ENDOPHYTES IN BIOTECHNOLOGY AND AGRICULTURE cost FA1103 Endophytes for plant protection: the state of the art, 27 – 29 May 2013 Berlin, Germany

# Endophytic bacteria from weeds promote growth of tomato plants *in vitro* and in greenhouse.

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## ABSTRACT

**Eight endophytic bacteria belonging to *Pseudomonas* spp. and *Bacillus* spp. that were isolated from spontaneous plants were tested for their ability to promote tomato plant growth. Experiments were carried out according to two protocols: i) tomato plants grown in vitro on MS medium supplemented with the bacterial filtrates. ii) Tomato plants obtained from bacterized seeds grown under greenhouse conditions. The phytostimulation was assessed by the evaluation of some growth parameters (seed germination percentage, length of the stem and of the main root, fresh and dry weight of aerial parts and roots). The endophytic bacteria positively affected seed germination and stimulated plant growth both *in vitro* and in greenhouse. Tomato seedlings treated with the bacterial filtrates in vitro and plants from bacterized seeds showed an increase in all growth parameters compared to controls, suggesting an effect of stimulation of nutrient acquisition and hormone production.**

## MATERIAL & METHODS

### Preparation of bacterial suspensions and filtrates

Six strains of *Bacillus* spp. and two *Pseudomonas* spp. strains (Krimi et al. 2012), were screened for plant growth promotion activity. Bacterial suspensions ($10^8$ cfu/ml) were used directly for seed bacterization in greenhouse experiments. For the *in vitro* test, sterile filtrates containing the bacterial metabolites were obtained from the suspensions by filtration through 0.2µm Millipore filters.

### *In vitro* experiments

Tomato seeds were placed onto the surface of MS tubes medium supplemented with the bacterial filtrates. The tubes were kept at room temperature (23 ± 2°C) under a 3000 lux light and a 12 hours photoperiod.

### Greenhouse experiments

Tomato seeds were bacterized with the suspension of bacteria ($10^8$ cfu/ml) and then incubated at 28°C for 72h. At the moment of sowing, seeds were again bacterized with one milliliter of bacterial suspensions and then covered with a soil substrate placed in plastic pots.

### Measurement of plant growth parameters

Percentage of seed germination was calculated at the end of *in vitro and* in greenhouse assays. Growth parameters (Length of the stem and of the main root, fresh and dry weight of shoot and root biomasses) were recorded four weeks after sowing. The vigor index was determined (mean root length + mean shoot length) × % germination (Abdul Baki & Anderson, 1973).

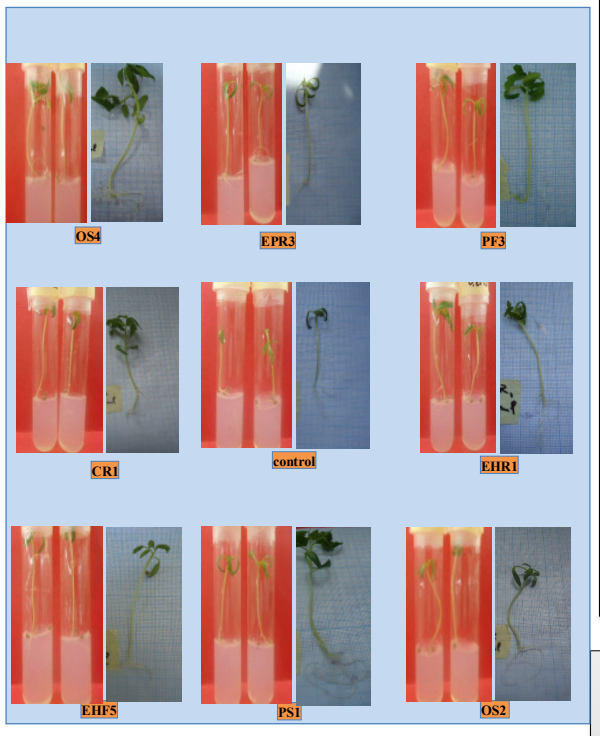


**Fig. 2. *In vitro* bioinoculation of tomato seedlings with endophytic bacterial filtrates .**

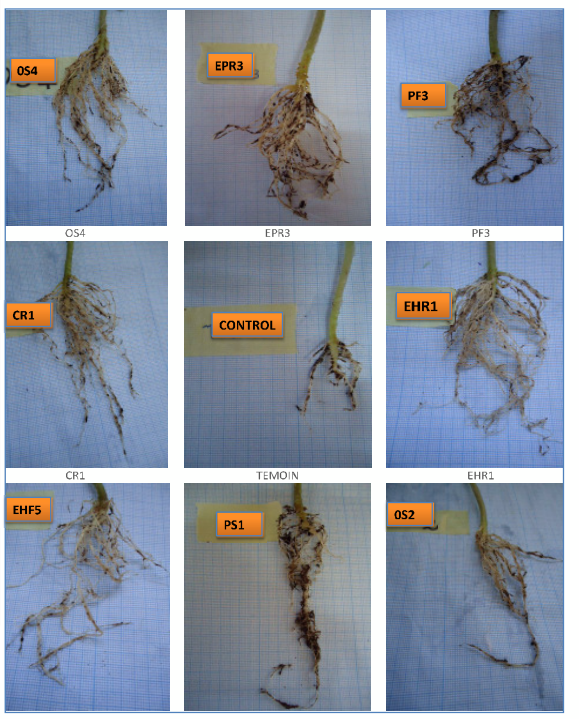


**Fig. 4. Tomato roots issued from plants inoculated with endophytic bacteria in pots experiments.**

## RESULTS

Treatment of seeds with bacterial filtrates and suspensions significantly increased germination percentage, stem height, fresh and dry weight of aerial parts and roots both in in vitro and in greenhouse conditions (Fig.1,2, 3, 4 and 5).

**Percentage of germination**

- All bacterial strains increased seed germination *in vitro* up to 83%, *Bacillus* spp. strain PF3 was the most stimulating (Fig.1).
- In pot trial, the percentage of germination increased up to 84% and *B. amyloliquefaciens* strain OS4, promoted the germination of all seeds (100%) (Fig.3).

**• Growth stimulation**

- The increase of dry and fresh weight biomasses for all inoculants
- The eight filtrates, such as the whole bacterial cells, induced a significant increase of root growth that may have positively influenced the development of the aerial part

**• Vigor index**

- In vitro, the highest enhancement of vigor index was determined by *Bacillus* spp. PF3 and *B. amyloliquefaciens* strain OS4, which recorded 1515 and 1456 values respectively (Fig.1).
- In pot trial, *Bacillus cereus* EHR1, *Pseudomonas* spp. PS1, and *B. amyloliquefaciens* OS4 showed the highest vigor indexes with respective values of 3603, 3247 and 3213 (Fig.3).

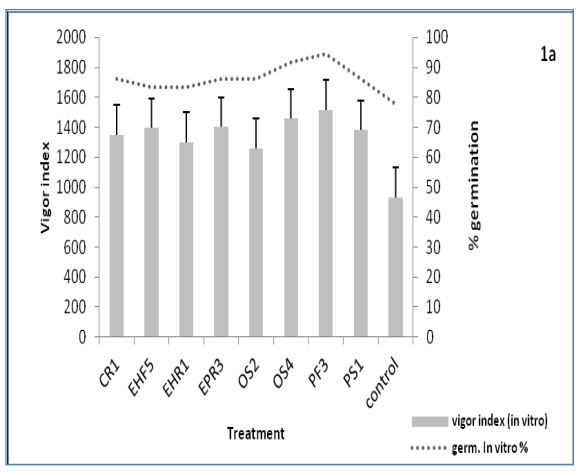


**Fig. 1. Effects of seed bacterization on germination and vigor of tomato plants in *in vitro conditions.***

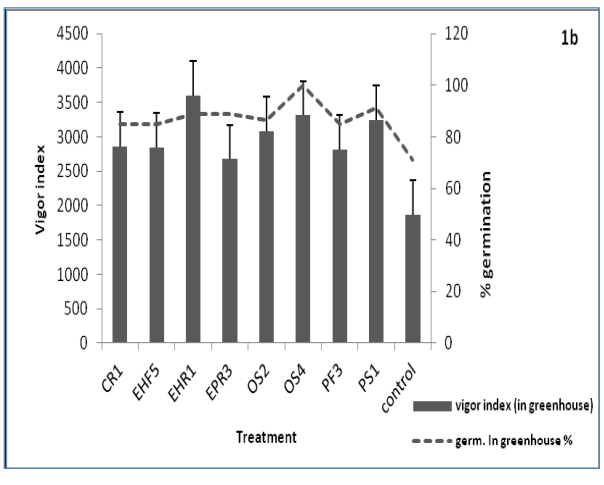


**Fig. 3. Effects of seed bacterization on germination and vigor of tomato plants in *greenhouse conditions* .**

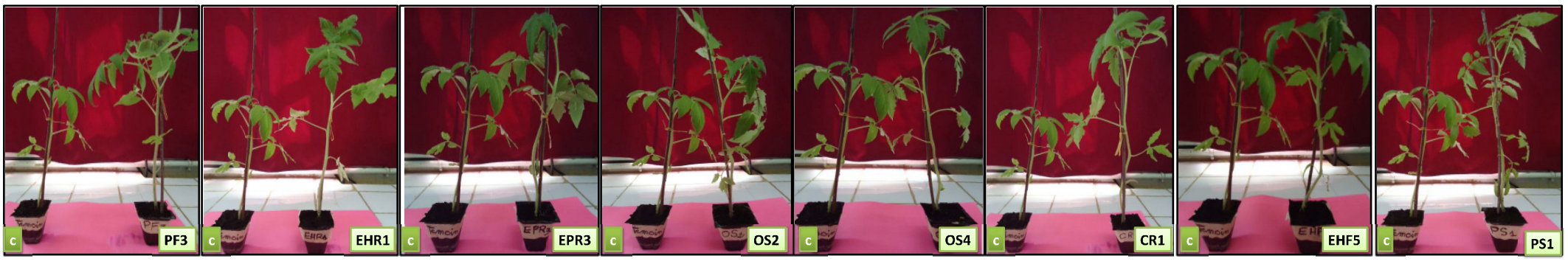


**Fig. 5. Tomato seedlings bioinoculated with the endophytic strains under greenhouse experiments.**

## CONCLUSION

The increase of dry and fresh weight biomasses as response to all inoculants *in vitro* and in greenhouse conditions, clearly showed the beneficial role of the bacterial endophytes originating from the native plant species. Our results suggest that bacteria inhabiting these plants may positively affect weed growth and in consequence their abundance in nature.
Besides, results showed that screening of endophytic bacteria for growth promotion under *in vitro* and greenhouse experiments may represent a useful tool to select bacteria potentially efficient as soil biofertilizers.

## REFERENCES

**Abdul Baki A A; Anderson J D (1973).** Vigor determination in soybean seed by multiple criteria. *Crop Science 13, 630–633.*

**Krimi Z; Raio A; Smain K; Khodja R; Djellout H; Alim D; Haddad N (2012).** Bacterial endophytes from diverse spontaneous plant species with biological activities. Proceedings of COST Action FA1103: *Endophytes in biotechnology and agriculture. November 14-16th, Trento (Italy). Pp. 33.*