Differences in common bean root colonization by distinct PGPRs and their fluorescent marked derivatives.

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ABSTRACT

PGPRs (Plant Growth-Promoting Rhizobacteria) play an important role in the promotion of plant development by several mechanisms, but the beneficial effect may be hampered due to constraints imposed by biotic and abiotic rhizosphere soil conditions. Thus, salinity and/or the presence of other microorganisms can modify early events of root colonization. Moreover, if the positive plant effect of several PGPRs is intended to be used for the elaboration of microbial inoculants, it is necessary to determine their mutual compatibility in the rhizosphere.

Key words: *Phaseolus vulgaris*, *Rhizobium etli*, PGPRs, root colonization

MATERIAL AND METHODS

Pseudomonas fluorescens Aur6, *Chryseobacterium balustinum* Aur9, *Aeromonas punctata* AMG272, *Enterobacter hormaechei* AMG443, *Rhizobium etli* ISP42 and derivative marked strains have been used. We have used the following plasmids carrying genes expressing different autofluorescent proteins (AFPs) (pMP2463, green), (pMP4516, cyan) and, (pMP4518, yellow) to mark Aur6, AMG443 and ISP42 strains; (pMp4655, green), (pMP4641, cyan) and, (pMP4518, yellow) to mark Aur9 and AMG272 strains (Stuurman et al., 2000). The culture media used were: yeast-mannitol agar (YMA) for *R. etli* and tryptic-soy agar (TSA) for the remainder strains. Common bean *Phaseolus vulgaris* cultivar Bush Blue Lake (BBL) was used.

Bean plants for the assessment of root colonization were grown in 200 ml glass-containers filled up with quartz sand, watered with a nutrient solution (Rigaud and Puppo, 1975). Surface disinfected and pre-germinated seeds were inoculated with 10^6 bacteria/ml. Plantlets were placed in a growth chamber (16h at 25 ºC/8 h 18 ºC

day/night, and 88% RH). Colonization assay was performed according to Albareda et al. (2006) but using the whole root system (6 days old), instead of root segments.

Results and Discussion

Results of bean root colonization are shown in Table 1. Three out of five rhizobacteria tested did not show significant differences in their root colonization capacity with respect to the corresponding marked-derivatives strains (AMG443, Aur6 and Aur9). However, all marked-derivatives strains of AMG272, and that expressing the cyan autofluorescent protein of ISP42 did show a significant reduction in their capacity to colonize bean roots (Table1). On the other hand, it is noteworthy that *R. etli* ISP42 - the microsimbionte strain of *Phaseolus* - showed a higher colonization degree than the others rhizobacteria. Root colonization of selected rhizobacteria able to alleviate salt stress was tested under control and salt (25 mM NaCl) conditions (Table 2). Most of the strains did show a better colonization degree under salt conditions.

Table 1. Root colonization of *Phaseolus vulgaris* by several rhizobacteria and their fluorescent-marked derivatives.

Genotype	Bacterial strains				
	AMG272	**AMG443**	**Aur6**	**Aur9**	**ISP42**
Wild type	7,85 aB	7,68 aCD	7,44 aBC	7,22 aD	8,57 aA
GFP	6,89 bC	7,53 aB	7,24 aB	7,32 aB	8,55 aA
YFP	6,84 bB	7,57 aB	7,25 aB	7,16 aB	8,61 aA
CFP	7,11 bC	7,58 aB	7,27 aC	7,39 aC	7,77 bA

Data (Log cfu/g root) are means of two independent assays, each one with 4 internal replicates. Significant differences between values within a column (lower case) and within a line (capital) are indicated by different letters.

Table 2. Root **c**olonization of *Phaseolus vulgaris* by different plant growth promoting rhizobacteria under control and salt conditions.

Plant growth conditions	Bacterial strains			
	AMG272	**Aur9**	**BB1**	**L81**
Control	7,8 aA	7,2 bB	7,3 bB	6,0 aC
Salt	7,9 aA	7,8 aA	7,7aA	6,2 aB

Data (Log cfu/g root) are means of two independent assays, each one with 4 internal replicates. Significant differences between values within a column (lower case) and within a line (capital) are indicated by different letters.

References

Albareda et al. (2006).FEMS Microbiol. Letters **259**, 67-73.

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