

Minireview

Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth

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Summary

Arbuscular mycorrhizal (AM) fungi and bacteria can interact synergistically to stimulate plant growth through a range of mechanisms that include improved nutrient acquisition and inhibition of fungal plant pathogens. These interactions may be of crucial importance within sustainable, low-input agricultural cropping systems that rely on biological processes rather than agrochemicals to maintain soil fertility and plant health. Although there are many studies concerning interactions between AM fungi and bacteria, the underlying mechanisms behind these associations are in general not very well understood, and their functional properties still require further experimental confirmation. Future mycorrhizal research should therefore strive towards an improved understanding of the functional mechanisms behind such microbial interactions, so that optimized combinations of microorganisms can be applied as effective inoculants within sustainable crop production systems. In this context, the present article seeks to review and discuss the current knowledge concerning interactions between AM fungi and plant growth-promoting rhizobacteria, the physical interactions between AM fungi and bacteria, enhancement of phosphorus and nitrogen bioavailability through such interactions, and finally the associations between AM fungi and their bacterial endosymbionts. Overall, this review summarizes what is known to date within the present field, and attempts to identify promising lines of future research.

Introduction

Many bacteria are known to be able to stimulate plant growth through direct or indirect interactions with plant roots and these have been classified as plant growth-promoting rhizobacteria (PGPR). In addition, most plant roots are colonized by mycorrhizal fungi and their presence also generally stimulates plant growth. However, the beneficial traits of root-colonizing bacteria and fungi have been mainly studied separately. Only recently have the synergistic effects of bacteria and mycorrhizal fungi been studied with respect to their combined beneficial impacts on plants.

Both ectomycorrhizal (Garbaye, 1994) and endomycorrhizal (Meyer and Linderman, 1986) fungi can interact with different bacterial species, however, this article will focus on the interactions involving arbuscular mycorrhizal (AM) fungi. These interactions occur in the zone of soil surrounding the roots and fungal hyphae; commonly referred to as the 'mycorrhizosphere' (Rambelli, 1973). The interactions between bacteria and AM fungi have potentially beneficial functions, including the majority of those where PGPR (Meyer and Linderman, 1986; von Alten *et al.*, 1993; Kloepper, 1994; 1996) including N₂-fixing bacteria (Secilia and Bagyaraj, 1987; Biró *et al.*, 2000) are involved.

To date, there is little information on the mechanisms controlling interactions of bacteria with AM fungi and plant roots in the mycorrhizosphere; however, a number of possible alternatives have been proposed. Some bacteria have been shown to directly affect AM fungal germination and growth rate (Mosse, 1959; Daniels and Trappe, 1980; Mayo *et al.*, 1986; Carpenter-Boggs *et al.*, 1995) and thus the beneficial impact to the plant could be through the AM association. Other bacteria can directly influence the physiology of the plants, for example, by increasing root cell permeability. In addition to interacting directly to beneficially influence the mycorrhizal relationship and/or plant growth (Linderman, 1988; 1992; Garbaye, 1994; Vivas *et al.*, 2003), specific bacteria together with AM fungi may create a more indirect synergism that supports plant growth (Barea, 1997), including nutrient acquisition (Barea *et al.*, 2002), inhibition of plant pathogenic fungi

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(Budi *et al.*, 1999), and enhancement of root branching (Gamalero *et al.*, 2004).

In addition to these effects of bacteria on AM fungi, the AM fungi themselves have also been shown to have an impact on the composition of bacterial communities (Artursson *et al.*, 2005). This impact may be relayed through the plant root because mycorrhizal establishment has been shown to change the chemical composition of root exudates and these are often a source of nutrients to associated bacteria in the mycorrhizosphere (Harley and Smith, 1983; Linderman, 1992; Azcón-Aguilar and Bago, 1994; Smith *et al.*, 1994; Barea, 1997; 2000; Gryndler, 2000; Linderman, 2000). However, changes in composition and activity of bacterial communities by AM fungi have also been ascribed to more direct interactions, including competition for inorganic nutrients (Christensen and Jakobsen, 1993). In addition, a few studies have shown that some bacterial species respond to the presence of certain AM fungi (Andrade *et al.*, 1997; Artursson *et al.*, 2005), suggesting a high degree of specificity between bacteria associated with AM fungi. One possible explanation for this noted stimulation of certain bacterial species by specific AM fungi may be that those bacteria are activated by species-specific fungal exudates.

Two main groups of bacteria interact with AM fungi in the mycorrhizosphere: saprophytes and symbionts, both groups potentially consisting of detrimental, neutral and beneficial bacteria (Barea *et al.*, 2002; Johansson *et al.*, 2004). In this article, we will focus on interactions between bacteria and AM fungi with proven or potentially synergistic properties that lead to stimulation of plant growth.

Plant growth-promoting rhizobacteria

Plant growth-promoting rhizobacteria are usually in contact with the root surface, or rhizoplane, and increase plant yield by one or more mechanisms such as improved mineral nutrition, disease suppression, or phytohormone production (Weller, 1988; Kloepper *et al.*, 1991; Lugtenberg *et al.*, 1991; Broek and Vanderleyden, 1995; Défago and Keel, 1995). An additional possibility is that the beneficial effects of some PGPR bacteria are due to their interactions with AM fungi. Some reports have shown that PGPR have a strong stimulatory impact on the growth of AM fungi (Linderman, 1997). For example, increased mycelial growth from *Glomus mosseae* spores caused by an unidentified PGPR has been reported by Azcón (1987). These results suggest that selected PGPR and AM fungi could be coinoculated to optimize the formation and functioning of the AM symbiosis.

Apart from having effects on AM fungal growth, PGPR have been suggested to possess a variety of other direct mechanisms to support the mycorrhizal symbiosis. Garbaye (1994) proposed the term 'mycorrhization helper

bacteria' for rhizobacteria that increased the ability of the root to establish symbiotic interactions with ectomycorrhizal fungi. He suggested a number of possible mechanisms for the helper effect, including stimulation of root development, enhanced susceptibility of the root to ectomycorrhizal fungal colonization, or enhancement of the recognition process between root and fungus. Several reports have also demonstrated enhanced AM fungal colonization levels in roots in the presence of PGPR. For example, association of *Pseudomonas putida* with indigenous AM fungi resulted in a clear growth enhancement of clover plants (Meyer and Linderman, 1986), suggesting that some PGPR may have properties that support both mycorrhizal establishment and function. In addition, Sanchez and colleagues (2004) showed that a fluorescent pseudomonad and an AM fungus (*G. mosseae*) had similar impacts on plant gene induction, supporting the hypothesis that some plant cell programmes may be shared during root colonization by these beneficial microorganisms. Specific interactions between AM fungi and PGPR most likely occur, and certain groups of bacteria have been shown to be established to a much higher extent in the mycorrhizosphere compared with other groups. This was shown, for example, by Andrade and colleagues (1997) who found that bacteria of the genera *Arthrobacter* and *Bacillus* were most frequent in the hyphosphere, the zone of soil surrounding individual AM fungal hyphae, whereas *Pseudomonas* spp. were most abundant in the rhizosphere of *Sorghum bicolor*. This study and others (Artursson *et al.*, 2005) suggest that Gram-positive bacteria may be more commonly associated with AM fungi than Gram-negative bacteria, but this possibility needs to be more rigorously confirmed. It is noteworthy, however, that the bacterial groups most commonly reported to interact synergistically with AM fungi are mainly Gram-positive bacteria and γ -proteobacteria (Table 1), supporting our hypothesis that some members of these phylogenetic groups are more integrally associated with AM fungi than others.

In the study by Andrade and colleagues (1997) the numbers of bacteria quantified by plate counting were higher in the rhizosphere than in the hyphosphere, suggesting that the bacteria benefit by a greater release of organic compounds from the roots compared with the AM fungal hyphae. However, possible changes in unculturable taxa were not evaluated in that study. Recently we used molecular tools to bypass the problems commonly encountered with culture-based approaches to visualize changes in actively growing bacterial community compositions as a result of *G. mosseae* inoculation or plant species (Artursson *et al.*, 2005). We found that mostly 'uncultured bacteria' and *Paenibacillus* sp. were active in the *G. mosseae* inoculated soil, suggesting that many species of interest may be missed if relying on culturing alone.

Table 1. Examples of synergistic interactions between bacteria and AM fungi, potentially leading to enhanced plant growth.

Bacterial species	AMF species	Effect	Reference
Gram +, low G+C			
<i>Bacillus pabuli</i>	<i>Glomus clarum</i>	↑ f.g., ↑ s.g., ↑ r.c.	Xavier and Germida (2003)
<i>Bacillus subtilis</i>	<i>G. intraradices</i>	↑ p.s., ↑ r.c.	Toro <i>et al.</i> (1997)
<i>Paenibacillus validus</i>	<i>G. intraradices</i>	↑ f.g.	Hildebrandt <i>et al.</i> (2002)
<i>Paenibacillus</i> sp.	<i>G. mosseae</i>	↑ f.g., ↑ s.g., ↑ r.c. + i.p.p.f.	Budi <i>et al.</i> (1999)
Gram +, high G+C			
<i>Corynebacterium</i> sp.	<i>G. versiforme</i>	↑ s.g.	Mayo <i>et al.</i> (1986)
<i>Streptomyces orientalis</i>	<i>Gigaspora margarita</i>	↑ s.g.	Carpenter-Boggs <i>et al.</i> (1995)
γ-Proteobacteria			
<i>Enterobacter</i> sp.	<i>G. intraradices</i>	↑ p.s., ↑ r.c.	Toro <i>et al.</i> (1997)
<i>Pseudomonas</i> sp.	<i>G. versiforme</i>	↑ s.g.	Mayo <i>et al.</i> (1986)
<i>Pseudomonas</i> sp.	<i>Endogone</i> sp.	↑ f.g., ↑ r.c.	Mosse (1962)
<i>Pseudomonas</i> sp.	<i>G. mosseae</i>	↑ f.g. + i.p.p.f.	Barea <i>et al.</i> (1998)
<i>Pseudomonas aeruginosa</i>	<i>G. intraradices</i>	↑ p.s.	Villegas and Fortin (2001; 2002)
<i>Pseudomonas putida</i>	<i>G. intraradices</i>	↑ p.s.	Villegas and Fortin (2001; 2002)
<i>Pseudomonas putida</i>	Indigenous mix of AMF	↑ r.c.	Meyer and Linderman (1986)
<i>Rhizobium meliloti</i>	<i>G. mosseae</i>	↑ Nitrogen fixation rates	Toro <i>et al.</i> (1998)

f.g., fungal growth; s.g., spore germination; r.c., AM fungal root colonization; p.s., phosphate solubilization; i.p.p.f., inhibition of plant pathogenic fungi.

In addition to direct stimulation or inhibition of particular bacterial taxa by organic compounds released from roots or hyphae, it is also possible that there are indirect effects. For example, it is known that AM fungi influence soil aggregates through exudation of glycoproteins such as glomalin (Zhu and Miller, 2003) and Andrade and colleagues (1998) demonstrated that there were differences in the bacteria associated with water-stable soil aggregates compared with the non-stable soil fraction.

Physical interactions between bacteria and AM fungi

Several PGPR have been shown to be excellent root colonizers (Lugtenberg and Dekkers, 1999; Barea *et al.*, 2002) and a number of surface components have been demonstrated to play a role in the physical interactions between such bacteria and plant roots (Bianciotto and Bonfante, 2002). However, little information is available concerning the extent to which PGPR colonize AM fungal hyphae. Bianciotto and colleagues (1996a) reported that some *Rhizobium* and *Pseudomonas* species attached to germinated AM fungal spores and hyphae under sterile conditions, and that the degree of attachment varied with the bacterial strain. However, no specificity for either fungal or inorganic surfaces could be detected among the bacteria tested. Based on their results, these authors suggested that interactions between rhizobacteria and AM fungi were mediated by soluble factors or physical contact.

We recently demonstrated that a *Bacillus cereus* strain, isolated from a Swedish soil containing abundant AM fungi, attached to hyphae of the AM fungus *Glomus dussii* at significantly higher levels than a number of bacterial control strains (Artursson and Jansson, 2003), indicating

that the colonization ability varies considerably between different bacteria. To further examine the biological features of bacterial attachment to AM fungal hyphae, we compared the attachment of five different *gfp*-tagged bacterial strains to vital and non-vital hyphae of the AM fungus *Glomus claroideum* (Toljander *et al.*, 2005). This study indicated major differences between the bacterial strains in their ability to attach to different physiological states of hyphae. As the effect of electrostatic attraction was diminished by washing the hyphae with strong salt solution before examination by microscopy, our results support those suggested by Bianciotto and colleagues (1996a) regarding a two-step mechanism. During the first stage of this proposed mechanism a weak binding will occur, often governed by general physicochemical parameters such as electrostatic attraction, whereas the second, more stable binding can be explained by mechanisms involving the production of cellulose fibrils or other bacterial extracellular polymers. In support of this hypothesis, Bianciotto and colleagues (2001) studied bacterial mutants inhibited in extracellular polysaccharide production and found that they were less able to attach to AM fungal hyphal surfaces compared with the wild-type strain.

Several bacteria reported to be good root colonizers, for example, some *Pseudomonas* spp., are also capable of adhering to AM fungal hyphal surfaces, suggesting that the mechanisms involved could be fairly similar. Close cell-to-cell contact between, for example, rhizobia and their host plant roots is an important prerequisite for the formation of the nodules during endosymbiosis, and one may speculate whether similar correlations exist between attachment of bacteria to AM fungal hyphal surfaces and changes in fungal growth or performance. To further eval-

uate this issue, a number of mycorrhiza associated PGPR were tested for their ability to attach to hyphae of ectomycorrhizal fungi. Although some of the bacteria adhered to the fungal mycelium (Sen *et al.*, 1996), in another study positive effects of other rhizobacteria on ectomycorrhizal fungal development and establishment were observed even when attachment did not occur (Garbaye, 1994). One possible way that attachment could benefit both partners, would be through facilitation of certain metabolic interactions, such as nutrient and carbon exchange and this would rely on close cell contact between the bacterial and fungal components.

Because the significance of bacterial attachment for mycorrhizal functioning, especially within the AM symbiosis, is still not clear, the next step would be to evaluate whether there generally is a clear correlation between bacterial attachment to mycorrhizal fungal hyphae and enhanced mycorrhizal fungal growth or performance. If that was shown to be the case, attachment properties should be an important feature to consider when screening for AM fungal compatible bacterial inoculants.

Enhancement of nitrogen availability

Nitrogen-fixing bacteria are known to improve the bioavailability of nitrogen to plants, and this capability may be enhanced when plants are also colonized by AM fungi (Barea *et al.*, 2002). For N_2 fixing rhizobia, the mycorrhizal and root nodule symbioses are typically synergistic both with regard to infection rate and their impact on mineral nutrition and growth of the plant. **Although, AM fungi may contribute to an increased nutrient status in the mycorrhizosphere, by decomposing organic N compounds,** plants may have a greater benefit through additional nitrogen provided through N_2 fixation. **Toro and colleagues (1998) used the $^{15}N/^{14}N$ ratio in plant shoots to show that N_2 fixation rates in *Rhizobium meliloti* inoculated mycorrhizal alfalfa plants were higher than the corresponding rates in non-mycorrhizal plants.** One explanation for increased N_2 fixation in mycorrhizal plants is that when both nitrogen and phosphorus are limiting, AM fungi can improve phosphorus uptake by the plant which in turn would result in more energy available for nitrogen fixation by rhizobia (Kucey and Paul, 1982; Fitter and Garbaye, 1994). In support of this hypothesis, it has been found that the enhanced N_2 -fixing ability in mycorrhizal plants compared with non-mycorrhizal plants, usually disappears if the non-mycorrhizal plants are supplied with a readily available P source (Smith and Read, 1997; Karandashov and Bucher, 2005). The uptake of other essential micronutrients from the soil by the AM fungal hyphae might also play a role in general plant growth improvement as well as in more indirect effects upon the N_2 -fixing system. However, although the main mycorrhizal effect in enhanc-

ing N_2 fixation is apparently mediated by a generalized stimulation of host nutrition, more specific effects may take place at the root or nodule level (Barea *et al.*, 1992). Interactions between AM fungi and rhizobia may, for example, occur at either the precolonization stages, when both microorganisms are localized in the mycorrhizosphere, or during the development of the tripartite symbiosis (Azcón-Aguilar and Barea, 1992). In addition, AM fungi may interact with both symbiotic and free-living N_2 -fixing bacteria (Barea, 1997).

Organic forms of nitrogen may also be made more available by bacteria associated with mycorrhizal fungal hyphae. Recent experiments by Hodge and colleagues (2001) demonstrated that the AM fungus *Glomus hoi* was able to enhance decomposition and increase plant N capture from grass leaves. However, further research is still needed to distinguish between the direct capacity of AM fungi to mobilize organic substrates and their possible, indirect effects on decomposition and plant nutrient uptake, caused by stimulation of decomposers and subsequent uptake of their decomposition products by mycorrhizal hyphae.

Enhancement of phosphorus availability

Bacteria may also support the AM symbiosis by increasing bioavailable phosphate. In soil with low P bioavailability, **free-living phosphate-solubilizing bacteria** may release phosphate ions from sparingly soluble inorganic and organic P compounds in soil (Kucey *et al.*, 1989), and thereby contribute with an increased soil phosphate pool available for the extraradical AM fungal hyphae to pass on to the plant (Smith and Read, 1997). The inorganic form of P may be held firmly in crystal lattices of largely insoluble forms, and may also be chemically bonded to the surface of clay minerals and unavailable to plants. Organic P is also largely unavailable to plants until it is converted to an inorganic form, for example, by phosphate-solubilizing bacteria. Soluble P entering the soil after mineralization by such bacteria results in localized and short-term increases in the concentration of phosphate ions in the soil solution, which AM fungal hyphae and subsequently plants may benefit from. **Organic P may be mineralized by bacteria that secrete phosphatases whereas inorganic P may be released by bacteria that excrete organic acids** (Smith and Read, 1997). Several studies have demonstrated synergistic interactions between phosphate-solubilizing bacteria and AM fungi (Barea *et al.*, 1997; Kim *et al.*, 1998). For example, Toro and colleagues (1997) studied phosphate limited systems containing plants, AM fungi and phosphate-solubilizing bacteria. Their study revealed that the bacteria promoted mycorrhizal establishment whereas the mycorrhizal symbiosis increased the size of the phosphate-solubilizing

bacterial population. The treatments inoculated with both AM fungi and bacteria significantly increased plant biomass and N and P accumulation in plant tissues, compared with their controls which were not dually inoculated. Using ^{32}P isotopic dilution approaches they found that dually inoculated plants displayed lower specific activities ($^{32}\text{P}/^{31}\text{P}$) than control plants, indicating that AM fungi and phosphate-solubilizing bacteria interacted to make use of P sources otherwise unavailable to plants.

Arbuscular mycorrhizal fungal endosymbiotic bacteria

Endocellular bacteria are reported in only a few fungi including some *Glomeromycota* species (AM fungi and *Geosiphon pyriforme*) (Scannerini and Bonfante, 1991; Bianciotto *et al.*, 1996b; 2000; Perotto and Bonfante, 1997; Schüssler and Kluge, 2001; de Boer *et al.*, 2005) and in the ectomycorrhizal basidiomycete *Laccaria bicolor* (Bertaux *et al.*, 2003). Regarding the AM fungi, their cytoplasm harbours bacteria-like organisms (Fig. 1), which have been observed by microscopy in several AM fungal species (*Glomus versiforme*, *Acaulospora laevis*, *Gigaspora margarita*) (Mosse, 1970; MacDonald and Chandler, 1981; Scannerini and Bonfante, 1991; Bonfante *et al.*, 1994). Further investigation of these structures, including the demonstration of their prokaryotic nature, was long regarded as a task too complicated because they could not be cultured. However, by using morphological observations in combination with molecular analyses, Bianciotto and colleagues (1996b) succeeded in showing that they actually were of true bacterial origin. They also demonstrated the AM fungal endosymbiotic properties of these bacteria, that they were able to complete their life cycles within fungal cells, and that the bacterial cells were Gram-negative and rod-shaped. Several additional characteristics of the endosymbiotic bacterial genome have since been reported (Ruiz-Lozano and Bonfante, 1999; 2000; Minerdi *et al.*, 2001; Minerdi *et al.*, 2002a; Minerdi *et al.*, 2002b).

Endosymbiotic bacteria have been detected in several members of the *Gigasporaceae*; actually the only fungal species in this family among the evaluated ones, reported not to contain such bacteria was *Gigaspora rosea* (Bianciotto *et al.*, 2000). In the five other species belonging to the *Gigasporaceae*, intracellular bacteria were detected through all the steps of the fungal life cycle: spores, germ tubes, and extra- and intraradical hyphae, except arbuscules (Bianciotto *et al.*, 1996b). The AM fungus most extensively studied for its endosymbiotic bacteria is *G. margarita* isolate BEG 34, which was also the first fungus in which these prokaryotic cells were further investigated (Bianciotto *et al.*, 1996b). Recent studies have indicated an average of about 20 000 bacteria per *G. margarita*

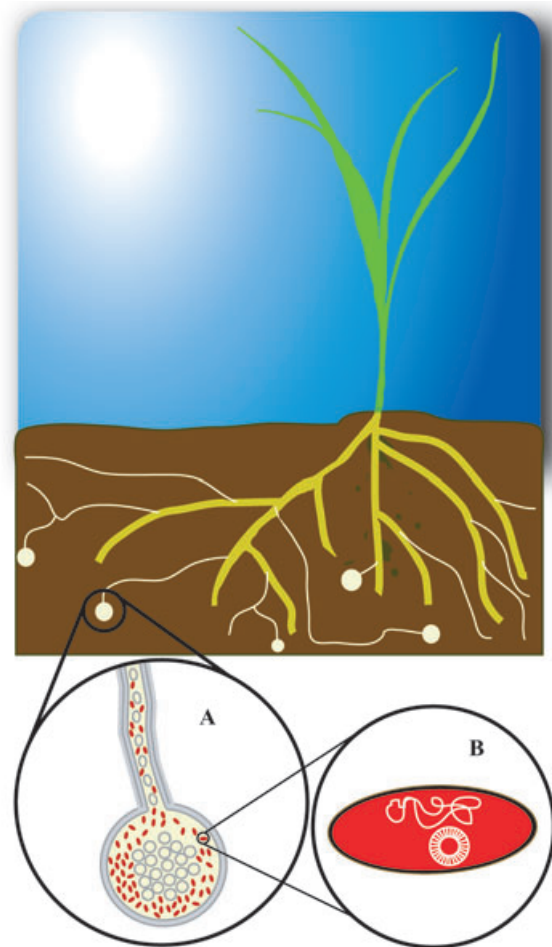


Fig. 1. Schematic view of endocellular bacteria *Ca. Glomeribacter gigasporarum* living in AM fungi. A. Bacteria (red rods) inside the cytoplasm of an AM fungal spore (circles within the spore represent its many nuclei). B. The expected total genome size of *Ca. Glomeribacter gigasporarum* is 1.4 Mb, consisting of an approximately 750-kb chromosome and an additional 650-kb replicon. The drawing is not to scale and underestimates the relative surface area of the extraradical mycorrhizal mycelium.

spore (Bianciotto *et al.*, 2004; Jargeat *et al.*, 2004). These bacteria were initially assigned to the genus *Burkholderia* on the basis of their 16S ribosomal RNA gene sequence, but were recently reassigned to a new taxon termed *Candidatus Glomeribacter gigasporarum* (Bianciotto *et al.*, 2003). In spite of several attempts, these bacteria have never been grown on cell-free media (MacDonald and Chandler, 1981; Scannerini and Bonfante, 1991; Bianciotto *et al.*, 2004; Jargeat *et al.*, 2004), which is the reason why they are assigned to the provisional *Candidatus* designation for uncultured bacteria (Murray and Schleifer, 1994; Murray and Stackebrandt, 1995).

The physiological role of the endosymbiotic bacteria in AM fungi is unknown, as is their potential role in the mycorrhizal symbiosis (Jargeat *et al.*, 2004). However,

some hints about such roles were derived from a genomic library developed from *G. margarita* spores, shown to also represent the genome of the bacterial endosymbiont (van Buuren *et al.*, 1999). Among the bacterial genes isolated from this library and from genomic spore DNA were several interesting finds, including a putative phosphate transporter gene, *pst* (Ruiz-Lozano and Bonfante, 1999), a *vacB*-like gene involved in host cell colonization by enteroinvasive, pathogenic bacteria (*Shigella flexneri* and *Escherichia coli*) (Ruiz-Lozano and Bonfante, 2000), three *nif*-genes (*nifH*, *nifD*, and *nifK*) (Minerdi *et al.*, 2001), the *mcpA* (Minerdi *et al.*, 2002b) and *cheY* (Minerdi *et al.*, 2002a) genes which are involved in chemotaxis, a kinase gene (*prkA*) and a *spoVR* gene (Minerdi *et al.*, 2002a) which is involved in coat formation of bacterial endospores. Jargeat and colleagues (2004) tried to verify the presence of these genes in *Ca. Glomeribacter gigasporarum*, using DNA obtained from pure genomic preparations, but were not able to PCR amplify several of the genes. They concluded that the original genomic library derived from *G. margarita* spores may have been contaminated with foreign bacterial DNA, which also seemed to be confirmed when further screening of the library was performed (Jargeat *et al.*, 2004). However, the *pst* and the *vacB* genes were still detected and these might be of particular interest for future determination of the potential role of the bacterium in the mycorrhizal symbiosis.

Until recently the mode of transmission of the endosymbionts to succeeding generations of the AM fungi was not established. Two alternatives include permanent and cyclical endosymbioses. A permanent symbiosis remains stable over time whereas a cyclical one involves regular reassociation events. For example, each AM fungal colonization event requires a reassociation of the fungal propagule with its host plant (Bianciotto *et al.*, 2004), and is therefore considered to represent a cyclical symbiosis. Similar modes of transmission are also found for *G. pyri-forme*, in which cyanobacteria penetrate the fungi through an endocytotic process (Schüssler and Kluge, 2001). Conversely, Bianciotto and colleagues (2004) demonstrated that cells of *Ca. Glomeribacter gigasporarum* were vertically transmitted through five fungal vegetative generations of *G. margarita* spores. The asexual reproduction typical of AM fungi and the coenocytic nature of their mycelium may facilitate the migration of the endosymbiotic bacteria from spores to hyphae, and thereby allow for the vertical transmission to take place. Active bacterial proliferation was demonstrated to occur in the fungal mycelium, and the authors suggested that these bacteria are obligate endocellular components of their AM fungal host, and thus represent a permanent endosymbiosis unlike the majority of endosymbioses present in the plant kingdom (Bianciotto *et al.*, 2004).

Candidatus Glomeribacter gigasporarum has a surprisingly small genome size for a bacterium, only around 1.4 Mb in total consisting of an approximately 750-kb chromosome and an additional replicon of approximately 650 kb (Jargeat *et al.*, 2004). However, small genomes are often a feature of obligate endocellular bacterial species, a fact which might lend additional support to the hypotheses discussed above regarding the vertical transmission of *Ca. Glomeribacter gigasporarum* bacteria within AM fungi, and their obligate endocellular nature. Considering processes like reductive evolution where only those genes absolutely essential for survival in an intracellular environment should be retained (Dale *et al.*, 2002), *Ca. Glomeribacter gigasporarum* represents a typical candidate for a permanent endosymbiont with its small genome size.

One of the major future challenges within this research area, is to reveal the functional significance of AM fungal endobacteria. One important step in this direction is to be able to remove the bacteria from the fungal cytoplasm, enabling comparisons of fungal effects on plants, in the presence and absence of the bacterial symbiont. Recently, Bonfante and coworkers obtained spores that were devoid of bacteria after successive vegetative generations, resulting in cured spores. These present an excellent tool for further elucidation of the impact of the endosymbiont on the fungus and subsequently on plants (P. Bonfante, pers. comm.).

Future prospects

Although there have been a substantial number of studies of interactions between AM fungi and bacteria, the underlying mechanisms of these associations are not very well understood, and the proposed mechanisms still need further experimental confirmation. More insight into these mechanisms will enable optimization of the effective use of AM fungi in combination with their bacterial partners as a tool for increasing crop yields.

Studies of interactions between AM fungi and bacteria, will greatly benefit from application of molecular approaches, which should enable valuable insights into the mechanisms of these associations, as well as important information regarding fungal and bacterial community structures and metabolic activities. In order to better study bacteria–AM fungal interactions in soil it is beneficial to have means to specifically identify the active bacterial populations in the complex soil community, because these have the potential to exert the greatest effect on their immediate environment. Although a random analysis of rRNA genes from a soil sample could lead to identification of the dominant organisms in the community this technique does not necessarily identify the organisms involved in a particular physiological

response, such as a plant growth promoting effect via AM fungi.

We recently demonstrated that bromodeoxyuridine (BrdU) incorporation and immunocapture was an efficient method for identification of actively growing bacteria independent of their ability to be cultured, in soil inoculated with specific AM fungi (Artursson *et al.*, 2005). This method relies on incorporation of the thymidine analogue BrdU, into growing cells during DNA replication, which is followed by a BrdU immunocapture procedure where the newly synthesized DNA is isolated (Borneman, 1999; Urbach *et al.*, 1999; Yin *et al.*, 2000; Artursson and Jansson, 2003; Artursson *et al.*, 2005). This approach permits identification of specific populations that grow in response to specified stimuli. In a recent study we coupled BrdU immunocapture with molecular fingerprinting techniques to identify bacterial species that were activated in the presence of specific AM fungi (Artursson *et al.*, 2005). The results of that study revealed distinct differences in active bacterial community compositions in response to *G. mosseae* inoculation and different plant species. The putative identities of the dominant bacterial species that were activated as a result of *G. mosseae* inoculation were found to be mostly uncultured bacteria and *Paenibacillus* species, suggesting that there remains a great amount of work to further our knowledge on the bacterial species associated with different AM fungal species.

Determination of the pattern by which certain bacterial species colonize different biotic surfaces, including roots and hyphae, may benefit from the use of marker genes to specifically track the bacteria of interest (Artursson and Jansson, 2003; von der Weid *et al.*, 2005). One particularly popular marker gene is that encoding the green fluorescent protein (GFP), that provides the cells with a green fluorescent phenotype enabling them to be easily detected even in non-sterile samples (Unge *et al.*, 1999; Jansson, 2003). We used the *gfp* gene to tag different Gram-positive bacteria (Artursson and Jansson, 2003; von der Weid *et al.*, 2005), to enable the cells to be visualized in association with AM fungal hyphae (Artursson and Jansson, 2003). We demonstrated that a GFP-tagged *B. cereus* bacterium, isolated as an actively growing strain from a Swedish field soil containing abundant AM fungi, clearly attached to hyphae of the AM fungus *G. dussii* when visualized by confocal microscopy (Fig. 2). Green fluorescent protein was also used to tag and visualize a *Paenibacillus brasilensis* strain, shown to have inhibitory effects on several plant pathogenic fungi (von der Weid *et al.*, 2005) while stimulating growth of certain AM fungi (V. Artursson and J.K. Jansson, unpublished).

Increasingly we are gaining understanding of the complexity of microbial interactions in the mycorrhizosphere. The application of molecular techniques, including those discussed above, should help to provide more information

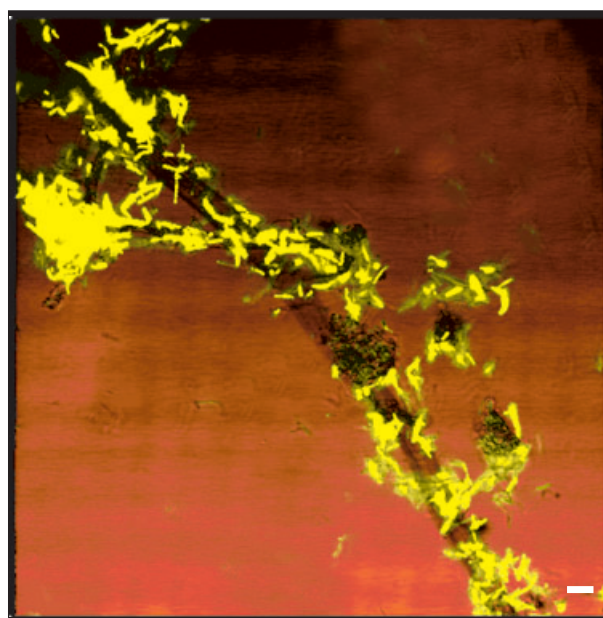


Fig. 2. Confocal image of GFP-tagged *Bacillus cereus* strain VA1 (yellow) attaching to hyphae of *Glomus dussii* (Artursson and Jansson, 2003). Size bar = 4 μ m.

about the role of each fungal/bacterial group on growth, survival and fitness of plants. This information should in turn be used to design better inoculants for maintenance of plant health and production within low-input, sustainable cropping systems. In addition, as more genome sequence information is produced and made available, the associations between AM fungi and bacteria can be evaluated in increasing detail. For example, the genome of the AM fungus *Glomus intraradices* is currently being sequenced, and this information will hopefully provide more clues about AM fungal genome organization, fungal evolution and AM fungal–bacterial specificity issues, including those discussed in the present article.

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