

ARBUSCULAR MYCORRHIZAE INFLUENCE THE OCCURRENCE OF SOIL FLUORESCENT PSEUDOMONADS HARBOURING TYPE III SECRETION SYSTEMS

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Type III secretion systems (T3SS) of pathogenic and symbiotic Gram negative bacteria allow direct interactions with eukaryotic cells. Saprophytic fluorescent *Pseudomonas* spp. harbouring T3SS genes (T3SS⁺) have been more frequently encountered in the rhizosphere than in bulk soil, which suggests a possible interaction between these bacteria and rhizospheric eukaryotes.

Our objectives were to compare, (i) the effect of mycorrhizal and of non-mycorrhizal roots of *Medicago truncatula* on the occurrence and diversity of T3SS⁺ fluorescent *Pseudomonas* spp. and (ii) the diversity of T3SS⁺ and T3SS⁻ bacterial genotypes.

Fluorescent pseudomonads from (i) the rhizosphere of mycorrhizal roots of *M. truncatula* Jemalong J5 (wild-type, Myc⁺ Nod⁺) and TRV48 (mutant Myc⁺ Nod⁻), (ii) the rhizosphere of non-mycorrhizal roots of TRV45 (mutant Myc⁻ Nod⁻), and (iii) the corresponding bulk soil were isolated.

Pseudomonas spp. strains harbouring T3SS genes were identified by PCR targeting a conserved *hrcRST* DNA fragment. Genotypic diversity of fluorescent pseudomonads has been described by a whole cell rep-PCR fingerprinting method (BOX-PCR) and 16S rRNA sequencing. For T3SS⁺ strains the polymorphism of *hrcRST* has been assessed by PCR-RFLP and sequencing.

The occurrence of T3SS⁺ fluorescent pseudomonads was significantly higher in the rhizosphere of mycorrhizal (J5 and TRV48) than in that of non-mycorrhizal roots (TRV45) or in the bulk soil. Four distinct *hrcRST* genotypes were described, two of which were specifically described for strains associated with mycorrhizal roots. Compared to *hrcRST* negative fluorescent pseudomonads, T3SS⁺ strains displayed specific BOX-PCR genotypes that were all ascribed to the phylogenetic group of *P. fluorescens* on the basis of 16S rRNA gene identity.

Mycorrhizal roots of *Medicago truncatula* clearly influence the population structure of root associated fluorescent pseudomonads in a different way than non-mycorrhizal roots. The occurrence of particular genotypes of fluorescent *Pseudomonas* spp. harbouring T3SS genes was highly increased in the rhizosphere of mycorrhizal roots suggesting that T3SS might be implicated in cellular interactions between fluorescent pseudomonads and arbuscular mycorrhizae.