Chapter 4

Microbial Interactions in the Rhizosphere

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4.1 INTRODUCTION

The diverse genetic and functional groups of the extensive soil microbial populations are known to carry out activities exerting a critical impact on soil functions (Barea et al., 2005b; Avis et al., 2008). Among other activities, soil microorganisms propel the biogeochemical cycling of nutrients and organic matter and improve plant performance and soil quality, key issues for agroecosystem self-sustainability (Giannazzi et al., 2010; Jeffries and Barea, 2012). These topics are relevant not only for optimizing the stability and productivity of the agroecosystems but also to prevent erosion and to minimize negative cultural and environmental stresses (Buscot, 2005).

Microbial activities are particularly relevant in the root-soil interface microhabitat, known as the rhizosphere, a dynamic environment where microorganisms interact with plant roots and soil constituents (Bowen and Rovira, 1999).

Strategic and applied research has demonstrated that microbial interactions in the rhizosphere can be managed, as a low input biotechnology, to help sustainable environmentally friendly agrotechnological practices (Ramos-Solano et al., 2009; Azcón and Barea, 2010). Thus, the formation, development, significance, functioning, and managing of rhizosphere microbial populations are topics of current research interest, which have been reviewed recently (Berg and Smalla, 2009; Jones et al., 2009; Lambers et al., 2009; Hartmann et al., 2009; Desseaux et al., 2010). Particularly, the molecular determinants involved in rhizosphere formation and functioning are receiving much attention (Matilla et al., 2007; Ferrer et al., 2008; Leitner et al., 2008; Faure et al., 2009; Mathesius, 2009; Tarkka et al., 2009; López-Ráez et al., 2012; Christensen and Kolomeets, 2011; Jousset et al., 2011; Santoro et al., 2011).

The soil microbiota is often separated into the so-called microorganisms and the larger "microfauna" (Bowen and Rovira, 1999). Although it is acknowledged that microfauna affect plant growth and above-ground food webs (Scheu et al., 2005), this review will concentrate on microorganisms and discuss the ecological and molecular aspects of rhizosphere microbial interactions, analyzing (i) trophic and functional groups of microorganisms involved in rhizosphere interactions; (ii) direct microbe–microbe interactions benefiting agroecosystems; and (iii) microbial interactions involving arbuscular mycorrhiza, the omnipresent fungal–plant (root) symbiosis. The research trends of this thematic area are thus outlined. As these topics were reviewed by us in Barea et al. (2005b), most information concerns new advances in rhizosphere interactions.

4.2 DIVERSITY OF TROPHIC AND FUNCTIONAL GROUPS OF RHIZOSPHERE MICROORGANISMS

A variety of microbial forms, either culturable or not, can be found in rhizosphere microhabitats as stimulated by
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root-released compounds and signal molecules (Roesch et al., 2007). A general view of the microbial types involved and their cooperative activities are discussed here.

4.2.1 Rhizosphere Microbial Populations

It is commonly accepted that any microbial group can develop important functions in the ecosystem (Giri et al. 2005). However, most studies on rhizosphere microbiology, especially those describing cooperative plant–microbial interactions, have focused only on bacteria and fungi. Accordingly, this review will focus on these two types of microbes.

Bacteria and fungi have different trophic/living habits, and a variety of saprophytic or symbiotic relationships, either detrimental (pathogens) or beneficial (mutualists), have been reported (Kobayashi and Crouch, 2009). Detrimental microbes include major plant pathogens and minor parasitizing and nonparasitizing deleterious rhizosphere organisms. Beneficial saprophytic bacteria and fungi can promote plant growth and health. Beneficial plant mutualistic symbionts include the N$_2$-fixing bacteria and the arbuscular mycorrhizal (AM) fungi (Barea et al., 2005b; see Section 6).

4.2.2 Nonsymbiotic Beneficial Rhizosphere Bacteria and Fungi

Particular attention has been devoted to the so-called plant-growth-promoting rhizobacteria, the PGPR (Kloepper et al., 1991; see Chapter 53). The molecular determinants of root colonization by PGPR are being investigated (Gamalero et al., 2004; Brozman et al., 2008; Barret et al., 2011; Liu et al., 2011; see Section 7). The PGPR participate in many important ecosystem processes, such as the biological control of plant pathogens, nutrient cycling and/or seedling development, and bioremediation of contaminated soils (Barea et al., 2005b; Adesemoye et al., 2009; Hayat et al., 2010). Figure 4.1 summarizes the main ecosystem roles of PGPR.

Numerous PGPR have been identified as biocontrol agents of plant pathogens, Pseudomonas sp. being one of the major groups involved (Haas and Defago, 2005; Mendes et al., 2011). Biological control of soil-borne diseases is known to result from (i) the reduction in the saprophytic growth of the pathogens followed by reduction in the frequency of the root infections through microbial antagonism; (ii) reduction of pathogen virulence; and (iii) the stimulation of "induced systemic resistance (ISR)" in the host plant (Zhang et al., 2004; Compant et al., 2005; see Chapters 54, 55). Microbial antagonism may be achieved through the release of antibiotics by the PGPR. Among the different antifungal factors produced by PGPR, acetylphloroglucinol (see Chapter 56) and phenazines (Schouten et al., 2004; Haas and Defago, 2005; Weller, 2007; Gross and Loper, 2007; Pierson and Pierson, 2010; see Chapter 54) are receiving most attention. The production of siderophores as pyoverdine and pyochelin contributes to pathogen control through competition for iron (Gross and Loper, 2007). Production of cell wall hydrolases with lytic activity against fungal pathogens has been shown to also contribute to biological control by PGPR (Chernin and Chet, 2002; Someya et al., 2007). The reduction of pathogen virulence, achieved through detoxification or degradation of virulence factors, including autoinducer signals (quenching thereby pathogen quorum-sensing capacity) is another relevant mechanism in biocontrol (Zhang and Birch, 1997; Dong et al., 2001, 2004; Molina et al., 2003; see Chapters 75, 76).

Bacterial determinants triggering ISR and mechanisms regulating resistance in the plant are under extensive scrutiny (Kloepper et al., 2004; Bakker et al., 2007, Ramos-Solano et al., 2008, 2010). Determinants already identified include lipopolysaccharides, antibiotics, diacetylphloroglucinol, 2,3-butanediol, and siderophores (Ryu et al., 2004; Høfte and Bakker, 2007; Bakker et al., 2007). The signaling pathways controlling the resistance response in the plant are being elucidated (Pozo and Azcón-Aguilar, 2007; Pozo et al., 2008; van Wees et al., 2008; Weller et al., 2012; see Chapter 54).

Among rhizosphere fungi described as biocontrol agents Trichoderma spp. is a representative example. Trichoderma controls fungal pathogens by acting both as antagonist, based on competition, antibiosis, and mycoparasitism, and by inducing localized and systemic defensive responses in the plant (Shoresh et al., 2010; Druzhinina et al., 2011; see Chapter 54). Trichoderma grows toward the fungal pathogen and releases a large variety of secondary metabolites such as the antibiotics.
gliotoxin, gliovirin, polyketides, pyrones, peptaibols (Mukherjee et al., 2012a), and a battery of lytic enzymes, mainly chitinases, glucanases, and proteases. These enzymes facilitate penetration of *Trichoderma* into the host and the utilization of the host components for nutrition (Kubitcek et al., 2011). Induction of plant defense mechanisms in biocontrol by *Trichoderma* has been highlighted (Hartman et al., 2004; Vinale et al., 2008; Shoresh et al., 2010; Hermosa et al., 2012), and the determinants and regulatory mechanisms involved are being investigated (Djonović et al., 2006, 2007; Segarra et al., 2009; Mukherjee et al., 2012a, 2012b).

Some endophytes and nonpathogenic strains of fungal pathogens can also act as biocontrol agents (Waller et al., 2005; Vallance et al., 2009; Kaur et al., 2010).

With regard to the role of PGPR in nutrient cycling, the main processes refer primarily to nitrogen fixation and phosphate solubilization (Richardson et al., 2010). Certain bacterial taxa are the only organisms able to fix N\(_2\), the first step in cycling N from the atmosphere to the biosphere, a key N input to plant productivity (Arrese-Igor, 2010; see Chapter 44). Many asymbiotic diazotrophic bacteria have been described and tested as biofertilizers under field conditions, but their effectiveness cannot be generalized (Ramos-Solano et al., 2009).

Diverse rhizobacterial (and rhizofungal) taxa are able to mineralize and/or solubilize sparingly available phosphate sources (Khan et al., 2010) largely by releasing specific enzymes and/or chelating organic acids (Marschner, 2008; Richardson et al., 2010; see Chapter 58). Phosphate-solubilizing bacteria (PSB) selected from PGPR populations have been assayed under field conditions but their effectiveness may be limited because reinstallation of solubilized phosphate ions on their way to the root (Barra et al., 2007; Zaidi et al., 2010).

*Azospirillum* species are also considered PGPR (Dobbelabrae and Okon, 2007; Gutiérrez-Mañero and Ramos-Solano, 2010; Hartman and Bashan, 2009; Couillerot et al., 2010; Bashan et al., 2011). A key action mechanism of these bacteria is the production of auxin-type phytohormones, which affect the rooting patterns whereby benefiting plant nutrient uptake (Baudoin et al., 2010; see Chapters 27, 29, 30).

Recent advances on the role and mechanisms of PGPR helping bioremediation of contaminated soils have been discussed (Zhuang et al., 2007; de Lorenzo, 2008; Wenzel, 2009; Dimkpa et al., 2009; Ruiz-Lozano and Azcón, 2011; Wenzel et al., 2011; see Section 12).

### 4.2.3 The Mutualistic Plant–Microbe Symbionts

Beneficial plant microbial symbionts include the N\(_2\)-fixing bacteria and the multifunctional mycorrhizal fungi.

The bacteria able to fix N\(_2\) in symbiosis with legumes belong to diverse genera, collectively termed as *rhizobia* (Willems, 2007; see Chapter 44). How these bacteria interact with legume roots leading to the formation of N\(_2\)-fixing nodules, the signaling processes involved, the evolutionary history, and, particularly, the molecular aspect determinants of host specificity in the rhizobial–legume symbiosis are described elsewhere in this book (see Chapter 45). Other bacteria, belonging to the genus *Frankia* (actinomycetes), also form N\(_2\)-fixing nodules on the roots of the so-called actinorhizal species (Normand et al., 2007a). The genome of *Frankia* sp. is being explored (Normand et al., 2007b). The genetic bases for their endosymbiosis establishment have been found to have common features with that of AM fungi and rhizobia (Gherbi et al., 2008; Markmann and Parmiske, 2009).

The other major groups of mutualistic microbial symbionts are the fungi that establish (mycorrhizal) associations with the roots of most plant species (Smith and Read, 2008; see Chapter 43). Mycorrhizal fungi engage in symbiotic, generally mutualistic, associations, established with most vascular plants where both partners exchange nutrients and energy. The soil-borne mycorrhizal fungi colonize the root cortex biotrophically and then develop an external mycelium, a bridge connecting the root with the surrounding soil microhabitats (Smith and Read 2008). Mycorrhizal symbioses can be found in almost all ecosystems worldwide to improve plant fitness and soil quality through key ecological processes (Azcón-Aguilar et al., 2009). Most of the major plant families form AM associations, the most common mycorrhizal type (Brundrett, 2009). The AM fungi are obligate symbionts, which are not able to complete their life cycle without colonizing a host plant (Bago and Cano, 2005). They are ubiquitous soil-borne microbial fungi, whose origin and divergence date back to more than 450 million years (Redecker et al., 2000; Bonfante and Genre, 2008; Dettl et al., 2009; Honrubia, 2009; Smith et al., 2010; Schüßler and Walker, 2011). Molecular analyses and the fossil record indicate that AM associations evolved as a symbiosis, facilitating the adaptation of plants to the terrestrial environment and suggest that AM fungi played a crucial role in land colonization by plants (Schüßler and Walker, 2011).

The AM fungi belong to the phylum *Glomeromycota* (Schüßler et al., 2001; Rosendahl, 2008; Helgason and Fitter 2009; Gamper et al. 2010; Schüßler and Walker, 2011).

Because of the microbial character of the AM fungi during their entire life cycle, this review will focus only on AM symbiosis and fungi. However, the importance of microbial interactions involving other mycorrhizal types must be recognized as well.

Earlier studies on the diversity of AM fungal communities were largely based on the morphological
characterization of their large multinucleate spores. However, molecular tools are now available for a challenging dissection of AM fungal population dynamics (Robinson-Roy et al., 2009). For molecular identification, the PCR-amplified ribosomal DNA (rDNA) fragments of the spores and/or the mycelia from AM fungi are usually subjected to cloning, fingerprinting, and sequencing (Hempel et al., 2007; Ópik et al., 2008, 2010; Toljander et al., 2008; Alguacil et al., 2009; 2011; Rosendahl et al., 2009; Songak et al., 2009; Krüger et al., 2011). Alternative molecular tools now exist to quantitatively analyze the effect of environment, management, or inoculation of soils on more diverse AM fungal communities. Q-PCR can be used for simultaneous specific and quantitative investigations of particular taxa of AM fungi in roots and soils colonized by several taxa (Gamper et al., 2008; Koenig et al., 2010). In addition, new techniques of high throughput sequencing (e.g., pyrosequencing) are rapidly developing (Roesh et al., 2007) and are now being used for AM fungi (Lumini et al., 2010; see Chapter 105). A lack of relationship between genetic and functional diversities has been shown (Munkvold et al., 2004; Croll et al., 2008; Ehinger et al., 2009).

Despite the advancement in molecular techniques, the identification approaches employed for AM fungi based on morphological characteristics are still valid and used, being considered complementary to the molecular methods (Morton, 2009; Oehl et al., 2009).

Recent advances in the genetics and genomics of the AM fungi have been reviewed (Ferrol et al., 2004; Gianinazzi-Pearson et al., 2004, 2009; Pawlowska, 2005; Parmiske, 2008; Sanders and Croll, 2010; see Chapter 43). The complete genome of the model AM fungus *Glomus intraradices* is being determined (Martin et al., 2008).

The AM fungi contribute to nutrient, particularly phosphate, acquisition and supply to plants thereby affecting rates and patterns of nutrient cycling in both agricultural and natural ecosystems (Barea et al., 2008). The AM symbiosis also improves plant health through increased protection against environmental stresses including biotic, as derived from pathogen attack (Pozo et al., 2010; López-Ráez et al., 2011), or abiotic, as caused by drought (Ruiz-Lozano et al., 2008; Areca et al., 2011), salinity (Porcel et al., 2012), heavy metals (HMs) (Tumau et al., 2006; Azcón et al., 2009b) or organic pollutants (Leyval et al., 2002). Additionally, AM associations improve soil structure through aggregate formation, necessary for appropriate soil quality (Rillig and Mummey, 2006). The role of flavonoids and strigolactones in root exudates as signals in mycorrhizal interactions has been evidenced (Akiyama et al., 2005; Steinkellner et al., 2007; López-Ráez et al., 2012; see Chapters 33, 34, 35, 51).

### 4.3 Microbe-Microbe Interactions Benefiting Agroecosystem Development

Direct interactions occurring between members of different microbial types often result in the promotion of key processes benefiting plant growth and health. It is obvious that all interactions taking place in the rhizosphere are, at least indirectly, plant mediated. However, this section deals with direct microbe–microbe interactions themselves, with the plant as a "supporting actor" in the rhizosphere. Three types of interactions have been selected for discussion here because of their relevance to the development of sustainable agroecosystems. These are (i) the cooperation between rhizobium and other PGPR for improving *N₂* fixation; (ii) microbial antagonism for the biocontrol of plant pathogens; and (iii) interactions between rhizosphere microbes and AM fungi to establish a functional mycorrhizosphere.

#### 4.3.1 PGPR Impact to Improve *N₂* Fixation by Rhizobia–Legumes Associations

As they share common microhabitats in the root–soil interface, rhizobia and other PGPR can interact during their processes of root colonization. It has been shown that some PGPR can improve nodulation and *N₂* fixation through mechanisms including production of plant hormones, flavonoids, *Nod* factors, or enzymes (Tilak et al., 2000; Remans et al., 2008; Dardanelli et al., 2008; Bansal, 2009; Mishra et al., 2009; Medeot et al., 2010; Ahmad et al., 2011; Fox et al., 2011; see Chapter 53).

Inoculation of PGPR that are able to solubilize phosphate (PSB) enhances legume nodulation and *N₂* fixation (*¹⁵N methods*) (Barea et al., 2008; Azcón and Barea, 2010; Zaidi et al., 2010).

Azcón et al. (2009a) demonstrated that PGPR isolated from a HMC-contaminated soil increased nodulation of legumes growing in these soils where control plants did not form nodules. The explanation may be related to the role of PGPR accumulating HM in soil and therefore reducing HM concentrations and uptake by plants and rhizobia, thereby preventing toxicity and enabling nodulation. In addition, an increase in soil enzymatic activities (phosphatase, β-glucosidase, dehydrogenase, etc.) and in auxin production around PGPR-inoculated roots could also be involved in the PGPR effect on nodulation.

Tolerant PGPR have been shown to improve nodulation, *N₂* fixation, and legume performance in stressed environments, including drought, salinity, nutrient deficiency, acidity, or alkalinity (Zahran, 2010; Ahmad et al., 2011).
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4.3.2 Microbial Antagonism for the Biological Control of Plant Pathogens

The role of microbial populations in the rhizosphere as the first barrier to pathogen infection was highlighted in the 1970s, when the term “suppressive soils” became established (Barea et al., 2005b). It is now widely known that some soils are naturally suppressive to soil-borne plant pathogens such as Fusarium, Gaecumannomyces, Rhizoctonia, Pythium, and Phytophthora. As the microorganisms with antagonistic properties toward plant pathogens are diverse (Heydari and Pessarakli, 2010), deciphering the metagenome of disease-suppressive soils is a major challenge in identifying the microbes and molecular mechanisms involved in such suppression (van Elsas et al., 2008; Mendes et al., 2011). The use of high-density 16S rDNA oligonucleotide microarrays (PhyloChip) has revealed that Proteobacteria, Firmicutes, and Actinobacteria are consistently associated with disease suppression. In addition to Pseudomonadaceae, species of Agrobacterium, Bacillus, Streptomyces, and Burkholderia have been shown to be effective antagonists of soil-borne pathogens (Whipps, 2001). Bacteriophages are recognized nowadays as biocontrol agents for bacterial diseases (Jones et al., 2007; Chandaie et al., 2010; see Chapter 57). A variety of fungal species and isolates have been applied in biocontrol, as nonpathogenic species of fungi as Pythium and Fusarium (Whipps 2001; Vallance et al., 2009; Kaur et al., 2010) but the ubiquitous Trichoderma species clearly dominate (Druzhinina et al., 2011; see Chapter 54). Trichoderma spp. efficiently controls a broad range of phytopathogenic fungi such as Rhizoctonia solani, Pythium ultimum, and Botrytis cinerea. Interactions between Trichoderma and mycotoxigenic Fusarium have been assayed for the biocontrol of Fusarium head blight (Matarese et al., 2012). Pseudomonas and Trichoderma spp. are examples of combination of multiple mechanisms for effective pathogen suppression (Heydari and Pessarakli, 2010).

The use of beneficial microorganisms for biocontrol strategies should aim to produce a high level of plant protection and wide range of effectiveness. To achieve that goal and reduce the variability in the results, biocontrol research has to face the challenges of finding appropriate screening procedures to select suitable microorganisms in diverse soil environments (Pliego et al., 2011). Understanding the impact of a changing environment on the biocontrol agent performance, and predicting the output of multiple interactions in the rhizosphere, is fundamental to develop effective combinations of antagonistic microorganisms (Xu et al., 2011).

4.3.3 Interactions Between AM Fungi and Other Rhizosphere Microbes for Mycorrhiza/Mychorhizosphere Establishment

Rhizosphere microorganisms can either interfere with AM fungi or benefit mycorrhiza establishment (Pivato et al. 2009; Larsen et al., 2009). A typical beneficial effect is that exerted by the so-called mycorrhiza-helper-bacteria (MHB), a term referring to those bacteria that enhance mycorrhiza formation (Frey-Klett et al., 2007; see Chapter 49). Soil microorganisms produce compounds that increase the rates of root exudation. This, in turn, stimulates AM fungal mycelia in the rhizosphere or facilitates root penetration by the fungus. Plant hormones, as produced by soil microorganisms, affect AM establishment (Barea et al., 2005b). Rhizosphere microorganisms influence the presymbiotic stages of AM development, such as spore germination rate and mycelial growth (Franco-Corra et al., 2010; Fernández-Bidondo et al., 2011). Conversely, the establishment of PGPR inoculants in the rhizosphere can be benefited by AM fungal co-inoculation (Toljander et al., 2007).

AM colonization changes the chemical composition of root exudates, whereas the AM soil mycelium introduces physical modifications into the environment surrounding the roots thereby affecting microbial structure and diversity (Barea et al., 2003b). In addition, there are specific modifications in the environment surrounding the AM mycelium itself, the mycorrhizosphere (Requena et al., 1999; Hooker et al., 2007; Toljander et al., 2007; Finlay, 2008; Vestergaard et al., 2008; Lioussanne et al., 2010).

A case of particular interest concerns the relationships between AM fungal structures and certain intimately associated endobacteria (Bonfante and Anca, 2009). The presence of endobacteria inside AM fungal cytoplasm has long been documented by electron microscopy, which distinguished two endobacterial morphotypes. The first, restricted to the Gigasporaceae, is an uncultured taxon, Candidatus Glomeribacter gigasporarum, related to Burkholderia (Lumini et al., 2007). The other bacterial type that has been detected inside AM fungal spores and hyphal colonizing plant roots sampled in the field is called bacterium-like organism (BLO) (MacDonald et al., 1982).

Recently, Naumann et al. (2010) analyzed 28 cultured AM fungi, from diverse evolutionary lineages and four continents, showing that most of the AM fungal species investigated possess BLOs. Analyzing the 16S rDNA they found that BLO sequences from divergent lineages all clustered in a well-supported monophyletic clade that was not closely related to any described bacterial group, but with the Mollicutes. The intracellular location of BLOs was revealed by confocal microscopy and
fluorescent *in situ* hybridization (FISH), and confirmed by pyrosequencing. These bacteria diverged from their sister group more than 400 million years ago, colonizing their fungal hosts before main AM fungal lineages separated. The BLO–AM fungal symbiosis can, therefore, be dated back at least to the time when AM fungi formed the ancestral symbiosis with emergent land plants.

Research aimed to isolate and characterize bacteria designated as "probable endobacteria" from AM fungal spores (*Gigaspora margarita*) has been recently published (Cruz and Ishii, 2011). Bacterial isolates were characterized by both morphological and molecular methods revealing the presence of *Bacillus* spp. and *Penibacillus* spp. These bacteria showed phosphate-solubilizing capacity and possessed nitrogenase activity. Besides, they were antagonistic to fungal plant pathogens but stimulated AM hyphal growth. The use of a new type of scanning electron microscope demonstrated the presence of bacterial aggregates, apparently similar to biofilms, on the surface of hyphae and spores.

### 4.4 INTERACTIONS BETWEEN SELECTED AM FUNGI AND PGPR FOR IMPROVING AGROECOSYSTEM SUSTAINABILITY

Managing microbial interactions involving selected AM fungi and PGPR (mycorrhizosphere tailoring) is recognized as a feasible biotechnological tool to improve plant growth and health, and soil quality, as summarized in Figure 4.2.

Many co-inoculation experiments using selected AM fungi and rhizosphere microorganisms have been reported. The main conclusions from key information will be summarized here with special emphasis on the ecological and molecular aspects of interactions related to (i) symbiotic N$_2$ fixation; (ii) phosphatic solubilization; (iii) phytoremediation of HM-contaminated soils; (iv) biological control of root pathogens; and (v) improvement of soil quality.

#### 4.4.1 Interactions with Symbiotic N$_2$-Fixing Bacteria

The widespread presence of the AM symbiosis in legumes and its role in improving nodulation and N$_2$ fixation by legume–rhizobia associations are both universally recognized processes (Azcón and Barca, 2010). Actually, rhizobial bacteria and AM fungi are known to interact among themselves and with their common legume host roots, either at the colonization stages or at the symbiotic functional level (Lesueur and Sarr, 2008). Particularly interesting are the studies (i) on the genetic and molecular relationships of AM fungi and rhizobia in establishing their endosymbioses; (ii) analyzing the physiological interactions related to the formation and functioning of the tripartite symbiosis; and (iii) using $^{15}$N to ascertain and quantify the AM role on N$_2$ fixation by legume–rhizobia associations.

Developmental genetics and evolution timing analysis of microbe–plant symbioses, including both mutualistic, either N$_2$-fixing or mycorrhizal, and pathogenic associations, have revealed a common developmental program for all of these compatible microbe–plant associations (Markmann et al., 2008; Parniske, 2008; Provorov and Vorobyov, 2009b; den Camp et al., 2011). As the rhizobia–legume symbiosis evolved much later than the AM symbiosis (Martínez-Romero, 2009; Provorov and Vorobyov, 2009a; Zhukov et al. 2009), the cellular and molecular events occurring during legume nodulation may have evolved from those already established in the AM symbiosis. The legume–rhizobia symbiosis seems to have evolved from a set of preadaptations during coevolution with AM fungi; thus some plant genes can modulate both legume symbioses (Parniske, 2004; Saito et al., 2007; Chen et al., 2009; Horváth et al., 2011; see Chapters 43, 45). A series of common plant genes required for early developmental stages of both AM and rhizobial symbioses have been identified by forward genetics approaches (Parniske, 2008). These include a receptor kinase, which is also required for actinorhizal symbiosis, and has been implicated in the evolution of nodulation (Markmann et al., 2008). The use of mycorrhiza-defective legume mutants (Myc$^-$)
has provided relevant information allowing the common cellular and genetic programs responsible for the legume root symbioses to be dissected (Gianinazzi-Pearson et al., 2009).

Both AM fungi and root-nodulating Frankia spp. coexist in the roots of some nonlegume (actinorrhizal) plant species and interact to facilitate establishment of their endosymbioses and to benefit plant-soil developments (He and Critchley, 2008; Orfanoudakis et al., 2010). Genomic and transcriptomic studies have shown that the actinorrhizal—Frankia symbioses also share some of the genes involved in the common SYM pathway described for AM fungi and legume—rhizobium symbioses (Gherbi et al., 2008; Hecher et al., 2011).

A great number of reports have focused on the physiological and biochemical basis of AM fungal—rhizobia interactions. The AM fungi are known to exert a general influence on plant nutrition, based on the supply of P by the AM fungi to satisfy the high P demand of symbiotic N_2 fixation, but more localized effects of AM fungi occur at the root, nodule, or bacteroid levels (Azcón and Barea, 2010).

The addition of a small amount of 15N-enriched inorganic fertilizer and an appropriate “nonfixing” reference crop is the basis to ascertain and quantify the amount of N, which is actually fixed by legume—rhizobia consortia in a particular situation and to measure the contribution of the AM symbiosis to the process. A lower 15N-to-14N ratio in the shoots of rhizobia-inoculated AM plants with respect to those achieved by the same rhizobial strain in nonmycorrhizal plants has been found. This indicates an enhancement of the N_2 fixation rates (an increase in 15N from the atmosphere), as induced by the AM activity (Barea et al., 2005a; Chalk et al. 2006).

### 4.4.2 Interactions with Phosphate-Solubilizing Bacteria

The interactions between AM fungi and phosphate-solubilizing microorganisms (PSM) are relevant to P cycling and plant nutrition. Because the Pi made available by PSM acting on sparingly soluble P source has limited diffusion in soil solution, and may not reach the root surface, AM fungi could tap the phosphate ions solubilized by the PSM and translocate them to plant roots (Barea et al., 2005a; Azcón and Barea, 2010). The microbial interaction of AM fungi and PSB has been tested in experiments using 32P-tracer methodologies (Barea et al., 2007). Upon adding a small amount of 32P to label the exchangeable soil P pool, the isotopic composition, or “specific activity” (SA = 32P/31P quotient), was determined in plant tissues. It was found that dual inoculation reduced the SA of the host plant, indicating that these plants acquired P from sources that were not directly available to noninoculated or singly inoculated plants. Microbial inoculation improved biomass production and P accumulation in plants, demonstrating the interactive effects of PSB and AM fungi on P capture, cycling, and supply in a tailored mycorrhizosphere (Barea et al., 2007).

Multi-microbial interactions, including those among locally isolated AM fungi, PSB, and Azospirillum, have also been reported, which indicate that microorganisms can act synergistically when co-inoculated (Azcón and Barea, 2010).

### 4.4.3 Interaction for Phytoremediation of Soil Contaminated with Heavy Metals

AM fungi improve phytoremediation of soils contaminated with HMs, radionuclides, or polycyclic aromatic hydrocarbons (Leyval et al., 2002). Most phytoremediation assays involving mycorrhizosphere interactions concern HMs and different strategies of phytoremediation have been investigated (Turnau et al., 2006; Ruiz-Lozano and Azcón, 2011; see Section 12). These studies mostly concentrated on Zn, Cu, Cd, Pb, or Ni, but remediation of As-contaminated soils has also been attempted (Dong et al., 2008).

Interactions between rhizobacteria and AM fungi have been investigated in diverse experiments to ascertain whether they cooperate to benefit phytoremediation (Azcón et al., 2009a, 2009b, 2010). The main achievements resulting from these experiments using HM-multiple contaminated soils and Trifolium as test plants were: (i) a number of bacteria and the AM fungi were isolated from an HM-contaminated soil and identified by 16S rDNA or 18S rDNA, respectively; (ii) the target bacteria were able to accumulate large amounts of metals; (iii) co-inoculation with an HM-adapted autochthonous bacteria and AM fungi increased biomass, N and P content as compared to noninoculated plants and also enhanced the establishment of symbiotic structures (nodule number and AM colonization), which were negatively affected as the level of HM in soil increased; (iv) dual inoculation lowered HM concentrations in Trifolium plants, inferring a phytostabilization-based activity; however, as the total HM content in plant shoots was higher in dually inoculated plants, due to the effect on biomass accumulation, a possible phytoextraction activity was suggested; and (v) inoculated HM-adapted bacteria increased dehydrogenase, phosphatase, and β-glucanase activities and auxin production in the mycorrhizosphere, indicating an enhancement of microbial activities related to plant development.

The physiological/biochemical mechanisms by which the tested bacterial isolates enhanced phytoremediation activity in AM plants include (i) improved rooting, and
AM formation and functioning; (ii) enhanced microbial activity in the mycorrhizosphere; and (iii) accumulation of metals in the root-soil environment, thus avoiding their transfer to the trophic chain, or to aquifers (Ruiz-Lozano and Azcón, 2011).

Inoculation of autochthonous AM fungi and bacteria, together with the application of treated agrowaste residue, changed bacterial community structure and enhanced phytoextraction to remediate HM-contaminated soils (Azcón et al., 2009a). An enhancement of antioxidant activities in plants inoculated with AM fungi and bacteria and agrowaste residue was evidenced (Azcón et al., 2009b). Such a mycorrhizosphere effect seems to help plants to limit oxidative damage to biomolecules in response to metal stress. The molecular mechanisms involved in HM tolerance in AM inoculated plants have been recently discussed (Cornejo et al., 2008; González-Guerrero et al., 2009; Zhang et al., 2009; Aloui et al., 2011).

4.4.4 Interactions for the Biological Control of Plant Pathogens

AM establishment has been shown to reduce damage caused by soil-borne plant pathogens with an enhancement of plant resistance/tolerance in mycorrhizal plants (Barea et al., 2005b; Pozo and Azcón-Aguilar, 2007; Pozo et al., 2009, 2010). As specific microorganisms antagonistic to plant pathogens are being used as biological control agents, a major aim in rhizosphere biotechnology is to exploit the prophylactic ability of AM fungi in association with these antagonists (Barea et al., 2005b). The mycorrhizosphere has been hypothesized to constitute an environment conductive to microorganisms antagonistic to soil-borne pathogen proliferation. Indeed, various antagonistic bacteria have been identified within AM extraradical structures or in the mycorrhizosphere of several AM species, thus favoring biological control of pathogens (Lioussanne, 2010).

A key point is to ascertain whether an antifungal biocontrol agent would negatively affect beneficial AM fungi. This is fundamental to exploit the possibilities of dual (AM fungi and microbial antagonists) inoculation to aid plant defense against root pathogens. In vitro and in field experiments aiming to evaluate the impact of Pseudomonas strains producing the antifungal diacetilphloroglucinol on AM formation and functioning confirmed the lack of effect of the bacteria on the numbers or diversity of the AM fungal population and performance. Moreover, the antifungal Pseudomonas improved the plant nutritional benefits from the AM association (Barea et al., 1998). Although some in vitro experiments show the capacity of Trichoderma to parasitize AM fungi, no inhibition or even promotion of plant colonization by AM fungi has been reported (Martínez-Medina et al., 2009). In some cases, although co-inoculation with other organisms resulted in a reduction in AM colonization, the effects on plant health were maintained or improved (Gamalero et al., 2010).

Most co-inoculation studies involving AM fungi and biocontrol agents have dealt with bioprotection against soil-borne pathogens such as Fusarium or Rhizoctonia (Saldajeno et al., 2008) and other fungi (Chandanie et al., 2009). However, neutral and even antagonistic effects of such interactions on the bioprotection against both, soil- and air-borne pathogens have also been described (Saldajeno et al., 2008).

The enhanced capacity of bioprotection achieved by dual inoculation usually results from the combination of the mechanisms used by each organism individually, such as competition, altered root exudates, morphological changes in the root system, antibiosis, and the activation plant defense responses (Saldajeno et al., 2008). This is illustrated in Figure 4.3.

As already mentioned, most antagonistic microorganisms trigger plant defense mechanisms that may differ depending on the signaling pathway activated. Triggering simultaneously different defense signaling pathways in the plant may alter the spectrum of pathogens to which the protection achieved is effective, as cross talk between phytohormones is a key element in the regulation of plant resistance (van Wees et al., 2008; Pieterse et al., 2009).

In summary, experimental evidence supports the argument that mycorrhizosphere management addressed at enhancing plant resistance/tolerance to pathogen attack is a promising biotechnological tool. However, as synergism and interference in the biocontrol properties of different organisms have been described, research on the optimal microbial combinations and conditions for synergism is essential for the successful application of microbial consortia in sustainable agricultural practices.

4.4.5 Interactions for Improving Soil Quality

Physical-chemical soil properties are fundamental for soil quality, with soil structure being one of the most influential factors (Buscot, 2005). Soil structure depends on the aggregation status of soil particles and well-aggregated soil ensures appropriate soil tilth, soil-plant water relations, aeration, root penetrability, and organic matter accumulation (Miller and Jastrow, 2000). The contribution of microbial interactions to the formation and stabilization of soil aggregates has been demonstrated (Miller and Jastrow, 2000).

As a result of degradation/desertification processes, disturbance of natural plant communities is often
Figure 4.3 Synergistic interactions among different antagonistic microorganisms and mycorrhizas for the biological control of plant pathogens: This is illustrated in this mycorrhizosphere model including plant roots (represented in brown), mycorrhizal hyphae (in blue), bacteria, fungi, nematodes and other microbes. Antagonistic bacteria are living on the root surface (A) or associated to spores or mycelia (B) from mycorrhizal plants (C). Trichoderma spp., a mycoparasitic fungus is also involved (D). These microorganisms synergistically interact and protect the plant against pathogens (E) through the combination of different mechanisms involving competition for nutrients and colonization sites, production of different antimicrobial compounds or siderophores, parasitism and, in some cases, priming of plant defenses, resulting in Induced Systemic Resistance.

4.5 CONCLUSIONS

There is considerable experimental evidence that bacteria and fungi living at the root-soil environments carry out a variety of interactive activities known to benefit plant growth and health, and also soil quality. In the past years, research has attempted to improve the understanding of diversity, dynamics, and significance of rhizosphere microbial populations, and to gain more information on the molecular determinants of their cooperative interactions.

From the practical/ecological point of view, the aims will be to improve sustainable plant productivity and food quality while preserving the environment. However, to achieve this, we need first to carry out basic and strategic studies to gain a better understanding of microbial interactions taking place in the rhizosphere. Only then can the corresponding agrobiotechnology be applied successfully. Hence, future investigations in the field of microbial cooperation in the rhizosphere will include: (i) advances in visualization technology; (ii) analysis of the molecular basis of root colonization; (iii) signaling processes in the rhizosphere; (iv) functional genomics; (v) mechanisms involved in beneficial cooperative microbial activities; (vi) engineering of microorganisms for beneficial purposes; and (vii) biotechnological developments for integrated management.

Nondisruptive in situ visualization techniques are already being used for detailed studies on the interactions of microorganisms within the rhizosphere, both between themselves and with the root. Improving these techniques, based on the use of confocal laser scanning microscopy and fluorescent proteins, will allow not only the simultaneous imaging of different populations of microbes in the rhizosphere but also the temporal–spatial visualization of gene expression. Novel research is needed to improve immunofluorescence techniques to assess gene transfer in rhizosphere environments without the need of cultivating the microorganisms.

Many traits of root colonization by rhizo-microbes have already been identified, but novel molecular approaches are being used to screen for new traits. These species in the natural succession. The improvement in the physical–chemical properties in the soil around the Anthyllis plants was shown by the increased levels of N, organic matter, and number of hydrostable soil aggregates. Glomalin-related glycol proteins, produced by the external hyphae of AM fungi, are seen to be involved in the initiation and stabilization of water-stable soil aggregates, due to its glue-like hydrophobic nature (Miller and Jastrow 2000; Rillig and Mummey, 2006; Bedini et al., 2009).

accompanied, or preceded by, loss of physical–chemical and biological soil properties, such as soil structure, plant nutrient availability, organic matter content, and microbial activity (Jeffries and Barea, 2012). Accordingly, management of AM fungi, together with rhizosphere bacteria, was proposed for the integral restoration of degraded ecosystems. This has been investigated in a desertified semi-arid ecosystem with Anthyllis cytisoides, a drought-tolerant legume, as a test plant (Requena et al., 2001). Anthyllis seedlings inoculated with indigenous rhizobia and AM fungi were transplanted to field plots for a 5-year trial. The tailored mycorrhizosphere enhanced seedling survival and growth, P acquisition, N fixation, and N transfer from N fixing to associated nonfixing.
are important to decipher the genes encoding proteins involved in transport or signal transduction pathways in colonizer. An increase in our knowledge on quorum-sensing systems will be important for understanding the ecodynamics of microbial populations in the rhizosphere, and the cellular and molecular aspects of signaling processes in microbe–microbe interactions.

Future functional genomics (including transcriptomics, proteomics, and metabolomics) developments will be useful to identify the genes expressed in the rhizosphere that play key roles in processes such as nutrient mobilization or suppression of plant diseases. The use of promoters to drive gene expression specifically at the root–soil interfaces will allow the engineering of microorganisms for beneficial purposes.

The specific management of mycorrhizal fungi/bacteria interactions, through the design of appropriate mycorhizospheres, should be one of the main objectives of applied studies in the future. The use of microbial inoculants must take into account the importance of retaining microbial diversity in the rhizosphere, and in achieving realistic and effective biotechnological applications (“rhizosphere technology”). The improvement of molecular-biology-based approaches will be fundamental for analyzing microbial diversity and community structure, and to predict responses to microbial inoculation/processes in the environment (“ecological engineering”). Further studies must address the consequences of the cooperation between microbes in the rhizosphere under field conditions to assess their ecological impacts and biotechnological applications.

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