RHIZOSPHERIC STRAINS OF *Pseudomonas fluorescens* WITH ANTAGONISTIC ACTIVITY AGAINST PHYTOPATHOGENIC FUNGI, PROMOTE GROWTH OF *Medicago truncatula* MAINLY TROUGH EMISSION OF VOLATILE ORGANIC COMPOUNDS

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Introduction. Rhizospheric bacteria can exhibit direct and indirect mechanisms to promote plant growth (Santoyo et al., 2012). The latter consists of methods through which bacteria synthesize antibiotics or other compounds, having an antagonistic action on phytopathogenic organisms in the rhizosphere, whereas direct mechanisms consists when bacteria can positively influence plant growth through synthesis and excretion of auxin or cytokinin type phytostimulating substances (Ortíz-Castro et al. 2009). Other direct methods include access to nutrients trough Fe reduction or emission of volatile organic compounds (VOCs) by Plant-Growth Promoting Rhizobacteria or PGPR (Orozco-Mosqueda et al. 2012). In this work, we isolated and characterized four novel, and highly antagonistic strains of *Pseudomonas fluorescens* against several phytopatogenic fungi, as well as their possible inhibitory mechanism. In addition, these strains also exhibited the capacity to promote plant growth of *M. truncatula* plants *in vitro* by VOCs emission, mainly.
Undoubtedly, these novel strains represent a great opportunity to develop biocontrol agents and will expand our current knowledge of the mechanisms to promote plant growth and antagonistic activity toward plant pathogens.

Methods. *P. fluorescens* strains were isolates from sorghum rhizospheric soil. Soil particles were separated by centrifugation and ten-fold serial dilutions were plated on nutrient agar (NA). Bacterial isolates were routinely maintained at 40C on NA. For evaluation of fungal antagonism it was followed as previously reported by Martínez-Absalón (2012). Genomic DNA was isolated from *P. fluorescens* strains to amplify by PCR the ribosomal 16S rDNA gene by using the primers Fd1 and Rd1 and sequence the products at LANGEBIO. *phlD* and *phzC* were also PCR amplified and sequenced. Siderophores production by *Pseudomonas* strains was also detected in CAS media and Indole-3-acetic acid (IAA) was detected by (GC-MS) analysis. Plant-growth promotion was analyzed as previously (Orozco-Mosqueda, et al. 2013). Briefly, *M. truncatula* plants were exposed to either diffusible compounds (DOCs) by direct contact with bacteria or inoculated separately to allow only VOCs exchange. Aerial and root plant fresh weight and chlorophyll content were measured. Currently, VOCs during interaction are being analyzed by gas chromatography.

Results. Four strains belonging to the *P. fluorescens* species (UM16, UM240, UM256 and UM270) were molecularly characterized and selected for showing high antagonistic activity against phytopatogenic fungi, including: *B. cinerea*, *F. oxysporum* and *R. solani*. We were able to amplify the DAPG antibiotic producing gene *phlD* and phenazine antibiotic gene *phzD* from genomic DNA. Also, siderophore and IAA production was detected; meanwhile no glucanase, cellulose or chitinase activities were found. Plant-growth promoting action was observed mainly by the emission of VOCs, since aerial and root fresh weight, as well as chlorophyll content was higher in those plants exposed to bacterial VOCs.

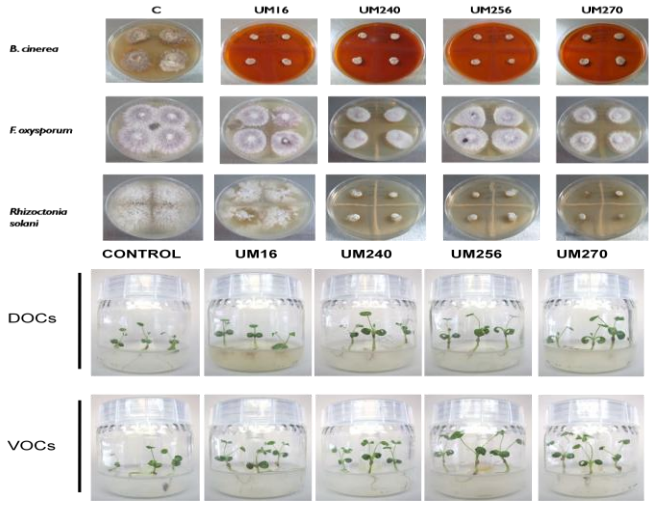

Fig.1 Upper panel shows the high antagonistic activity of the *P. fluorescens* trains against phytopatogenic fungi. Lower panel shows the *Pseudomonas* DOCs and VOCs beneficial effects of growth promotion on *M. truncatula*, compared to plant controls.

Conclusion. These new *P. fluoresces* strains exhibit direct and indirect PGP activities, either by inhibiting phytopatogenic fungi or by producing phytohormones and represent excellent biocontrol and biopromoting agents.

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References.

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