# Studies of plant growth promoting bacteria associated to rapeseed (Brassica napus) roots

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## Introduction

Numerous species of soil bacteria grow around or within plant tissues, stimulating their growth through a variety of mechanisms. These microorganisms are collectively known as plant growth promoting bacteria (PGPBs). The study of these bacteria is important not only for the understanding of their ecological role in the interaction with plants but also for their biotechnological application. The aim of this study was to isolate and identify bacteria from the rhizosphere of rapeseed plants able to fix nitrogen and solubilize phosphates, promoting in this way plant growth.

## Materials y methods

Bacteria isolation: Epiphytic and endophytic bacteria of rapeseed plants growing at fields in the localities of Rio Cuarto (Cordoba, Argentina) and Tres Arroyos (Buenos Aires, Argentina) were obtained. For the isolation of epiphytic bacteria roots were separated, washed with water and resuspended in buffered saline solution (PBS). Dilutions of this solution were spread on plates containing 20 ml of 10% TSA medium supplemented with 50 µg/ml cycloheximide. For the isolation of endophytes, surface-sterilized roots were cut and homogenized in sterile distilled water. Dilutions of this solution were analysed as described above (Kuklinsky-Sobral et al. 2004). Morphological and staining properties of bacteria from different colonies were determined and bacteria were identified according to Bergey's manual. Bacteria from morphologically different colonies were choosing to evaluate their ability to fix nitrogen and to solubilize phosphate.

Phosphate solubilization ability: It was evaluated in the medium described by Nauyital (1999). The solubilization halo was measured after 7 days of incubation at 28 ˚C.

Nitrogen fixation ability: It was determined by evaluating bacterial growth below the surfaces of tubes containing nitrogen-free semisolid media JNFb, NFB or JMV (Döbereiner, 1995).

Growth chamber trials: The isolates able to solubilize phosphates were individually inoculated on rapeseed seedlings growing under controlled conditions in pots containing volcanic sand, supplemented with 40 ml of 0.2% tricalcium phosphate. They were watered with phosphorus-free Hoagland solution. Isolates able to solubilize phosphate obtained from peanut plants (*Klebsiella* sp. NTI31, *Serratia* sp. S119 and J49) were also inoculated. A phosphate solubilizing *Pseudomonas fluorescens* strain used as biofertilizer for wheat and maize (Rizobacter) was used as positive control. Plants were harvested at 5 weeks after inoculation and their aerial fresh and dry weigth and number of leaves were determined.

## Results:

The proportion of Gram-positive and Gram-negative bacteria was similar in both endophytic and epiphytic populations. Gram-positive cocci were only isolated in epiphytic association (Fig. 1). Based on the phenotypic and physiological characteristics evaluated, the bacterial genera *Pesudomonas*, *Enterobacter*, *Staphyloccocus* and *Bacillus* were identified. Interestingly, *Serratia*, *Aeromonas*, *Corynebacterium* and *Lactobacillus* genera were also found and, until our knowledge, this is the first time they are reported as associated with the rhizosphere of *Brassica napus* (Fig. 2 and Table 1).

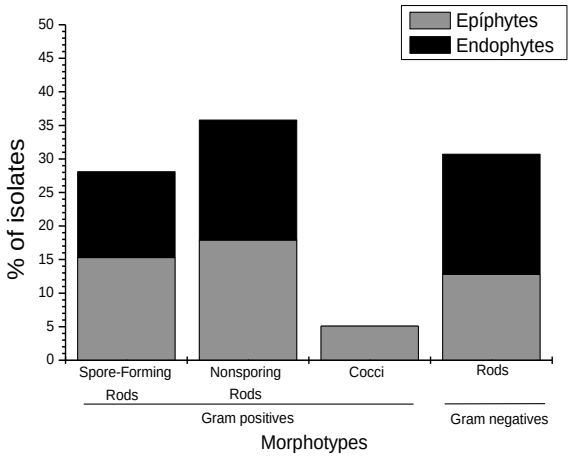


Figure 1. Proportion of morphotypes related

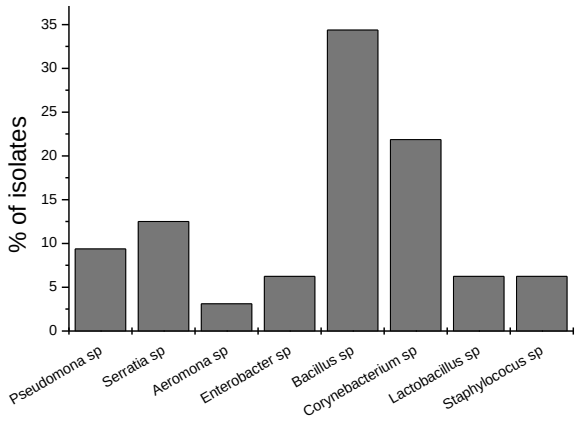


Figure 2. Percentage of isolates from the different genera

Table 1. Genera assigned to different isolates

| Isolates | Genus | Isolates | Genus |
|---|---|---|---|
| LRCP-1 | *Bacillus sp.* | LRCP-40 | Unidentified |
| LRCP-2 | *Bacillus sp.* | LTAP-41 | *Corynebacterium sp.* |
| LRCP-3 | *Bacillus sp.* | LTAP-42 | *Corynebacterium sp.* |
| LRCP-4 | *Bacillus sp.* | LTAP-43 | *Pseudomona sp.* |
| LRCP-5 | Unidentified | LTAP-44 | *Lactobacillus sp.* |
| LRCP-6 | *Pseudomona sp.* | LTAP-46 | *Corynebacterium sp.* |
| LRCP-7 | *Staphylococus sp.* | LTAP-48 | *Bacillus sp.* |
| LRCP-9 | *Corynebacterium sp.* | LTAP-49 | *Serratia sp.* |
| LRCP-10 | *Staphylococus sp.* | LTAP-50 | *Bacillus sp.* |
| LRCP-11 | Unidentified | LTAD-52 | *Aeromina sp.* |
| LRCD-13 | Unidentified | LTAD-53 | *Bacillus sp.* |
| LRCD-14 | *Corynebacterium sp.* | LTAD-54 | Unidentified |
| LRCD-16 | *Serratia sp.* | LTAD-55 | *Bacillus sp.* |
| LRCP-17 | *Corynebacterium sp.* | LTAD-58 | *Corynebacterium sp.* |
| LRCD-22 | *Serratia sp.* | LTAD-59 | *Pseudomona sp.* |
| LRCD-23 | Unidentified | LTAD-60 | *Bacillus sp.* |
| LRCD-25 | *Serratia sp.* | LTAD-61 | *Lactobacillus sp.* |
| LRCP-29 | *Enterobacter sp.* | LTAD-62 | *Bacillus sp.* |
| LRCD-32 | Unidentified | LTAD-63 | *Bacillus sp.* |
| LRCP-37 | *Enterobacter sp.* | LTAD-65 | Unidentified |

The 10,8 % of the morphotypes obtained did not show any of the PGPB activities evaluated. Furthermore, 37,8% of the isolates was able to solubilize phosphate (mostly epiphytic isolates), and 62,1% was able to fix nitrogen. Bacteria from the genera *Staphyloccocus* and *Enterobacter* were only able to solubilize phosphate while *Pseudomona, Serratia* and *Lactobacillus* were only able to fix nitrogen. Strains from the genera *Bacillus, Corynebacterium* and *Aeromona* showed both features (Fig 3).

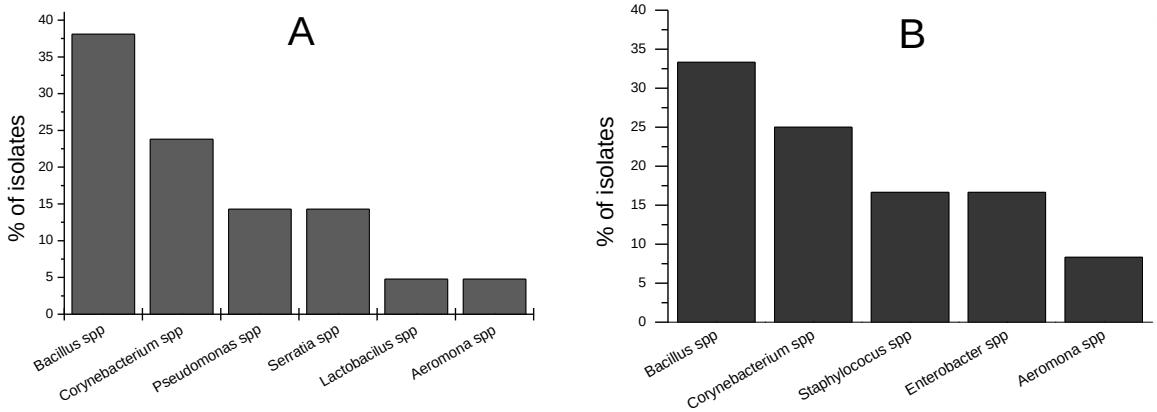


Figure 3. Diversity of bacteria associated with *Brassica napus* able to fix nitrogen (A) or to solubilize phosphate (B)

The epiphytic isolates LRCP-17, LRCP-37 and LRCP-29 showed the highest phosphate solubizing activities considering the diameters of the halos that they produced in Nauyital medium.

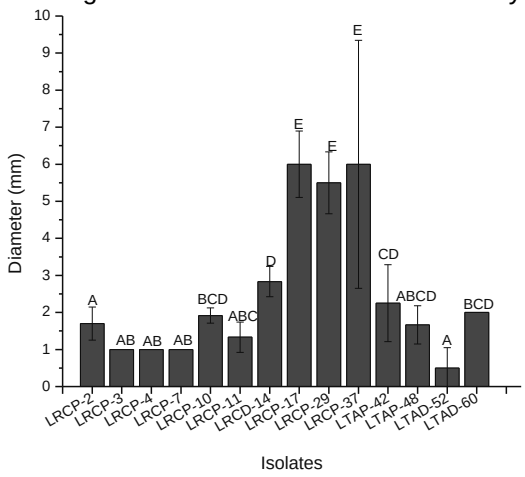


Figure 4. Phosphate solubilization ability. Different letters indicate significant differences ($p < 0.05$) according to the LSD statistical test

It was found that isolates LRCP-2, 3, 4, 11, 17, 29 and LTAD-52 significantly increased the plant shoot dry weight (in a range from 40% to 55%) compared to uninoculated plants (Fig. 5). These increases are consitent whit the larger plant development observed. Moreover, in general, there was a positive correlation between the increase in the plant shoot fresh weight and the number of leaves developed (Fig. 6 and 7).

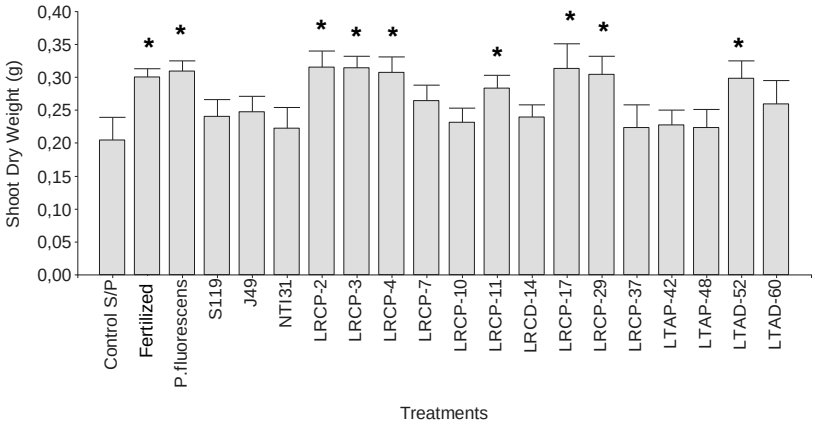


Figure 5. Effect of inoculation of phosphate solubilizing bacteria on shoot dry weight of *Brassica napus*. Data represent the mean ± SE of two replications with n = 5. * indicates significant differences ($p < 0.05$) according to the LSD statistical test with respect to the negative control.

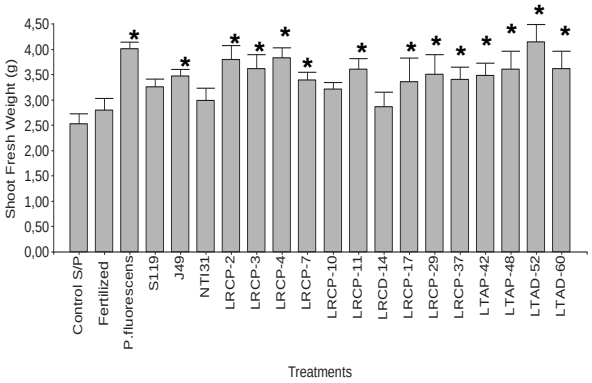


Figure 6. Effect of inoculation of phosphate solubilizing bacteria on shoot fresh weight of *Brassica napus* plants. Data represent the mean ± SE of two replications with n = 5. * indicates significant differences (p <0.05) according to the LSD statistical test with respect to the negative control.

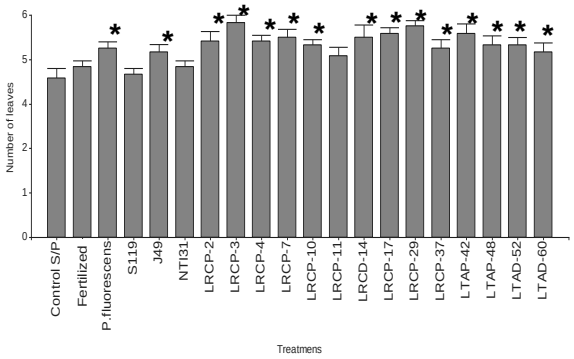


Figure 7. Effect of inoculation of phosphate solubilizing acteria on the number of leaves developed in *Brassica napus* plants. Data represent the mean ± E of two replications with n = 5. *indicates significant differences (p <0.05) according to the LSD statistical test with respect to the negative control.

## Conclusion

There is a wide diversity of bacteria associated with *Brassica napus* roots and rhizosphere and they have a great potential to promote this plant growth probably related to their ability to solubilize phosphates. In this work the bacterial genera *Aeromona, Corynebacterium, Lactobacillus* and *Serratia* were described by the first time associated with Brassica napus roots and rhizosphere. Further studies to evaluate the behaviour of these PGPB under field conditions will be done.

## References

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