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The bacterial community of tomato rhizosphere is modified by inoculation with arbuscular mycorrhizal fungi but unaffected by soil enrichment with mycorrhizal root exudates or inoculation with *Phytophthora nicotianae*

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi have been shown to induce the biocontrol of soilborne diseases, to change the composition of root exudates and to modify the bacterial community structure of the rhizosphere, leading to the formation of the mycorrhizosphere. Tomato plants were grown in a compartmentalized soil system and were either submitted to direct mycorrhizal colonization or to enrichment of the soil with exudates collected from mycorrhizal tomato plants, with the corresponding negative controls. Three weeks after planting, the plants were inoculated or not with the soilborne pathogen *Phytophthora nicotianae* growing through a membrane from an adjacent infected compartment. At harvest, a PCR-Denaturing gradient gel electrophoresis analysis of 16S rRNA gene fragments amplified from the total DNA extracted from each plant rhizosphere was performed. Root colonization with the AM fungi *Glomus intraradices* or *Glomus mosseae* induced significant changes in the bacterial community structure of tomato rhizosphere, compared to non-mycorrhizal plants, while enrichment with root exudates collected from mycorrhizal or non-mycorrhizal plants had no effect. Our results support that the effect of AM fungi on rhizosphere bacteria would not be mediated by compounds present in root exudates of mycorrhizal plants but rather by physical or chemical factors associated with the mycelium, volatiles and/or root surface bound substrates. Moreover, infection of mycorrhizal or non-mycorrhizal plants with *P. nicotianae* did not significantly affect the bacterial community structure suggesting that rhizosphere bacteria would be less sensitive to the pathogen invasion than to mycorrhizal colonization. Of 96 unique sequences detected in the tomato rhizosphere, eight were specific to mycorrhizal fungi, including two *Pseudomonas*, a *Bacillus simplex*, an *Herbaspirilium* and an Acidobacterium. One *Verrucomicrobium* was common to rhizospheres of mycorrhizal plants and of plants watered with mycorrhizal root exudates.

1. Introduction

AM fungi have been shown to reduce disease development in a wide range of plant-pathogen associations (St-Arnaud and Vujanovic, 2007). These ubiquitous fungi are grouped into the phylum *Glomeromycota* (Schüßler et al., 2001) and form a mutualistic symbiosis with most land plants, receiving carbon from their host, and delivering minerals and water back (Smith and Read, 2008). They benefit plant growth in a large part through their ability to colonize a wider soil volume and to exploit resources more efficiently than roots. Recently, Maherali and Klironomos (2007) provided evidences supporting that functional ability to protect from soil pathogens may be conserved within AM fungi evolutionary lineages.

AM fungi impact the other soil microorganisms development leading to the formation of the specific zone of soil called the mycorrhizosphere (Linderman, 1988). Root colonization with AM fungi has most of the time been shown to decrease (Christensen and Jakobsen, 1993; Bansal and Mukerji, 1994; Cavagnaro et al., 2006), but also to increase (Posta et al., 1994; Albertsen et al., 2006) or have no effect (Olsson et al., 1996) on the microbial biomass within not only the rhizosphere but also within the mycosphere, the zone of soil under the influence of the mycorrhizal mycelium only. They were also shown to have species-specific impacts by stimulating or inhibiting the growth of specific microbial taxa (Marschner and Timonen, 2006). As some rhizobacteria are known to inhibit pathogen proliferation through various mechanisms (Bowen and Rovira, 1999; Whipps, 2001), one way AM fungi may reduce disease development is therefore by inducing the formation of a bacterial community unfavourable to pathogens development

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(Li et al., 2007). However, the mechanisms controlling the interaction between bacteria and AM fungi in soil are still poorly understood.

AM fungi have been shown to quantitatively change the amount of root exudates but also to induce some qualitative modifications of root exudate composition (Bansal and Mukerji, 1994; Azaizeh et al., 1995; Marschner et al., 1997; Sood, 2003). Carbohydrates (Hooker et al., 2007; Toljander et al., 2007) and citric acid (Tawaraya et al., 2006) were detected in mycorrhizal hyphae exudates. In this manner, the decrease of soil microbial biomass and the modification of the soil microbial community induced by mycorrhizal colonization were suggested to depend on quantitative and qualitative changes of root exudates (Bansal and Mukerji, 1994; Marschner et al., 1997). Filion et al. (1999) observed that extracts from *Glomus intraradices* mycelium grown *in vitro* in a root free compartment had differential effects on soil microbes, stimulating *Pseudomonas chlororaphis* and *Trichoderma harzianum*, reducing conidial germination of *Fusarium oxysporum* f. sp. *chrysanthemi* and having no effect on the growth of *Clavibacter michiganensis*. In addition, chemotactic responses of the plant-growth-promoting rhizobacteria *Azotobacter chroococcum* and *Pseudomonas fluorescens* to exudates of tomato plants colonized with *G. fasciculatum* were significantly stronger than the response to non-mycorrhizal root exudates (Sood, 2003). Furthermore, exudates produced by AM extraradical mycelia influenced the vitality and the community of bacteria extracted from soil, *in vitro*, but this varied with the time of incubation (Toljander et al., 2007).

Phytophthora nicotianae (an Oomycete) is a soilborne pathogen inducing root rot diseases on a huge host range, infecting more than 72 genera from 42 plant families (Erwin and Ribeiro, 1996). It is responsible for large yield losses in many important crops, including tomato. Reduction of the detrimental effect of *P. nicotianae* on tomato plants colonized by the AM fungus *Glomus mosseae* (Trotta et al., 1996; Vigo et al., 2000; Pozo et al., 2002) but also by *G. intraradices* (Lioussanne et al., 2009) has been described. Cell defense responses and the induction of new isoforms of defense-related enzymes after colonization with *G. mosseae* were described and would contribute to limit pathogen proliferation within host roots (Pozo et al., 2002). Vigo et al. (2000) however reported that *G. mosseae* clearly reduced the number of infection loci formed by *P. nicotianae* on tomato roots which supports the hypothesis that the pathogen's ability to reach and penetrate roots may also be affected before root infection.

It has been estimated that only 0.1–10% of the microorganisms found on typical agricultural soils would be culturable using current culture media formulations while culture independent methods based on 16S rRNA gene amplification permit the detection of over 90% of microorganisms that can be observed microscopically *in situ* (Hill et al., 2000). Denaturing gradient gel electrophoresis (DGGE) is a method by which fragments of DNA of the same length but with different sequences can be resolved electrophoretically and used to infer about the microbial diversity within samples (Muyzer et al., 1993). This approach led to observations that microbial communities within different plant rhizosphere were changed by mycorrhizal colonization (Marschner et al., 2001; Wamberg et al., 2003; Marschner and Timonen, 2005).

The aim of the present study was first to verify that inoculation with *G. intraradices* Schenck and Smith and *G. mosseae* (Nicol. and Gerd.) Gerdemann and Trappe modified the microbial community structure of tomato rhizosphere within our experimental conditions, and to compare the effect of the two AM fungal species. Secondly, we tested if enrichment of the rhizosphere soil with root exudates from plants colonized with the same AM fungi would induce changes similar to direct inoculation. Lastly, we assessed if the bacterial community structure of the AM-inoculated and root exudate-enriched non-mycorrhizal plants was modified by inoculation with *P. nicotianae* Breda de Haan. To this end, tomato plants were grown individually in a compartmentalized soil microcosm and submitted to direct mycorrhizal colonization or supplied with exudates from mycorrhizal plants, with the corresponding negative controls. Plants were then inoculated or not with *P. nicotianae*. At harvest, the bacterial community structure was characterized by PCR-DGGE analysis of 16S rRNA gene fragments amplified from DNA directly extracted from the rhizosphere soil.

2. Methods

2.1. Experimental design

Using a compartmentalized microcosm (described below), twelve tomato plants were, in a first step, either submitted to direct root colonization with AM fungi or to enrichment with root exudates from mycorrhizal tomato plants, with the corresponding negative controls. Thus, half of the plants was supplied with sterilized pure water (E−) and either colonized with *G. intraradices* (Gi), with *G. mosseae* (Gm) or not colonized (G-). The other half of the plants received 2 mL daily of a standardized tomato root exudate solution collected from plants colonized with *G. intraradices* (E^{Gi}), *G. mosseae* (E^{Gm}) or not colonized (E^{G-}). Three weeks later, in a second step, plants were either inoculated (P+) or not inoculated (P−) with *P. nicotianae*. Therefore, the experiment included the twelve following treatment combinations: E-G-P-, E-GiP-, E-GmP-, E^{G-}G-P-, E^{Gi}G-P-, E^{Gm}G-P-, E-G-P+, E-GiP+, E-GmP+, E^{G-}G-P+, E^{Gi}G-P+, E^{Gm}G-P+. The experimental design was a split-plot with the six mycorrhizal inoculation/exudate application treatments randomized in the main plots, and *P. nicotianae* inoculation treatments randomized in the subplots. There were four blocks, each containing one experimental system with a full complement of treatment combinations, giving therefore four independent replicates per treatment. Two blocks were set out first, and the other two blocks were set out three weeks later.

2.2. Biological material and growth conditions

The growth substrate, a 2:2:1 mix of field sandy loam soil, sand and a commercial potting mix (Tropical Plant Soil, Modugno-Hortibec Inc., St-Laurent, QC, pH 5.3–6.3, 0.06% N, 0.1% P, 0.4% K, conductivity 0.5–2.0 mmhos cm^{-1}), was autoclaved twice for 60 min at 121 °C. To reintroduce a microbial community exempt of mycorrhizal fungi in the growth substrate, a 500 g subsample of the sandy loam soil was mixed in 1.5 L of sterilized Milli-Q water (Milli-Q synthesis, RiOs, Millipore, Mississauga, ON), agitated for 30 min, passed through Whatman No. 1 and 42 filters, and added to 5 kg of growth substrate. The mix was homogenised daily at 26 °C for two weeks before use.

Leek (*Allium porrum* L. cv. Farinto) and tomato (*Solanum lycopersicum* L. cv. Cobra) seeds were surface-sterilized 15 min in 70% ethanol, followed by 20 min in 1.5% sodium hypochlorite plus 1% Triton X100 and rinsed three times in sterilized Milli-Q water. Seeds were germinated 48–96 h on Tryptic Soy Agar (TSA, Quélab, Montreal, QC) and transferred to the experimental units. Seedlings were thinned to one per compartment and grown in a greenhouse with 16 h daylight (22–20 °C). Plants were fertilized with 20 mL of 5× Long Ashton nutrient solution (Hewitt, 1966) per week and watered with deionized water as needed.

Ri T-DNA-transformed *Daucus carota* L. roots colonized with *G. intraradices* Schenck and Smith (DAOM 181602) were grown in minimal medium solidified with 0.4% (w/v) gellan gum (Gel Gro, ICN Biochemical, Cleveland, OH), as described in Fortin et al. (2002), for six months in the dark at 26 °C. The spores were separated from the gel in sodium citrate buffer (Doner and Bécard, 1991) and suspended in sterile water. *G. mosseae* (Nicol. & Gerd.) Gerdemann and Trappe (BEG 12) spore production and disinfection were performed as described in Budi et al. (1999) with modifications. Spores were recovered by wet sieving and decanting, and purification was