



# Arbuscular mycorrhizae influence the occurrence of soil fluorescent pseudomonads harbouring type III secretion systems

Amandine Viollet, Thérèse Corberand, Adeline Robin, Christophe Mougel, Philippe P. Lemanceau, Sylvie S. Mazurier

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Introduction

Type III secretion systems (T3SS) of Gram negative bacteria mediate direct cellular interactions with eukaryotes.

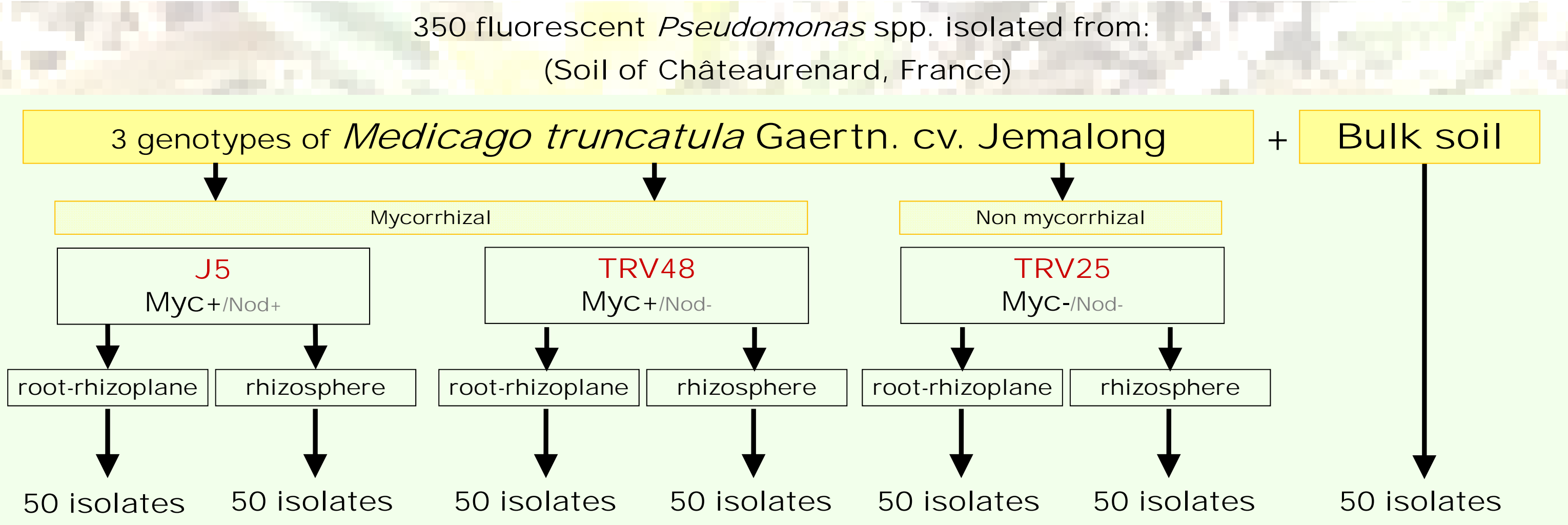
Numerous *Pseudomonas* spp. harbouring T3SS genes have been previously encountered among rhizosphere isolates<sup>1</sup>.

This suggests a possible interaction between these bacteria and rhizosphere eukaryotes.

Objective

To evaluate the impact of arbuscular mycorrhization on the occurrence and diversity of T3SS+ pseudomonads.

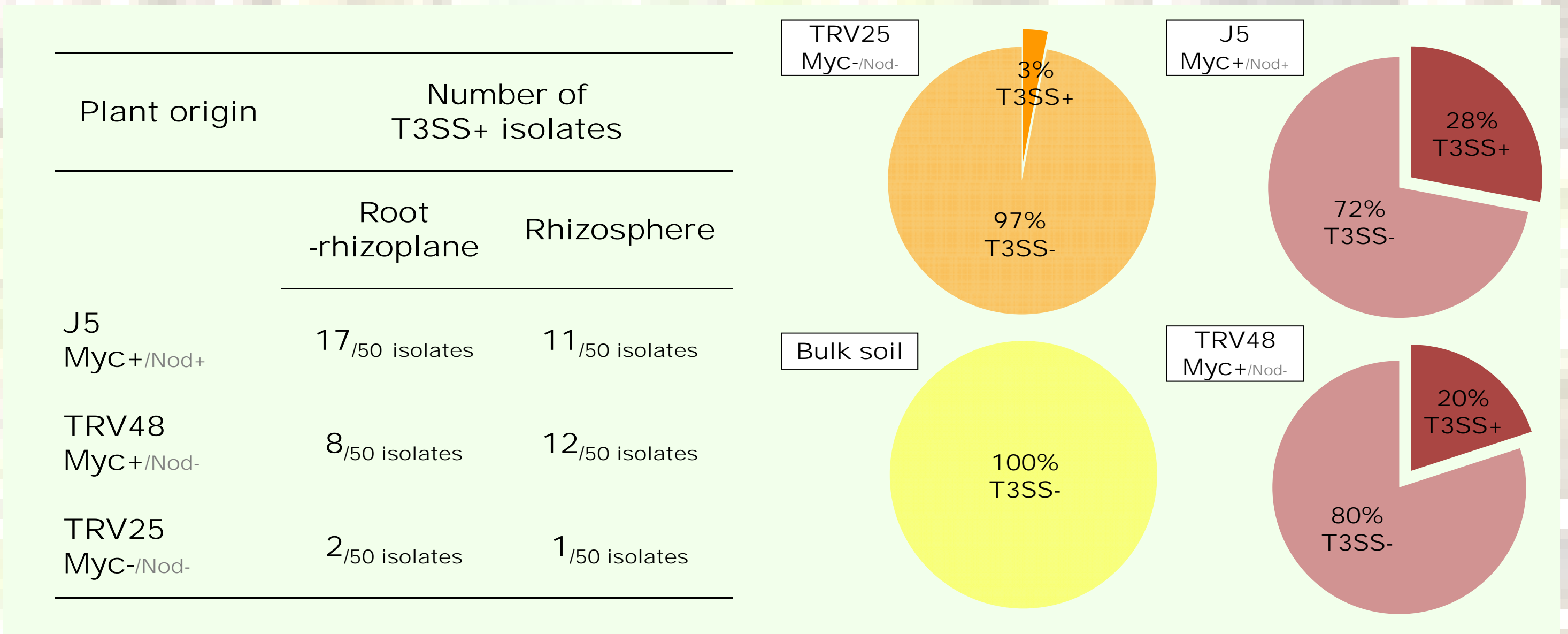
Materials and methods



- Occurrence of fluorescent pseudomonads T3SS+ evaluated by PCR of *hrcRST* genes.
- Diversity of *hrcRST* genes studied by PCR-RFLP, cloning and sequencing.
- Genotypic background of fluorescent pseudomonads described by whole cell rep-PCR fingerprinting (BOX-PCR).

Results

Frequency and occurrence of T3SS+ fluorescent pseudomonads



- No T3SS+ isolates were detected in the bulk soil.
- Distribution of T3SS+ isolates in the root+rhizoplane and in the rhizosphere was not significantly different (Chi-2=2,25; p=0,37).
- 94 % of the T3SS+ pseudomonads were isolated from plant mycorrhizal genotypes (J5 and TRV48).
- The representation of T3SS+ isolates among pseudomonads from both plant mycorrhizal genotypes was equivalent (Chi-2=1,34; p=0,15).

Diversity of *hrcRST* sequences and genotypic background of fluorescent pseudomonads

PCR <i>hrcRST</i> <sup>a</sup>	<i>hrcRST</i> -RFLP genotypes	BOX-PCR genotypes	Number of isolates			
			Myc+		Myc-	Bulk soil
			J5	TRV48	TRV25	
Total			100	100	100	50
+	1 (AAA) <sup>b</sup>	P	6			
+	2 (ADA)	J		6		
+	3 (BBB)	A	8	4		
+	3 (BBB)	B	1	5	2	
+	3 (BBB)	Uncl. <sup>c</sup>	1			
+	4 (CCC)	L	9	5		
+	4 (CCC)	Uncl.	1		1	
+ /-	ND <sup>d</sup>	Uncl.	2			
Total <i>hrcRST</i> <sup>+</sup>			28	20		
			48		3	0
-	-	C			8	
-	-	D		2	6	
-	-	E	8	3	8	19
-	-	F	1	1	3	
-	-	G	4			
-	-	H			2	2
-	-	I	1		1	
-	-	K	5		8	
-	-	M	33	48	31	2
-	-	N	3	16		
-	-	O	5	4	2	
-	-	Q	1	1		
-	-	R		1	2	1
-	-	S				2
-	-	T				2
-	-	U			2	
-	-	V	5	1		2
-	-	W	1		4	
-	-	X			7	1
-	-	Y			3	2
-	-	Uncl.	5	3	10	17
Total <i>hrcRST</i> <sup>-</sup>			72	80	97	50

<sup>a</sup> , +,strong PCR product ; +/-, weak PCR product ; -, no visible PCR product. <sup>b</sup> , numbers designate the *hrcRST*-RFLP genotypes and letters designate the patterns obtained with the restriction enzymes *AluI*, *RsaI*, and *TaqI*, respectively. <sup>c</sup> , Uncl., unique *hrcRST*-RFLP genotypes and BOX-PCR genotypes that remained unclustered. <sup>d</sup> , ND, not determined.

- 4 *hrcRST*-RFLP distinct genotypes were described (1, 2, 3 and 4).
- hrcRST*-RFLP genotypes 1 and 2 were only described for pseudomonads isolated from mycorrhizal roots.
- No common BOX-PCR genotypes were observed between T3SS+ and T3SS- isolates.
- hrcRST* sequences analysis confirmed that T3SS+ pseudomonads belonged to 4 different genotypes.
- hrcRST* genotypes 1 and 2 clustered together, with pathogenic pseudomonads and with *P. fluorescens* C7R12, biocontrol agent and Mycorrhizal Helper Bacteria<sup>2</sup> (green frame A).
- hrcRST* genotypes 3 and 4 clustered together, with the biocontrol agent *P. fluorescens* F113 and with 2 saprophytic isolates (green frame B).
- Incongruencies were observed between phylogenies of *hrcRST* and 16SrRNA sequences.

Conclusions

- Fluorescent *Pseudomonas* spp. harbouring T3SSs appeared preferentially associated with mycorrhizal roots of *Medicago truncatula*. Further studies are in progress to evaluate the role of T3SS+ pseudomonads in *M. truncatula*-mycorrhizal arbuscular fungi (MA) interactions.
- T3SS+ strains displayed specific genotypic backgrounds and were all ascribed to the *P. fluorescens* phylogenetic group on the basis of 16SrRNA gene identity.
- Incongruencies observed between the *hrcRST* and the 16SrRNA phylogenies are compatible with previous findings made on pathogenic pseudomonads and suggesting that HGT (Horizontal Gene Transfer) is a major force in T3SS evolution.

<sup>1</sup>Mazurier S, Lemunier M, Siblot S, Mougel C and Lemanceau P (2004). Distribution and diversity of type III secretion-like genes in saprophytic and phytopathogenic fluorescent pseudomonads. *FEMS Microbiology Ecology*, 49:455-467.

<sup>2</sup>Pivato B, Offre P, Marchelli S, Barbonaglia B, Mougel C, Lemanceau P and Berta G (2009). Bacterial effects on arbuscular mycorrhizal fungi and mycorrhiza development as influenced by the bacteria, fungi, and host plant. *Mycorrhiza*, 19:81-90.