Chapter

Arbuscular Mycorrhiza-Associated Rhizobacteria and Biocontrol of Soilborne Phytopathogens

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Abstract

The mutualistic symbiosis of most land plants with arbuscular mycorrhizal (AM) fungi has been shown to favor mineral and water nutrition and to increase resistance to abiotic and biotic stresses. The main mechanisms involved in the control of the disease symptoms and intraradical proliferation of soilborne phytopathogens are due to root colonization with AM fungi. The role of the rhizobacteria is shown to be specifically associated with extraradical network of the AM and mycorrhizosphere. The mycorrhizosphere can form a favorable environment for microorganisms which have potentiality to act antagonistic to pathogen abundance. It makes an additional advantage in identifying rhizobacteria from AM fungi structures or mycorrhizosphere, which often lead to the isolation of organisms having strong properties of antagonism on various soilborne pathogens. The ability of AM fungi to control soilborne diseases is mainly related to their capacity to stimulate the establishment of rhizobacteria against the favorable environment of pathogen within the mycorrhizosphere prior to the root infection. Recent advancement in scientific research has provided more clear picture in understanding the mechanisms involved in AM fungi/rhizobacteria interactions. Herein, this chapter includes the mechanisms of the AM fungi-mediated biocontrol, interactions between AM-associated bacteria and AM fungus extraradical network, AM-associated bacteria and biocontrol activities and unfavorable zone to pathogen development: the mycorrhizosphere.

Keywords: AM-associated bacteria (AMB), arbuscular mycorrhizal fungi, biocontrol, mycorrhizosphere, soilborne pathogens

1. Introduction

A majority of land plants in nature are growing symbiotically in relationship with AM fungi. This relationship is well established with the roots of these plants. Soil exploration by the external mycelium of AM fungi increases the nutrient absorptive root surface area and thus favors the host plant in access to nutrients and water [1, 2]. Moreover, as the largest component of the soil microbial biomass [3, 4], AM fungi form widespread mycelial networks within the soil atmosphere, and hyphae harbour important sites for interactions with other soilborne microorganisms. The constricted zone adjacent to soil-living roots is called the

rhizosphere [5]. It is characterized by increased microbial activity and by a specific microbial community structure [6, 7]. Along with root-AM fungi associations, factors influencing the community structure and the biomass of soil microorganisms lead to the establishment of a zone called mycorrhizosphere [8–12]. The zone of soil influenced by only AM fungi is called mycosphere. In the mycorrhizosphere, AM fungi structures and various rhizobacteria (AM fungi-associated rhizobacteria or AMB, e.g. *Paenibacilli*, *Bacilli* and *Pseudomonas* spp.) are generally identified by classical culture-dependent methods [13, 14]. It includes phospholipid fatty acid analysis (PLFA) [15] and polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) [13, 16, 17] which reinforce the hypothesis that AM fungi structures constitute important nutrient-rich niches for soilborne microorganisms. *Glomeribacter gigasporarum* (a new taxon of *Burkholderiaceae*) was even described as a Gram-non-cultivable (obligatory) bacterial endosymbiont of spore vacuoles, mycelium and intraradical hyphae of *Gigaspora margarita* [18]. *Glomeribacter gigasporarum* described in detail shows to be widespread within *Gigasporaceae*; it transmitted vertically and contains nitrogen fixation genes [19–21], while in *Gigaspora margarita*, it has been suggested and observed that this AM fungus might fix nitrogen and then deliver it to the symbiotic plant through the associated bacterial population [22]. The effects of this on host plant physiology can be recognized in mycorrhizal root colonization because of the consequence of the activity of specifically AM fungi-associated rhizobacteria.

The beneficial effects of AM fungi on the host plant physiology, in the decrease of intraradical and mycorrhizosphere population and in the decrease of disease symptoms of soilborne pathogens were reported in many biological systems, probably due to synergistic mechanisms [23–25]. The use of chemical pesticides are now avoided and not advocated in fields due to its risks to human health and the environment, and thus the implementation of sustainable agriculture has become essential in crop industry. The perception of the mechanisms involved in the AM fungi-mediated biocontrol will allow to maximize the performance of management of such sustainable agroecosystems and thus authorize the use of AM fungi and its benefits [26]. The main mechanisms involved in the biological control of diseases induced by soilborne phytopathogens start after root colonization with AM fungi especially due to its association with rhizobacteria which constitutes major element for this biocontrol.

2. Mechanisms of the AM fungi-mediated biocontrol

Reduction in the detrimental effects of soilborne pathogens after root colonization with AM fungi was described a long time ago [27, 28] and has been observed on various fungi, stramenopiles, nematodes and bacteria [12, 29]. Carlsen et al. [30] reported the total check of infectivity caused by *Pythium ultimum* on clover plants cv. Sonja by using *Glomus mosseae* as a symbiotic relation partner. For the biological control of pathogen, AM fungus or AM fungi/plant taxa association, conditions of culture, level of root colonization, time of AM fungus or pathogen inoculation and harvest, the mechanisms hypothesized, etc. should be involved [12, 23, 24, 29, 31–35]. The disease symptoms induced by pathogens can systemically be reduced in non-mycorrhizal roots of plants grown in AM fungi-inoculated split-root systems [36]. Various hypotheses have been suggested in an endeavor to elucidate the AM fungi-mediated biocontrol of soilborne phytopathogens. The fact that pathogen-induced symptoms are systemically regulated by AM fungi colonization is related to the establishment of induced systemic resistance (ISR) [37]. ISR is a resistance mechanism induced or acquired in plants which were already undergone

for pretreatment with a variety of organisms and compounds [e.g. superoxide dismutases (SOD) and peroxidases, pathogenesis-related type 1 proteins (PR-1 proteins)].

Further, higher concentrations of phenolic acids could be detectable in plants which are colonized with AM fungi species subjected for biocontrol activities. Accumulation of jasmonic acid involved in the rhizobacteria-mediated ISR in mycorrhizal roots could be related to the systemic pathogen biocontrol [38, 39]. Cordier et al. [40] identified local cell wall modifications (callose accumulation around arbuscule-containing cortical cells of tomato roots). The synthesis of constitutive and additional isoforms of defense-related enzymes (e.g. chininases, chitosanases, β-1,3-glucanases, peroxidases and SOD) has also been locally detected in mycorrhizal roots [41–43]. The level of production of these enzymes or flavonoids was reported to be unrelated to the capacity of biocontrol of the AM fungi species [30, 44]. The transcript profiling and real-time quantitative PCR used to explore the transcriptional changes triggered by AM fungus colonization revealed a complex pattern of local and systemic changes in gene expression in roots of *Medicago truncatula* [45], and transcripts for defense-related proteins were reported to expressed locally. Furthermore, increase in concentrations of defense-related compounds such as rosmarinic acid, caffeic acids, phenolics and essential oils has not been recorded in colonization with *Glomus mosseae* which was reported for its role in protecting basil plants against *Fusarium oxysporum* f. sp. *basilica.* It highlights and indicates the role of other possible mechanisms in the AM fungus-mediated biocontrol activity which differs to stimulation of systemic and localized plant defense mechanisms [46].

The most commonly documented response to AM fungi colonization is an increase in phosphorus nutrition to the host plants which subsequently imparts more dynamic and more resistant properties against pathogen invasion. However, AM fungi-mediated biocontrol is unrelated to the soil phosphorus (P) availability and to the phosphorus status in plant tissues, thus possibly more dependent on other mechanisms [46–49].

Arbuscular mycorrhizal fungi normally compete for space and nutrients with soilborne pathogens within the zone of mycorrhizosphere and the host roots. Larsen and Bodker [50], using signature fatty acid profiles, demonstrated the decrease in biomass and energy reserves of both *Glomus mosseae* and *Aphanomyces euteiches* co-occupying pea roots; however *Phytophthora nicotianae* and *Glomus mosseae* never reported to occupy simultaneously in the same tomato root tissues [40]. A reduction in the extent of mycorrhizal colonization by different plant pathogens has been reported [51–54] indicating the possible occurrence of competitive interactions. The AM fungus is often inoculated before the attack of pathogen in order to favor biocontrol efficiency [54]. However, *Fusarium solani* f. sp. *phaseoli* genomic DNA quantified using quantitative real-time PCR was significantly reduced not only in the mycorrhizosphere and mycosphere but also in the bulk soil of a compartmentalized soil-root system which was inoculated with *Glomus intraradices*, whereas the AM fungus genomic DNA was not significantly modified by the pathogens in the soil [55]. Reduction in *Fusarium solani* f. sp. *phaseoli* growth as well as decrease in root rot symptoms as a result of colonization with *Glomus intraradices* could not be attributed to the competition for resources and habitat between the two fungi but mostly to the biotic or abiotic characteristic factors of the established mycorrhizosphere.

The extraradical network formed by *Glomus intraradices* around the roots of the plants has been reported to show a decrease in the growth of nematodes (e.g. *Radopholus similis* and *Pratylenchus coffeae*) and conidial formation of *Fusarium oxysporum* f. sp. *chrysanthemi*. In vitro aseptic conditions and the above-stated

negative impacts are not important to affect the developmental stages of all nematodes, and it is also unrelated to the mycelial or spore densities of AM fungus [56–58]. Additionally, in the presence of the AM fungi, significant increase in spore germination and hyphal growth by *Fusarium oxysporum* f. sp. *chrysanthemi* was also reported, and thus, direct inhibition of pathogen by AM fungi structures could not properly be explained for biocontrol [56].

In vitro results of impact studies of the exudates of extraradical AM fungi network or by the mycorrhizal roots on pathogens are in contradiction. Crude extracts from the extraradical network of *Glomus intraradices* is clearly reported for the reduced germination of conidia of *Fusarium oxysporum* f. sp. *chrysanthemi* [59]. Similarly, inhibition in sporulation of pathogen *Phytophthora fragariae* is reported with exudates of strawberry roots which were colonized by *Glomus etunicatum* and *Glomus monosporum* [60]. During the harvest, compared to the exudates of non-AM-inoculated tomato roots, the exudates from in vitro grown AM (*Glomus intraradices*)-inoculated roots were reported either repulsive or more attractive for the zoospores of *Phytophthora nicotianae* [61].

Another example can be seen in the exudates of tomato roots which are reported to double the microconidia germination of *Fusarium oxysporum* f. sp. *lycopersici* in the presence of AM fungi *Glomus mosseae* compared to the exudates from non-mycorrhizal roots [54, 62]. The direct impact of exudates from mycorrhizal plants in the AM fungus-mediated biocontrol activity can directly be measured in soil conditions by quantification of the capacity of root infection by the pathogen [63]. Application of root exudates of tomato plants which are colonized with *Glomus intraradices* or *Glomus mosseae* has not been reported for any positive impact on another tomato plant for the control of pathogen *Phytophthora nicotianae,* while direct inoculation of these AM fungi (i.e. *Glomus intraradices* or *Glomus mosseae*) significantly reduced or controlled the growth of pathogen *Phytophthora nicotianae* in these other tomato plants. Thus, it suggests that exudates from one's mycorrhizal plant will not directly or indirectly inhibit the capacity of pathogen intraradical proliferation on other plants.

From the above it is evident that none of the cited mechanisms is involved in the AM fungus-mediated biocontrol, but it has been shown to happen in every plant-fungi system. These mechanisms might act in synergistic way with each other, with one mechanism becoming preponderant depending on the environmental conditions and the plant cultivar-pathogen/AM fungus strain. However, the mechanism related to the capacity of interaction of AM fungi with other soil microorganisms can significantly be attributed as one of the main reasons involved in the control of soilborne diseases.

3. Interactions between AM-associated bacteria and AM fungus extraradical network

The bacterial communities associated with various AM fungal inoculum or spores have been reported to differ from one another based on their association as one found in mycorrhizal isolate and others largely encountered in the mycosphere [15]. The species assemblages of cultivable bacteria from surface-disinfected spores of *Glomus mosseae* and *Glomus intraradices* were influenced both by fungal and plant species where 'spore type' is the important factor. This specificity of interaction in AM fungal species is usually hypothesized to be related to spore size and surface roughness. Under sterile conditions the bacterial adherence to spores or hyphae of AM fungi was demonstrated to be species-specific or depends on bacterial isolate and the fungal vitality [64]. The association competence of rhizobacteria to AM fungal surfaces could be dependent on their ability to form biofilms [65].

The roots colonized with *Gigaspora margarita* and its extraradical hyphae demonstrate that extracellular polysaccharides are involved in the in vitro association of *Pseudomonas fluorescens* CHAO to these biological surfaces [66]. *Pseudomonas fluorescens* CHAO have the abilities to form light spots, while two mucoid mutants of this strain by increased production of acidic extracellular polysaccharides formed a large number of clusters on non-mycorrhizal carrot roots, and mutants of *Azospirillum brasilense* and *Rhizobium leguminosarum* affected in extracellular polysaccharide production were strongly impaired in the capacity to attach to mycorrhizal root [67]. Strains of *Burkholderia* on *Gigaspora decipiens* were able to colonize the interior of the spores, and it demonstrates that AM fungal colonization does not occur on AM surfaces only through the biofilm formation [68]. Saprophytic activity of the bacteria was also observed by scanning electron microscopy (SEM) observations of *Glomus geosporum* spores [69]. The growth of *Pseudomonas chlororaphis* was also stimulated in presence of crude extracts, containing AM fungus exudates and mycelial compounds of AM fungi from the extraradical network of in vitro grown *Glomus intraradices* [59].

Arbuscular mycorrhizal fungi can stimulate the growth of rhizobacteria by providing nutritional resource through the release of exudates. Exudates collected from tomato roots which were colonized by *Glomus fasciculatum* were reported to attract *Azotobacter chroococcum* and *Pseudomonas fluorescens* more strongly than those collected from non-colonized roots [70]. According to Toljander et al. [71], a bacterial community extracted from soil was significantly affected after 48 h when inoculated with exudates produced by AM fungus mycelia in comparison to a control composed of culture medium.

The reduction in exudation through defoliation of pea plants did not change the PCR-DGGE profile of rhizosphere bacteria, while missing and supplementary bands were observed from the rhizosphere of plants which were pre-colonized with *Glomus intraradices* [72]. PCR-DGGE analysis reported to show no effect on the bacterial community structure of tomato rhizosphere which was treated with pre-colonized (with *Glomus intraradices* or *G. mosseae*) root exudates however direct colonization of root with these AM fungi-induced significant changes [24]. The rhizobacterial community structure modification by AM fungal colonization is usually related poorly to exudate liberation by mycorrhizal roots or by the AM fungal mycelium, and importantly it may be dependant on their physical presence or on direct species-specific interactions [24]. It has been noticed that the impact of AM fungus colonization on other soil microorganisms is negative. The overall decrease of microbial activity described after root colonization with AM fungi has been proposed to be due to competition for substrates [73]. In association with cucumber, *Glomus intraradices* possess negative effect on the population of *Pseudomonas fluorescens* DF57. This negative effect was reported in both rhizosphere and in mycosphere [74].

4. AM-associated bacteria and biocontrol activities

Most of AM-associated bacteria (AMB) described so far in detail showed antagonistic characteristics towards soilborne pathogens or behaved as mycorrhization helper [16]. Similar studies have been performed by various researchers in aiming to identify AMB with biocontrol activities. A bacterial strain of *Paenibacillus* sp. B2 has been isolated from the mycorrhizosphere of *Glomus mosseae* and identified by phylogeny of its 16S rRNA gene sequence and analytical profile index (API) system. It has been found that it acts antagonistic to various soilborne pathogens under in vitro conditions and reduces necrosis in tomato roots (necrosis caused by *Phytophthora nicotianae*) [75]. This isolate (i.e. bacteria) displayed

cellulolytic, proteolytic, chitinolytic and pectinolytic activities and was reported for antibiotic polymyxin B1 and two other polymyxin-like compounds [76–78]. Moreover, its presence resulted in disorganization of cell walls and/or cell contents of *Phytophthora nicotianae* and *Fusarium oxysporum* as observed in electron microscope. It also increases the root and shoot fresh weights of mycorrhized tomato plants and stimulated *Glomus mosseae* to colonize tomato roots [75].

Under compartmentalized growth system, Mansfeld-Giese et al. [78] identified *Paenibacillus polymyxa* and *P. macerans* from the three different regions, namely mycorrhizosphere, hyphosphere (root-free soil and sand compartments) and from a root-free sand compartment. It was found to be closely associated with *Glomus intraradices*. All *Paenibacilli* strains tested from these AM fungi influenced soil zones and helped in preventing pre-emergence damping-off (caused by *Pythium aphanidermatum*) [79]. Out of 18 cultivable isolates from surface-disinfected spores of *Glomus mosseae*, 14 isolates were identified. These identified isolates were mainly composed of *Bacillus simplex*, *B. niacini*, *B. drententis*, *Paenibacillus* spp. and *Methylobacterium* sp. which were reported to show antagonism to various soilborne pathogens (e.g. *Phytophthora nicotianae*, *Fusarium solani*, *Fusarium oxysporum*, etc.) [80]. Bacteria isolated from surface-decontaminated spores of *Glomus intraradices* and *Glomus mosseae* which were extracted from rhizospheres of *Festuca ovina* and *Leucanthemum vulgare* were classified within two phylogenetic clusters: one corresponding to *Proteobacteria* and the other corresponding to *Actinobacteria* and *Firmicutes* [14]. Under dual culture in vitro assays, bacteria from both clusters were reported antagonistic to *Rhizoctonia solani*. Further, selected bacteria, two isolates of *Stenotrophomonas maltophilia*, three isolates of *Pseudomonas* spp., one isolate each of *Bacillus subtilis* and *Arthro bacterilicis*, were reported to act as antagonistic to *Erwinia carotovora* var. *carotovora*, *Verticillium dahliae*, *Phytophthora infestans* and *Rhizoctonia solani*. In vitro studies revealed that these isolates are responsible for producing siderophores and proteases and thus decrease the weight of rotten potato tissues [81]. The ability of AM fungi to specifically harbor and then to stimulate rhizobacteria with biocontrol properties suggests that these bacteria can directly reduce pathogen development within the mycorrhizosphere and they can strongly contribute to the biocontrol of soilborne diseases.

5. Unfavorable zone to pathogen development: the mycorrhizosphere

The mycorrhizosphere has been hypothesized to comprise of favorable surroundings for the growth and development of microorganisms which works antagonistic to soilborne pathogens proliferation. Undeniably, co-culture of the non-mycorrhizal species (e.g. *Dianthus caryophyllus*) with the mycorrhizal species (e.g. *Tagetes patula*) in the presence of AM fungi (e.g. *Glomus intraradices*) clearly reduces the disease caused by *F*usarium *oxysporum* f. sp. *dianthi* in the plant *Dianthus caryophyllus*. It occurs in a manner which differs in providing nutrition to plants and thus suggests a decline in the pathogen development within the mycorrhizosphere [82]. Moreover, a reduction in the number of infection loci in tomato roots (pre-colonized with *Glomus mosseae* and also inoculated with *Phytophthora nicotianae* zoospores) infers that the pathogen may be affected prior to root penetration in the mycorrhizosphere [83].

The mycorrhizosphere influenced by the rhizobacteria + AM fungus + root tripartite associations presents specific characteristics, in which individual factor influences the others' growth and health. Remarkably in the presence of glycoproteins such as glomalin, AM fungi favor the formation of aggregates which provide

stable microsites favorable to root and microbe establishment [84, 85]. The AM fungi extraradical network also constitutes specific microsites which favor the growth of some bacteria. Among different plant growth-promoting rhizobacteria, P-solubilizing and N-fixing bacteria has been reported for more efficient synergistic interaction with AM fungi. Increased P and N availability to the plants promotes its growth and probably favors its capacity to counteract pathogen impact [11, 86–88].

Plant growth-promoting rhizobacteria can also display biocontrol properties and impact pathogen proliferation through direct liberation of toxic compounds or by competing for space and nutrients, reduction of Fe and Mn availability, modification of the plant hormone balance and stimulation of plant defense mechanisms [89, 90]. A synergistic or additive impact by dual inoculation of AM fungi with rhizobacteria in controlling pathogens reflects the dependence of biocontrol properties on the combinations of bacterial and fungal species used, nutritional status in soil and probably other environmental conditions [87].

Reduction in gall formation and nematode multiplication (which are usually responsible for causing root rot in chick pea) was significantly reported in the tomato plants when its roots were inoculated together with *Glomus intraradices* and bacteria *Pseudomonas striata* and *Rhizobium* sp. [91]. Similar positive reports have been recorded when dual inoculation of *Glomus mosseae* with *Pseudomonas fluorescens* was done [92]. Jaderlund et al. [93] reported the interactions of two plant growth-promoting rhizobacteria, namely, *Pseudomonas fluorescens* SBW25 and *Paenibacillus brasilensis* PB177, with AM fungi *Glomus mosseae* and *Glomus intraradices,* respectively; he investigated it on winter wheat which was infested with *Microdochium nivale* and concluded that this interactions are species-specific between fungi and bacteria. From the above and several other studies, it is clear that microbial antagonist to pathogens, and fungi-plant growth-promoting rhizobacteria, do not exert any negative effect against AM fungi [87]. Thus, such mycorrhization helper bacteria (MHB) are important in promoting mycorrhizal development and may even increase AM fungi impact on pathogens.

6. Conclusion

The competence of AM fungi to control disease symptoms and the intraradical and rhizosphere proliferation of soilborne pathogens is multifaceted and influenced by different mechanisms possibly acting in a synergetic way with each other. Among these mechanisms, the capacity of extraradical network of AM fungi to stimulate beneficial microorganisms is possibly a strongly responsible factor involved. Different bacteria with high capacities of antagonistic activities against several soilborne pathogens have been reported within AM fungal extraradical structures and in the mycorrhizosphere of several AM fungi species. The AM fungi-mediated biocontrol activities can not solely be due to the AM fungus function but also related strongly to the capacity of the AM fungi to constitute an environment which favors the establishment of rhizobacteria with potential biocontrol abilities.

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