Pseudomonas fluorescens PICF7, an olive root endophyte with biocontrol and plant-growth promotion abilities

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Figure 1. Olive trees severely affected by Verticillium wilt. For details on epidemiology and integrated disease management of this disease, see López-Escudero & Mercado-Blanco (2011) [2].

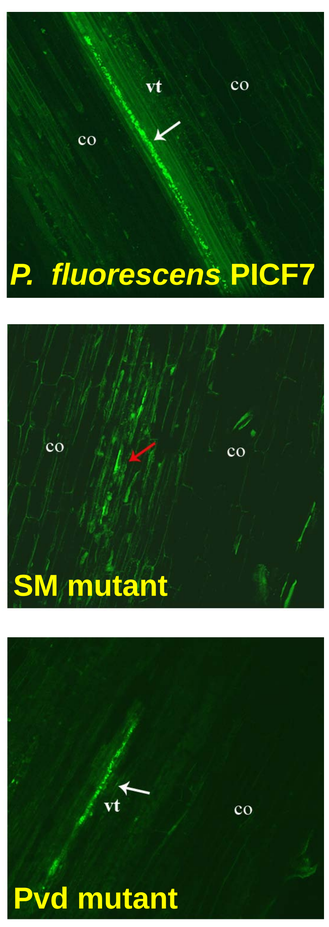
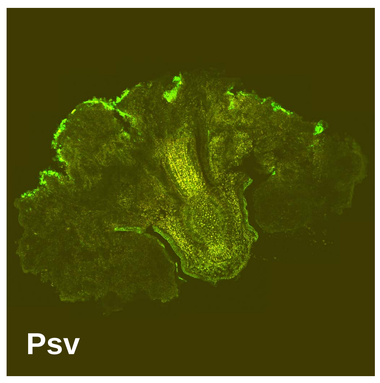


Figure 3. CLSM images of olive root sections (40 µm thick) showing localization of *Pseudomonas fluorescens* PICF7 and its GFP-labeled derivatives defective in swimming motility (SM mutant) and siderophore pyoverdine production (Pvd mutant). All strains were able to colonize inner olive root tissues such as vascular vessels (white arrows) and the intercellular spaces of the cortex (red arrow). co, cortical cells; vt, vascular tissue. For details, see Maldonado-González *et al.*, (2015) [5].



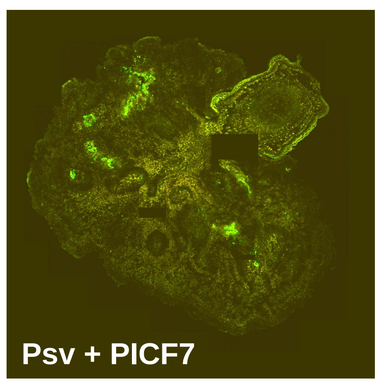


Figure 5. CLSM images showing localization of GFP-tagged *Pseudomonas savastanoi* NCPPB3335 in the absence (Psv) or presence (Psv+PICF7) of *Pseudomonas fluorescens* PICF7. Transversal tumor sections (40 µm thick) were made to show inner colonization. Each panel is a composition of several images to show complete knots. In the absence of PICF7 (up), Psv-GFP is mainly visualized at the knots surface. In the presence of PICF7 (down), Psv-GFP is visualized mostly at inner cavities of the tumor. CLSM analysis was performed 9 weeks after inoculation. See Maldonado González *et al.*, (2013) [8].

Pseudomonas fluorescens PICF7 is a natural colonizer of olive (*Olea europaea* L.) roots [1]. This strain is an effective biocontrol agent (BCA) against Verticillium wilt of olive (VWO), one of the most important diseases affecting this woody crop caused by the soil-borne fungal pathogen *Verticillium dahliae* (Figure 1) [2].

Endophytic lifestyle of strain PICF7 in olive root tissues was demonstrated under different experimental conditions. Root hairs were shown to play a key role in the endophytic colonization of root tissues by this rhizobacteria (Figure 2) [3]. Surface and endophytic colonization of intact olive roots by PICF7 are required for effective control of VWO [4].

Mechanism(s) underlying PICF7-mediated biocontrol of *V. dahliae* and endophytism are poorly understood. Mutant analysis has revealed that bacterial traits such as siderophore pyoverdine production and swimming motility are needed neither for biocontrol performance nor endophytic lifestyle (Figure 3) [5].

Colonization of olive roots by PICF7 triggers a broad range of transcriptomic changes, mostly related to defense responses to different (a)biotic stresses. Some of these responses occur at both local (roots) [6] and systemic (stems) [7] level (Figure 4). The way these responses are modulated along time could explain biocontrol effectiveness of strain PICF7 and how olive tissues recognize this bacterium as a 'non-hostile' invader.

Presence of strain PICF7 in roots does not hinder olive knot development caused by *Pseudomonas savastanoi* pv. savastanoi (Psv), another relevant olive pathogen. However, when the BCA and Psv are co-inoculated in stems, PICF7 altered the appearance and the anatomy of tumors as well as the localization of the pathogen within the hyperplasic tissue (Figure 5) [8].

Strain PICF7 is also able to colonize roots of barley (*Hordeum vulgare* L.), even endophytically, and to promote plant growth and yield increase in this species (Table 1).

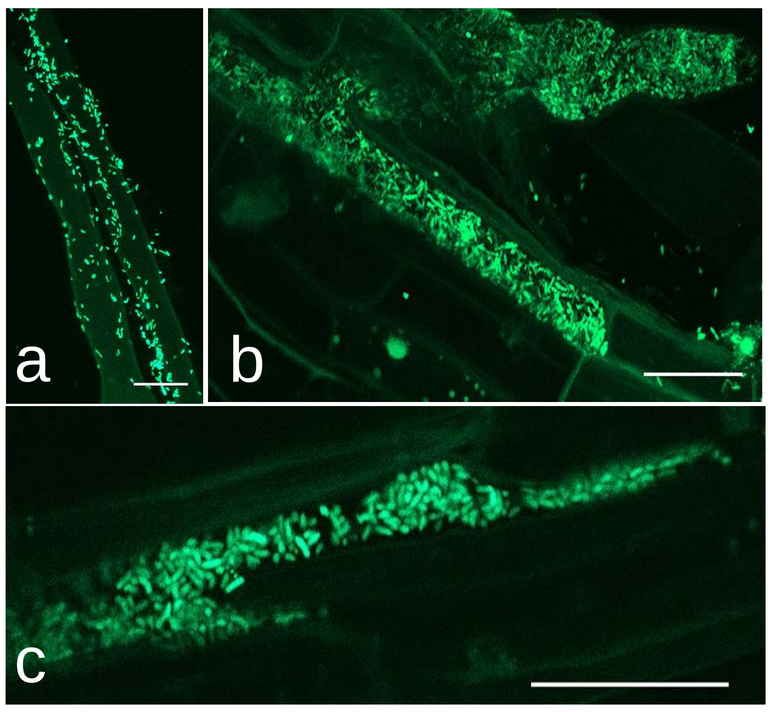


Figure 2. Confocal laser scanning microscopy (CLSM) images of the colonization process of *in vitro* olive cv. Manzanilla roots by *Pseudomonas fluorescens* PICF7. **a**, Surface EGFP-tagged PICF7 colonization of two root hairs; **b**, An epidermal cell with root hair entirely colonized by PICF7. **c**, Intercellular colonization of the cortical tissue of the root differentiation zone by PICF7 cells. Scale bar represents 50 µm in **a** and 20 µm in **b** an **c**. See Prieto *et al.*, (2011) [3].

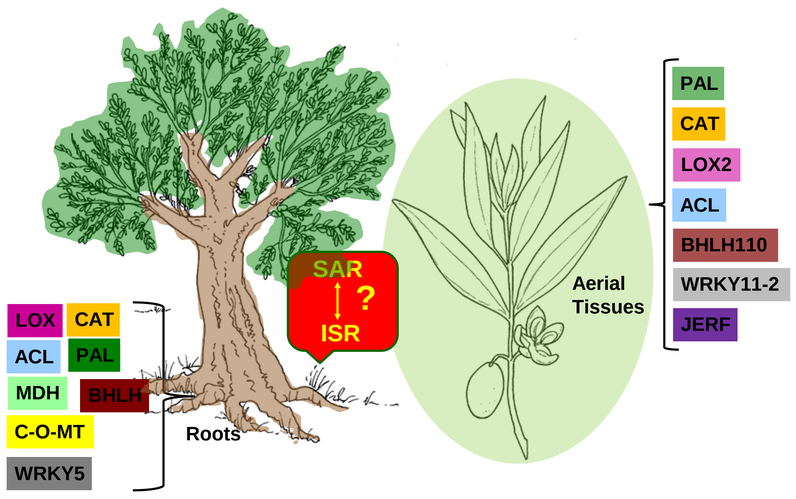


Figure 4. Scheme showing some genes involved in defense responses which are induced in roots and aerial tissues during strain PICF7-olive root interaction. ACL: Acetone cyanohydrin lyase; CAT: catalase; C-O-MT: Caffeoyl-O-methyltransferase; BHLH110 and BHLH: basic helix-loop-helix transcription factors; JERF: transcription factor JERF; LOX: lipoxygenase; MDH: Malate dehydrogenase; PAL: Phenylalanine ammonia-lyase; WRKY5 and WRKY11-2: WRKY transcription factors; SAR: systemic acquired resistance; ISR: induced systemic resistance. Based on Schilirò *et al.* (2011) [6] and Gómez-Lama Cabanás *et al.*, (2015) [7]

	Number of grains per plant	Grain weight per plant (g)
Experiment 1		
Control	121.38 ± 27.05 b	4.63 ± 0.95 b
PICF7	145.00 ± 19.62 a	5.58 ± 0.69 a
P-value	*0.0473*	*0.0302*
Experiment 2		
Control	130.35 ± 26.54 b	5.35 ± 1.34 b
PICF7	151.96± 35.46 a	6.35 ± 1.75 a
P-value	*0.0298*	*0.0419*

Table 1. Yield parameters scored in barley plants grown from strain PICF7-inoculated seeds (135 days after seed bacterization). Values are means ± standard error. ANOVA was based on a completely randomized design. Means were separated using the LSD test with a probability level of 0.05, (n=20).

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CONCLUSION:

***Pseudomonas fluorescens* PICF7 is a versatile root endophyte providing a range of benefits to the hosts that is able to colonize**

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