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Description of a strategy for analyzing the feedback loop of the interactions between plants, arbuscular mycorrhizal fungi and bacteria

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Arbuscular mycorrhiza (AM) are ancient, dating back to 400 millions years. Despite the long joint evolutions of plants and AM fungi, AM are generally assumed to be non-specific associations. However, the diversity of AM fungi has been shown to affect the plant community composition. Furthermore, this long joint evolution is expected to not have occurred independently of the resident bacterioflora.

Here, we report the strategy followed in our group over the last years to increase knowledge in the multitrophic interactions between medics, AM fungi and bacteria.

This general strategy can be assigned to a feedback loop relying on successive steps.

The first step consisted in modifying the rhizosphere environment via the cultivation of different medic genotypes in order (i) to assess if plant species affect AM fungal community as AM fungi do on plant community composition and (ii) to determine if specific bacterial groups and populations are promoted by AM. For that purpose, AM fungi associated with four medic species were compared. As expected, AM fungal diversity did not vary from a plant species to the other, however the abundance of specific fungal OTUs did, indicating preferential associations between fungal and plant genotypes. Bacteria from mycorrhizal *Medicago truncatula* wild-type and non-mycorrhizal mutant impaired in its ability to establish symbiosis were compared. This comparison indicated that Comamonadaceae and Oxalobacteraceae were preferentially associated with AM, and that pseudomonads harboring type three secretion systems (T3SS) were significantly enhanced by AM.

The second step consisted in testing if the bacterial groups and populations selected by AM would in return be beneficial to the fungal symbiosis. Representative strains of these groups and a T3SS+ pseudomonad model strain were tested for their effect on the mycorrhization. Oxalobacteraceae strain and the T3SS+ pseudomonad strain indeed promoted mycorrhization of *M. truncatula* in gnotobiotic conditions. The use of a T3SS- mutant further demonstrated, even in non-gnotobiotic conditions, that pseudomonad promotion was related to this secretion system.

Taken together these data give insight to the reciprocal interactions between plants, AM fungi and bacteria.

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