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

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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[1].

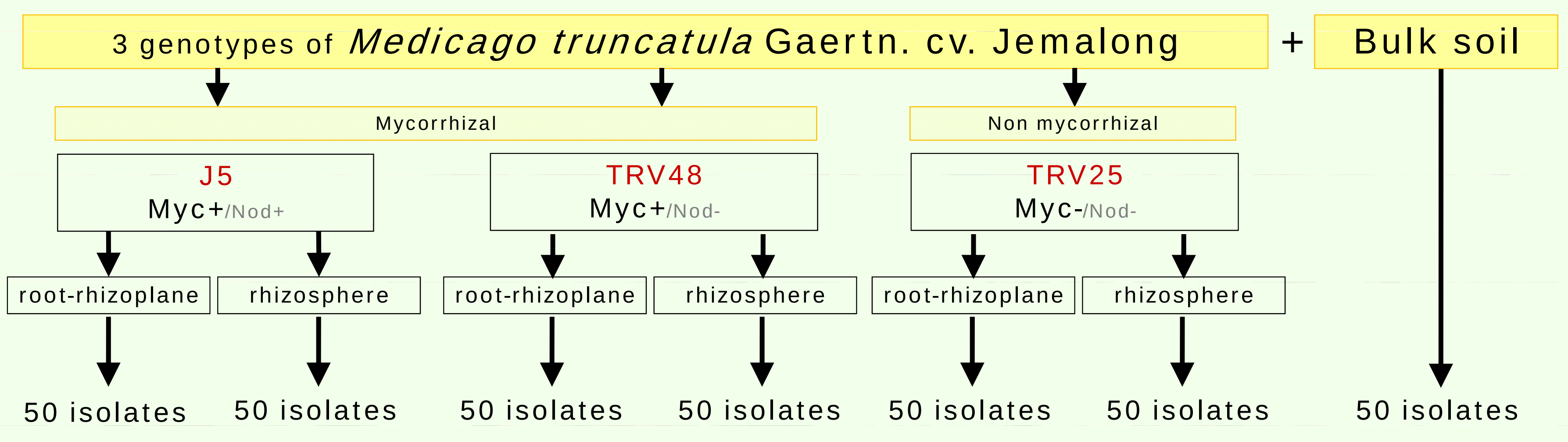
This suggests a possible interaction between these bacteria and rhizosphere eukaryotes.

Objective

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

Materials and methods

350 fluorescent *Pseudomonas* spp. isolated from:
(Soil of Châteaurenard, France)



3 genotypes of *Medicago truncatula* Gaertn. cv. Jemalong + Bulk soil

Mycorrhizal: J5 Myc+/Nod+; TRV48 Myc+/Nod-

Non mycorrhizal: TRV25 Myc-/Nod-

root-rhizoplane → 50 isolates; rhizosphere → 50 isolates (for each genotype)

Bulk soil → 50 isolates

-Occurrence of fluorescent pseudomonads T3SS+ evaluated by PCR of *hrc*RST genes.

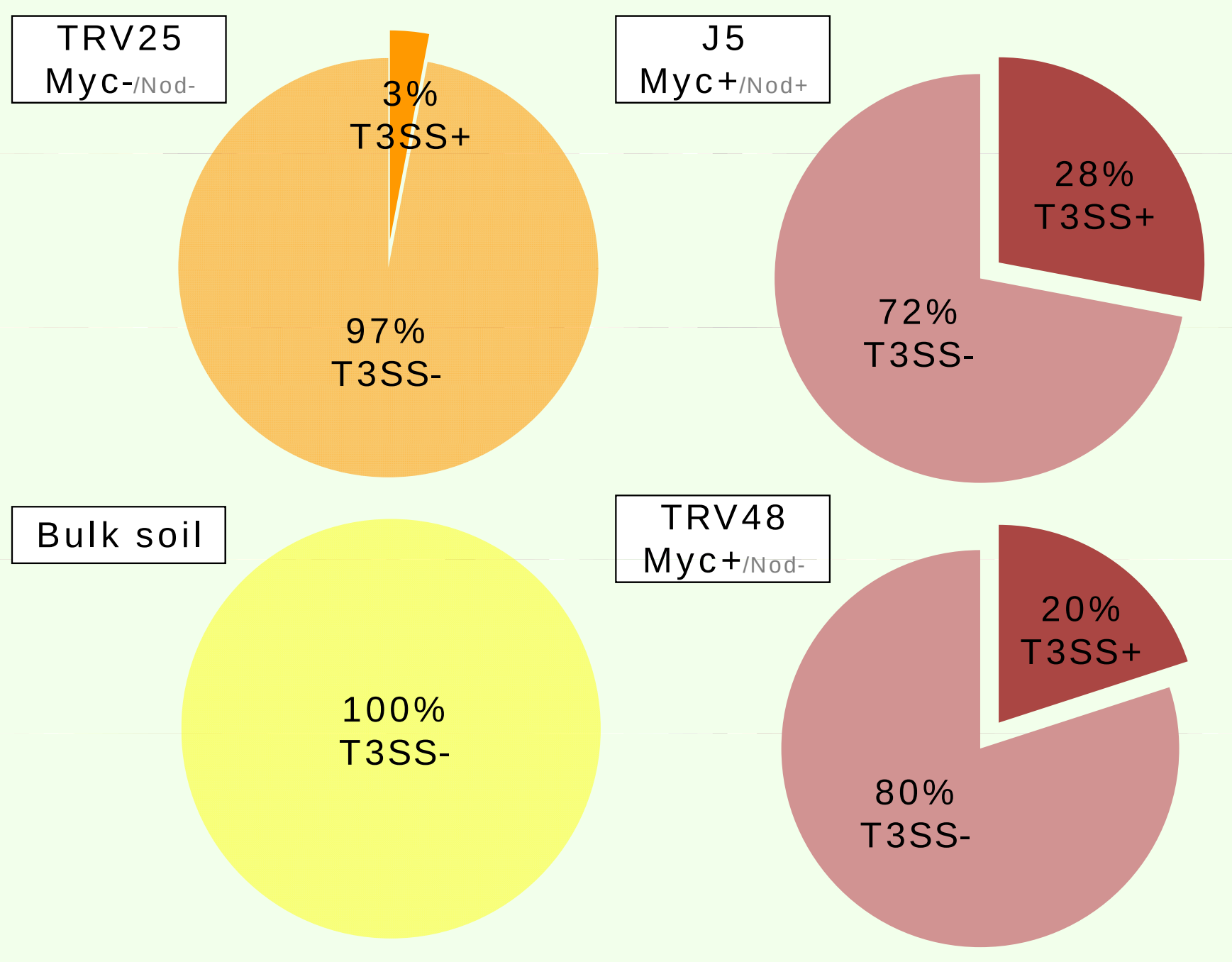
-Diversity of *hrc*RST genes studied by PCR-RFLP, cloning and sequencing.

-Genotypic background of fluorescent pseudomonads described by whole cell rep-PCR fingerprinting (BOX-PCR).

Results

Frequency and occurrence of T3SS+ fluorescent pseudomonads

Plant origin	Number of T3SS+ isolates Root -rhizoplane	 Rhizosphere
J5 Myc+/Nod+	17/50 isolates	11/50 isolates
TRV48 Myc+/Nod-	8/50 isolates	12/50 isolates
TRV25 Myc-/Nod-	2/50 isolates	1/50 isolates



-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 *hrc*RST sequences and genotypic background of fluorescent pseudomonads

PCR *hrc*RST[a]	*hrc*RST-RFLP genotypes	BOX-PCR genotypes		Number of isolates Myc+ J5	 TRV48	 Myc- TRV25	 Bulk soil
			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 *hrc*RST+	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 *hrc*RST-	72	80		
				152		97	50

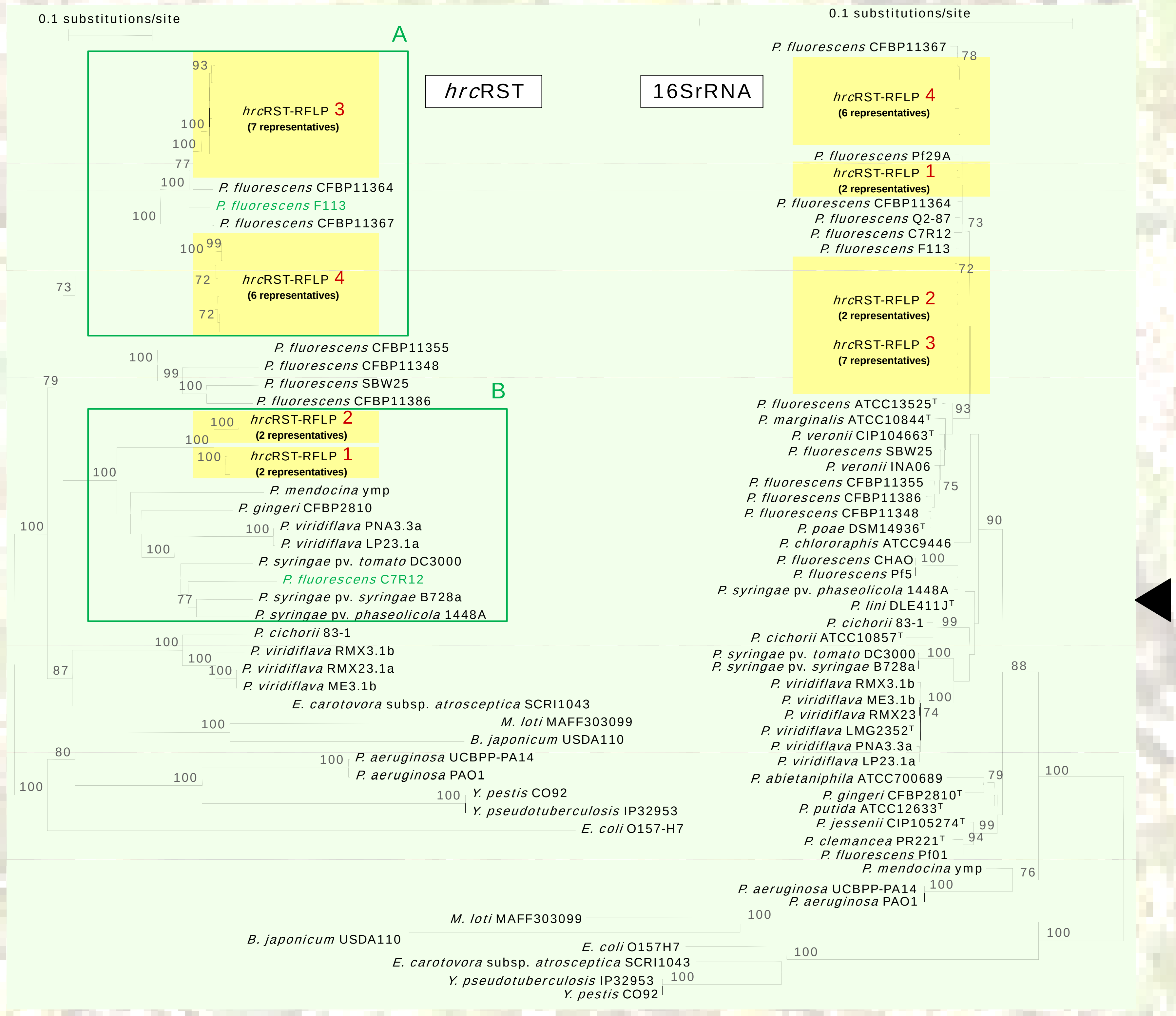
[a] , +,strong PCR product ; +/-, weak PCR product ; -, no visible PCR product. [b], numbers designate the *hrc*RST-RFLP genotypes and letters designate the patterns obtained with the restriction enzymes *Alu*I, *Rsa*I, and *Taq*I, respectively. [c], Uncl., unique *hrc*RST-RFLP genotypes and BOX-PCR genotypes that remained unclustered. [d], ND, not determined.

▲

-4 *hrc*RST-RFLP distinct genotypes were described (1, 2, 3 and 4).

-*hrc*RST-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.



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-*hrc*RST sequences analysis confirmed that T3SS+ pseudomonads belonged to 4 different genotypes.

-*hrc*RST genotypes 1 and 2 clustered together, with pathogenic pseudomonads and with *P. fluorescens* C7R12, biocontrol agent and Mycorrhizal Helper Bacteria[2] (green frame A).

-*hrc*RST 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 *hrc*RST 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 *hrc*RST 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.

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[1] **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.**

[2] **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.**