8 Bacterial Community Composition and Activity in Rhizosphere of Roots Colonized by Arbuscular Mycorrhizal Fungi

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8.1 Introduction

Rhizosphere microbial communities can be regarded as a subset of the soil microbial community. As the root tip grows through the soil, microorganisms in its pathway will be the first colonisers. During rapid root growth, the zone of elongation immediately behind the root tips is only sparsely colonised by soil microorganisms. Thereafter, microbial population densities increase rapidly in the zone behind the root tips, where high concentrations of soluble, insoluble and volatile root exudates can be utilised for microbial growth and metabolism. In contrast, along the older root parts, the compounds present in the rhizosphere are dominated by cellulose and other recalcitrant cell wall materials from sloughed root cortex tissues. Here the population density is often lower than in the younger root regions closer to the rot tip. The species composition of microbial communities in rhizosphere differs from that in the bulk soil (Foster 1986; Marilley and Aragno 1999). This is a clear indication that plants have a strong influence on the microbial populations on their roots. Indeed, in many cases the rhizosphere communities of different plant species growing in the same soil are distinct (Ibekwe and Kennedy 1998) and plants may even have very similar microbial community composition in different soils (Grayston et al. 1998; Miethling et al. 2000).

Plant roots release 1–25% of the net photosynthesis as soluble and insoluble compounds into the rhizosphere (Merbach et al. 1999). Among rhizosphere microbial ecologists there is currently a consensus that differences in exudate amount and composition are likely to affect microbial community composition because microbial species differ in their ability to metabolise and compete for different carbon sources. Therefore, differences in exudate amount and composition will affect the competitiveness and hence the survival of microbial species. A wide range of factors have

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been shown to affect root exudation, including plant genotype (Rovira 1959; Rengel 1997; Grayston et al. 1998), plant age (Martin 1971; van Veen et al. 1991; Marschner et al. 2001), nutritional status (Hoffland et al. 1989; Liljeroth et al. 1990; Marschner and Crowley 1998; Fan et al. 2001) and colonization by mycorrhizal fungi (Po and Cumming 1997; Marschner et al. 1997). In addition to being easily available substrates for soil microorganisms, certain components of root exudates can also have a selective influence on rhizosphere microorganisms by repelling some species and increasing the competitive ability of others (Geurts and Franssen 1996).

Mycorrhizal plants transfer more assimilates to the roots than non-mycorrhizal plants (Wang et al. 1989; Eissenstat et al. 1993), which may be explained by the carbon demand of the fungus for growth and respiration (Fitter 1991; Kucey and Paul 1982). Arbuscular mycorrhizal (AM) colonization has been shown to decrease root exudation (Dixon et al. 1989; Graham et al. 1981; Marschner et al. 1997) although no effect on exudation has also been reported (Azaizeh et al. 1995). Mycorrhizal colonization may also affect root exudate composition (Marschner et al. 1997; Po and Cumming 1997) and the carbohydrate metabolism of the roots (Buwalda and Goh 1982; Shachar-Hill et al. 1995). These changes could be related to the carbon uptake by the fungus and/or the effect of mycorrhizal colonization on host plant physiology. And mycorrhizal fungi themselves may release exudates that selectively influence the microorganisms in the rhizosphere. Exudates from mycorrhizal fungi have not yet been investigated in detail; however there are reports of compounds such as glomalin, which may increase soil aggregation (Rillig et al. 2002). As microorganisms are mainly found in soil aggregates, glomalin can have positive effects on microbial population density (Andrade et al. 1998b).

Mycorrhizal colonization could also indirectly affect the microbial community in the rhizosphere by its effects on root morphology (Berta et al. 1990, 1993; Hetrick 1991), rhizosphere pH (Bago and Azcón-Aguillar 1997), nutrient content (Li et al. 1991) and enzyme activity (Tarafdar and Marschner 1994) as well as on soil structure (Neergaard-Bearden and Petersen 2000; Rillig et al. 2002; Tisdall 1991). As discussed in Chap. 9, the hyphae of AM fungi create an additional habitat for soil microorganisms which is distinct from that of the rhizosphere of non-mycorrhizal roots and will exert its own influence on the microbial communities.

Creating a mycorrhizosphere habitat may be beneficial for mycorrhizal fungi because some microorganisms specific for the rhizosphere of mycorrhizal roots can stimulate mycorrhizal formation and change mycorrhizal gene expression (Becker et al. 1999; Poole et al. 2001). If plant growth is increased by certain rhizosphere microorganisms this would also benefit the AM fungus because the larger plants could supply the fungus with more carbohydrates.

In this chapter, we will outline the effect of AM colonization on bacterial rhizosphere colonization, community structure and activity and the possible causes of these effects.

8.2 Rhizosphere Colonization by Bacteria

8.2.1 Soil Bacteria

AM colonization affects the colonization pattern of roots by bacteria, resulting in a greater spatial variability of bacterial distribution on AM roots (Christensen and Jacobsen 1993). It can increase the population density of bacteria in the rhizosphere (Abdel-Fatah and Mohamedin 2000; Andrade et al. 1998a; Bagyaraj and Menge 1978; Medina et al. 2003; van Aarle et al. 2003), have no effect on bacterial density (Andrade et al. 1997; Mansfeld-Giese et al. 2002; Meyer and Lindermann 1986; Olsson et al. 1998), or decrease it (Ames et al. 1984; Christensen and Jakobsen 1993). These apparently contradictory findings may be due to AM fungal species-specific interactions, because, as shown in a number of studies (Krishnaraj and Sreenivasa 1992; Marschner et al. 2001; Marschner and Baumann 2003; Secilia and Bagyaraj 1987), AM fungal species differ in their effect on the microorganisms in the rhizosphere. There are also indications that the interactions between AM and rhizosphere bacteria are plant species-specific (Marschner and Timonen 2004; Medina et al. 2003; Vancura et al. 1989) (Fig. 8.1). AM

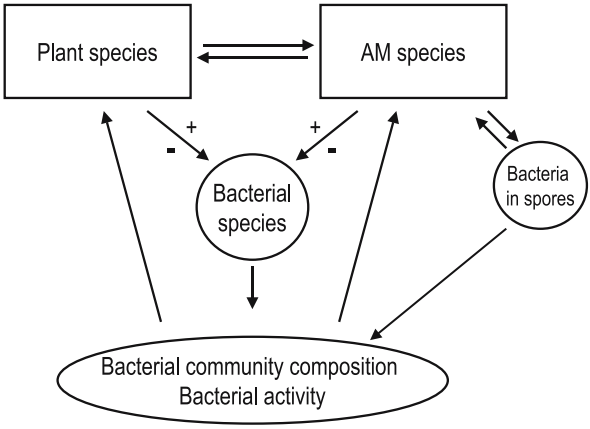

Fig. 8.1. Interactions between plant species, AM colonization and bacteria in the rhizosphere (see text for details)

colonization may also indirectly affect bacteria via changes in population density of bacterial predators such as protozoa (Wamberg et al. 2003).

Compared to non-mycorrhizal roots, roots colonized by AM fungi offer soil microorganisms an additional habitat: the extraradical fungal structures (see Chap. 9). Bianciotto et al. (1996) showed that bacteria form biofilms on spores and hyphae, indicating that these fungal structures can be important habitats for soil bacteria. Our own results (Marschner and Timonen 2004) suggest that the bacterial community colonizing the external mycelium of the outer mycorrhizosphere may be different from that colonizing the inner mycorrhizosphere of AM plants.

8.2.2 Pseudomonads

Of the many soil bacterial genera, the interactions between AM colonization and pseudomonads has received the most interest. The reasons for this are that pseudomonads are considered to be typical rhizosphere bacteria, easily cultured in laboratory media and many are known to be pathogens, biocontrol agents or plant growth-promoting bacteria.

AM colonization often decreases the rhizosphere colonization by *Pseudomonas* sp. (Marschner and Crowley 1996; Marschner et al. 1997; Meyer and Linderman 1986; Paulitz and Linderman 1989). In the study by Ravenskov et al. (1999), the population density of a fluorescent pseudomonad was not affected by AM colonization but its culturability was decreased; suggesting that the cells are more starved in the rhizosphere of AM roots (Ramos et al. 2000). This confirmed the studies by Marschner and Crowley (1996) and Marschner et al. (1997) who reported that AM colonization can reduce the physiological activity of a fluorescent pseudomonad in the rhizosphere. Ravnskov et al. (1999) found that their isolate did not attach to AM hyphae. AM fungal species differ in their suppressive effect towards *Pseudomonas* sp. (Marschner and Crowley 1996; Marschner et al. 1997; Paulitz and Linderman 1989). However, Paulitz and Linderman (1989) argued that this apparent AM fungal species effect may be related to differences in the extent of root colonization by the different AM fungal species. Hence, AM species with a greater percentage root length colonized would be expected to have a stronger suppressive effect.

8.2.3 N_2 Fixing Bacteria and P Solubilizers

Only very few studies have examined the effect of AM colonization on associative N_2 fixing bacteria. Klyuchnikov and Kozhevin (1990) found that

AM colonization increased the rhizosphere population density of *Azospirillum brasiliense*. Far more studies investigated the interactions between AM colonization and the agronomically very important symbiotic N_2 fixers.

In legumes colonized by AM fungi and *Rhizobium* both symbionts represent a significant carbon sink (Kucey and Paul 1982) and competition for host carbohydrates may explain negative interactions between AM fungi and *Rhizobium*. For example, Reinhard et al. (1992) found that the presence of *Rhizobium* decreased AM colonization. The competition between the two symbionts may be particularly expressed under low light conditions when less carbohydrates are translocated to the roots (Bethlenfalvay et al. 1982).

On the other hand, positive interactions between AM and *Rhizobium* have also been frequently reported. AM colonization can increase nodulation (Abbott and Robson 1977) and enhance plant yield and N uptake (Barea et al. 1987; Xavier and Germida 2003). AM colonization can also stimulate colonization of alder by *Frankia*, the N_2 fixing actinomycete (Fraga-Beddiar and Le Tacon 1990).

The apparent contrasting results, negative interactions on the one hand and positive interactions on the other, could be due to compatibility between the two symbionts as well as between the microbial partners and the host plant. Evidence for the former was given by Xavier and Germida (2003), who showed recently that some combinations of AM fungal species and *Rhizobium* species had a negative effect on yield and N uptake while others have a positive effect. The importance of the combination of AM species and N_2 species was also evident in a study by Subba Rao et al. (1985) where the extent of synergism between *A. brasiliense* and AM colonization in terms of plant growth strongly depended on the AM fungal species. The contrasting results may also be due to the benefit gained by AM colonization for P uptake. Under conditions of low P supply, AM colonization can increase P supply to plants and nodules and thus positively affect nodulation and N_2 fixation. If P is not limiting growth of plants and *Rhizobium*, AM fungi will represent a carbon drain with little or no benefit. Then, negative interactions between *Rhizobium* and AM colonization may be expected. It should, however, be noted that AM fungi not only increase uptake of P, but also of other poorly mobile nutrients such as Zn (George et al. 1994; Ryan 2003) and can improve soil structural stability (Andrade et al. 1998b; Neergaard-Bearden and Petersen 2000), thus improving plant growth and thereby also carbohydrate supply to *Rhizobium*. Additionally, AM colonization can suppress soil-borne plant pathogens (see Chap. 9), which would also result in improved plant growth. Positive effects of AM on *Rhizobium* could be related to the suppression of microorganisms that inhibit root colonization by *Rhizobium,* while negative interactions may be expected if such microorganisms are stimulated by the presence of AM.

Many soil microorganisms can increase the solubility of sparingly soluble P minerals. If AM fungi have a stimulating effect on such microorganisms, plant P uptake could potentially be increased. Indeed, AM colonization can increase the population density of P solubilizers in the rhizosphere and co-inoculation of plants with AM fungi and P solubilizers can increase plant P uptake compared to inoculation with AM fungi alone (Sreenivasa and Krishnaraj 1992) or P solubilizers alone (Azcón et al. 1976).

8.2.4 Biological Control Organisms

Biocontrol agents such as pseudomonads, which produce antibiotics or lyse fungal cell walls could potentially have a negative impact on mycorrhizal colonization. There are reports that biocontrol agents such as *Azospirillum*, *Pseudomonas* or *Trichoderma* have no negative effect on AM colonization (Barea et al. 1998; Vazquez et al. 2000). On the other hand, Wyss et al. (1992) showed that two biological control organisms, *Trichoderma harzianum* and *Streptomyces griseoviridis*, decreased colonization by *Glomus mosseae*. This was also the case for *Glomus intraradices* (Green et al. 1999). With respect to the effect of AM colonization on biocontrol organisms, Green et al. (1999) showed that *G. intraradices* decreased the population density and activity of *Trichoderma harzianum*. From the data presented so far in this chapter, it seems very likely that the interaction between biocontrol agents with AM fungi would by highly specific for a given biocontrol agent/AM fungus combination which may be further affected by the plant species.

8.3 Bacterial Community Composition

The studies with single isolates suggest that the density of some bacterial species is lower in AM roots than in non-mycorrhizal roots. However, these studies are highly artificial because only one bacterial species is used. This is in contrast to the soil environment, where the microbial community is highly complex and consists of species with different growth rates and substrate preferences. Nevertheless, experiments conducted with complex microbial communities show that AM colonization can change the bacterial community composition in the rhizosphere by stimulating the population density of certain bacterial species or functional groups, while depressing others (Amoralazcano et al. 1998; Andrade et al. 1997; Marschner et al. 2001; Marschner and Baumann 2003; Meyer and Linderman 1986; Posta et al. 1994; Secilia and Bagyaraj 1987; Wamberg et al. 2003) (Table 8.1). In many

Table 8.1. Interactions between plant species, AM colonization and bacteria in the rhizosphere (see text for details)

Microorganism	AM fungus	Effect of AM	Reference
Indigenous bacteria	*Indigenous fungi*	Increase	van Aarle et al. (2003)
Gram-positive *Bacillus* sp	*Glomus sp.*	Decrease	Secilia and Bagyaraj (1987)
	Glomus intraradices	Decrease	Posta et al. (1994)
Gram-negative	*Glomus sp.*	Increase	Secilia and Bagyaraj (1987)
	Glomus mosseae	Increase	Posta et al. (1994)
Fluorescent pseudomonads	*Glomus desterticola*	Decrease	Marschner et al. (1997); Vázquez et al. (2000)
	Glomus fasciculatus	Decrease	Meyer and Linderman (1986)
	Glomus mosseae	Decrease	Waschkies et al. (1994); Posta et al. (1994); Vázquez et al. (2000); Marschner et al. (1997)
Azotobacter	*Glomus fasciculatus*	Increase	Bagyaraj and Menge (1978)
Azospirillum brasiliense	*Glomus mosseae*	Increase	Klyuchnikov and Kozhevin (1990)
Actinomycetes	*Glomus fasciculatus*	Increase	Bagyaraj and Menge (1978)
	Glomus intraradices	Increase	Abdel-Fatah and Mohamedin (2000)
	Glomus mosseae	Increase	Posta et al. (1994)
	Glomus sp.	Increase	Secilia and Bagyaraj (1987)
Streptomyces sp	*Glomus deserticola*	No effect	Vázquez et al. (2000)
	Glomus fasciculatus	Decrease	Meyer and Linderman (1986)
	Glomus mosseae	No effect	Vázquez et al. (2000)
Ammonia oxidizers	*Glomus mosseae*	Increase	Amoralazcano et al. (1998)
	Glomus fasciculatus	Increase	Amoralazcano et al. (1998)
Ammonifiers	*Glomus fasciculatus*	Decrease	Amoralazcano et al. (1998)
	Glomus mosseae	Decrease	Amoralazcano et al. (1998)
Chitinase producers	*Glomus fasciculatus*	Decrease	Meyer and Linderman (1986)
Mn reducers	*Glomus mosseae*	Increase	Posta et al. (1994); Kothari et al. (1991)
N_2 fixers	*Glomus sp.*	Increase	Secilia and Bagyaraj (1987)
Nitrifiers	Glomus fasciculatus	Decrease	Amoralazcano et al. (1998)
	Glomus mosseae	Decrease	Amoralazcano et al. (1998)

studies it has been shown that the population density of Gram-negative bacteria (Secilia and Bagyaraj 1987; Posta et al. 1994) and actinomycetes (Bagyaraj and Menge 1978; Abdel-Fatah and Mohamedin 2000) is increased in the rhizosphere of AM roots. Kothari et al. (1991) and Posta et al. (1994) found that AM colonization increased the population density of Mn reducers in the rhizosphere, thus increasing Mn availability to the plants and plant Mn uptake. In agreement with the studies with single isolates mentioned above, fluorescent pseudomonads, are generally inhibited by AM colonization (Meyer and Linderman 1986; Posta et al. 1994; Waschkies et al. 1994). In the study by Waschkies et al. (1994) inoculation with AM fungi was associated with an alleviation of grape vine replant disease and the authors argued that this was due to the decreased population density of fluorescent pseudomonads which appear to be one of the causative agents of the disease.

AM colonization can also affect microorganisms involved in N mineralisation in soil. The population density of ammonia oxidizers was higher, while those of ammonifiers and nitrifiers was lower in pot cultures of *Glomus mosseae* and *G. fasciculatum* than in non-mycorrhizal pot cultures (Amoralazcano et al. 1998).

It should be noted that most studies investigating the effect of AM colonization on bacteria in the rhizosphere have relied on culture-dependent methods such as dilution plating. However, less than 5% of soil microorganisms are assessed with culture-dependent methods (Bakken 1985). The main reasons for this low recovery are that (i) many microbial species do not grow or grow very slowly on conventional culture media (Janssen et al. 2002) and (ii) a large fraction of cells are in a viable but non-culturable state (Oliver 1993) or in a state of starvation (Ramos et al. 2000) and therefore do not form visible colonies on standard laboratory media.

Bacterial community composition as affected by AM colonization has also been studied using culture-independent methods such as those based on differences in gene sequence or fatty acid profiles. In maize, Marschner et al. (2001) showed that the bacterial community composition in the rhizosphere (assessed by denaturing gradient gel electrophoresis) of plants inoculated with *Glomus mosseae* or *G. intraradices* differed from that of non-mycorrhizal plants. The two fungal species differed in their effect on bacterial community composition and this was not related to the P status of the plant. In this study, the effect of AM colonization was more pronounced after six weeks than after three weeks and during this time the percentage root length colonized by the fungi increased more than twofold. This suggests that the extent of AM effect maybe related to the percent root length colonized (Paulitz and Linderman 1989). However, we found recently that AM colonization had a strong effect on bacterial community composition in canola (*Brassica napus*) with less than 10% of root length colonized while

it had no effect in clover (*Trifolium subterraneum*) where more than 50% root length were colonized (Marschner and Timonen 2004). Hence, the bacterial community composition can even be affected when only a small fraction of the root system is colonized by AM fungi.

The results of a study with split-root maize plants (Marschner and Baumann 2003) indicate that the AM effect is, at least in part, plant-mediated because AM colonization changed the bacterial community composition in the rhizosphere on both the root half colonized by AM and the non-mycorrhizal half. In agreement with the earlier study (Marschner et al. 2001), the rhizosphere bacterial community composition was fungal species-specific.

On the other hand, there are reports that AM colonization has no effect on bacterial community composition (Mansfeld-Giese et al. 2002; Olsson et al. 1996; Søderberg et al. 2002). As mentioned above, these contrasting results indicate that the effect may be fungal species-specific (Marschner and Baumann 2003; Marschner et al. 2001; Secilia and Bagyaraj 1987) or plant species-specific (Vancura et al. 1989). This is supported by our own results (Marschner and Timonen 2004), which showed complex interactions of plant and AM fungal species on the bacterial community composition in the rhizosphere.

8.4 Bacterial Activity

As mentioned above, AM colonization can either increase N_2 fixation (Azcon et al. 1991; Barea et al. 1987) or decrease it (Reinhard et al. 1992). Bethlenfalvay et al. (1982) showed that although nodule dry weight may be decreased by AM colonization under low light conditions, specific activity of nodules (N_2 fixation per dry weight of nodule) is increased. Thus, the development of nodules was inhibited by the presence of AM fungi, but once the nodules had reached maturity they were capable of competing effectively with the AM fungus for host assimilates and may have benefited from the improved P nutrition of AM plants. This suggests that the interactions between AM fungi and *Rhizobium* are complex and may change during the development of the nodules.

Besides the well-studied effects on N_2 fixation there are only a limited number of studies that have examined the effect of AM colonization on other microbial activities in the rhizosphere. AM colonization increased chitinase activity in the rhizosphere (Abdel-Fatah and Mohamedin 2000), suggesting that the presence of AM hyphae stimulates the capacity of the rhizosphere microflora to decompose fungal cell walls. Christensen and Jacobsen (1993) found that AM colonization decreased the growth rate of

bacteria in the rhizosphere. In agreement, the studies by Marschner and Crowley (1996) and Marschner et al. (1997) indicate that AM colonization induces a state of starvation in a genetically modified bioluminescent pseudomonad and the degree of inhibition was fungal species-specific. Søderberg et al. (2002) showed that this decrease may be also be plant-species specific.

The increased P uptake by plants inoculated by both P solubilizers and AM fungi compared to inoculation with each microorganism separately (Azcon-Aguilar et al. 1986b; Sreenivasa and Krishnaraj 1992) suggests that presence of AM fungi may stimulate the activity of P solubilizers.

8.5 Effects of Bacteria on AM Fungi

The effects of bacteria on AM fungi will only be briefly outlined here. For a more detailed discussion the reader is referred to Duponnois (Chap. 15) on mycorrhizal helper bacteria.

Certain bacterial species may stimulate AM spore germination (Azcon-Aguilar et al. 1986a, Hildebrand et al. 2002), AM colonization (Fester et al. 1999; Vivas et al. 2003) or the proliferation of the extraradical mycelium (Gryndler et al. 2002). It appears, however, that this effect is bacterial and fungal species-specific (Gryndler et al. 2002; Medina et al. 2003). Bacteria isolated from spores can also inhibit spore germination (Xavier and Germida 2003).

8.6 Bacteria in AM Fungi

Evidence is now emerging that bacteria can also live within AM fungi. Bacteria-like objects (BLOs) in AM fungi were first reported by Mosse (1970) and MacDonald et al. (1983). More recently it was confirmed that these BLOs are indeed bacteria (Scannerini and Bonfante 1991). Xavier and Germida (2003) found Gram-negative and Gram-positive bacteria on the surface of AM spores, but only Gram-positive bacteria within the spores. Bacteria appear to colonize fungal spores intracellularly (Bianciotto et al. 2000), where they are associated with protein and lipid bodies (Minerdi et al. 2002). AM hyphae also contain bacteria intracellularly (Bianciotto et al. 1996).

Some bacterial species found in spores seem to be ubiquitous; they are found in the spores of the same fungal species isolated from different areas (Minerdi et al. 2002) and even in spores of different AM fungal species

(Bianciotto et al. 2000). Minerdi et al. (2002) argued that this suggests co-evolution of the intracellular bacteria and AM fungi. These bacteria may be transferred from one generation of AM fungi to the next via asexual AM spores. Interestingly, the spores of some AM species do not seem to contain bacteria (Bianciotto et al. 2000).

The information on the bacterial species found in AM fungi is just emerging. *Burkholderia* sp. appear to be one of the major groups of bacteria found inside AM spores (Minerdi et al. 2002). Xavier and Germida identified one spore isolate as *Bacillus patsuli*. *Burkholderia* sp. carry *nif* genes, could therefore potentially fix N_2 and thus contribute to the N nutrition of the fungus (Minerdi et al. 2001). However it remains to be shown that the bacteria in spores actually fix nitrogen. The chitinolytic bacteria isolated from spores (Filippi et al. 1998) could have a role in spore germination. Clearly more information about bacterial species from within spores and their role and significance for spore survival and germination is needed (Filippi et al. 1998; Bianciotto et al. 2000).

8.7 Conclusions

Root colonization by AM fungi can affect the bacterial community composition in the rhizosphere by stimulating some species while suppressing others. These effects may be due directly to the fungus or could be plant and/or soil-mediated. They appear to be the result of complex fungus-plant-environment interactions which we are just beginning to understand (Fig. 8.1).

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