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Arbuscular mycorrhizal fungal spore-associated bacteria affect mycorrhizal colonization, plant growth and potato pathogens

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi and their bacterial associates are essential living components of the soil microbiota. From a total of 385 bacteria previously isolated from spores of AM fungi (AMB), 10 were selected based on ability to inhibit growth of plant pathogens. Effects of these isolates on AM fungal colonization, plant growth in potato (*Solanum tuberosum* L.) and inhibition of pathogens was investigated. AM fungal root colonization of potato was 7-fold higher in the presence of the *Pseudomonas* FWC70 isolate in a greenhouse and was 6–9-fold higher in the presence of the three isolates *Pseudomonas* FWC70, *Stenotrophomonas* FWC94 and *Arthrobacter* FWC110 in an outdoor pot experiment. Several growth traits of potato were stimulated by the *Pseudomonas* isolates FWC16, FWC30 and FWC70 and by the *Stenotrophomonas* isolate FWC14. All three *Pseudomonas* isolates showed inhibition against *Erwinia carotovora*, *Phytophthora infestans* and *Verticillium dahliae* but *Stenotrophomonas* isolates were variable. Protease(s), siderophores and indole acetic acid were produced by all isolates. Chitinase(s) were produced by all *Stenotrophomonas* and phosphate-solubilizing activity by all *Pseudomonas* isolates, the *Stenotrophomonas* FWC14 isolate and the *Arthrobacter* FWC110 isolate. We conclude that some AMB are multifunctional and production of extracellular enzymes and bioactive compounds are likely mechanisms for their multifunctional activities. Our results show that some AMB are likely to contribute to the often described ability of AM fungi to inhibit pathogens, acquire mineral nutrients and modify plant root growth.

1. Introduction

Arbuscular mycorrhizal (AM) fungi have the capacity to form a mutualistic symbiotic association with approximately 80% of terrestrial plant species. In AM fungal symbiosis, the fungus receives carbon from the host and the host receives phosphorus and other nutrients of poor mobility in soils through the fungus (Smith and Read, 1997). Some plant species belonging to the Cruciferae are not known to form this symbiosis and are therefore considered non-hosts (Smith and Read, 1997). In the case of AM crucifers, the association appears mostly non-functional (Ocampo et al., 1980).

The AM fungi coexist with other soil organisms in the rhizosphere, a competitive and dynamic habitat. For survival in the rhizosphere, AM fungi must be able to produce a large number of propagules in the form of extra radical hyphae, mycorrhizal root debris and spores. The production and viability of spores varies depending upon the plant species, AM fungal species and various abiotic and biotic factors. Among the biotic factors, several kinds of bacteria associated with AM fungal spores (denoted here as AMB, Bharadwaj et al., 2008) have been reported. They may have positive or negative effects on the AM fungi. The bacteria *Pseudomonas* sp. and *Corynebacterium* sp. isolated from non-disinfected AM fungal spores have been shown to stimulate the germination of *Glomus versiforme* spores (Mayo et al., 1986), while *P. putida* isolated from AM fungal spores inhibits the germination of *Glomus clarum* NT4 spores (Walley and Germida, 1997). Certain AMB isolates also stimulate mycorrhizal formation. *Paenibacillus* sp. isolated from surface-sterilized *Glomus mosseae* spores stimulates mycorrhizal formation in *Sorghum bicolor* (Budi et al., 1999), while *Bacillus pabuli* isolated from *G. clarum* spores enhances *G. clarum* colonization in pea roots (Xavier and Germida, 2003). It seems that AMB play an important role in the development of AM fungi. Those AMB that help in the development of mycorrhizal symbiosis are termed mycorrhiza helper bacteria (MHB, Garbaye, 1994). It has been suggested that AMB can also function as plant growth-promoting bacteria (PGPB) because they improve the nutrient acquisition of plants (Artursson et al., 2006).

The ecological role of AMB with regard to their interaction with plants and plant pathogens is far from understood. Budi et al. (1999) reported that AMB have the potential for biocontrol of plant

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pathogens and showed that the AMB *Paenibacillus* sp. possesses a broad spectrum of antagonistic activity towards different fungal pathogens including *Phytophthora infestans*, *Fusarium oxysporum* and *Rhizoctonia solani*. Recently Li et al. (2007) also found that AM fungi-associated bacteria from the genus *Paenibacillus* have biocontrol ability against *Pythium*, which causes damping-off of cucumber. The possible antagonistic mechanisms of AMB against plant pathogens have been suggested to be the same as those of PGPB, i.e. competition for nutrients such as Fe, production of antibiotics or production of fungal cell wall-degrading enzymes (Whipps, 2001; Compant et al., 2005)

Soil-borne pathogens are the causal agents of many plant diseases that in severe attacks can lead to dramatic yield losses in a wide range of plant species. For example, the pathogens *Verticillium dahliae* (*V. dahliae*), which causes wilt, and *Erwinia carotovora* var *carotovora* (*Ecc*), which causes soft rot, have a broad host range (Bhat and Subbarao, 1999; Perombelon, 2002). The role of AM fungi in the reduction of plant pathogens is relatively well known. The mode of action of this biocontrol activity could be direct interactions between AM fungi and pathogens but mycorrhiza-mediated triggering of plant defence reactions has also been proposed (Azcon-Aguilar and Barea, 1996; Whipps, 2004). In addition, antagonism from bacteria inhabiting the mycorrhizosphere has also been suggested as a possible mechanism (Budi et al., 1999). Utilization of agrochemicals to control soil-borne pathogens is one of the major problems with modern crop production due to increasing concern for human health and environmental safety. One of the alternative approaches currently being given high priority is biological control. In addition to the use of AM fungi, utilization of microorganisms such as AMB antagonistic to several pathogens combined with their possible positive effects on AM fungal colonization and plant growth offer an important but yet to be fully exploited resource.

To understand the various roles of AMB and possible mechanisms used, we investigated their (1) effects on potato growth and colonization of potato roots by *G. mosseae* in greenhouse conditions and by native AM fungi in natural conditions, (2) effects against plant pathogens and (3) production of extracellular enzymes and secondary metabolites.

2. Materials and methods

2.1. Microorganisms and culturing conditions

The 10 arbuscular mycorrhiza-associated bacteria (AMB) used here were previously isolated from *Glomus intraradices* and *G. mosseae* spores (Bharadwaj et al., 2008). They were selected based on their ability to inhibit growth of two fungal plant pathogens, *R. solani* and *Phytophthora infestans* (Bharadwaj et al., 2008; unpublished observations). The identity and source of these isolates are summarized in Table 1. The isolates were multiplied on diluted tryptic soy broth agar (TSA10, 10 g tryptic soy broth 10, 15 g agar, Difco Ltd) plates for different experiments.

Three plant pathogens, *Erwinia carotovora* var *carotovora* (*Ecc*), *P. infestans* and *Verticillium dahliae*, selected for their potential to cause damage to potato plants, were used for the interaction study. They were obtained from our own culture collection and their pathogenicity on potato was confirmed before setting up different experiments. *Ecc* was routinely multiplied on King's Medium B agar (KBA, King et al., 1954), *V. dahliae* on potato dextrose agar (PDA, Oxoid Ltd) and *P. infestans* on rye agar (Caten and Jinks, 1968).

A crude soil inoculum of the AM fungal isolate *G. mosseae* BEG 85 was obtained from Prof. Søren Rosendahl, University of Copenhagen, Denmark, for the study on interaction with AMB in the greenhouse.

2.2. Plant material and growth conditions

Potato (*Solanum tuberosum* L) plantlets, tubers or true seeds of cv King Edward and winter oilseed rape (*Brassica napus* L) cv Banjo were used for the study on interaction with AMB in gnotobiotic conditions and in greenhouse experiments. Potato plantlets were obtained from Svenskt Potatisutsäde AB, Umeå, Sweden and propagated aseptically via nodal culture on Murashige and Skoog medium (Murashige and Skoog, 1962) in a moist growth chamber at 20 °C. Potato tubers of cv Matilda were used for the outdoor pot experiment.

2.3. Effect of AMB on root colonization by G. mosseae in greenhouse

Five isolates (FWC14, FWC16, FWC30, FWC42 and FWC70) that showed the strongest *in vitro* inhibition of *R. solani* (Bharadwaj et al., 2008, Table 1) were used in order to study the interaction of AMB on AM fungi colonization in potato. The bacteria were cultured on KBA for 48 h at 22 °C and fresh cells were suspended in 0.1% peptone water, yielding approximately 10^7 CFU ml^{-1}. The suspensions were then inoculated onto potato and oilseed rape.

Before bacterial inoculation, potato plantlets were grown for 5 weeks in *in vitro* conditions and then transferred to a growth substrate containing sterile sand and soil (1:1 v/v) in a moist growth chamber for preconditioning. The growth conditions were: temperature 20–22 °C, relative humidity 70% and light and dark

Table 1
Identity and functional characteristics of 10 isolates of arbuscular mycorrhizal fungi (AMF)-associated bacteria (AMB)

AMB isolate no.[a]	Identity[b]	*In vitro* inhibition[c] of:				Extracellular production[c] of:							
		Ecc[d]	*Vd*	*Pi*	*Rs*[b]	Fl	Si	Pr	Ch	Ce	P	HCN	IAA
FWC14	*Stenotrophomonas maltophilia*	+	+	+	+++	−	+	+	+	−	+	−	+
FWC16	*Pseudomonas putida* biotype B	++	+	+	+++	+	+	+	−	−	+	+	+
FWC30	*P. fluorescens* biotype F	++	++	++	+++	+	+	+	−	−	+	−	+
FWC42	*Bacillus subtilis*	+	+++	++	+++	−	+	+	−	+	−	−	+
FWC70	*P. putida* biotype A	+++	++	++	+++	+	+	+	−	−	+	−	+
FWC94	*S. maltophilia*	++	−	−	−	−	+	+	+	−	−	−	+
FWC101	*S. maltophilia*	+++	−	+	−	−	+	+	+	−	−	−	+
FWC110	*Arthrobacter ilicis*	++	+	+	++	−	+	+	+	−	+	−	+
LWC2	*S. maltophilia*	++	+++	++	+++	−	+	+	+	−	−	−	+
LYC39	*S. maltophilia*	+	−	−	−	−	+	+	+	−	−	−	+

Ecc, *Erwinia carotovora* var *carotovora*; Vd, *Verticillium dahliae*; Pi, *Phytophthora infestans*; Rs, *Rhizoctonia solani*; Fl, fluorescent; Si, siderophore; Pr, protease; Ch, chitinase; Ce, cellulase; P, phosphate-solubilizing activity; HCN, hydrogen cyanide producing; IAA, indole acetic acid.

[a] F, *Festuca ovina*; L, *Leucanthemum vulgare*; W, white spore (*G. intraradices*); Y, yellow spore (*G. mosseae*).

[b] Data from Bharadwaj et al (2008).

[c] −, absence; +, presence; ++, moderate; +++, strong inhibition.

[d] Based on Table 2.