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Effects of mycorrhization and nodulation on the structure and diversity of fluorescent pseudomonads associated with *Medicago truncatula* roots

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1- Introduction

Despite the importance of mycorrhizae and *Rhizobium* on plant growth and health of leguminous plants, little is known on their interaction with other rhizospheric microorganisms. Among them, some populations of fluorescent pseudomonads are known to affect the saprophytic (microbial antagonism) and parasitic (ISR) growth of phytopathogenic organisms and are then likely to affect also symbiotic microorganisms in the rhizosphere.

The aim of our study was to assess the effect of mycorrhization and nodulation on the structure and diversity of indigenous populations of fluorescent pseudomonads. Our working hypothesis was indeed that populations, preferentially associated with mycorrhizae and /or nodules, would have no deleterious effects on these symbiotic relations or even more would promote them.

2- Materials and methods

The study strategy consisted in comparing the populations of fluorescent pseudomonads associated with the roots of *M. truncatula* wild-type J5 mycorrhized and nodulated (Nod⁺Myc⁺) to that of mutants impaired in their ability to establish symbiotic relations: mutant TRV48 (Nod⁻Myc⁺) and mutant TRV25 (Nod⁻Myc⁻) (Sagan and *al.*, 1995).

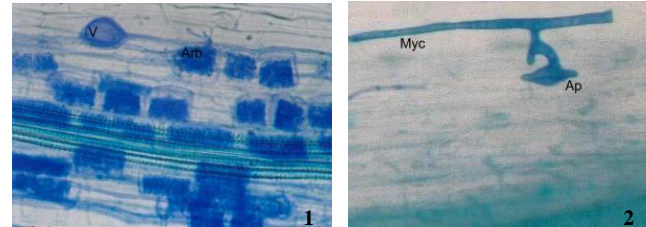
The experimental procedure consisted in:

- Cultivating the wild-type J5 and the mutants TRV48 and TRV25 in a soil showing a high nodulating and mycorrhizal potential.
- Characterizing the phenotype of the host plants (J5, TRV48 and TRV25).
- Numerating and isolating fluorescent pseudomonads from 3 compartments : rhizospheric soil (108 strains), root tissues (103 strains) and uncultivated soil (33 strains).
- Characterizing these populations by AFLP (Amplified Fragments Length Polymorphism).
- Analyzing the data (AMOVA, Chi2 test).

3- Results

3.1- Phenotypic characterization of plants

- ✓ No significant difference in plant growth between J5, TRV48 and TRV25 ($p \leq 0.05$)
- ✓ Presence of nodules only for the wild-type phenotype J5 (Nod⁺Myc⁺)
- ✓ Intensive mycorrhizal colonization of the roots of the wild-type phenotype J5 (Nod⁺Myc⁺) and of the mutant TRV48 (Nod⁻Myc⁺) whereas mycorrhizal mycelium colonized only the root surface of the mutant TRV25 (Nod⁻Myc⁻)
- ✓ Carbon flows (cf. poster of Talon *et al.*)



(1) Mycelium differentiation in Vesicular (V) and Arbuscular (Arb) structures J5 and TRV48 tissues
(2) Mycelial colonisation blocked at appressoria stage (Ap) and no penetration of mycelium in TRV25 tissues

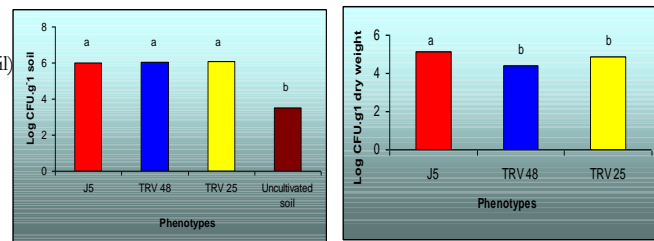
3.2- Compared densities of populations of fluorescent pseudomonads

➤ Compartment effect

- ✓ Density significantly higher ($p \leq 0.05$) in the rhizospheric soils (10^6 CFU.g⁻¹ soil) than in the uncultivated soil (3.3×10^3 CFU.g⁻¹ soil).

➤ Phenotype effect

- ✓ Similar densities in the rhizospheric soil of all plant phenotypes.
- ✓ Densities significantly lower ($p \leq 0.1$) in root tissues of mutants TRV48 and TRV25 than in those of the wild-type phenotype J5.



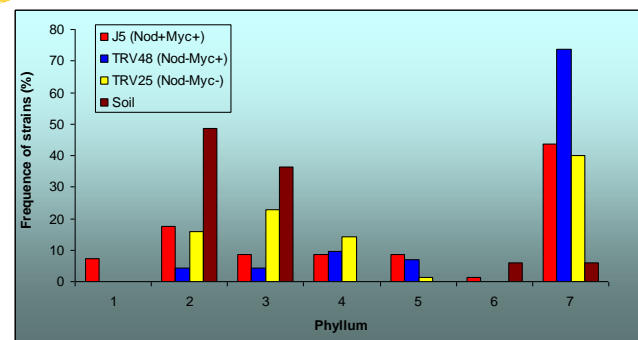
3.3- Structure and diversity of populations of fluorescent pseudomonads

➤ Structure

- ✓ Frequency distributions of populations associated with roots and soil are significantly different (Chi2 test).
- ✓ Populations associated with plants and soil, and populations associated with J5, TRV48 and TRV25 are no significantly different (AMOVA, $p=0.13$; $p=0.26$).

➤ Diversity

- ✓ Significant variations between replicates within a given experimental condition (AMOVA).
- ✓ Diversity of rhizospheric populations is higher than that of soil populations.
- ✓ The highest diversity was observed within the populations associated with the wild-type phenotype J5.



4- Conclusions

- Similar growth for the three plant phenotypes \Rightarrow experimental conditions not favorable for the expression of the beneficial effect of the symbiotic relations.
- Confirmation of rhizosphere effect in our experimental conditions.
- Similar carrying capacities of the rhizosphere of the three plant phenotypes for fluorescent pseudomonads.
- The root tissues of the mutants Nod⁻ harbored lower fluorescent pseudomonads populations than those of the wild-type (Nod⁺) \Rightarrow analogy of recognition between plant/*Rhizobium* and plant/pseudomonads?
- No identification of populations of fluorescent pseudomonads preferentially associated with mycorrhized and/or nodulated roots.