Use of model plants for monitoring colonization of several PGPR isolated from an organic olive grove

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ABSTRACT

Indol acetic acid (IAA) production is a common PGPR feature. It has been reported that bacteria producing IAA can promote rooting and their effects on plants mimic that of exogenous IAA, a common practise in nursery industries. In a previous work, 500 strains were isolated from an organic olive grove and tested for PGPR activities. Some of them were selected by their ability for auxin production and checked for rooting induction on olive cuttings.
The goal of this work is the monitoring of bacterial root colonization through a green protein fluorescence marker (GFP) using model plants under axenic conditions. For that, we have designed rooting induction assays on *Vigna radiata* (mung bean) cuttings and *Brassica napus* (canola) seeds. Number and root length, as well as bacterial colonization were determined. In both model plants, root colonization has been observed by means of Laser Scanning Microscope.
KEYWORDS: Olive cuttings, organic agriculture, PGPR, IAA, root colonization, GFP.

1. INTRODUCTION

Plant growth promoting rhizobacteria (PGPR) are indigenous to soil and plant rhizosphere. It is well known that some of them can affect the growth of a plant root system since they are able to produce plant hormones like auxin. Therefore, these microorganisms are potential tools for a sustainable agriculture.
The use of canola seeds and mung bean cuttings as an approximation to understand the effect of the PGPR bacteria in nursery setting to promote adventitious root formation has been widely used (Tang *et al*., 95; Mayac *et al*., 99). Confocal Laser Scanning Microscopic (CLSM) approaches combined with fluorescent tagging of bacteria permit to analyse the colonization behaviour of our strains on model plants.

2. METHODS

2.1. **Bacterial strains and culture conditions**

The strains used in this work (Montero-Calasanz *et al*., 2008) are summarized in Table 1. Strains were grown for 72 hours at 28ºC with shaking (180 rpm) in Nutrient Broth supplemented with 100 ppm Tryptophane.

2.2. **Rooting induction and elongation assays with mung bean cuttings and canola seeds.**

Vigna radiata assays were performed according to Kutter *et al*., 2006. Twelve days after sowing, stems were cut under sterile conditions and placed in other tubes according the protocol described by Patten and Glick, 2002. *Brassica napus* assays were made as described by Patten and Glick, 2002. Determination of CFU number and the monitoring of root colonization were carried out

according to Kutter *et al*., 2006. Statistical analysis were performed using SPSS ver.10.0 software for Windows.

Strain	Microbial group and similarity	Auxin production[a] (ppm)
CT364 GFP	*Pseudomonas sp 99.4%*	45±13
M16 GFP	*Pantoea agglomerans* 99.6%	75 ±14.5
CT363 GFP	*Pseudomonas sp 99.9%*	12 ± 3
CT42 GFP	*Pseudomonas sp* 99.6%	55 ± 10

Table 1. Strains used in this work.
[a] Mean + standard deviation from six separate experiment.

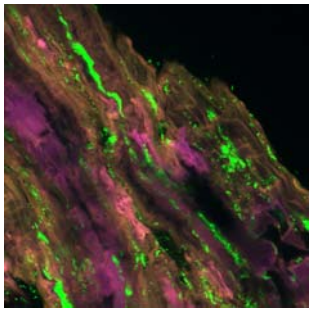

Fig 1. CLSM image of CT42 GFP. Orthogonal view created from a Z-stack of xy-scans.

3. RESULTS AND DISCUSSION

The use of monoxenic model systems has help us to demonstrate the potential of our strains as substitute of synthetic auxin. The effect of several bacterial isolates on adventitious roots of mung bean cuttings and canola seeds are summarized in Table 2. All of the used strains unleash both, higher rooting induction and root elongation than negative control in mung bean and canola assays, except strain CT363 that didn't increase the root length in canola plants.

	Vigna radiata			*Brassica napus*	
STRAINS	Root number	Root length (cm)	CFU	Root length (cm)	CFU
Water (negative control)	7.46+2.29a	1.01+0.34a		6.05±1.35a	
IAA (positive control)	51±12.02c	29±9.65c		--	
CT363 GFP	28.66±3.32b	17.13±3.65cb	$2.1x10^{7}$±2.3	5.79±0.88a	$3x\ 10^{5}$±1
CT42 GFP	21.23±8.51b	12.15±5.75b	$1.6x10^{9}$±0.3	6.27±1.06b	$3.5x10^{3}$±5.3
CT364 GFP	26.40±6.55b	15.34±6.26cb	$7x10^{9}$±0.8	8.02±1.21c	$1.5x10^{4}$±2.3
M16 GFP	44.06±15.70c	18.21±5.92c	$3x10^{9}$±1.5	7.37±0.82b	$1x10^{6}$±3

Table 2. Effects of strains on the number and length of adventitious roots on mung bean cuttings and lengths of roots from canola seeds and CFU determination of root-associated bacteria at the end of the assays. One-way analysis of variance (ANOVA) was done and comparison among treatments was performed by using Duncan´s test at the P= 0.05 level.

On the other hand, it has been demonstrated that these strains were able to colonize the plant roots permanently since a good bacterial population at the end of the assays was found (Table 2) and stable root associations (microcolonies and cell cordons) colonizing the surface of main roots, side roots and root hairs were observed by means of CLSM. The use of CLSM and nondiffusible fluorescent markers to tag bacterial cells allows the *in situ* bacterial localization without plant tissue manipulation. We have observed a similar bacterial colonization pattern as they preferentially colonize the surface of the differentiation zone and around root hairs (Fig 1). Perhaps, this root region might be favoured by either being a more protective microenvironment for the bacteria or better source of nutrients and/or bacterial chemoattractants (Prieto *et al*., 2008).

4. REFERENCES

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