

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 isolates¹.

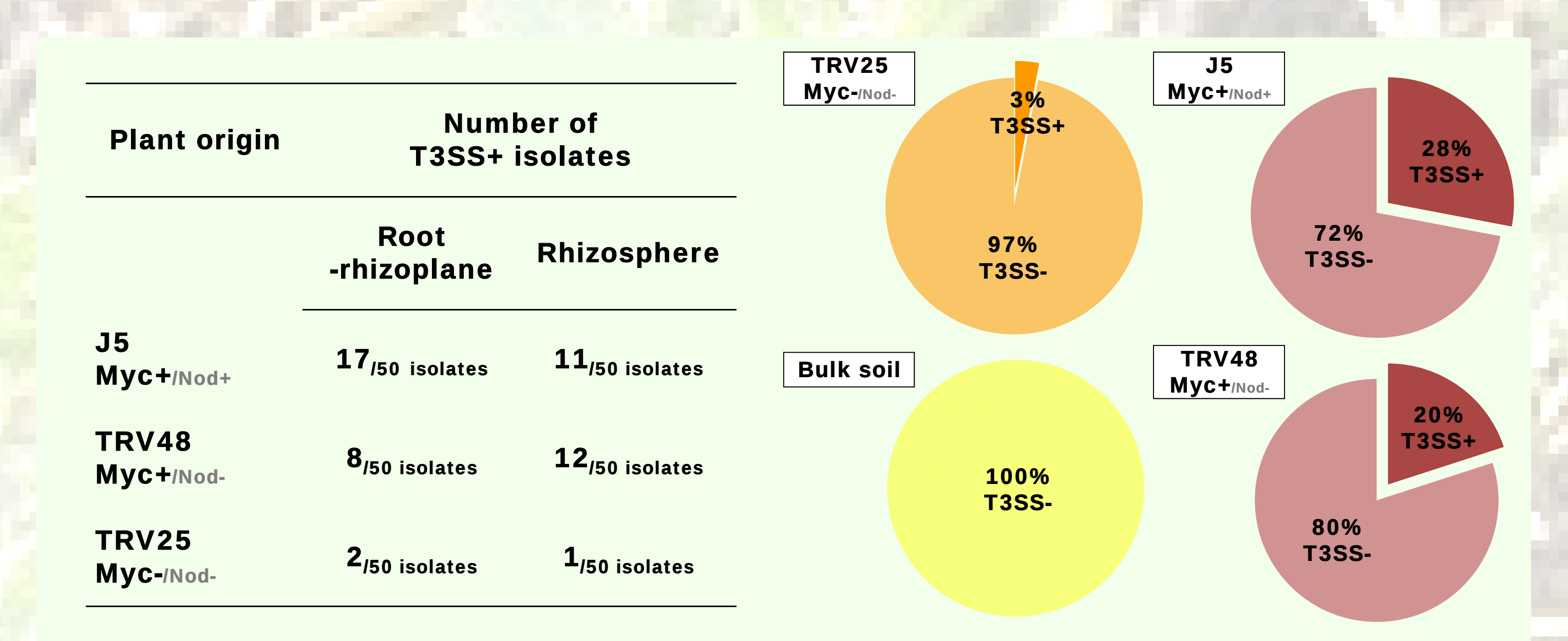
This suggests a possible interaction between these bacteria and rhizosphere eukaryotes.

Objective

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

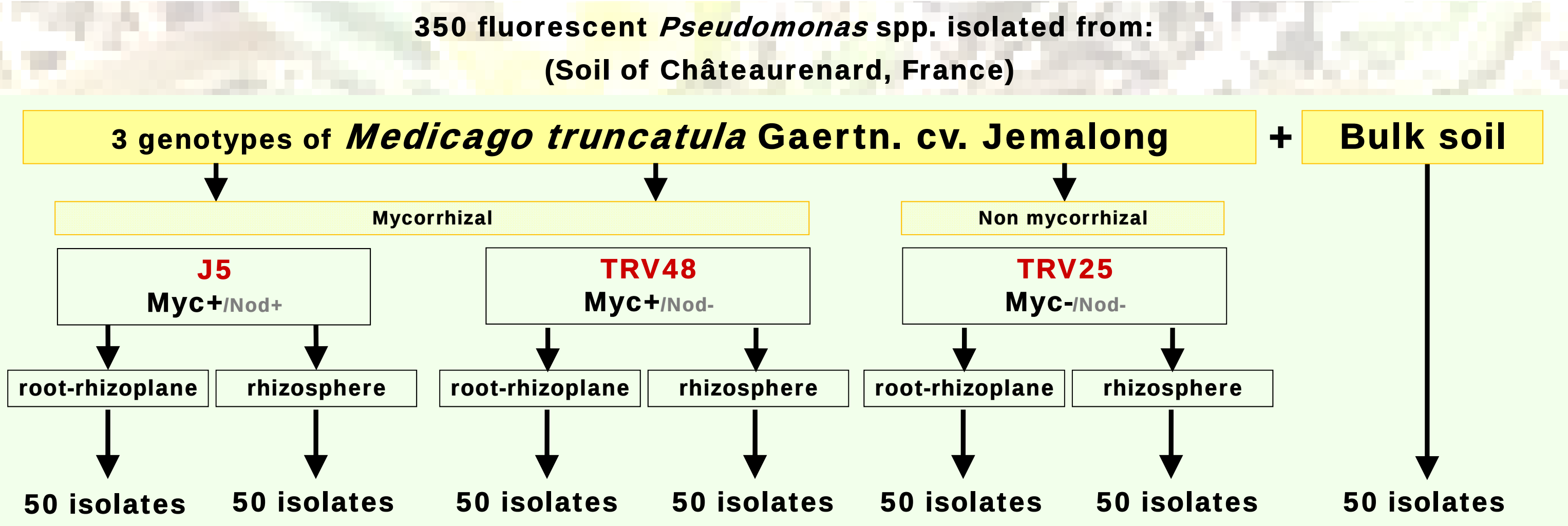
Results

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).

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).

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

PCR <i>hrcRST</i> ^a	<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) ^b	P		6			
+	2 (ADA)	J			6		
+	3 (BBB)	A		8	4		
+	3 (BBB)	B		1	5	2	
+	3 (BBB)	Uncl. ^c		1			
+	4 (CCC)	L		9	5		
+	4 (CCC)	Uncl.		1		1	
+ /-	ND ^d	Uncl.		2			
			Total	28	20		
		<i>hrcRST</i> +		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	72	80		
		<i>hrcRST</i> -		152		97	50

^a, +, strong PCR product ; +/-, weak PCR product ; -, no visible PCR product. ^b, numbers designate the *hrcRST*-RFLP genotypes and letters designate the patterns obtained with the restriction enzymes *AluI*, *RsaI*, and *TaqI*, respectively. ^c, Uncl., unique *hrcRST*-RFLP genotypes and BOX-PCR genotypes that remained unclustered. ^d, 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 Bacteria² (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.

¹Mazurier 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.

²Pivato 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.