative growth in comparison to control plants having no mycorrhiza. Seedlings of custard apple inoculated with *Glomus fasciculatum* showed significant higher growth characteristic like height and fresh and dry weight than to non-inoculated seedlings (Ojha et al. 2008). However, it was found that the growth in mycorrhizal plants is higher in comparison to control medicinal plants (Goussous and Mohammad 2009; Karagiannidis et al. 2012; Shuab et al. 2014, 2016; Lone et al. 2015b) and increases biomass of pepper, parsley and carrot (Regvar et al. 2003). Morphology of root systems upon AMF associations can show modifications in its structural, spatial, quantitative and temporal manner (Kapoor et al. 2008). It has been shown that AMF colonization can impact root development, with definite effects on the root anatomy, physiology and morphology (Gutjahr and Parniske 2013); thus it could alter the root growth in structural, quantitative, spatial and temporal manner, and same results have been obtained when rhizobium and mycorrhizal inoculation were given together (Nzanza et al. 2011). The increment in production of dry matter might be due to increased photosynthesis, as there is increased leaf canopy surface area because of biological nitrogen fixation and mycorrhizal colonization which enhances carbon dioxide fixation. The assimilated carbon derived from photosynthesis might have translocated to host plant roots, thereby promoting mycorrhizal population. Mycorrhizae inoculation along with *Rhizobium* significantly increased the number as well as dry weight of nodules over control (Subramanian et al. 2011). Mycorrhizal inoculation regardless of phosphorus addition increases the height, shoot and root biomass and shoot phosphorus (Pasqualini et al. 2007).

Most land plants showed symbiotic association with AMF, which helps the plant in nutrient uptake from soil and exchange with plant for photosynthetically fixed carbon skeleton. This exchange is a significant factor in nutrient cycles globally and in the ecology, evolution and physiology of plants (Redecker 2000). These mycorrhizal fungi improve crop growth, development and productivity, forming an important component of sustainable soil-plant systems (Van Der Heijden et al. 1998). There are reports that mycorrhizal plants contain higher concentration of growth

hormones than their non-mycorrhizal equivalents (Skimmer 1985). Smith et al. (1985) studied the effect on the activity of glutamate synthetase and glutamate dehydrogenase enzymes by the AMF inoculation and phosphate fertilization in shoots and roots of *Allium cepa* and *Trifolium subterraneum*. Similar responses were observed in onion plants; increased enzymatic activity (glutamate synthetase) directly followed the intensity of AMF colonization and soil phosphorous level. Gianinazzi et al. (1992) reported that the activity of alkaline phosphatases is increased in AMF-associated plant when grown in phosphorous-deficient substrates. This may explain the importance of phosphate ion on the physiological aspects of all mycorrhiza, not only on those of the arbuscular type. Lone et al. (2016) reported that in saffron plant nitrogen-assimilating enzyme and antioxidant enzyme activity gets affected by the influence of AMF.

AMF can also influence the nutrient uptake of other essential nutrients necessary for plant growth, development and yield production including nitrogen. AMF affects the cycling of nitrogen, plant growth and ecosystem functioning (Miransari 2010). McFarland et al. (2010) reported that more than 50% of nitrogen requirement in plants was supplied by mycorrhizal associations. Radioisotopic studies have indicated that extraradical mycelium can efficiently utilize the inorganic nitrogen in soil (Johansen et al. 1994). Arbuscular mycorrhizal plants have easy access to all forms of nitrogen, unavailable to non-arbuscular mycorrhizal plants (Tobar et al. 1994a, b). Inorganic nitrogen can be absorbed by the external hyphae of AMF in the form of both NO_3^- and ammonium (NH_4^+) (Bago et al. 1997). The fungal hyphae are able to absorb NH_4^+ at lower concentrations than roots. In addition, the development of the external hyphae of *Glomus intraradices* has been shown to be favoured by the addition of NH_4^+ to soil (Johnson et al. 1996). Activity of enzyme nitrate reductase in leaf was found significantly lower in AMF-associated plants conversely to non-arbuscular mycorrhizal fungi plants (Cartmill et al. 2008).

Under high alkalinity the AMF increased the antioxidant activity, due to which the plants become capable of maintaining their detoxifying activity, because of the enhanced micronutrient status. In AMF-inoculated plants, the activity of nitrate reductase enzyme on exposing to higher alkalinity was higher on a whole plant basis, maintaining leaf nitrogen content when HCO^{3-} increased (Cartmill et al. 2008). The tripartite association of rhizobium-mycorrhiza-soybean enhances activity of the enzyme acid phosphatase which helps in the acidification of the rhizosphere that facilitates availability of phosphorous besides triggering activity of nitrate reductase (Subramanian et al. 2011). Ferrera-Cerrato and Villerias (1985) reported that roots and shoots of plants inoculated with *Glomus* species showed a huge increment in nitrogen content and higher activity of enzymes peroxidase, phosphatises, catalase, and nitrate reductase and level of growth hormones indole-acetic acid, cytokinins and gibberellin in contrast to those inoculated only with *Rhizobium*.

Assimilation of ammonium by the glutamine synthetase/glutamate synthase pathway has also been reported for associations of corn with *Glomus fasciculatum* and of *Allium cepa* with *Glomus mosseae* (Smith et al. 1985). The hyphae of *Glomus intraradices* possess the glutamine synthetase/glutamate synthase enzymatic

system for the assimilation of inorganic nitrogen in the form of NH_4^+. Morte et al. (2000) reported that the potassium accumulation increased in shoots and roots of AMF-inoculated plants than the control plants having no AMF inoculation.

AMF influences both the glutamine synthetase/glutamate synthase and glutamate dehydrogenase enzymes (Lone et al. 2016). Activities of enzymes, namely, glutamine synthetase and nitrate reductase, were enhanced in roots as well as in shoots of *Zea mays* infected with *Glomus fasciculatum* compared with non-inoculated plants, and glutamate dehydrogenase activity increased only in roots (Cliquet and Stewart 1993). AMF increase carbohydrate accumulation by increasing the net photosynthesis due to increased chlorophyll and carotenoids. AMF inoculation that made reduction in malondialdehyde content conversely to salinized plants showed lower oxidative damage in the colonized plants (Latef and Chaoxing 2011). Santos et al. (2001) reported that there is an increase in peroxidase activity in the AMF-inoculated passion fruit roots than the non-mycorrhizal plants. Inoculation of AMF together with *Rhizobium* increased the activity of nitrate reductase than in either of the rhizobium and AMF alone in plants. Activity of nitrate reductase in AMF alone or combined inoculation of *Rhizobium* with *Glomus intraradices* treatments was considerably higher as compared to control.

AMF enhanced activity of nitrate reductase in shoot and root of *Juniperus oxycedrus* (Alguacil et al. 2006). Roots infected with mycorrhiza possess easy and greater access to transport and accumulate nitrogen derived from nitrate which is usually unavailable to uninoculated plants (Subramanian et al. 2011). Besides having set genomes for regulating nitrate reductase activity, mycorrhizal colonization increased nitrate reductase activity in maize roots due to transport of nitrate nitrogen by the external mycelium, serving as substrate for nitrate reductase (Subramanian and Charest 1999). Cluster bean infected with combined *Rhizobium* and AMF inoculums showed higher enzyme activity (Tarafdar and Rao 2001). The increased salt resistance in arbuscular mycorrhizal symbiotic association was mainly confined with the enhanced ascorbate peroxidase, peroxidase and superoxide dismutase activity by arbuscular mycorrhizal fungi which abated more reactive oxygen species to ease the damages caused to cell membrane under salt stress conditions, whereas the towering activity of superoxide dismutase, ascorbate peroxidase and peroxidase due to AMF depended on the salinity condition (ZhongQun et al. 2007). Activity of enzyme was higher in *Ziziphus xylopyrus* in all arbuscular mycorrhiza-inoculated seedlings when compared with the control (Mathur and Vyas 1996). Concentration of phenols and polyphenol oxidase activity were higher in tomato, when inoculated with mycorrhiza and or/in combination with root-aggregated nematode *Meloidogyne incognita* (Shreenivasa et al. 2011). During acclimatization rapid AMF colonization elevated physiological adjustments, thereby helping plantlets to recover rapidly and also helping in obtaining greater growth during post-acclimatization (Estrada-Luna and Davies 2003).

AMF colonization has a great impact on root development because of their consequential effects on host plant root anatomy, morphology and physiology. Their effects on net photosynthesis rates, enzymatic activity, water uptake and carbohydrate accumulation result in an overall increment in fresh and dry matter content of host plants.

25.9 Arbuscular Mycorrhizal Fungi Effect on Storage Metabolites

Arines et al. (1993) observed that colonized roots of *Trifolium pratense* by *Glomus mosseae* showed high-protein content. Proteins can be either covalently coupled or non-covalently attached, the former within the fungal cell wall while the latter case with the wall, by either forming insoluble complexes or being loosely embedded (Carlile et al. 2001). Members of the *Glomeromycota* within walls possess both soluble and insoluble proteins (Bonfante-Fasolo and Grippolo 1984), which constitute β-glucan complexes and cross-linked chitin (or chitosan) (Bago et al. 1997). Freitas et al. (2004) also noticed that inoculation of AMF in mint resulted into a huge increase as much as 89% in the contents of menthol and essential oil. Gianinazzi-pearson and Gianinazzi (1995) and Santos et al. (2001) reported that root extracts of AMF-associated plants show increase in protein concentration.

Borges and Chaney (1993) have noticed that although mycorrhizal seedlings possess higher concentrations of soluble sugars in roots in contrast to non-mycorrhizal seedlings, the increased photosynthetic content resulted into huge increment in the overall concentrations of soluble sugars and starch in roots. Onion being highly responsive to several AMF species, after association, results in amendment of plant growth resistance to soil salinity and water stress conditions (Mahaveer et al. 2000; Bolandnazar et al. 2007; Bolandnazar and Hakiminia 2013). Hajra et al. (2009) that made a comparative assay of sunflower indicate that amount of carbohydrates, proteins, amino acids and chlorophyll **a** and **b** considerably varied in mycorrhizal plants in contrast to non-mycorrhizal plants. In *Aloe vera* plantlets, the concentration of flavonols and flavanones increased when *Aloe vera* plantlets were inoculated with *Glomus claroideum* and/or *Glomus fasciculatum* (Mota-Fernández et al. 2011). Ferrera-Cerrato and Villerias (1985) reported that plants inoculated with *Glomus* species show an increased nitrogen content along with increase in their dry weight content, rate of photosynthesis, amino acids, lipids, protein content and sugars in roots and shoots in contrast to those inoculated only with *Rhizobium*.

AMF increase the metabolite mobilization in host plants, therefore enhancing levels of starch, amino acids, lipids, sugars, proteins and other biomolecules of the inoculated plants.

25.10 Arbuscular Mycorrhizal Fungi and Plant Tolerance and Resistance

AMF help their associated host plants by providing protection to them against environmental abiotic (Auge 2001; Abdel-Fattah and Shabana 2002; Ruiz-Lozano 2003; Azcon et al. 2009) and biotic environmental stresses (Khaosaad et al. 2007). Improved root hydraulic conductivity (Robert et al. 2008), better water uptake at low soil moisture levels as a result of extraradical hyphae (Fagbola et al. 2001), stomatal regulation or transpiration rate (Allen and Boosalis 1983), osmotic adjustment which promotes turgor maintenance even at low tissue water potential (Auge

et al. 1986) promoting increased photosynthetic activity, carbohydrate and proline accumulation and increased nutritional status in mycorrhizal plants (Kandowangko et al. 2009; Lone et al. 2015a, 2016) may lead to feasible mechanisms for better tolerance of mycorrhizal plants against drought conditions. Wu and Xia (2006) reported that osmotic adjustment improved under mycorrhizal colonization which arises from total nonstructural carbohydrates, K^+, Mg^{2+} and Ca^{2+} but not from proline results in the enhancement of drought tolerance. Such mechanisms may prove vital in adaptation of mycorrhizal plants to drought environment. The AMF symbiosis with plant roots is acknowledged among the most ancient and widespread plant tactics to enrich nutrient acquisition which survives under the environmental stresses (Brachmann and Parniske 2006). Extraradical arbuscular mycorrhizal hyphae diffuse in rhizosphere and provide a large surface area through which the AMF absorb nutritional elements such as phosphorus, nitrogen, zinc or copper and transport and transfer them to the host plant (Smith and Read 2008).

It has been reported that with the AMF-colonized roots, the above-ground effect against phytopathogen is quite apparent (Sensoy et al. 2007; Kapoor et al. 2008; Ozgonen et al. 2010; Al-Askar and Rashad 2010). Mycorrhizal plants are often more tolerant and well suited to deal with different environmental stresses, e.g. heavy metal, soil compaction, salinity and drought (Porras-Soriano et al. 2009; Miransari 2010). Proline augmentation correlates with drought resistance in various plant species. At the end of water stress period, plants associated with AMF comprise lower free proline concentration in their leaves as compared to non-mycorrhizal plants, pointing towards a distinct water stress tolerance in corn (Mcumiller and Hofner 1991). It has been reported that amino and imino acid in drought plants either increase (Subramanian and Charest 1995; Schellembaum et al. 1998) or decrease (Auge et al. 1992; Subramanian and Charest 1995) with AM symbiosis. It has been shown in roots; mycorrhizal colonization and drought interact for bringing modifications in free amino acids and sugar pools (Auge et al. 1992). The net accumulation of sugar and carbohydrates suggested that arbuscular mycorrhizal colonization promoted tolerance of the AMF-associated plant against drought stress (Porcel and Ruiz-Lozano 2004). Sorial (2001) observed that arbuscular mycorrhizal inoculation recorded highly significant induction in chemical constituents, e.g. chlorophyll a, b; total chlorophylls; nitrogen, phosphorous, potassium uptakes; total sugars; total amino acids; as well as proline concentration under water stress conditions, which increased the osmoregulation of wheat plants exposed to water stress. Subramanian and Charest (1997) also provided evidence that mycorrhizal plants after drought having higher levels of foliar concentrations of soluble sugars suggest sustenance of greater photosynthetic capacity. Higher starch levels in arbuscular mycorrhizal plants indicate the perpetuation of greater photosynthetic capacity during drought in contrast to non-arbuscular mycorrhizal plants (Auge et al. 1987; Davies et al. 1993, 2005). AMF have also enhanced stomatal resistance, thereby reducing the rate of transpiration (Mathur and Vyas 1995). Under drought stress conditions, significant differences in the fresh weight, chlorophyll content and leaf area are seen, which are higher in mycorrhiza-associated plants than in non-mycorrhizal plants. AMF plants had a higher chlorophyll concentration (Cartmill

et al. 2008). Growth, pigment content, phosphorous content and flower quality are positively affected by mycorrhiza (Asrar and Elhindi 2010).

Mycorrhizal plants are generally more effective and more combative to deal with environmental stresses. This enhanced tolerance or resistance is mycorrhizal responsive and may be due to the increase in root hydraulic conductivity, stomatal regulation or transpiration rate, enhanced water uptake, osmotic adjustment, increases photosynthetic activity, carbohydrate accumulation, proline production and accreted nutritional status of mycorrhizal host plants.

25.11 Other Functions of Arbuscular Mycorrhizal Fungi

Sustainable plant growth and development is governed by AMF (Agarwal et al. 2009). Mycorrhiza in AMF-plant association is managed by water-soluble mono- and disaccharides, organic acids, flavonoids, amino acids, enzymes and nucleotides (Rovira 1996) by volatile exudates (alcohols, ketones, esters, phenols, terpenoids, organic acids) and by surface-bound recognition molecules. Studies have revealed that AMF have an impact on phytohormone levels of jasmonate (Hause et al. 2002), terpenoids and carotenoids (Akiyama et al. 2002; Fester et al. 2002) and phenols (Zhu and Yao 2004). Other functions attributed to AMF may either be beneficial or can sometimes be antagonistic that comprise plant growth hormone production, increase in the activities of defence-related enzymes (phenylalanine ammonia-lyase, polyphenol oxidase and peroxidase), uptake of heavy metals and uptake of radionuclides and also protecting ions of plant from radioactivity (Jones and Smith 2004; Selvaraj et al. 2005). The balance between carbon costs and phosphorous uptake is a negative correlation of most of the arbuscular mycorrhizal activities (Li et al. 2008). The AMF develop properties of rhizospheric soil, enlarge root areas of host plants by means of hyphal penetrations into deeper soil areas and improve its efficiency of water absorption, phosphorous and other nutritional elements and then improve nutritional status of host plant and quickly actifies defence mechanisms of host plant. AMF associations protect plants against oxidative damage generated by drought and also affect the expression of genetic material and are some of the possible mechanisms by which the AMF can improve resistance against drought stress.

The plant growth is enhanced by the mycorrhizal association by promoting translocation of essential nutrients and water between the root and shoot of host plant (Osonubi 1994). Arbuscular mycorrhizal fungi considerably make an increment in the net photosynthesis by elevating total chlorophyll and carotenoid contents that ultimately increases accumulation of carbohydrate. The AMF are considered to be eco-friendly as they are used as biofertilizers, bioprotectors and biocontrolling agents (Azcón-Aguilar et al. 2002). The AMF can influence insect herbivores by altering plant growth and chemistry (Reidinger et al. 2012). With an aim of boosting crop productivity and also lowering the application of chemical fertilizers and pesticides, AMF as a biofertilizer have been highly recommended (Schwartz et al. 2006). Higher levels of heavy metals like zinc, lead, etc., which directly enter our

food chain, might be effectively mitigated by the filtering property of mycorrhiza (Celik and Arcak 2002). One of the most promising applications of symbiotic association of AMF is the phytoremediation of heavy metals from adulterated soils (Upadhyaya et al. 2010). AMF play a key role in the absorption of phosphorous, which is the second most essential macronutrient after nitrogen, and its runoff leads to eutrophication.

Regardless of the above-discussed diverse functionalities of AMF, these have also been found to elevate levels of plant phytohormones. These can also act as environment friendly biofertilizers, bioprotectors and biocontrolling agents. They can also be used positively for phytoremediation, controlling eutrophication and many more.

Conclusion

The establishment of a functional symbiosis between AMF and associated host plants involves a series of recognition events advancing to the morphological and physiological alliance of the two symbionts. The developmental shifts in the fungi are triggered by host signals which induce some changes in gene expression and a process resulting into unequivocal identification among the two partners of symbiosis. Widespread distribution both in terms of habitats and host species, evolution of symbiosis due to terrestrialization, host protection and growth promotiveness obligate nature and nonspecificity for host; positive interaction with other rhizosphere microbes and several other characteristics of AMF have obviously made plant biologists to work out their practical aspects. With advancements in isolation techniques, mass production methods and inoculation, AMF have been looked as a perk for agriculture, forestry, horticulture and rehabilitation of spastic niches.

References

Abdel-Fattah GM, Shabana YM (2002) Efficacy of the Arbuscular mycorrhizal fungus *Glomus clarum* in protection of cowpea plants against root rot pathogen *Rhizoctoniasolani.* Zeitschrift Fur *Pflanzenkrankheiten* Und Pflanzenschutz. J Plant Dis Prot 109(2):207–215

Agarwal S, Koul KK, Lone R (2009) Arbuscular mycorrhizal fungi- essential partners in sustainable plant growth. In: Lakshman HC (ed) Bioinoculants for integrated plant growth. M D Publications Pvt Ltd, New Delhi, pp 53–76

Agarwal S, Lone R, Farooq F, Shuab R, Koul KK (2013) In vitro establishment of Arbuscular mycorrhizal fungi (AMF) in cucumber (Cucumis sativus) roots. Adv Biores 4(4):155–159

Akiyama K, Matsuoka H, Hayashi H (2002) Isolation and identification of a phosphate deficiency-induced *C*-glycosyl flavonoid that stimulates Arbuscular mycorrhiza formation in melon roots. Mol Plant Microbe Interact 15:334–340

Akthar MS, Siddiqui ZA (2008) Arbuscular mycorrhizal fungi as potential bioprotectants against plant pathogens. In: Siddiqui ZA, Akhtar MS, Futai K (eds) Mycorrhizae: sustainable agriculture and forestry. Springer, Dordrecht, pp 61–97

Al-Agel A, Sylvia DM, Ma LQ (2005) Mycorrhizae increase arsenic uptake by the hyperaccumulator Chinese brake fern (*Pteris vittata* L.) J Environ Qual 34:2181–2186

Al-Askar AA, Rashad YM (2010) Arbuscular mycorrhizal fungi: a biocontrol agent against common bean *Fusarium* root rot disease. Plant Pathol J 9:31–38

Alguacil M, Caravaca F, Diaz-Vivancos P, Hernandez JA, Roldan A (2006) Effect of arbuscular mycorrhizae and induced drought stress on antioxidant enzyme and nitrate reductase activities in Juniperus oxycedrus L. grown in a composted sewage sludge-amended semi-arid soil. Plant Soil 279:209–218

Al-Karaki G, McMicheal B, Zak J (2004) Field response of wheat to Arbuscular mycorrhizal fungi and drought stress. Mycorrhiza 14:263–269

Allen MF (1991) The ecology of mycorrhizae. Cambridge University Press, Cambridge

Allen MF, Boosalis MG (1983) Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat (Glomus fasciculatus, Glomus mosseae). New Phytol 93(1):67–76

Arines J, Palma MJ, Vilarino A (1993) Comparison of protein patterns in non-mycorrhizal and vesicular-Arbuscular mycorrhizal roots of red clover. New Phytol 123:763–768

Artursson V, Finlay RD, Jansson JK (2006) Interactions between Arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environ Microbiol 8:1–10

Asrar AA, Elhindi KM (2010) Alleviation of drought stress of marigold (*Tageteserecta*) plants by using Arbuscular mycorrhizal fungi. Saudi J Biol Sci. doi:10.1016/j.sjbs.2010.06.007

Auge RM (2001) Water relations, drought and vesicular-Arbuscular mycorrhizal symbiosis. Mycorrhiza 11:3–42

Auge RM, Scheikel KA, Warmple RL (1986) Osmotic adjustment in leaves of VA-mycorrhiza and non-mycorrhizal rose plants in response to drought stress. Plant Physiol 82:765–770

Auge RM, Schekel KA, Wample RL (1987) Leaf water and carbohydrate status of VA mycorrhizal rose exposed to drought stress. Plant Soil 99:291–302

Auge RM, Foster JG, Lasher WH, Stodola AW (1992) Symplastic sugar and free amino acid molality of *Rosa* roots with regarded to mycorrhizal colonization and drought. Symbiosis 12:1–17

Azcon R, Peralvarez MC, Biro B, Roldan A, Ruiz-Lozano JM (2009) Antioxidant activities and metal acquisition in Mycorrhizal plants growing in a heavy-metal multicontaminated soil amended with treated lignocellulosicagrowaste. Appl Soil Ecol 41:168–177

Azcon-Aguilar C, Barea JM (1996) Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an over view of the mechanisms involved. Mycorrhiza 6:457–464

Azcón-Aguilar C, Jaizme-Vega MC, Calvet C (2002) The contribution of Arbuscular mycorrhizal fungi to the control of soil-borne plant pathogens. In: Gianinazzi S, Schüepp H, Barea JM, Haselwandler K (eds) Mycorrhizal technology in agriculture. Birkäuser Verlag, Switzerland, pp 187–197

Baas R, Kuiper D (1989) Effects of vesicular-Arbuscular mycorrhizal infection and phosphate on Plantago major spp. pleiosperma in relation to internal cytokinin concentrations. Physiol Plant 76:211–215

Bago B, Donaire JP, Azcón-Aguilar C (1997) ATPase activities of root microsomes from mycorrhizal sunflower (*Helianthus annuus*) and onion (*Allium cepa*) plants. New Phytol 236(2):305–311

Bagyaraj DJ (1984) Biological interactions with mycorrhizal fungi. In: Powell CL, Bagyaraj DJ (eds) VA mycorrhiza. CRC Press, New York, pp 131–153

Bagyaraj DJ, Varma AK (1995) Interaction between VA mycorrhizal fungi and plants and their importance in sustainable agriculture in arid and semi arid tropics. Adv Microb Ecol 14:119–142

Barea JM, Azcón R, Azcon-Aguilar C (2002) Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie Van Leeuwenhoek 81:343–351

Bhat RP, Kaveriappa KM (1997) Occurrence of vesicular Arbuscular mycorrhizal fungi in the tubers of *Colocasia esculenta* (L.) Schott. Mycorrhiza News 9:12–13

Biro B, Koves-Pechy K, Voros I, Takacs T, Eggenberg P, Strasser RJ (2000) Interrelations between Azospirillum and Rhizobium nitrogen-fixers and Arbuscular mycorrhizal fungi in the rhizosphere of alfalfa in sterile, AMF-free or normal soil conditions. Appl Soil Ecol 15:159–168

Bohra A, Mathur N, Bohra S, Singh J, Vyas A (2007) Influence of AM fungi on physiological changes in *Terminalia arjuna* L.: an endangered tree of Indian thar desert. Indian Forester 133(11):1558–1562

Bolandnazar S, Hakiminia I (2013) Impact of Mycorrhizal Fungi on P acquisition, yield and water use efficiency of onion under regulated deficit irrigation. Res Plant Biol 3(1):321–328

Bolandnazar S, Aliasgarzad N, Neishabury MR, Chaparzadeh N (2007) Mycorrhizal colonization improves onion (*Allium cepa* L.) yield and water use efficiency under water deficit condition. Sci Hortic 114(1):11–15

Bonfante P, Perotto S (1995) Strategies of Arbuscular mycorrhizal fungi when infecting host plants. New Phytol 130:3–21

Bonfante-Fasolo P, Grippolo R (1984) Cytochemical and biochemical observations on the cell wall of the spore of Glomus epigaeum. Protoplasma 123:140–151

Borges RG, Chaney WR (1993) Solar irradiance and the development of endomycorrhizal green ash seedlings. Tree Physiol 13:227–238

Borowicz VA (2001) Do Arbuscular mycorrhizal fungi alter plant-pathogen relations. Ecology 82:3057–3068

Bossuyt H, Denef K, Six J, Frey SD, Merckx R, Paustian K (2001) Influence of microbial populations and residue quality on aggregate stability. Appl Soil Ecol 16:195–208

Brachmann A, Parniske M (2006) The most important symbiosis on earth. Soil Biol 4:239

Brundrett M, Beegher N, Dell B, Groov T, Malajczuk N (1996) Working with mycorrhizas in forestry and agriculture. ACIAR Monograph 32

Buee M, Rossignol M, Jauneau A, Ranjeva R, Becard G (2000) The pre-symbiotic growth of Arbuscular mycorrhizal fungi is induced by a branching factor partially purified from plant root exudates. Mol Plant Microbe Interact 13:693–698

Carlile MJ, Watkinson SC, Gooday GW (2001) The fungi, 2nd ed. Academic, London, p 578

Cartmill AD, Valdez-Aguilar LA, Bryan DL, Alarcon A (2008) Arbuscular mycorrhizal fungi enhance tolerance of *vinca* to high alkalinity in irrigation water. Sci Hortic 115:275–284

Celik S, Arcak S (2002) Effects of vesicular-arbuscular mycorrhizae on the growth and uptake of some heavy metals by oat (*Avena Sativa* L.). In: International conference on sustainable land use and management, Soil Science Society of Turkey, Çanakkale

Charles P, Raj ADS, Kiruba S (2006) Arbuscular mycorrhizal fungi in the reclamation and restoration of soil fertility. Mycorrhiza News 18(2):13–14

Chellappan P, Anitha Christy SA, Mahadevan A (2002) Multiplication of Arbuscular mycorrhizal fungi on roots. In: Mukerji KG, Manoharachary C, Chamola BP (eds) Techniques in mycorrhizal studies. Kluwer, Dordrecht, pp 285–297

Cliquet JB, Stewart GR (1993) Ammonia assimilation in *Zea mays* L. infected with a vesicular-Arbuscular mycorrhizal fungus *Glomus fasciculatum*. Plant Physiol 101(3):865–871

Dalpe Y (2005) Mycorrhizae: a potential tool for plant protection but not a panacea. Phytoprotection 86:53–59

David-Schwart R, Gadkar V, Wininger S, Bendov R, Gallic G, Levy AA, Kapulnik Y (2003) Isolation of a premycorrhizal infection (*Pmi*2) mutant of tomato, resistant to Arbuscular mycorrhizal fungal colonization. Mol Plant Microbe Interact 16:382–388

Davies FT, Potter JR, Linderman RG (1993) Drought resistance of mycorrhizal pepper plants independent of leaf P concentration – response in gas exchange and water relations. Plant Physiol 87:45–53

Davies FT, Calderon C, Huaman Z (2005) Influence of Arbuscular mycorrhiza indigenous to Peru and a flavonoid on growth, yield and leaf elemental concentration of 'Yungay' potatoes (*Solanum tuberosum* L.) Hortic Sci 40(2):381–385

Devi MC, Reddy MN (2002) Phenolic acid metabolism of groundnut (*Arachis hypogea* L.) plants inoculated with VAM fungus and Rhizobium. Plant Growth Regul 37:151–156

Duponnois R (2006) Bacteria helping mycorrhiza development. In: Mukerji KG, Manoharachary C, Singh J (eds) Microbial activity in the rhizosphere. Springer, Berlin, pp 297–310

Edwards D, Duckett JG, Richardson JB (1995) Hepatic characters in the earliest land plants. Nature 374:635–636

Elahi FE, Mridha MAU, Aminuzzaman FM (2010) Influence of AMF inoculation on growth, nutrient uptake, arsenic toxicity and chlorophyll content of eggplant grown in arsenic amended soil. Adv Nat Appl Sci 4(2):184–192

Estrada-Luna AA, Davies FT (2003) Arbuscular mycorrhizal fungi influence water relations, gas exchange, abscisic acid and growth of micro propagated Chile Ancho pepper (*Capsicum annuum*) plantlets during acclimatization and post acclimatization. J Plant Physiol 160:1073–1083

Fagbola O, Osonubi O, Mulongox K, Odunfa SA (2001) Effects of drought stress and Arbuscular mycorrhiza on the growth of *Gliricidia sepium* (Jacq). Walp, *Leucaena leucocephala* (Lam). Dewit. In simulated eroded soil conditions. Mycorrhiza 11:215–223

Farahani HA, Valadabadi SA, Khalvati MA (2009) Interactive effects of P supply and drought on root growth of the mycorrhizal coriander (*Coriandrum sativum* L.) J Plant Breed Crop Sci 1(5):217–222

Farzaneh M, Wichmann S, Vierheilig H, Kaul HP (2009) The effects of arbuscular mycorrhiza and nitrogen nutrition on growth of chickpea and barley. Pflanzenbauwissenschaften 13:15–22

Farzaneh M, Wichmann S, Vierheilig H, Kaul HP (2011) The effects of arbuscular mycorrhiza and nitrogen nutrition. Plant Soil Environ 57(10):465–470

Ferrera-Cerrato R, Villerias SJ (1985) The VA endomycorrhiza and its effect of the development of three arboreous legumes. In: Proceedings of the Sixth North American Conference on *Mycorrhizae*, Bend, p 328

Fester T, Schmidt D, Lohse S, Walter MH, Guiliano G, Bramley PM, Frase PD, Hause B, Strack D (2002) Stimulation of carotenoid metabolism in Arbuscular mycorrhizal roots. Planta 216:148–154

Filion M, St-Arnaud M, Fortin JA (1999) Direct interaction between the Arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. New Phytol 141:525–533

Filion M, St-Arnaud M, Jabaji-Hare SH (2003) Quantification of *Fusarium solani* f. sp *phaseoli* in mycorrhizal bean plants and surrounding mycorrhizosphere soil using real-time polymerase chain reaction and direct isolations on selective media. Phytopathology 93:229–235

Frank AB (1885) Über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäum durch unterirdische Pilze. Berichte der Deutchen Botanishen Gesellschaft 3:128–145

Freitas MS, Martins MA, Vieira IJC (2004) Produção e qualidade de óleos essenciais de *Mentha arvensis* em resposta à inoculação de fungos micorrízicos arbusculares. Pesquisa Agropecuária Brasileira, v. 39, p. 887-894.

Frey-Klett P, Garbaye J, Tarkka M (2007) The mycorrhiza helper bacteria revisited. New Phytol 176:22–36

Ganesan V, Mahadevan A (1998) The role of mycorrhizae in the improvement of tuber crops in pot and field conditions. In: Prakash A (ed) Fungi in biotechnology. CBS Publishers, New Delhi, pp 51–58

Garbaye J (1994) Helper bacteria: a new dimension to the mycorrhizal symbiosis (Tansley Review, 76). New Phytol 128:197–210

Garcia-Garrido JM, Ocampo JA (2002) Regulation of the plant defence response in Arbuscular mycorrhizal symbiosis. J Exp Bot 53:1377–1386

Garrido E, Bennett AE, Fornoni J, Strauss SY (2010) Variation in Arbuscular mycorrhizal fungi colonization modifies the expression of tolerance to aboveground defoliation. J Ecol 98:43–49

Gianinazzi S, Gianinazzi-Pearson V, Tisserant B, Lemoine MC (1992) Protein activities as potential markers of functional endomycorrhizas. In: Read DJ, Lewis DH, Fitter AH, Alexander ID (eds) Mycorrhizas in ecosystems. CAB International, Wallingford, pp 333–339

Gianinazzi-Pearson V (1996) Plant cell responses to Arbuscular mycorrhizal fungi: getting to the roots of symbiosis. Plant Cell 8:1871–1883

Gianinazzi-pearson V, Gianinazzi S (1995) Proteins and proteins activities in endomycorrhizal symbioses. In: Varna V, Hock B (eds) Mycorrhiza. Springer, Berlin, pp 251–266

Goussous SJ, Mohammad MJ (2009) Comparative effect of two arbuscular mycorrhizae and N and P fertilizers on growth and nutrient uptake of onions. Int J Agric Biol 11:463–467

Graham JH (2001) What do root pathogens see in mycorrhizas. New Phytol 148:357–359

Guissou T (2009) Contribution of Arbuscular mycorrhizal fungi to growth and nutrient uptake by jujube and tamarind seedlings in a phosphate (P)-deficient soil. Afr J Microbiol Res 3(5):297–304

Gutjahr C, Parniske M (2013) Cell and developmental biology of arbuscular mycorrhiza symbiosis. Annu Rev Cell Dev Biol 29:593–617
Hajra N, Firoza K, Shahina F (2009) Effects of VAM and nematode interaction on some biochemical parameters of sunflower. Pak J Nematol 27(2):193–201
Halder S, Ray MB (2006) Effect of VAM soil Containing *Glomus fasciculatum* on Growth of *Withania somnifera* Dun. Asian J Exp Sci 2(2):261–268
Harley JL, Smith SE (1983) Mycorrhizal symbiosis. Academic, London, p 483
Harrier L (2001) The Arbuscular mycorrhizal symbiosis: a molecular review of the fungal dimension. J Exp Bot 52:469–478
Harris PM (1978) Mineral nutrition. In: Harris PM (ed) The potato crop. Chapman and Hall, London, pp 195–243
Harrison MJ (2005) Signaling in the Arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 59:19–42
Harrison MJ, Dixon RA (1993) Isoflavanoid accumulation and expression of defense gene transcripts during the establishment of vesicular Arbuscular mycorrhizal associations in roots of Medicago truncatula. Mol Plant Microbe Interact 6:643–654
Hart MM, Forsythe JA (2012) Using Arbuscular mycorrhizal fungi to improve the nutrient quality of crops; nutritional benefits in addition to phosphorus. Sci Hortic 148:206–214
Hart MM, Reader RJ (2002) Taxonomic basis for variation in the colonization strategy of Arbuscular mycorrhizal fungi. New Phytol 153:335–344
Hause B, Maier W, Miersch O, Kramell R, Strack D (2002) Induction of jasmonate biosynthesis in Arbuscular mycorrhizal barley roots. Plant Physiol 130:1213–1220
Hirrel MC, Mehravaran H, Gerdemann JW (1978) Vesicular–arbuscular mycorrhiza in Chenopodiaceae and Cruciferae: do they occur? Can J Bot 56:2813–2817
Hodge A (2000) Microbial ecology of the Arbuscular mycorrhizal. FEMS Microbiol Ecol 32:91–96
Hodge A, Campbell CD, Fitter AH (2001) An Arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. Nature 413:297–299
Jackson ML (1984) Soil chemical analysis. Prentice Hall of India Private Limited, New Delhi, pp 205–256
Janos DP (1996) Mycorrhizas, succession and rehabilitation of deforested lands in the humid tropics. In: Frankland JC, Gadd GM (eds) Fungi and environmental change. Cambridge University Press, Cambridge, pp 1–18
Javaid A (2007) Allelopathic interactions in mycorrhizal associations. Allelopathy J 20:29–42
Javaid A (2009) Mycorrhizal mediated nutrition in plants. J Plant Nutr 32:1595–1618
Javaid A, Riaz T (2009) Mycorrhizal colonization in different varieties of *Gladiolus* and its relation with plant vegetative and reproductive growth. Int J Agric Biol 10:278–282
Jeffries P, Gianinazzi S, Perotto S, Turnau K, Barea JM (2003) The contribution of Arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. Biol Fertil Soils 37:1–16
Johansen A, Jakobsen I, Jensen ES (1994) Hyphal transport of ^{15}N-labelled nitrogen by a vesicular-Arbuscular mycorrhizal fungus and its effect on depletion of inorganic soil N. New Phytol 122:281–288
Johansson JF, Paul LR, Finlay RD (2004) Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiol Ecol 48:1–13
Johnson JF, Vance CP, Allan DL (1996) Phosphorus deficiency in *Lupinus albus*. Altered lateral root development and enhanced expression of phosphoenolpyruvate carboxylase. Plant Physiol 112:31–41
Jones MD, Smith SE (2004) Exploring functional definitions of mycorrhizas: are mycorrhizas always mutualisms. Can J Bot 82:1089–1109
Kamienski F (1881) Die Vegetationsorgane der *Monotro pahypopitys* L. Bot Zeitschr 39:225–234
Kandowangko NY, Suryatmana G, Nurlaeny N, Simanungkalit RDM (2009) Proline and abscisic acid content in droughted corn plant inoculated with *Azospirillum* sp. and Arbuscular mycorrhizae fungi. Hayati J Biosci 16(1):15–20

Kapoor R, Sharma D, Bhatnagar AK (2008) Arbuscular mycorrhizae in micropropagation systems and their potential applications. Sci Hortic 116:227–239

Karagiannidis N, Thomidis T, Panou-Filotheou E (2012) Effects of *Glomus lamellosum* on growth, essential oil production and nutrients uptake in selected medicinal plants. J Agric Sci 4(3):137–144

Kavanova M, Grimoldi AA, Lattanzi FA, Schnyder H (2006) Phosphorus nutrition and mycorrhiza effects on grass leaf growth. P status- and size-mediated effects on growth zone kinematics. Plant Cell Environ 29:511–520

Khade SW, Rodrigues BF (2003) Incidence of Arbuscular mycorrhizal colonization in tubers of *Gloriosa superba* L. Mycorrhiza News 15:14–16

Khaliq A, Sanders FE (2000) Effect of vesicular- Arbuscular mycorrhizal in oculation on the yield and phosphorous uptake of field – grown barley. Soil Biol Biochem 32:1691–1696

Khan AG, Kuek C, Chaudhry TM, Khoo CS, Hayes WJ (2000) Role of plants, mycorrhizae and phytochelators in heavy metal contaminated land remediation. Chemosphere 41:197–207

Khaosaad T, Vierheilig H, Nell M, Zitterl-Eglseer K, Novak J (2006) Arbuscular mycorrhiza alter the concentration of essential oils in oregano (Origanum sp., Lamiaceae). Mycorrhiza 16:443–446

Khaosaad T, Garcia-Garrido JM, Steinkellner S, Vierheilig H (2007) Take all disease is systemically reduced in roots of Mycorrhizal barley plants. Soil Biol Biochem 39:727–734

Kobae Y, Tamura Y, Takai S, Banba M, Hata S (2010) Localized expression of Arbuscular mycorrhiza-inducible ammonium transporters in soybean. Plant Cell Physiol 51(9):1411–1415

Kunwar IK, Reddy PJM, Manoharachary C (1999) Occurrence and distribution of AMF associated with garlic rhizosphere soil. Mycorrhiza News 11:4–6

Lambert DH, Weidensaul TC (1991) Element uptake by mycorrhizal soybean from sewage-sludge-treated soil. Soil Sci Soc Am J 55:393–398

Latef AAHA, Chaoxing H (2011) Arbuscular mycorrhizal influence on growth, photosynthetic pigments, osmotic adjustment and oxidative stress in tomato plants subjected to low temperature stress. Acta Physiol Plant 33(4):1217–1225

Li H, Smith FA, Dickson S, Holloway RO, Smith S (2008) Plant growth depressions in Arbuscular mycorrhizal symbioses: not just caused by carbon drain. New Phytol 178:852–862

Linderman RG, Davis EA (2004) Varied response of marigold (*Tagetes* spp.) genotypes to inoculation with different Arbuscular mycorrhizal fungi. Sci Hortic 99:67–78

Lone R, Shuab R, Wani KA, Ganaie MA, Tiwari AK, Koul KK (2015a) Mycorrhizal influence on metabolites, indigestible oligosaccharides, mineral nutrition and phytochemical constituents in onion (*Allium cepa* L.) plant. Sci Hortic 193:55–61

Lone R, Shuab R, Sharma V, Kumar V, Mir R, Koul KK (2015b) Effect of Arbuscular mycorrhizal fungi on growth and development of potato (*Solanum tuberosum*) plant. Asian J Crop Sci 7:233–243

Lone R, Shuab R, Koul KK (2016) AMF association and their effect on metabolite mobilization, mineral nutrition and nitrogen assimilating enzymes in saffron (*Crocus sativus L.)* plant. J Plant Nutr 39(13):1852:1862

MacKay DC, Carefoot JM, Entz T (1988) Detection and correction of midseason P deficiency in irrigated potatoes. Can J Plant Sci 68:523–534

Maherali H, Klironomos J (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316:1746–1748

Mahaveer PS, Singh R, Adholeya A (2000) Laboratory manual for basic techniques in arbuscular mycorrhizal research, Center for Mycorrhizal Research, Tata Energy Research Institute New Dehli, India.

Mathur N, Vyas A (1995) Changes in enzymes of nitrogen metabolism by VA mycorrhizae in *Ziziphusnummularia*. J Plant Physiol 147(3–4):331–333

Mathur N, Vyas A (1996) Biochemical changes in *Ziziphus xylopyrus* by VA mycorrhizae. Bot Bull Acad Sin 37:209–212

Mathur, N., Vyas, A (2000) Influence of arbuscular mycorrhizae on biomass production, nutrient uptake and physiological changes in Ziziphus mauritiana Lam. under water stress. J. Arid Envir., 45:191–195.

McFarland JW, Ruess RW, Kielland K, Pregitzer K, Hendrick R, Allen M (2010) Cross ecosystem comparison of *in situ* plant uptake of amino acid-N and NH_4. Ecosystem 13:177–193

Mcumiller I, Hofner W (1991) Influence of the VAM on P-uptake and recovery potential of corn (*Zea mays* L.) under water stress conditions. Z P flanz Bodenk 154:321–322

Miller RM, Jastrow JD (1990) Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biol Biochem 22:579–584

Miransari M (2010) Contribution of Arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress. Plant Biol 12:563–569

Morandi D, Gianinazzi-Pearson V (1986) Influence of mycorrhiza and phosphate nutrition on secondary metabolite contents of soybean roots. In: Gianinazzi-Pearson V, Gianinazzi S (eds) Physiological and genetical aspects of mycorrhizae. INRA, Paris, pp 787–791

Morandi D, Bailey JA, Gianinazzi-Person V (1984) Isoflavonoid accumulation in soybean roots infected with vesicular-Arbuscular mycorrhizal fungi. Physiol Plant Pathol 24:357–364

Morte A, Lovisola C, Schubert A (2000) Effect of drought stress on growth and water relations of the mycorrhizal association *Helianthemum almeriense*-Terfezia clavery. Mycorrhiza 10(3):115–119

Mota-Fernández S, Alvarez-Solis JD, Abud-Archila M, Dendooven L, Gutierrez-Miceli FA (2011) Effect of Arbuscular mycorrhizal fungi and phosphorus concentration on plant growth and phenols in micropropagated *Aloe vera* L. plantlets. J Med Plant Res 5(27):6260–6266

Nair PKR (1998) Directions in tropical agroforestry research: past, present, and future. Agrofor Syst 38:223–245

Nasim G (2005) The role of symbiotic soil fungi in controlling roadside erosion and the establishment of plant communities. Caderno de PesquisaserieBiologia 17(1):119–136

Newsham KK, Fitter AH, Watkinson AR (1995a) Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. J Ecol 83:991–1000

Newsham KK, Fitter AH, Watkinson AR (1995b) Multi-functionality and biodiversity in arbuscularmycorrhizas. Trends Ecol Evol 10:407–411

Nicolson TH (1967) Vesicular Arbuscular mycorrhiza – a universal plant symbiosis. Sci Prog 55:561–581

Nisha MC, Rajeshkumar S (2010) Influence of Arbuscular mycorrhizal fungi on biochemical changes in *Wedilla chinensis* (Osbeck) merril. Anc Sci Life 29(3):26–29

Nzanza B, Marais D, Soundy P (2011) Tomato (*Solanum lycopersicum* L.) seedling growth and development as influenced by *Trichoderma harzianum* and Arbuscular mycorrhizal fungi. Afr J Microbiol Res 5(4):425–431

Ojha S, Chakraborty MR, Dutta S, Chatterjee NC (2008) Influence of VAM on nutrient uptake and growth of custard-apple. Asian J Exp Sci 22(3):221–224

Okon IE, Osonubi O, Sanginga N (1996) Vesicular-arbuscular Mycorrhiza effects on *Gliricidia sepium* and *Senna siamea* in a fallowed alley cropping system. Agrofor Syst 33:165–175

Osonubi O (1994) Comparative effects of vesicular Arbuscular mycorrhizal inoculation and phosphorus fertilization on growth and phosphorus uptake of maize and sorghum plant under drought stressed conditions. Biol Fertil Soils 14:159–165

Ozgonen H, Akgul DS, Erkilic A (2010) The effects of Arbuscular mycorrhizal fungi on yield and stem rot caused by *Sclerotium rolfsii* Sacc. In peanut. Afr J Agric Res 5:128–132

Pasqualini D, Uhlmann A, Sturmer SL (2007) Arbuscular mycorrhizal fungal communities influence growth and phosphorus concentration of woody plants species from the Atlantic rain forest in South Brazil. For Ecol Manage 245:148–155

Pirozynski KA, Mulloch DW (1975) The origin of land plants: a matter of mycotrophism. Biosystems 6(3):153–164

Porcel R, Ruiz-Lozano JM (2004) Arbuscular mycorrhizal influence on leaf water potential, solute accumulation, and oxidative stress in soybean plants subjected to drought stress. J Exp Bot 55:1743–1750

Porras-Soriano A, Soriano-Martın M, Porras-Piedra A, Azcon R (2009) Arbuscular mycorrhizal fungi increased growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. J Plant Physiol 166:1350–1359

Potty VP (1985) Cassava as alternate host for multiplication of VAM fungi. Plant Soil 88:135–137
Pozo MJ, Azcon-Aguilar C (2007) Unraveling mycorrhiza-induced resistance. Curr Opin Plant Biol 10:393–398
Pursglove JD, Sanders FE (1981) The growth and phosphorus economy of the early potato (*Solanum tuberosum*). Commun Soil Sci Plant Anal 12:1105–1121
Qui YL, Lee J (2000) Transition to a land flora: a molecular phylogenetic perspective. J Phycol 36:799–802
Raja P, Mahadevan A (1991) Axenic cultivation of VAM fungi – a review. J Plant Res 7:1–6
Read DJ, Duckett JG, Francis R, Ligrone R, Russell A (2000) Symbiotic fungal association in lower land plants. Philos Trans R Soc Lond B 355:815–831
Reddy N, Raghavender CR, Sreevani A (2006) Approach for enhancing Mycorrhiza mediated disease resistance of tomato damping-off. Indian Phytopathol 59(3):299–304
Redecker D (2000) Specific PCR primers to identify Arbuscular mycorrhizal fungi (Glomales) with in colonized roots. Mycorrhiza 10:73–80
Regvar M, Vogel-Mikus K, Severkar T (2003) Effect of AMF inoculum from field isolates on the yield of green pepper, parsley, carrot, and tomato. J Folia Geobotanica 38(2):223–234
Reidinger S, Eschen R, Gange AC, Finch P, Bezemer TM (2012) Arbuscular mycorrhizal colonization, plant chemistry, and aboveground herbivory on *Senecio jacobaea*. Acta Oecol 38:8–16
Remy W, Taylor TN, Hass H, Kerp H (1994) Four hundred-million-year-old vesicular Arbuscular mycorrhizae. Proc Natl Acad Sci U S A 91:11841–11843
Rigamonte TA, Pylro VS, Duarte GF (2010) The role of mycorrhization helper bacteria in the establishment and action of ectomycorrhizae associations. Braz J Microbiol 41:832–840
Rillig MC, Mummey DL (2006) Mycorrhizas and soil structure. New Phytol 171(1):41–53
Rillig MC, Wendt S, Antonovics J, Hempel S, Kohler J, Wehner J, Caruso T (2014) Interactive effects of root endophytes and Arbuscular mycorrhizal fungi on an experimental plant community. Oecologia 174:263–270
Robert M, Auge RM, Heather D, Carl F, Sams EA, Ghazala N (2008) Hydraulic conductance and water potential gradients in squash leaves showing mycorrhiza-induced increases in stomatal conductance. Mycorrhiza 18:115–121
Rodríguez Y, de la Noval B, Fernández F, Rodríguez P (2004) Estudio comparativo delcomportamiento de seiscepas de hongosmicorrícicosarbusculares en su interacción con el tomate (*Lycopersicon esculentumMill*. var. “Amalia”). Ecología Aplicada 3:162–171
Rovira AD (1996) Plant root exudates. Bot Rev 35:35–58
Ruiz-Lozano JM (2003) Arbuscular mycorrhizal symbiosis and alleviation of osmotic stress. New perspectives for molecular studies. Mycorrhiza 13:309–317
Santi MM, Dipjyoti C, Satyahari D (2010) Phenolic acids act as signaling molecules in plant-microbe symbioses. Plant Signal Behav 5(4):359–368
Santos BA, Maia LC, Cavalcante UM, Correia MTS, Coelho LCBB (2001) Effect of Arbuscular mycorrhizal fungi and soil phosphorus level on expression of protein and activity of peroxidase on passion fruit roots. Bras J Biol 61(4):693–700
Scharrff AM, Jakobsen I, Rosendahl L (1997) The effect of symbiotic microorganisms on phytoalexin contents of soybean roots. J Plant Physiol 151:716–723
Schellembaum L, Muller J, Boller T, Wienken A, Schuepp H (1998) Effects of drought on non-mycorrhizal and mycorrhizal maize: Changes in the pools of non-structural carbohydrates, in “The activities of invertase and trehalase, and in the pools of amino acids and imino acids”. New Phytol 138:59–66
Schubler A (2002) Molecular phyogeny, taxonomy, and evolution of *Gossiphora pyriformis* and Arbuscular mycorrhizal fungi. Plant Soil 244:75–83
Schwartz MW, Hoeksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbott LK, Pringle A (2006) The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. Ecol Lett 9:501–515
Selosse MA, Richard F, He X, Simard SW (2006) Mycorrhizal networks: des liaisons dangereuses? Trends Ecol Evol 21:621–628
Selvaraj T, Kim H (2004) Ecology of vesicular Arbuscular mycorrhizal (VAM) fungi in coastal areas of India. Agric Chem Biotechnol 47:71–76

Selvaraj T, Chellappan P, Jeong YJ, Kim H (2005) Occurrence and quantification of vesicular Arbuscular mycorrhizal (VAM) fungi in industrial polluted soils. J Microbiol Biotechnol 15(1):147–154

Selvaraj T, Nisha MC, Rajeshkumar S (2009) Effect of indigenous arbuscular mycorrhizal fungi on some growth parameters and phytochemical constituents of Pogostemon patchouli Pellet. Maejo Int J Sci Technol 3(1):222–234

Sensoy S, Demir S, Turkmen O, Erdinc C, Savur OB (2007) Responses of some different pepper (*Capsicum annuum* L.) genotypes to inoculation with two different Arbuscular mycorrhizal fungi. Sci Hortic 113:92–95

Sharma MP, Adholeya A (2000) Benefits of inoculation of indigenous AM fungi upon growth and productivity of four onion (*Allium cepa* L.) varieties in an Alfisol. Biol Agric Hortic 18(1):1–14

Sharma AK, Srivastava PC, Johri BN (1991) Zinc transport in VA mycorrhizal plants. Mycorrhiza News 3:1–4

Shreenivasa KR, Krishnappa K, Rekha D (2011) Interaction effect of arbuscular mycorrhizal fungus, glomus fasciculatum and root knot nematode meloidogyne incognita on biochemical parameters in tomato. IJSN 2(3):534–537

Shuab R, Lone R, Naidu J, Sharma V, Imtiyaz S, Koul KK (2014) Benefits of inoculation of Arbuscular mycorrhizal fungi on growth and development of onion (Allium cepa) plant. Am-Eurasian J Agric Environ Sci 14(6):527–535

Shuab R, Malla NA, Ahmad J, Lone R, Koul KK (2016) Arbuscular mycorrhizal fungal symbiosis with saffron (*Crocus sativus* L.) plant. J New Biol Rep 5(1):59–67

Sieverding E (1991) Vesicular-Arbuscular mycorrhiza management in tropical agrosystems. DeutcheGesellschaftfurrTechnischeZusammenarbeit, GTZ No. 224. Eschborn. 371

Sikes BA, Cottenie K, Klironomos JN (2009) Plant and fungal identity determines pathogen protection of plant roots by arbuscular mycorrhizas. J Ecol 97:1274–1280

Silva MF, Pescador R, Rebelo RA, Sturmer SL (2008) The effect of arbuscular mycorrhizal fungal isolates on the development and oleoresin production of micropropagated Zingiber officinale. Braz J Plant Physiol 20(2):119–130

Simon L, Bousquet J, Levesque RC, Lalonde M (1993) Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363:67–69

Skimmer FA (1985) Agriculture and biotechnology. In: Higgins IJ, Best DJ, Jones J (eds) Biotechnology: principles and applications. Blackwell Scientific Publication

Smith SE (1995) Discoveries, discussions and directions in mycorrhizal research. In: Mycorrhiza: structure, function, molecular biology and biotechnology. Springer, Berlin, pp 3–24

Smith SE, Read DJ (1997a) Mycorrhizal symbiosis. Academic, San Diego

Smith S, Read DJ (1997b) Mycorrhizal symbiosis. Academic, London

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3rd edn. Elsevier Academic Publishers, New York

Smith SE, Smith FA (2011) Roles of Arbuscular mycorrhizas in plant nutrition and growth. New paradigms from cellular to ecosystem scales. Annu Rev Plant Biol 62:227–250

Smith SE, Smith FA (2012) Fresh perspectives on the roles of Arbuscular mycorrhizal fungi in plant nutrition and growth. Mycologia 104(1):1–13

Smith SE, St John J, Smith FA, Nicholas DJD (1985) Activity of glutamine synthetase and glutamate dehydrogenase in *Trifolium subterraneum* L. and *Allium cepa* L.: effects of mycorrhizal infection and phosphate nutrition. New Phytol 99:211–227

Smith SE, Gianinazzi-Pearson V, Koide R, Cairney JWG (1993) Nutrient transport in mycorrhizae; structure, physiology and consequences for efficiency of the symbiosis. Plant Soil 159:103–113

Sohn BK, Kim KY, Chung SJ, Kim WS, Park SM, Kang JG, Rim YS, Cho JS, Kim TH, Lee JH (2003) Effect of the different timing of AMF inoculation on plant growth and flower quality of chrysanthemum. Sci Hortic, v. 98, p. 173-183.

Solanki AS, Kumar V, Sharma S (2011) AM fungi and *Azotobacter chroococcum* affecting yield, nutrient uptake and cost efficacy of *Chlorophytum borivilianum* in Indian Arid Region. J Agric Technol 7(4):983–991

Sorial ME (2001) Growth, phosphorus uptake and water relations of wheat infected with an arbuscular mycorrhizal fungus under water stress. Ann Agric Sci Moshtohor 39(2):909–931
Spanu P, Boller T, Ludwig A, Wiemken A, Faccio A, Bonfante-Fasolo P (1989) Chitinase in roots of mycorrhizal *Alfium porrum*: regulation and localization. Planta 177:447–455
Subramanian KS, Charest C (1995) Influence of Arbuscular mycorrhizae on the metabolism of maize under drought stress. Mycorrhiza 5:273–278
Subramanian KS, Charest C (1997) Nutritional, growth, and reproductive responses of maize (*Zea mays* L.) to arbuscular mycorrhizal inoculation during and after drought stress at tasselling. Mycorrhiza 7:25–32
Subramanian KS, Charest C (1998) Arbuscular mycorrhizae and nitrogen assimilation in maize after drought and recovery. Plant Physiol 102 285–296
Subramanian KS, Jegan RA, Gomathy M, Vijayakumar S (2011) Biochemical and nutritional responses of tripartite soybean Rhizobium-Glomus association under low and high P fertilization. Madras Agric J 98(7–9):224–228
Taber RA, Trappe JM (1982) Vesicular Arbuscular mycorrhiza in rhizomes scale-like leaves, roots and xylem of ginger. Mycologia 74:156–161
Tarafdar JC, Rao AV (2001) Response of cluster bean to *Glomus mosseae* and Rhizobium in an arid soil fertilized with nitrogen, phosphorus and farm yard manure. J Indian Soc Soil Sci 49:751–755
Taylor TN, Remy W, Hass H, Kerp H (1993) Fossil Arbuscular mycorrhizae from the early devonian. Mycologia 87:560–573
Tesdall JM, Oades JM (1979) Stabilisation of soil aggregates by the root system of rye grass. Aust J Soil Sci 17:429–441
Timonen S, Peterson RL (2002) Cytoskeleton in mycorrhizal symbiosis. Plant Soil 244:199–210
Tobar R, Azcon R, Barea JM (1994a) Improved nitrogen uptake and transport from 15N-labelled nitrate by external hyphae of arbuscular mycorrhiza under water-stressed conditions. New Phytol 126:119–122
Tobar R, Azcon R, Barea JM (1994b) The improvement of plant N acquisition from an ammonium-treated, drought-stressed soil by the fungal symbiont in arbuscular mycorrhiza. Mycorrhiza 4:105–108
Tong-jian X, Qing-song Y, Wei R, Guo-hua X, Qi-rong S (2010) Effect of inoculation with Arbuscular mycorrhizal fungus on nitrogen and phosphorus utilization in upland rice-mungbean intercropping system. Agric Sci China 9(4):528–535
Treseder KK, Turner KM (2007) Glomalin in ecosystems. Soil Sci Soc Am J 71:1257–1266
Upadhyaya H, Panda SK, Bhatacharjee MK, Dutta S (2010) Role of Arbuscular mycorrhiza in heavy metal tolerance in plants: prospects for phytotolerance in plants: prospects for phytoremediation. J Phytology 2(7):16–27
Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72
VanEtten HD, Mansfield JW, Bailey JA, Farmer JA (1994) Two classes of plant antibiotics: phytoalexins versus "Phytoanticipins". Plant Cell 6:1191–1192
Vazquez-Prieto MA, Miatello RM (2010) Organosulfur compounds and cardiovascular disease. Mol Aspects Med 31(6):540–545
Verma NK (1998) Effect of VA mycorrhiza on the growth and P uptake in *Eupatorium adenophorum* Spring. (Asteraceae) grown in soil amended with soluble phosphate. J Natl Bot Soc 52:41–45
Vierheiling H, Alt M, Mohr U, Boller T, Wiemken A (1994) Ethylene biosynthesis and activities of chitinases and β-1, 3 – glucanase in the roots of host and non host plants of vesicular- Arbuscular mycorrhizal fungi after inoculation with *Glomus mosseae*. J Plant Physiol 143:337–343
Volpin H, Elkind Y, Okon Y, Kapulnik Y (1994) A vesicular –arbuscular mycorrhizal fungus (*Glomusintraradix*) induces a defense response in mycorrhizal alfalfa roots. Plant Physiol 108:1449–1454

Ward NI, Stead K, Reeves J (2001) Impact of endomycorrhizal fungi on plant trace element uptake and nutrition. Nutr Pract 32:30–31

Wehner J, Antunes PM, Powell JR, Mazukatow J, Rillig MC (2009) Plant pathogen protection by arbuscular mycorrhizas: a role for fungal diversity? Pedobiologia. doi:10.1016/j. pedobi. 2009.10.002

Wellman CH, Osterloff PL, Mohiuddin U (2003) Fragments of the earliest land plants. Nature 425:282–285

Whipps JM (2004) Prospects and limitations for mycorrhizas in biocontrol of root pathogens. Can J Bot 82:1198–1227

Wilson GWT, Rice CW, Rillig MC, Springer A, Hartnett DC (2009) Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. Ecol Lett 12:452–461

Wood T (1992) VA mycorrhizal fungi: challenges for commercialization. In: Arora DK, Elander P, Mukerji KG (eds) Handbook of applied mycology, fungal biotechnology IV. Marcel Dekker Inc., New York, pp 823–848

Wu Q, Xia R (2006) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J Plant Physiol 163:417–425

Zhang R, Zhub H, Zhao H, Yao Q (2013) Arbuscular mycorrhizal fungal inoculation increases phenolic synthesis in clover roots via hydrogen peroxide, salicylic acid and nitric oxide signaling pathways. J Plant Physiol 170(1):74–79

Zhongqun H, Chaoxing H, Zhibin Z, Zhirong Z, Huaisong W (2007) Changes of antioxidative enzymes and cell membrane osmosis in tomato colonized by arbuscular

Zhu HH, Yao Q (2004) Localized and systematic increase of phenols in tomato roots induced by *Glomus versiforme* inhibits *Ralstonia solanacearum*. J Phytopathol 152:537

Microbial Options for Improved Crop Production

26

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Abstract

Crop plants are always unceasingly confronted by the biosphere around them, so if we want to improve crop production, it becomes obligatory to reinforce agricultural production in a workable manner and to find solutions to handle various problems like environmental stress responses and nutrient availability. Furthermore, due to increase in cost of synthetic fertilizers and chemical pesticides, the crop production has been reduced to a drastic level, and also these chemicals are being added to the food chain which is having very adverse effect on flora and fauna because of their toxicity. So, alternative methods have been looked upon by the researchers to find effective solutions, and, thus, the approach of using microflora for crop improvement programs offers a better, cost-effective, and eco-friendly answer to almost all the existing problems in modern agriculture. Plant growth-promoting microorganisms could be applied to the crops for getting mass production, but strategies should be made for their own regulated production programs so that better results could be obtained and this technique become more practical, feasible, and available for the farmers.

26.1 Introduction

Human beings and animals are largely dependent on the plants for meeting their energy requirements. Also, continuous change in the environmental conditions due to climate change and global warming is having adverse effects on agricultural

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crops in commercial sectors. Conditions for plants become very drastic which are not suitable for their survival. So, at this stage, i.e., in the changing environment, it becomes essential to improve our agriculture system in such a way that we should not just aim for higher production but should also be looking for betterment in plant protection and adaptability, simultaneously.

In the recent decade, we have seen huge increase in production in the agriculture sector which is largely attributed to the extensive use of synthetic chemicals (Oerke 2006), but this is not a long-term strategy for getting sustainable production. Due to high consumer pressure and new policies made by regulatory authorities, the withdrawal of these chemicals had been seen on a larger scale in order to reduce toxic residue in soil. In addition to this, the production, development, and registration cost for these synthetic chemicals has been inclining very rapidly (Glare et al. 2012), which is further limiting available control strategies for the growers. The pursuit for substitute solutions for agriculture has encouraged researchers to have a second look at the variety of microorganisms, recognized earlier to impart assistances to agricultural production, and is providing options for biocontrol agents (Lehr 2010) and plant growth-promoting microbes (Berg 2009). In spite of the purpose for these microorganisms applied to crops, they must be produced at commercial or large scale and used in a way that maintains their activity and functionality in the target conditions also. So far, these formulations are available as liquids (sprays, drenches, root dips) mainly for experimentation or as dehydrated powder forms, which are supplied at the time of plantation. But most of these strategies do not work on mass production scale, due to a huge amount of microbial inoculum which is required.

The beneficial microbes can be applied to the seeds for placing the microbial flora into the soil, where they will start colonizing and will provide protection by interacting against different insects and pests which are feeding on plant roots and reducing the yield. In addition, they will also synthesize essential nutrients which are required for proper growth and development of plants. This is not a new technique and is already established and demonstrated at small scale by different researchers in different conditions (Graham and Vance 2003). However, despite the fact that they have been well demonstrated and available in various formulations in association with legume crops, they are still not used on commercial scale by the farmers due to lack of knowledge and interests.

Plant-microbiome interactions represent a very promising solution for providing protection and improving agricultural yield sustainably. In this chapter, we tried to merge the fundamental basics and applied aspects of beneficial plant-microbial interactions effectively. This is our humble approach and sincere effort for advancing the agriculture by providing details about available microbial solutions (Fig. 26.1).

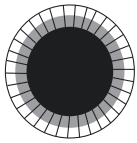
	Bioprimed	*Film coated*	*Slurry coated*	*Pelleted*
	 *Inoculant within seed*	 *Inoculant in thin layer on seed surface*	 *Inoculant in (peat) carrier stuck to outside of seed*	 *Inoculant applied to seed along with conventional seed additives*
Method	Seed soaked in saline / inoculant suspension	Inoculant suspended (e.g. sugar. methyl cellulose) and dried	Inoculant grown in solid carrier medium applied to seed using sticker Often dusted with lime to ensure flowability	Typical commercial process
Utility	Experimental limited commercial use	Mainly for experimental use only	Widely used for rhizobial inoculants prior to sowing	Not yet but desired by seed companies and growers
Inoculant survival	Good long term survival	Short term survival	Variable	Poor survival unless resistant (spore-former) inoculants used

Fig. 26.1 Methods and preparations available for microbial seed inoculation

26.2 Microorganisms for Sustainable Plant Growth

Plants are unsurprisingly accompanying multifaceted microbiomes, which are known to boost plant growth and stress tolerance, backing plant nutrition and antagonizing plant pathogens. The main properties of microorganisms for subsidiary plant growth and development are shown in Fig. 26.2.

26.2.1 Harmonizing Soil Ecology

Microorganisms are a fundamental part of almost every soil ecosystem. Soil is a hub of various kinds of biological and biochemical activities, and most of them are carried out by microorganisms. Metabolic activities of PGPR, mycorrhiza, cyanobacteria, and certain soil fauna have been reported to improve soil health and increase crop productivity.

Most of the beneficial microorganisms need carbon as a sole source of energy, so that's why it has been observed that the soils which are poor in organic matter have less microbial activities. Moreover, the extensive use of synthetic fertilizers, insecticides, and pesticides in the last decades further lowered down the level of

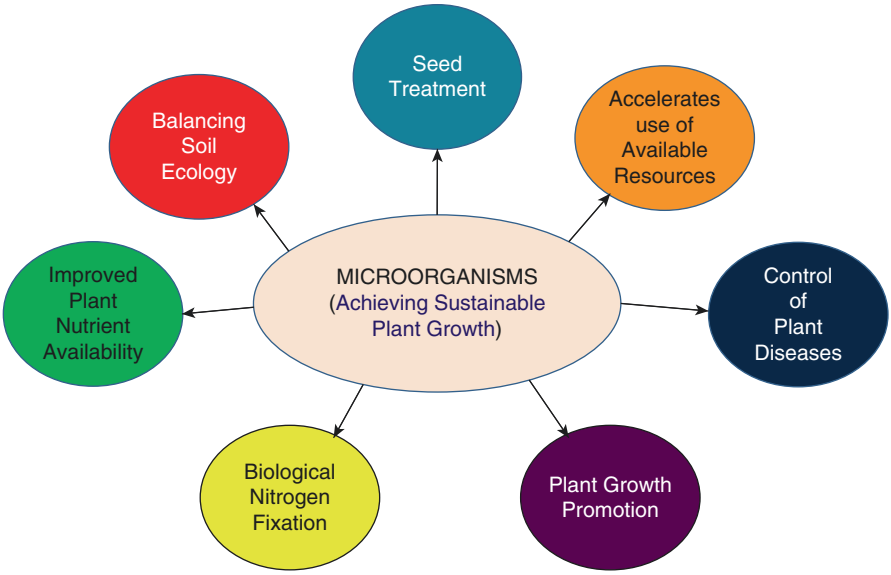


Fig. 26.2 Role of microorganism in plant growth

organic matter and even worsens the situation by having adverse effects on microbial activities. This led to decrease, or in many cases, a number of beneficial soil microorganisms such as PGPR, fungi, earthworms, actinomycetes, etc. have been extinct from those areas. Similar kind of situation prevails in almost all parts across the country.

26.2.2 Biological Fixation of Nitrogen

Nitrogen is the most abundant gas on earth, but it is impossible to use nitrogen in its gaseous form by the plants. Also, nitrogen is a primary essential macronutrient which is required by the plants and is a part of most of the biomolecules which has a role in physiological function and metabolism. There are many microorganisms which have the potential to convert gaseous nitrogen to its usable form that is nitrates through a process called as biological nitrogen fixation (BNF). BNF is the main process and source of nitrogen for legumes and other important crops. BNF provides the largest input of nitrogen to agricultural soils worldwide. *Rhizobium* inoculation as biofertilizer in the crops like groundnut, pigeon pea, soybean, etc. reported to provide 19–22 kg of nitrogen per hectare with 17–33% of total increase in crop yield. Likewise, the use of *Azotobacter*, which is a nonsymbiotic bacterium, and *Azospirillum* in wheat, sorghum, tomato, cotton, and sugarcane contributed nitrogen supply to crops to an extent of 20–30 kg per hectare providing

10–30% increased crop yield. Wherever water, sunlight, and carbon dioxide are available, phototrophic microorganisms like blue-green algae or cyanobacteria can grow. Therefore, rice ecosystem provides an ideal environment for the growth and development of these self-supporting organisms such as *Anabaena*, *Nostoc*, *Aulosira*, *Calothrix*, *Tolypothrix*, etc. They colonize the rice field soils, compete well with the native strains, thus grow profusely near the rhizosphere, and release fixed nitrogen through exudation or through microbial decomposition after the algae dies. So, in rice fields, the degradation of algal biomass most frequently results in maintenance of soil fertility. The residual effects influence the succeeding crops also.

Apart from fixing nitrogen and adding organic matter to soil, BGA are also known to produce and excrete plant promoting substances like indoleacetic acid. Also, the continuous use of the BGA biofertilizers for 2–3 years adequately builds up the population of these organisms in the soil. The relative contribution of BGA as a percentage of total nitrogen fixed in paddy fields varies widely and is estimated to be 15–35 kg nitrogen per hectare in India. In areas where chemical nitrogen is not used for various reasons, algal inoculation enhances minimum of 4% to a maximum of 32.8% crop yield with an overall average of 16.1%. Even at the levels of chemical nitrogen fertilizers being used in different states, the application of BGA biofertilizers resulted in an increased crop yield of 8.85%. Plant growth-promoting rhizobacteria (PGPR) are low-cost input from nature; besides nitrogen fixers, many bacteria colonize plant roots. Some of them promote plant growth significantly. They help in mobilization of the soil nutrients and production of phytohormones or growth-regulating substances. These phytohormone-producing microbes have been classified as PGPR. Of the many such bacteria identified, the role of fluorescent *Pseudomonas* and *Bacillus* species has attracted much attention. The substances produced by them have natural biocontrol and plant growth-promoting capabilities. Increased amount of nutrient uptake by plants inoculated by *Pseudomonas putida* has been attributed to the production of growth regulators by the bacterium at root surface which stimulates root development. Pseudomonad (group of *Pseudomonas* sp.) inoculants produce indoleacetic acid-like substances (plant hormone) in the rhizosphere of wheat grown in field conditions.

Many PGPR, for example, *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*, also produce substances such as siderophores and saponins, which are responsible for the removal of heavy metal toxicity. These organisms are also responsible for enhancement of rhizospheric competitive ability by antagonistic effects on other harmful bacteria; control of plant diseases that affect root density; and production of chemicals that interfere with the organisms infecting plant roots, enhancing the availability of nutrients that improve the efficacy of plants. PGPR are therefore being widely evaluated for their role in sustainable resource management as biocontrol agent and biofertilizer (Table 26.1).

Table 26.1 Growth-promoting substances released by some important plant growth-promoting bacteria

Beneficial bacteria	Growth-promoting properties/compounds
Pseudomonas putida	Siderophores, IAA, ammonia, phosphate solubilization, HCN, exo-polysaccharides
Pseudomonas aeruginosa	Siderophores, IAA, ammonia, phosphate solubilization, HCN, exo-polysaccharides
Klebsiella sp.	Siderophores, IAA, ammonia, phosphate solubilization, HCN, exo-polysaccharides
Enterobacter asburiae	Siderophores, IAA, ammonia, phosphate solubilization, HCN, exo-polysaccharides
Mesorhizobium sp.	HCN, ammonia, IAA, exo-polysaccharides, siderophores
Acinetobacter sp.	Phosphate solubilization, IAA, siderophores
Rhizobium sp.(pea)	HCN, ammonia, IAA, exo-polysaccharides, siderophores
Rhizobium sp.(lentil)	HCN, ammonia, IAA, exo-polysaccharides, siderophores
Pseudomonas sp. A3R3	IAA, siderophores
Psychrobacter sp. SRS8	Heavy metal mobilization
Bradyrhizobium sp.	HCN, ammonia, IAA, exo-polysaccharides, siderophores
Pseudomonas aeruginosa 4EA	Siderophores
Bradyrhizobium sp.750	Heavy metal mobilization
Bacillus species PSB10	Ammonia, IAA, siderophores, HCN
Paenibacillus polymyxa	Siderophores, IAA
Rhizobium phaseoli	IAA
Stenotrophomonas maltophilia	Nitrogenase activity, phosphate solubilization, IAA, ACC deaminase
Rahnella aquatilis	ACC deaminase, phosphate solubilization, IAA
Proteus vulgaris	Siderophores
Pseudomonas sp.	Siderophore, phosphate solubilization, IAA
Azospirillum amazonense	Biocontrol potentials, nitrogenase activity, HCN, IAA
Mesorhizobium sp.	IAA, siderophores, HCN, ammonia
Pseudomonas sp.	ACC deaminase, IAA, siderophore
Serratia marcescens	IAA, siderophore, HCN
Pseudomonas fluorescens	ACC deaminase, phosphate solubilization
Enterobacter sp.	Phosphate solubilization, siderophore, N2 fixation, phosphate solubilization, ACC deaminase, IAA

IAA indole-3-acetic acid, *HCN* hydrogen cyanide, *ACC* 1-aminocyclopropane-1-carboxylate

26.3 Increasing the Potential Gain of Available Resources

Mycorrhizae play a dominant role in making unavailable soil nutrients available to plant roots and increasing the potential gain of available resources. These organisms ensure easy availability of organic carbon and complex organic nitrogen and phosphorus sources and increase phosphorus solubilization and availability in clay soils. These fungi work upon large volumes of soil. Their hyphae extend outwardly from

the roots ranging from a few centimeters to several meters in the soil. This results in increasing the effective absorbing surface of the host root by as much as ten times, resulting in enhanced absorption of immobile nutrients such as phosphorus, zinc, copper, etc. in the soil by 60 times.

Mycorrhizal fungi also transport many other nutrients including calcium, magnesium, sodium, sulfur, iron, chlorine, etc., all essential for plant growth and development. It has been reported that plants with mycorrhizal association are more tolerant to heavy metal toxicity. These plants survive well in drought and arid conditions as improved water movement is facilitated by mycorrhiza. Theoretically, the most efficient level of nutrients is the concentration of mineral elements in the plant tissue just above the "critical level" necessary for optimum growth. Further addition of chemical fertilizers may be taken up by plants, as "luxury concentration." This adds very little to plant growth. Now, these microorganisms help in constituting the "optimum level" of minerals in the plant tissue even at low level of fertilizer inputs. They fix nitrogen, solubilize phosphorus, and facilitate uptake of minerals by roots. Thus, these microorganisms in the form of biofertilizers are essential for maintaining good soil fertility, better soil conditions, and sustainable agricultural productivity.

26.4 Seed Treatments

There is a growing curiosity in the use of soil microorganisms which are beneficial for plant development as potential substitutes to synthetic fertilizers and pesticides in agricultural production.

Seed inoculation techniques developed for research purposes are often not possible to be implemented at a commercial scale because of significant obstacles or challenges like technical aspects for maintaining viable microbial inocula throughout complete seed treatment process and seed storage. Further research advances in these technologies are required for imparting benefits of a wide range of environmentally sensitive potential seed inoculants in the field of agriculture.

Presently, there are no solutions available for commercialization of seed inoculation treatments at commercial scale. So, there is an urgent need for association of scientific fields like soil science, microbiology, biotechnology, agriculture, and adjuvant chemistry to develop a sustainable protocol for making these technologies commercially viable and available to farmers.

26.5 Control of Plant Diseases and Plant Growth Promotion

Seeds are exposed to fungicides and bactericides in order to prevent crop failure because of seed- or soil-borne pathogens. Normally these treatments are chemical techniques which are cytotoxic; it means they can also have adverse effects on the viability of seeds and their germination potential. Microbial inocula, which are antagonistic to soil-borne pathogens, is an ideal delivery system as it directly introduces inoculum to the rhizosphere of the plant where plant pathogens like *Pythium*

and *Rhizoctonia* are active, causing diseases like seed rots in the spermosphere and damping-off disease in seedling.

Various bacterial and fungal antagonists have been identified and developed experimentally and commercially for this purpose (Butt and Copping 2000; Nelson 2004; Berg 2009), but their use as seed treatments is still very limited (McQuilken et al. 1998).

26.6 Challenges in the Development of Microbial Inoculant Seed Treatments

In the past decades, so much of research have been done on importance of microorganisms which are beneficial to agriculture; only few of them are used, for instance, the cropping systems in the plants like cotton. Many of beneficial microorganisms have been reported to act as biofertilizers and biocontrol agents, but still not even a single product is marketed as microbial seed treatment on commercial purpose. Currently, our objective is to treat seeds with inoculants to increase their cost effectiveness and time effectiveness and to gain more and more profit, but there is still no permanent solution available to cope up with these problems and meet up the improvement criteria. There are certain seed companies which are treating legume seeds on demand. If we want to remove the problem of increasing shelf life of inoculum, then the seeds are exposed to microbial inoculum just before sowing. But still in the present scenario, farmers and companies prefer pre-inoculated seeds often months prior to sowing rather than using inoculation just before sowing. Commercial seed treatments which are available are having almost similar shelf life as that of non-inoculated seeds, but the problem related to shelf life of microbial inocula still persists. Accomplishment of certain commercialized products like Cedomon®-based treatments on *P. chlororaphis* (BioAgri AB) may be related to microbial compatibility. Researchers reported and demonstrated that seeds treated with Cedomon® can withstand prolonged storage, transportation, and handling exactly similar as that of untreated and traditionally treated seeds. In addition to practical expectations by farmers, the companies, which are dealing with the production of such seeds, expect lower production costs, increased shelf life of seed and microbial inocula, and broader applicability and availability. In order to meet these ever-rising expectations, there are so many obstacles and challenges, but these also offer huge amount of opportunities to develop novel methods for treatment of seeds with microbes, as listed below:

1. Economical production microbial inocula
2. Formulation and seed treatment processes
3. Shelf life and storage conditions
4. Quality control
5. Product safety and registration
6. Consistent field performance
7. Microbial compatibility issues

References

Berg G (2009) Plant-microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Appl Microbiol Biotechnol 84:11–18

Butt TM, Copping LG (2000) Fungal biological control agents. Pesticide Outlook – October 2000. http://www.researchinformation.co.uk/pest/2000/B008009H.PDF. Accessed 25 Apr 2016

Glare T, Caradus J, Gelernter W, Jackson T, Keyhani N, Köhl J, Marrone P, Morin L, Stewart A (2012) Have biopesticides come of age? Trends Biotechnol 30:250–258

Graham PH, Vance CP (2003) Legumes: importance and constraints to greater use. Plant Physiol 131:872–877

Lehr P (2010) Biopesticides: the global market. Report code CHM029B, BCC Research, Wellesley

McQuilken MP, Halmer P, Rhodes DJ (1998) Application of microorganisms to seeds. In: Burges HD (ed) Formulation of microbial biopesticides: beneficial microorganisms, nematodes and seed treatments. Kluwer Academic Publishers, Dordrecht, pp 255–285

Nelson EB (2004) Biological control of oomycetes and fungal pathogens. In: Goodman RM (ed) Encyclopedia of plant and crop science. Marcel Dekker, USA, pp 137–140

Oerke EC (2006) Crop losses to pests. J Agric Sci 144:31–43

Plant Growth-Promoting Rhizobacteria: A Probiotic for Plant Health and Productivity

27

S.K. Gosal, Jupinder Kaur, and Jaspreet Kaur

Abstract

Probiotics are beneficial microorganisms that provide health benefits when consumed in adequate amount. The positive influence of probiotics is not confined to human health sector; even the plants can get benefit from the microbes residing in their native habitat. The beneficial microorganisms which exert health-promoting and nutritional benefits on plants are called plant probiotics. Plant growth-promoting rhizobacteria (PGPR) are right choice to be used as probiotics for agricultural crops as they not only stimulate plant growth and productivity but they also protect plants from diseases and various types of stresses. Plant probiotic PGPR facilitate the plant growth directly by helping in acquisition of essential nutrients and production of phytohormones or indirectly by acting as biocontrol agent. Beneficial effects of inoculation of plant probiotics in agricultural crops include increases in yield, chlorophyll content, protein content, nutrient uptake, and seed germination rate, improved soil health, biocontrol, tolerance to abiotic stress, etc. The use of plant growth-promoting rhizobacteria as plant probiotics contributes to increasing the agronomic efficiency by improving plant health and productivity in an environmental friendly and sustainable manner, decreasing dependence on chemical fertilizers. The use of plant growth-promoting rhizobacteria with probiotic potential as microbial inoculants will be a sustainable approach to improve plant health and productivity in an eco-friendly manner.

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27.1 Introduction

Soil is a dynamic, living, natural body that is very important to the function of terrestrial ecosystems. It represents a unique balance between different physical, chemical, and biological factors. It is a nonrenewable resource whose condition influences food production, environmental efficiency, and global balance. Different bacterial genera are vital components of soil and are involved in various activities of the soil ecosystem. They are dominant driving forces in recycling the nutrients, and consequently, they are crucial for plant health, plant productivity, and soil fertility. The bacteria present in the rhizosphere or colonizing any plant part are more efficient and versatile to carry out transformation and mineralization of the nutrients as compared to the bacteria present in bulk soils. Nowadays, the biological approaches for improving plant health and crop production are gaining strong status among agronomists and environmentalists due to negative impacts of extensive use of chemical fertilizers. In this context, there is an ongoing research with a greater emphasis on a wide range of beneficial rhizobacteria possessing novel traits like degradation of pesticides, detoxification of heavy metals, salinity tolerance, and biocontrol of various plant pathogens along with the plant growth-promoting activities such as production of growth hormones, siderophore (iron chelator) production, production of hydrogen cyanate, ammonia production, nitrogenase activity, and phosphate solubilization, (Ahemad and Kibret 2014) to use them as probiotics for the improvement of plant health and productivity. Hence, diverse symbiotic (*Rhizobium*, *Mesorhizobium*, *Bradyrhizobium*), associative (*Azospirillum*), and nonsymbiotic (*Pseudomonas*, *Bacillus*, *Klebsiella*, *Azotobacter*, *Azomonas*) rhizobacteria are now being used worldwide as plant probiotics to promote plant growth and development under various stresses (Ahemad and Khan 2011). In this chapter, we will elucidate the concept of beneficial rhizobacteria as probiotics, the underlying mechanisms of plant growth promotion, and the positive influence of their inoculation on soil fertility, plant health, and plant productivity.

27.2 Rhizosphere

The region of soil surrounding the root system of plant is called rhizosphere and this term was first introduced by Hiltner (1904). Rhizosphere is also known as a storehouse of microbial activity as it is a rich source of microbes and microbial activity. The term “rhizobacteria” is widely used for a group of useful bacteria present in rhizosphere competent in colonizing the root environment. The roots of plant provides mechanical support and facilitates nutrient as well as water uptake. Apart from this, plant roots also synthesize, accumulate, and secrete a diverse array of compounds commonly known as root exudates. The compositions of these exudates change with the physiological status and species of plants (Kang et al. 2010). The wide range of chemical compounds (sugars, flavonoids, amino acids, etc.) secreted

as root exudates modifies the physical and chemical properties of soil and, thus, regulates the structure of microbial communities of soil present in the rhizospheric region (Dakora and Phillips 2002). Thus, the composition of rhizo-microbiome (microbes present in rhizosphere) is distinct from that of the microbial community of the surrounding soil or bulk soil. Some of the compounds secreted by plant roots act as repellants against microorganisms, while others act as attractants to lodge the microbes.

The microorganisms and their products also interact with plant roots in a variety of ways. Microbes can show positive, negative, and neutral interaction with plants. Microbial activity in the rhizosphere affects patterns of roots and the supply of various essential nutrients to plants, thereby modifying the quantity and quality of root exudates. Such interactions can influence growth and development of plants, modify nutrient dynamics, and alter a plant's susceptibility to disease and abiotic stress. These beneficial rhizobacteria can be exploited to improve the health and productivity of crop plants.

27.3 Plant Probiotics

Probiotics are generally defined as a food or dietary supplement containing live bacteria for therapeutic reasons. Yogurt, sauerkraut, dark chocolate, and pickles are the foods that contain beneficial probiotics which help in digestion along with other health benefits. The importance of probiotics is mostly focused on the human food, but the ongoing research related to agriculture is focusing on the relationship between probiotics and plants. The ability of plants to adapt genetically to rapid changes in environment (nutrient deficiency, heat, toxins, and droughts) is very less. So, they may use microorganisms that have the ability to rapidly evolve because of their vastly shorter life cycles. Soil is rich with amazing diversity of microbes. A broad range of plant species can benefit from the microbes residing in their native habitats. By having the right microbes for the conditions, the plants can be healthier and more productive. This is similar to humans taking probiotics to improve their health. Studies conducted by various researchers have proved that the inoculation of beneficial bacteria to soil can reduce the dependence on chemical fertilizers in addition to the stimulation of plant growth and productivity. The microbial inoculants help the plant not only with nutrients, but they increase growth and tolerance to stresses.

27.4 Plant Growth-Promoting Rhizobacteria

A number of beneficial microorganisms that promote the growth of plants are found in the rhizosphere. Bacteria that colonize plant roots or any other plant part which enhance plant growth and protect it from diseases and abiotic stresses through a wide variety of mechanisms are referred to as plant growth-promoting rhizobacteria

(PGPR). The term PGPR was first coined by Kloepper et al. in 1980. The plant growth-promoting rhizobacteria are characterized by these three intrinsic characteristics:

(a) They must be able to colonize the root or any other plant part.
(b) When used as probiotic (bio-inoculant), they must survive, multiply, and compete with other native microflora at least for the time needed to express their plant promotion activities.
(c) They must promote plant growth.

Nowadays, agricultural production is dependent on the large-scale use of chemical fertilizers. Chemical fertilizers have become integral and necessary components of modern agriculture because they provide essential plant nutrients (macro- as well as micronutrients) such as nitrogen (N), phosphorus (P), and potassium (K). However, the excess use of chemical fertilizers for increased crop production leads to harmful environmental impacts (Adesemoye et al. 2009). The use of efficient PGPR inoculants is an important strategy to achieve maximum benefits in terms of fertilizer savings and better growth and for reducing environmental problems caused by the use of chemical fertilizers (Hungria et al. 2013).

27.5 PGPR as Probiotics

Plant growth-promoting rhizobacteria possess the ability to improve plant growth and productivity as they provide various essential nutrients (macro- as well as micronutrients) to plants. They are good candidates to be used as probiotics for plants as they live in close association with plants and possess all the traits of good inoculant. So, PGPR act as probiotic for plants. PGPR have gained considerable interest in research as plant probiotics because they stimulate plant growth, increase crop yield in an environment friendly and sustainable manner, and reduce the cost of chemical fertilizers. The use of PGPR as probiotic for plants is a better way to grow plants with reduced pollution from fertilizers and pesticides.

27.6 Types of Probiotic Plant Growth-Promoting Rhizobacteria

The plant beneficial rhizobacteria act as probiotic both for plant and soil because in addition to improving plant health they also improve the fertility and texture of soil. Plant growth-promoting rhizobacteria can interact with plant roots through different types of associations with different degrees of proximity with the roots and the surrounding soil (Fig. 27.1). Depending upon the association of the bacteria with plant, the PGPR may be categorized into following:

1. **Endophytic**
2. **Symbiotic**
3. **Associative**
4. **Free living**

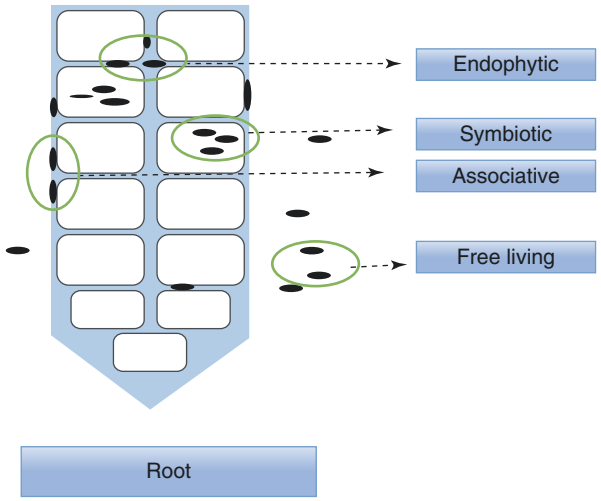

Fig. 27.1 Schematic illustration of different types of associations between plant roots and PGPR

27.6.1 Endophytic PGPR

Endophytic bacteria are the bacteria that live in plant tissue. These bacteria do not cause any harm to their host plant in which they reside and establish mutualistic association (Badri et al. 2009). Plant constitutes vast and diverse niches for endophytic PGPR and these rhizobacteria are found in all species of plants. Endophytes have low population density and constitute bacterial populations different from those encountered in the rhizosphere and the soil, thus indicating that there is selection of the bacteria that may inhabit plants. Endophytic bacteria are good inoculant candidates to be used as probiotics, because they colonize roots, promote plant growth, and create a favorable environment for development and function.

27.6.2 Symbiotic PGPR

Symbiosis is a close ecological relationship and was first defined by Anton de Bary in 1869 in a work entitled "Die Erscheinung der Symbiose." Symbiotic PGPR live in symbiosis with another organism or plants. This kind of interaction is beneficial for both the partners. Rhizobia–legume symbiosis is one of the examples, and this has been reported to provide over half of the biological source of fixed nitrogen and is the primary source of fixed nitrogen in land-based systems. Symbiotic PGPR are in obligate relationship with plants. These can also be used as probiotics as they are beneficial to the host plant.

27.6.3 Associative PGPR

Associative rhizobacteria are loosely associated with plants and are widespread in soil. Beijerinck described an associative nitrogen-fixing PGPR under the name

Spirillum lipoferum in 1925. The nomenclature of this PGPR was revised and designated as *Azospirillum*. Various enrichment procedures are available for the isolation of associative rhizobacteria from plant roots as well as soil samples. These associative PGPR possess various plant growth-promoting traits that made them probiotic for various agricultural crops.

27.6.4 Free-Living PGPR

Free-living PGPR have independent existence in soil and work without the assistance of any host plant. The most common and widely studied free-living PGPR with probiotic potential is *Azotobacter*. It is a nonsymbiotic nitrogen fixer along with cyanobacteria. Beijerinck was the first to isolate and describe *Azotobacter*. Free-living rhizobacteria act as probiotic for soil because their inoculation results in improved soil health and quality. Apart from the *Azotobacter*, the other beneficial free-living PGPR that can also be used as probiotics for plants due to their plant growth-promoting activities include *Pseudomonas*, *Bacillus*, *Klebsiella*, *Azomonas*, etc.

27.7 Probiotic PGPR: Mechanism of Action

There are a wide range of mechanisms by which PGPR act as probiotic for the growth of plants and improve productivity. These mechanisms are divided into two categories—direct and indirect mechanisms (Fig. 27.2). Probiotic PGPR stimulate plant growth directly by providing the plant with any compound which is

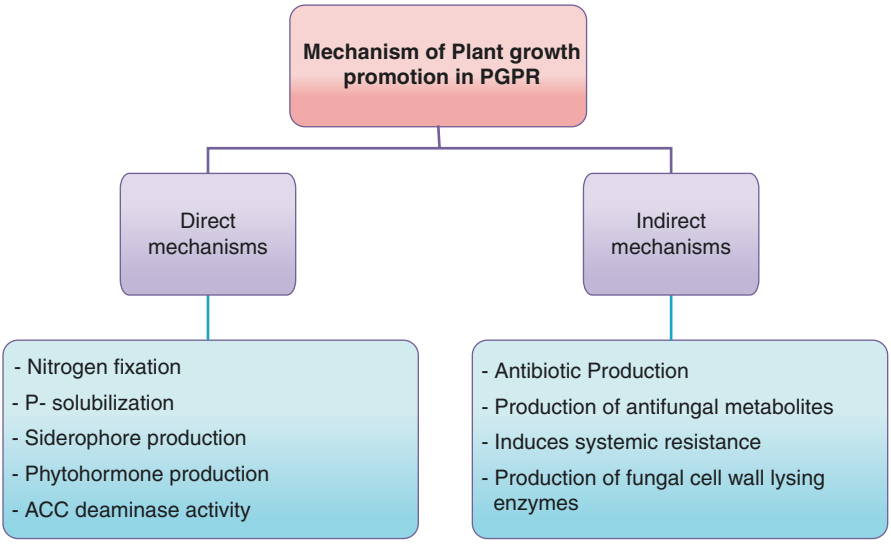

Fig. 27.2 Mechanisms of plant growth promotion in PGPR

synthesized by the bacterium, for example, growth-enhancing hormones (phytohormones), or facilitating the uptake of essential nutrients like N, P, and K from the environment (Glick 1995). These rhizobacteria promote the growth of plants in an indirect way also. In case of indirect promotion of plant growth, PGPR lessen or prevent the negative effects of one or more pathogenic organisms that are harmful to plants. This can happen by producing antagonistic substances or by inducing resistance to pathogens (Glick 2012). There is no single mechanism for promoting the growth of plants. The mechanisms by which bacteria can influence plant growth differ among species and strains. The use of PGPR as probiotic inoculants offers an environmentally sustainable approach to increasing crop productivity and soil health as we can select efficient beneficial microbes involved in biological nitrogen fixation, solubilization of insoluble phosphates, production of plant growth hormones, and disease suppression. Beneficial effects of PGPR inoculation in agricultural crops include increases in yield, chlorophyll content, protein content, nutrient uptake, seed germination rate, and leaf area, delayed senescence, biocontrol, tolerance to abiotic stress, etc. (Bashan et al. 2004).

27.7.1 Direct Mechanisms

Most of the agricultural soils suffer from the deficiency of one or more essential nutrients. Deficiency of essential nutrients in soil made the soil unsuitable for crop production as plant growth will be suboptimal. To get higher productivity and reduce this problem, farmers are extensively using chemical fertilizers as the sources of macro- as well as micronutrients (especially nitrogen and phosphorous). Chemical fertilizers are expensive and their production depletes natural resources. Indiscriminate use of chemical fertilizers also poses human and environmental hazards. It would be economical and advantageous, if efficient biological means can be used to provide essential nutrients to plants. By this way, we can substitute for at least a portion of the chemical fertilizers that is currently used. These mechanisms positively influence the plant growth activity directly. So, the direct mechanism of PGPR is the major step involved to support growth and development of plants. Direct mechanism includes fixation of atmospheric nitrogen, solubilization of inorganic phosphate, production of growth hormones, and increase in iron availability.

27.7.2 Indirect Mechanisms

The major indirect mechanism of plant growth promotion by probiotic rhizobacteria is through acting as biocontrol agents (Glick 2012). There are various modes of biocontrol activity in plant probiotics. It includes antibiotic production, competition for nutrients, niche exclusion, antifungal metabolite production, and induced systemic resistance. Many rhizobacteria with probiotic potential have been reported to produce antifungal metabolites like hydrogen cyanide, pyrrolnitrin, DAPG (2,4-diacetylphloroglucinol), pyoluteorin, and tensin (Bhattacharyya and Jha 2012).

Interaction of some plant probiotics with the roots of plant can result in plant resistance against some pathogenic organisms which are harmful to plants. This phenomenon is called induced systemic resistance (Lugtenberg and Kamilova 2009). Thus plant growth-promoting rhizobacteria function as probiotics for plants as they improve the plant growth and productivity by combination of various direct and indirect mechanisms.

27.8 Inoculation of PGPR as Probiotics

Microbial inoculants popularly known as "biofertilizers" or "bio-inoculants" are substances which contain living microorganisms which, when applied to seed, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and promote growth by increasing the supply or availability of primary nutrients to the host plant. Biofertilizers have a natural mechanism to supply nutrients to plants by nitrogen fixation, phosphorus solubilization, and synthesis of plant growth-promoting substances. The microbes present in biofertilizers increase the soil natural nutrient cycle and help in building soil organic matter and maintain the soil fertility. PGPR have gained worldwide acceptance as plant probiotics and are one of the preferred microorganisms to be used as microbial inoculants. The main advantage of using PGPR as microbial inoculants for probiotic action is that they are cheaper and safer than chemical pesticides. In order to have beneficial bacteria to act as probiotic for plants, it is essential to introduce them into the soil. PGPR strains when inoculated to soil act as probiotics and positively influence the growth of plant as well as soil health. The means by which PGPR act as probiotic for plant health and crop productivity is by acting as biofertilizer for growth promotion and biocontrol agent for controlling disease management.

27.9 Impact of Inoculation of PGPR with Probiotic Potential on Plant Health and Crop Productivity

The beneficial bacteria which exert health-promoting and nutritional benefits are termed as plant probiotics. PGPR have been documented to promote plant health and productivity by acting as probiotic for plants. The growth stimulation by the plant probiotics can be a result of nitrogen fixation, phosphate solubilization, production of phytohormones, biocontrol of phytopathogens in the root zone, etc. The probiotic potential of PGPR isolates was observed for maize crop under glasshouse experiment as well as under field conditions. Various rhizobacteria with plant growth-promoting activities were screened for their probiotic potential under glasshouse conditions using maize as host after their biochemical and functional characterization. The rhizobacteria that stimulate plant growth and productivity under glasshouse conditions were then characterized using partial sequencing of 16s rDNA. These PGPR with probiotic potential, identified as *Pseudomonas* sp., *Azotobacter*, *Bacillus* sp., *Delftia*, and *Agrobacterium*, were further tested under

field conditions. Improvement in plant growth and yield attributes was observed after inoculation with PGPR. It was also observed that these rhizobacteria on inoculation act as probiotic and perform better when two or more beneficial bacteria are co-inoculated (consortium). The beneficial rhizobacteria with plant growth-promoting activities had a profound effect on different growth and yield parameters of crop plant as their inoculation improves the nutrient status of the soil. The positive influence of inoculation of PGPR on plant health and productivity has also been reported in case of other agricultural crops.

27.10 Impact of PGPR on Soil Health

To meet the food demands of increasing human population, the crop productivity needs to be increased. But due to decrease in the cultivable land due to rapid urbanization, farmers are dependent on fertilizers to get higher productivity. For this, they use chemical fertilizers which degrade soil quality. The inoculation of beneficial rhizobacteria not only improves plant growth and yield attributes but it also improves the soil health. The beneficial rhizobacteria on inoculation improve the nutrient status of soil by providing essential nutrients as they are involved in transformation of nutrients. Plant growth-promoting rhizobacteria fix atmospheric nitrogen, solubilize organic phosphorous, and detoxify heavy metals. These traits of PGPR help them to improve soil health.

27.11 Effect of Inoculation of Probiotic PGPR on Soil Biological Activities

The plant growth-promoting rhizobacteria act as probiotic both for plant and soil because in addition to improving plant health they also improve the fertility and biological activity of soil. The effect of inoculation of PGPR as probiotics along with the differential doses of inorganic fertilizers and organic manures was studied on soil biological activities in case of potato crop. It was observed that the application of various organic sources and beneficial rhizobacteria significantly improves microbial population, soil enzyme activity, and physicochemical properties of soil, which in turn improved the nutrient uptake, yield attributes, and yield of potato crop.

27.12 Factors Affecting the Efficiency of Inoculated PGPR

There are various factors which can influence the success and efficiency of PGPR as probiotic inoculants for agricultural crops. Among all the factors, the ability of these bacteria to colonize plant roots, the soil health and the exudation by plant roots play the most important role (Fig. 27.3). The root colonization efficiency of probiotic PGPR is closely associated with microbial competition and survival in the soil, as

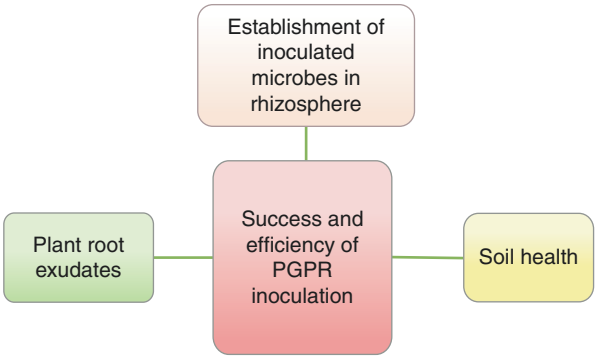

Fig. 27.3 Factors affecting success and efficiency of plant probiotics

well as on the expression of several genes and cell-cell communication (Beauregard et al. 2013). Soil health is another important factor that affects the inoculation success and efficiency, due to several characteristics such as soil texture, soil moisture, soil pH, microbial diversity, nutrient availability, toxic metal concentrations, and soil disturbances caused by management practices. Plant roots respond to different environmental conditions through the secretion of a wide range of chemical compounds (root exudates) which interfere with the plant–bacteria interaction and are considered as an important factor in the efficiency of the inoculants (Carvalhais et al. 2013). Climatic variations can also influence the effectiveness of plant probiotics.

Some examples of potential PGPR that can be used as probiotic inoculants for agricultural crops include *Azotobacter*, *Pseudomonas*, *Arthrobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Serratia*, *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, and *Rhizobium*. Numerous actinomycetes are also one of the major components of rhizospheric microbial communities. They display enormous potential plant growth beneficial traits and are used as biocontrol agents against different root fungal pathogens (Bhattacharyya and Jha 2012).

27.13 Impact of Plant Probiotics on Native Microflora of Soil

Modifications in the soil–plant–microorganism partnership bring about intricate reaction mechanisms. When a plant probiotic is introduced at high levels in the rhizosphere of a crop, the interaction within and between indigenous populations may lead to enhanced or repressed growth of native rhizospheric microorganisms. In response to the inoculated plant probiotics, certain groups may be enhanced, while others may be inhibited, or the introduced PGPR may not affect population structure (Dobbelaere et al. 2003). Inoculation with plant probiotics can affect the environment. As a consequence of higher microbial densities and higher metabolic (enzymatic) activities, carbon, phosphorous, and nitrogen turnover is increased in the rhizosphere (Johansen and Binnerup 2002).

Plant probiotics with antibiotic production potential can alter rhizobacterial communities. Niche overlap between an inoculant and resident bacteria appears to be

limited, even with resident organisms that are phylogenetically closely related to the inoculant. Spatial separation and nutrient versatility are certainly important dimensions contributing to this restricted overlap. Nevertheless, some studies do point to longer-term residual effects of antibiotic-producing PGPR on resident bacteria such as when 2,4-diacetylphoroglucinol production by *Pseudomonas fluorescens* F113Rif appeared to cause a reduction in rhizobial diversity (Walsh et al. 2001). Other studies point to strong shifts in the community structure of some specific bacterial groups.

27.14 Future Prospects and Challenges

Plant probiotics are now considered as safe means of agriculture as they increase soil fertility, promote plant health, and are safe for the environment. PGPR with potential of probiotics can also improve yield as they control pests and plant diseases which are responsible for one third of plant losses. PGPR have shown their probiotic potential in laboratory as well as greenhouse experiments. An emerging field to improve and explore the strains of plant beneficial bacteria is by genetic engineering which enables to overexpress the traits so that strains with required characters are obtained. Besides being beneficial there are several challenges faced by PGPR, such as environmental barriers and adverse conditions that can influence the activity of PGPR. The natural variation is an issue because it is difficult to predict how bacteria will act in laboratory and when placed in field. Under field conditions, PGPR need to be propagated to regain their viability and biological activity. The effectiveness of plant probiotics in field conditions can also be affected by climatic conditions.

Conclusion

Soil is an unpredictable environment and intended results are sometimes difficult to achieve. Thus, the effect of inoculated PGPR with probiotic potential on plant health and crop productivity can vary under laboratory, greenhouse, and field trials. But, due to the existing reluctance worldwide to embrace foods produced by genetically modified plants, probiotic PGPR can be advantageous as a means of promoting plant growth. The wide-scale application of probiotic PGPR may decrease the global dependence on agricultural chemicals. Furthermore, it is a technology which is readily accessible to farmers in both developed and developing countries.

References

Adesemoye AO, Torbert HA, Kloepper JW (2009) Plant growth-promoting rhizobacteria allow reduced application rates of chemical fertilizers. Microb Ecol 58:921–929

Ahemad M, Khan MS (2011) Response of greengram (*Vigna radiata* L.) grown in herbicide-amended soil to quizalafop-p-ethyl and clodinafop tolerant plant growth promoting Bradyrhizobium sp. (vigna) MRM6. J Agric Sci Technol 13:1209–1222

Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26:1–20

Badri DV, Weir TL, van der Lelie D, Vivanco JM (2009) Rhizosphere chemical dialogues: plant–microbe interactions. Curr Opin Biotechnol 20(6):642–650

Bashan Y, Holguin G, De-Bashan LE (2004) *Azospirillum*-plant relations physiological, molecular, agricultural, and environmental advances (1997–2003). Can J Microbiol 50:521–577

Beauregard PB, Chai Y, Vlamakis H, Losick R, Kolter R (2013) *Bacillus subtilis* biofilm induction by plant polysaccharides. Proc Natl Acad Sci U S A 110:1621–1630

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiol Biotechnol 28:1327–1350

Carvalhais LC, Dennis PG, Fan B, Fedoseyenko D, Kierul K, Becker A, Von Wiren N, Borriss R (2013) Linking plant nutritional status to plant-microbe interactions. PLoS One 8:6855

Dakora FD, Phillips DA (2002) Root exudates as mediators of mineral acquisition in low-nutrient environments. Plant Soil 245:35–47

Dobbelaere S, Vanderleyden J, Okon Y (2003) Plant growth promoting effects of diazotrophs in the rhizosphere. Crit Rev Plant Sci 22:107–149

Glick BR (1995) The enhancement of plant growth by free-living bacteria. Can J Microbiol 41:109–117

Glick B (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012:1–15

Hiltner L (1904) Over recent experiences and problems in the field of soil bacteriology and special those into account the Grundungung and fallow. Arb Deutsche Agricultural Enges 98:59–78

Hungria M, Nogueira MA, Araujo RS (2013) Co-inoculation of soybeans and common beans with rhizobia and azospirilla: strategies to improve sustainability. Biol Fertil Soils 49:791–801

Johansen JE, Binnerup SJ (2002) Contribution of cytophaga like bacteria to the potential of turnover of carbon, nitrogen, and phosphorus by bacteria in the rhizosphere of barley (*Hordeum vulgare* L). Microb Ecol 43:298–306

Kang BG, Kim WT, Yun HS, Chang SC (2010) Use of plant growth-promoting rhizobacteria to control stress responses of plant roots. Plant Biotechnol Rep 4:179–183

Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhancing plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286:885–886

Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Annu Rev Microbiol 63:541–556

Walsh UF, Morrissey F, Gara O (2001) Pseudomonas for biocontrol of phytopathogens: from functional genomics to commercial exploitation. Curr Opin Biotechnol 12:289–295

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Please note that the author sequence was published incorrectly as Adivitiya, Babbal, and Yogender Pal Khasa in the original version. It has now been corrected to Babbal, Adivitiya, and Yogender Pal Khasa.

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