Plant growth-promoting rhizobacteria associated to candelilla rhizosphere (Euphorbia antisyphilitica) and its effects on Arabidopsis thaliana seedlings

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Abstract

In the communities of Sierra Mojada and Viesca, Coahuila, Mexico of Coahuila desert, two rhizosphere samplings of candelilla (Euphorbia antisyphilitica Zucc) were collected to isolate, characterize, and identifying plant growth-promoting rhizobacteria (PGPR). 165 rhizobacteria were tested in vitro with Arabidopsis thaliana seedlings to evaluate their potential as plant growth promoters, and obtaining 21 strains with best results in the variables of the number of secondary roots and fresh weight concerning the uninoculated control. Their salinity tolerance was evaluated at concentrations from 0.85 M, 1.7 M and 2.55 M of NaCl. Biochemical tests were accomplishing such as siderophores production, phosphates solubilization, production of Indole-3-acetic acid (IAA), and the activity of the ACC deaminase enzyme. The results obtained from 21 strains selected, high activities were obtained in organic substances like a siderophores since they developed a translucent orange halo around their growth; four rhizobacteria developed a clear halo around the bacterial growth with a thickness between 1.487 mm ± 0.667 mm and 5.267 mm ± 0.704 mm in phosphates solubilization; in the production of Indole-3-acetic acid (IAA), the bacterial strains showed the presence of this phytohormone, with values from 4.444 μg mL⁻¹ to 19.286 μg mL⁻¹; and according to the activity of the ACC deaminase enzyme, values from 0.424 to 1.306 μmol α-KB/h/mg Pr were showed. 16S rRNA sequencing was carried out and genus identified were Bacillus, Staphylococcus, Acinetobacter, Cronobacter and Siccibacter. The results obtained show the potential of the isolated rhizobacteria as growth promoters and the increase in the biomass of the Arabidopsis thaliana seedlings is evident. This is a first indication to proceed to carry out tests in different phenological stages in crops of agricultural importance.

Keywords: halophilic rhizobacteria; Indole-3-acetic acid; phosphates solubilization; salinity; siderophores
Introduction

The rhizosphere is the area determined between the roots of the plant and the soil that surrounds it (Dijkstra et al., 2014), which is subdivided into three areas: Ectorhizosphere, rhizoplane, and endorhizosphere (Johansson et al., 2004). The microorganisms that inhabit the rhizosphere act in the nutrient cycles of the soil-plant system (Singh and Mukerji, 2006). The bacterial populations of this microenvironment are mainly favored by the organic compounds produced by the plant such as organic acids, amino acids, sugars, phenolic acids, flavonoids, enzymes, among others, as well as by the presence of nutrients, pH, and soil texture (Stafforsuch, 2005; Singh and Mukerji, 2006; Raaijmakers et al., 2009). The microorganisms attached to the root establish interactions with plants in two main ways: those that form a symbiotic relationship (Rhizobium-Leguminous) and those of free life, which can be in the soil, on or inside the tissues of the plant (Kloeper et al., 1988; Frommel et al., 1991). The free-living bacteria that benefit plant development and even intervene as biological control of phytopathogens are commonly known as plant growth-promoting rhizobacteria (PGPR) (Palacio-Rodríguez et al., 2016).

Rhizobacteria have various mechanisms to promote plant growth, including an increase in nutrient mobilization, nitrogen fixation, improved nutrient absorption, and biological control of pathogens (Bais et al., 2006; De-Bashan et al., 2007; Dias et al., 2009; Hariprasad and Niranjana, 2009; Altomare and Tringovska, 2011). Also, they synthesize growth-regulating substances such as gibberellins, cytokinins, and auxins, they stimulate the density and length of root hairs, thereby increasing the absorption capacity of water and nutrients, which results in greater growth and adaptation to conditions of drought, acidity, and alkalinity (Sandhya et al., 2010; Saraf et al., 2011). Some rhizobacteria have antagonistic results on other microorganisms and induce systemic resistance in plants (Sharma et al., 2007).

Currently, the agricultural sector in dry arid zones suffers from serious abiotic factors such as salinity, which is exacerbated by the high applications of chemical agro-inputs by farmers. Malik et al. (2018), explains that an option to chemical fertilization is the use of biofertilizers from beneficial microorganisms. However, these microorganisms must have the ability to benefit plants under saline conditions and high temperatures because they are conditions that prevail in agriculture in arid-saline environments.

The central area of Coahuila desert is a region located in the central north-western part of Mexico country. It has an area of 61,706 km². The prevailing edaphoclimatic conditions are extreme and its vegetation is widely adapted to them (temperatures above 50 °C in summer and below -4 °C in winter; rainfall below 100 mm per year) and soils low in organic matter (<0.3%). Among the main plants that can be observed in its soils with a high concentration of salts (> 20 gr / kg of dry soil) are candelilla, lechiguilla, sotol, ocotillo, and various cactis (CONAFOR, 2009). Some of this kind of plants in others deserts, showed the presence of beneficial microorganisms which play an important role providing nutrients to this kind of plants. According the above, the aim of this research was to isolate, characterize, and identify strains of rhizobacteria (ecto and endo) that are present in the rhizosphere of candelilla (Euphorbia antisyphilitica) and evaluate their potential to promote growth in Arabidopsis thaliana seedlings, studying their capacity to growth under salinity conditions (0.85 M, 1.7 M and 2.55 M) of NaCl and biochemical tests such as siderophores production, phosphates solubilization, production of Indole-3-acetic acid (IAA), and the activity of the ACC deaminase enzyme.
Materials and Methods

Collection of samples and isolation of bacteria from endorhizosphere and ectorhizosphere

The rhizosphere and root samples were collected in two study areas of Coahuila desert, the first area in the Sierra Mojada (Ejido San Jose de Madero, Coahuila) with coordinates 27° 0' 54" N and 103° 34' 33" W and the second in Sierra Viesca (Rancho Penjamo, Coahuila) with coordinates 25° 10' 41" N and 102° 39' 12" W (Figure 1).

On each sampling site, root and soil samples were collected 10 cm away from the stem of five wild plants of *E. antisyphilitica* in flowering stage. Five subsamples were taken for each plant (500 g of soil and roots) and at a depth of 10, 20 and 40 cm. Candelilla plants were selected for their robustness (0.80 ± 10 cm wide), height (0.90 ± 10 cm) and color (deep opaque green). The collected material was deposited in dark plastic bags, labeled with their corresponding collection data and stored in a thermal container with ice that had a temperature of 4 ± 1 °C for transferring to the Microbial Ecology Laboratory of the Faculty of Biological Sciences – Universidad Juarez del Estado de Durango (UJED).

For the isolation of the rhizobacteria from the endorhizosphere, 1 g of the root was weighed, washed with 70% ethanol, and rinsed with 0.5X sterile PBS solution, then it was macerated with a sterile mortar with 10 mL of 0.5X PBS solution.

For the isolation of the rhizobacteria from the ectorhizosphere, 1 g of rhizospheric soil adhered to root system was suspended in 10 mL of sterile 0.5X PBS solution and placed in the shaking incubator for 5 min at 200 rpm.

Aliquots (100 µL) of serial dilutions (10⁻¹, 10⁻², and 10⁻³) were inoculated from both procedures (endorhizosphere and ectorhizosphere) in Petri dishes with culture media: Free Nitrogen (NFb) for nitrogen-fixing bacteria (Dobereiner and Day, 1975), Luria Bertani (LB) is a generalist medium (Bertani 1951), and Chrome Azurol S (CAS) for siderophore-producing bacteria (Schwyn and Neilands, 1987). Then, all Petri dishes were incubated at 30 °C for 5 days. Finally, with the support of a sterile loop, the colonies were taken separately and conserved in 700 µL of bacterial culture with 300 µL of glycerol for their conservation and they were stored at -70 °C. Afterwards, each bacterial isolate was isolated and differentiated bacterial strains according to colonial morphology. The bacterial strains isolated in this study were named with codes that
represent the medium where they were isolated (NFb, LB, CAS), the area of the rhizosphere where they were isolated (endorhizosphere and ectorhizosphere) and the strain number (1, 2, 3, etc.) (Example: NFbEndo3).

Also, the soil was analyzed; it consisted of detecting in ppm (mg kg soil): N-NO$_3$, P, Ca$^{+2}$, Mg$^{+2}$, Na$, \text{C.I.C. in mEq} \times 100\text{mg} \times \% \text{Exchangeable Cations: Ca}^{+2}$, Mg$^{+2}$, Na$^+$ and K$^+$ were based on Aguilar et al. (2013).

**Evaluation of the growth promoting capacity of rhizobacteria in Arabidopsis thaliana**

Seeds of Arabidopsis thaliana (Col-0) firstly were disinfected in an Eppendorf tube with 20% sodium hypochlorite, stirring for 5 min in the vortex, then they were rinsed with sterile distilled water four times and seeds were placed at 4 $^\circ$C for 24 h for stratification. Subsequently, 45 seeds were placed in Petri dishes with MS 0.2X medium; Petri dishes were placed in a bioclimatic chamber (Torrey@ RV26TVC26) with photoperiods of 16 h light and 8 h of darkness at 25 $^\circ$C. Then after 4 days the germinated A. thaliana seedlings were inoculated and placed at a rate of 7 seedlings per Petri dish.

The inoculation consisted in firstly rhizobacteria isolated and selected from the endo and ectorhizosphere of the candelilla were independently developed in the liquid medium from which they were isolated (NFb, LB and CAS). The incubation conditions were continuous shaking (120 rpm) and a temperature at 30 $^\circ$C (Reinhold et al., 1987). Between 14 and 24 h (logarithmic phase) elapsed, the concentration of the culture of each bacterium was determined, using the following procedure: 1 mL of the culture was poured into a cell for spectrophotometer (master spectrum FISHER SCIENTIFIC 415), taking the reading of absorbance at a wavelength of 540 nm against a medium control (NFb, LB and CAS without bacteria). The bacterial was diluted until obtaining an absorbance of 1.00 unit, which corresponds to a suspension of $2.5 \times 10^8$ CFU mL$^{-1}$. To each of the cultures (Table 1) amount of germinated seed (radicle emerged in 1 gr) $(390 \pm 7)$ of A. thaliana were added in a 50 mL Kitazato flask, they were subjected to vacuum at 100 mm Hg for 1 m (Carrillo et al., 1988). Root growth measurements were made every 48 h for 12 days; each of the experiments were performed in quadruplicate; each Petri dishes contained seven seedlings, throwing 588 experimental units. The variables evaluated were lateral root number (secondary roots) and fresh weight. The number of secondary roots was counted using an AllScience stereoscope 12 days after sowed. For the fresh weight the plant was removed from the petri dish with tweezers and weighed on an Ohaus brand analytical balance (Lopez-Bucio et al., 2007).

**Salinity tolerance experiments, the phosphates solubilization, production of IAA, siderophores and ACC deaminasa activity**

The bacterial strains that were characterized by their ability for plant growth-promoting were exposed to different concentrations of NaCl to see their ability to develop. LB solid medium supplemented with different concentrations of NaCl 0.85 M, 1.7 M, and 2.55 M was used; the rhizobacteria were streaked in LB with NaCl and incubated at 30 $^\circ$C, after three days the development of the bacterial strains was observed, the growth diameter reading was taken to evaluate the tests with a millimeter vernier (Mitutoyo America Corporation). Also was considered in the presence of colony a classification: $(+++)$ maximum development; $(++)$ medium development; $(+)$ minimum development; $(−)$ no development (Palacio-Rodríguez et al., 2016).

The phosphates solubilization were evaluated through the solid medium Pikovskaya; according to what was reported by Goldstein (1986). The evaluation of the production of Indole-3-acetic acid (IAA) of the rhizobacteria was carried out according to what is reported by Bric et al. (1991). The siderophores production was determined by the method Schwyn and Neilands (1987) using Chromium Azurol S (CAS) medium. The activity of the ACC deaminase enzyme was determined according to the method described by Penrose and Glick (2003).

**Molecular Identification**

The identification of PGPR was carried out by DNA extraction using the CTAB technique, according to the method of Doyle and Doyle (1990), then the partial amplification of the 16S rRNA gene was carried out...
by means of PCR using the oligonucleotides 27F 5´AGAGTTTGATCMTGGCTCAG 3´ and 1492R 5´GGTTACCTTGTTACGACTT 3`, the PCR product was sent to McLAB in San Francisco, CA, USA for sequencing. The sequences obtained were subjected to comparison using BLAST (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine the taxonomy of bacterial strains (Weisburg et al., 1991; Jha et al., 2011).

**Statistical analysis**

The development parameters (secondary roots and fresh weight) of the seedlings were analyzed in a random experimental design, a statistical analysis of ANOVA was carried out followed by a test of Tukey’s T (HSD), with a P value = 0.05. Analyzes were performed using Graph Pad Prism 6 program.

**Results**

**Candelilla rhizobacteria isolation and soil analysis**

For the area in Sierra Mojada, Ejido San Jose de Madero, Coahuila, a total of 77 bacterial strains were isolated (Figure 2a), and in the second area Sierra Viesca, Rancho Penjamo, Coahuila, a total of 88 bacterial strains were isolated (Figure 2b). In Table 1, we can observe that the area of the Sierra Mojada vs the Viesca area, showed numerical differences in the microorganisms isolated from the CAS medium (ecto and endorhizosphere), numerically favoring the area of Viesca. Another appreciation was with those developed on the LB medium, where at the ectorhizophere level, the Sierra Mojada area stood out numerically; the opposite occurred in the NFb medium where the Viesca area the values of ectorizospheric microorganisms were higher than those of the Sierra Mojada area (Figure 2ab). To another hand, considering morphologically identical colonies from the 165 isolates, some of them showed morphological duplication, reducing to 21 strains (Table 1).

![Figure 2. Culture media used and the number of strains isolated in the study area: (a) Sierra Mojada, (b) Sierra Viesca](image)
In relation to soil analyses in the two areas sampled, results showed that candelilla plants develops in soils with a sandy loam texture (sand: 62.13 ± 3.22; silt: 15.33 ± 2.47; clay: 22.54 ± 2.09); EC: 263 ± 33.12 g kg\(^{-1}\); field capacity = 13.11±2.99 g kg\(^{-1}\); O.M. 0.041 ± 0.010 g kg\(^{-1}\); N-NO\(_3\) = 0.05 ± 0.01 mg kg\(^{-1}\); P = 2.09 ± 0.11 mg kg\(^{-1}\); permeability K\(^+\) (cm h\(^{-1}\)) = high to excessive 14.9 ± 1.01; and exchangeable cations: Ca\(^{2+}\) = 263 ± 33.12 g kg\(^{-1}\); Na\(^+\) = 176 ± 30.282 kg\(^{-1}\)). These physical-chemical characteristics indicate a halotolerance of candelilla plants according to C.E. obtained in the analyzes and a low content of O.M. present in both areas.

**Effect of inoculation with candelilla rhizobacteria on the growth of Arabidopsis thaliana seedlings**

**Strains isolated from Sierra Mojada, Ejido San Jose de Madero, Coahuila**

In this test, two bacterial isolates (LbEcto8 and NFbEndo16) from 77 endo and ectorizosphere were positive to show results in variables evaluated, considering their respective control without inoculation. In lateral root number variable at 12 days after sowing (das), those treatments of seed germinated of A. thaliana and inoculated with LbEcto8 and NFbEndo16 rhizobacteria’s showed a significant difference (Tukey, P≤0.05) vs treatment non inoculated. The treatment with the LbEcto8 strain obtained an average of 27 secondary roots while the NFbEndo16 strain obtained an average of 22 for the number of secondary roots; 13 secondary roots was to the control without rhizobacteria. According to fresh weight variable, similar development with lateral root number was observed in this variable. The results indicated 77.35 mg in fresh weight, and 80.55 mg and fresh weight respectively to LbEcto8 and NFbEndo16 rhizobacteria, compared with the control without inoculation which obtained an average of 75.48 mg of fresh weight (Figure 3). The seedlings inoculated with the remaining bacterial isolates, among the observed effects were a rotting, turning first brown and then black.

Figure 4 show the effect of inoculation in the number of lateral roots and root fresh weight on which it is observed their abundance compared to the non-inoculated control plants.
Figure 3. (a) Effect of the inoculation of rhizobacteria (LbEcto8 and NFbEndo16 strain) from Sierra Mojada in A. thaliana seedlings, in root growth at 12 days after inoculation; (a) lateral root number, (b) fresh weight

Different letters indicate a significant difference (P≤0.05) between inoculated seedlings and the uninoculated control.

Figure 4. Effect of inoculation of rhizobacteria from Sierra Mojada, Ejido San Jose de Madero, Coahuila, on the growth of A. thaliana seedlings 12 days after inoculation in Petri dishes on 0.2× MS medium agar

a) Control, b) LbEcto8 and c) NFbEndo16.

Strains isolated from Sierra Viesca, Rancho Penjamo, Coahuila

In lateral root number and fresh weight variables carried out for this sampling zone, 19 rhizobacteria were tested (LbEndo2, LbEndo6, LbEndo7, LbEndo13, LbEndo15, CASEndo4, CASEndo7, CASEndo10, CASEndo17, NFbEndo12, LbEcto1, CASEcto2, CASEcto4, CASEcto11, CASEcto12, CASEcto13, NFbEcto1, NFbEcto8, NFbEcto18) (Figure 5 and 6). The results indicate in the lateral roots number a significant difference (P≤0.05), compared with the uninoculated control; a similar behaviour was to fresh weight variable.

In this comparison, the rhizobacteria inoculated in A. thaliana seedlings, which showed a significant difference (Tukey, P≤0.05) with respect to the uninoculated control, which in the variable of number of secondary roots obtained an average of 9, were the strains CASEcto2 with 22, CASEcto4, CASEndo10 and CASEcto12 with 19 secondary roots on average. For the fresh weight variable, the uninoculated control obtained an average weight of 76.1 mg and the rhizobacteria that showed a significant difference (Tukey, P≤0.05) were NFbEcto18 and CASEcto12 with 130.0 mg and 180.9 mg of fresh weight respectively (Figures 5 and 6).

In other form of representation, Figure 7, showed the control is surpassed by LbEndo7, CASEndo4, NFbEndo12, CASEcto4, CASEcto13, NFbEcto1 and NFbEcto18 treatments. However, same Figure 7 show us the control stands out those treatments based on the rhizobacteria CASEcto11 and NFbEcto8.
Figure 5. Effect of the inoculation of rhizobacteria in *A. thaliana* seedlings, where it is observed that the strains LbEndo6, LbEndo7, LbEndo13, CASEndo4, CASEndo7, CASEndo10, CASEndo17, NFbEndo12, CASEcto2, CASEcto4, CASEcto12, CASEcto13, NFbEcto18, increased the number of secondary roots of the plant 12 days after inoculation. Different letters indicate a significant difference (P ≤ 0.05) between inoculated seedlings and the uninoculated control.

Figure 6. Effect of the inoculation of the rhizobacteria in *A. thaliana* seedlings, on fresh weight (mg) variable. Different letters indicate a significant difference (P ≤ 0.05) between inoculated seedlings and the uninoculated control.
Figure 7. Effect of rhizobacteria inoculation on the growth of *A. thaliana* seedlings 12 days after inoculation in Petri dishes on 0.2× MS medium agar. a) Uninoculated control, b) LbEndo2, c) LbEndo6, d) LbEndo7, e) LbEndo13, f) LbEndo15, g) CASEndo4, h) CASEndo7, i) CASEndo10, j) CASEndo17, k) NFbEndo12, l) LbEcto1, m) CASEcto2, n) CASEcto4, o) CASEcto11, p) CASEcto12, q) CASEcto13, r) NFbEcto1, s) NFbEcto8, t) NFbEcto18

Tolerance of rhizobacteria to different concentrations of NaCl

The rhizobacteria selected from the two areas sampled (LbEndo2, LbEndo6, LbEndo7, LbEndo13, LbEndo15, CASEndo4, CASEndo7, CASEndo10, CASEndo17, NFbEndo12, NFbEndo16, LbEcto1, LbEcto8, CASEcto2, CASEcto4, CASEcto11, CASEcto12, CASEcto13, NFbEcto1, NFbEcto8, NFbEcto18), tested under salinity conditions of NaCl (0.85 M, 1.7 M and 2.55 M), all isolates had the ability to grow optimally in LB medium at 1.7 M, however, the rhizobacteria LbEcto8, CASEcto4, CASEcto13, CASEndo10 and CASEndo7 showed greater development up to the concentration at 2.55 M of NaCl (Table 2). All isolated had the opportunity to growth at 0.85M (5%) of NaCl with a maximum development. Our results showed that isolated bacterial could get in the classification as some halophilic bacteria (2% to 5% NaCl), moderately halophilic (5% to 20% NaCl), and extreme halophilic (20% to 30% NaCl), depending on their salt requirement according to Oren (2008), Kanekar *et al.* (2011) and Faraj *et al.* (2016).
Table 2. Growth bacterial under different salinity concentrations of NaCl of rhizobacteria (endorhizosphere/ectorhizosphere) of *E. antisyphilitica* from Sierra Mojada, Ejido San Jose de Madero, Coahuila and Sierra Viesca, Rancho Penjamo, Coahuila

| Rhizobacteria ID | Salinity tolerance |
|-----------------|--------------------|
|                 | 0.85M (5%) | 1.7M (10%) | 2.55M (15%) | 3.4M (20%) |
| LbEndo2         | +++      | +         | -          | -          |
| LbEndo6         | +++      | ++        | +          | -          |
| LbEndo7         | +++      | +++       | +          | -          |
| LbEndo13        | +++      | +++       | +          | -          |
| LbEndo15        | ++       | +         | -          | -          |
| CASEndo4        | ++       | +         | +          | -          |
| CASEndo7        | +++      | +++       | +++        | -          |
| CASEndo10       | +++      | +++       | +++        | -          |
| CASEndo17       | +        | +         | -          | -          |
| NFbEndo12       | +++      | +         | +          | -          |
| NFbEndo16       | +        | +         | +          | -          |
| LbEcto1         | +++      | +         | +          | -          |
| LBEcto8         | +++      | +++       | +          | -          |
| CASEcto2        | +++      | +         | +          | -          |
| CASEcto4        | +++      | +++       | +++        | +          |
| CASEcto11       | +        | +         | +          | -          |
| CASEcto12       | +++      | +         | +          | -          |
| CASEcto13       | +++      | +++       | +++        | +          |
| NFbEcto1        | +++      | ++        | +          | -          |
| NFbEcto8        | +++      | +         | +          | -          |
| NFbEcto18       | +++      | ++        | +          | -          |

(+++) maximum development; (++) medium development; (+) minimum development; (-) no development

**Phosphate solubilization**

For the determination of phosphate solubilization, it was observed that the rhizobacteria isolated from the candelilla root CASEcto2, CASEcto4, CASEcto13 and LBEcto8 (Figure 8), showed growth potential in Pikovskaya solid medium, determined by the formation of a clear halo around the bacterial growth with a thickness of 1.487 mm ± 0.667 mm, 2.133 mm ± 0.399 mm, 2.933 mm ± 0.530 mm, 5.267 mm ± 0.704 mm, respectively (Table 3). This result represents 19% of microorganisms selected and isolated in this study.

**Indole-3-acetic acid production (IAA)**

The results showed that in the production of IAA in all the selected bacterial strains showed the presence of this phytohormone, with values from 3.175 μg mL⁻¹ to 22.619 μg mL⁻¹ (Table 3).
Table 3. Results of the biochemical characterization of isolated rhizobacteria

| Rhizobacteria ID | Phosphate solubilization (mm) | IAA production (μg mL⁻¹) | Siderophores production | ACC deaminase (μmol α-KB/h/mg Pr) |
|-----------------|-------------------------------|--------------------------|-------------------------|----------------------------------|
| LbEndo2         | -                             | 13.016                   | -                       | 0.614                            |
| LbEndo6         | -                             | 13.889                   | -                       | 0.747                            |
| LbEndo7         | -                             | 16.772                   | +                       | 1.082                            |
| LbEndo13        | -                             | 12.778                   | +                       | 1.154                            |
| LbEndo15        | -                             | 4.630                    | +                       | 1.306                            |
| CASEndo4        | -                             | 17.460                   | +                       | 0.518                            |
| CASEndo7        | 1.33 ± 0.488                  | 15.000                   | +                       | 0.748                            |
| CASEndo10       | 1.400 ± 0.507                 | 11.984                   | -                       | 0.157                            |
| CASEndo17       | -                             | 3.175                    | +                       | 0.424                            |
| NFbEndo12       | -                             | 22.619                   | +                       | 0.720                            |
| NFbEndo16       | -                             | 5.397                    | +                       | 0.665                            |
| LbEcto1         | -                             | 12.937                   | +                       | 0.388                            |
| LBEcto8         | 5.267 ± 0.704                 | 14.127                   | +                       | 0.757                            |
| CASEcto2        | 1.487 ± 0.667                 | 17.249                   | +                       | 0.657                            |
| CASEcto4        | 2.133 ± 0.399                 | 6.429                    | +                       | 0.457                            |
| CASEcto11       | -                             | 11.455                   | +                       | 0.550                            |
| CASEcto12       | 2.667 ± 0.900                 | 11.905                   | +                       | 0.344                            |
| CASEcto13       | 2.933 ± 0.530                 | 5.026                    | +                       | 0.685                            |
| NFbEcto1        | -                             | 13.862                   | +                       | 0.537                            |
| NFbEcto8        | -                             | 3.757                    | +                       | 0.902                            |
| NFbEcto18       | -                             | 14.630                   | +                       | 0.878                            |

Arithmetic means ± standard deviation of triplicate experiments

(+) Development (-) No development

**Siderophores production**

In the assay carried out to demonstrate the production of siderophores; all the rhizobacteria isolated from the candelilla root (LbEndo2, LbEndo6, LbEndo7, LbEndo13, LbEndo15, CASEndo4, CASEndo7, CASEndo10, CASEndo17, NFbEndo12, NFbEndo16, LbEcto1, LbEcto8, CASEcto2, CASEcto4, CASEcto11, CASEcto12, CASEcto13, NFbEcto1, NFbEcto8, NFbEcto18 showed a translucent orange halo around their colony, suggesting, that these isolates have the ability to produce these low molecular weight organic substances.

**ACC deaminase activity**

The results showed that in the ACC deaminase activity all of the selected bacterial strains showed its presence, ranging from 0.157 to 1.306 μmol α-KB/h/mg Pr (Table 3), standing out that isolated LbEndo7, LbEndo13 and the LbEndo15 with values above of 1 μmol α-KB / h / mg Pr, while the least productive was CASEndo10.

**Molecular identification**

The characterization using the 16S ribosomal gene shows that, from the isolated bacterial microorganisms, and based on the biological properties, the microbial isolated corresponded to those indicated in Table 4, being *Bacillus* the most frequent of the genera.
Table 4. Molecular identification by gen 16Sr of the rhizobacteria isolated from candelilla
ID = identification, bp= base pairs

| ID       | Taxon                      | bp   | Identity | Acces Number |
|----------|----------------------------|------|----------|--------------|
| LbEndo2  | Bacillus paramycoidei      | 1403 | 99       | NR_157734.1  |
| LbEndo6  | Bacillus mojavensis        | 466  | 99       | NR_118290.1  |
| LbEndo7  | Staphylococcus epidermidis | 1396 | 99       | NR_113957.1  |
| LbEndo13 | Bacillus subtilis          | 1366 | 95       | NR_104873.1  |
| LbEndo15 | Bacillus pseudomycoidei    | 1379 | 99       | NR_113991.1  |
| CASEndo4 | Bacillus pacificus         | 1365 | 93       | NR_157733.1  |
| CASEndo10| Bacillus cereus            | 1398 | 99       | NR_074540.1  |
| CASEndo17| Bacillus subtilis          | 1379 | 97       | NR_027552.1  |
| NFbEndo12| Cronobacter muytiensis     | 724  | 98       | NR_118088.1  |
| NFbEndo16| Staphylococcus epidermidis | 1398 | 99       | NR_113957.1  |
| LbEcto1  | Siccibacter colleas        | 1360 | 99       | NR_134807.1  |
| LbEcto8  | Acinetobacter lactuae      | 242  | 80       | NR_152082.1  |
| CASEcto2 | Siccibacter colletis       | 1371 | 99       | NR_134807.1  |
| CASEcto4 | Staphylococcus epidermidis | 1402 | 99       | NR_036904.1  |
| CASEcto11| Acinetobacter lwoftii      | 1344 | 97       | NR_113346.1  |
| CASEcto12| Mixta gaviniae             | 641  | 99       | NR_117305.1  |
| CASEcto13| Staphylococcus epidermidis | 1377 | 95       | NR_113957.1  |
| NFbEcto1 | Bacillus subtilis          | 657  | 99       | NR_104873.1  |
| NFbEcto8 | Siccibacter colletis       | 1371 | 99       | NR_134807.1  |
| NFbEcto18| Acinetobacter johnstonii   | 353  | 82       | NR_164627.1  |

Discussion

In the present study, 21 rhizobacteria isolated from the candelilla rhizosphere were characterized, with the ability to stimulate growth in Arabidopsis thaliana. Our results agree with those study’s conducted by Palacio-Rodriguez et al. (2017) and Ramos-Acosta et al. (2015) where the beneficial microbial are presented in plants from Chihuahuan Desert at Coahuila State. Palacio-Rodriguez et al. (2017) from the rhizosphere of Distichlis spicata isolated 75 bacterial strains, 31 of which were isolated from the endorizosphere and 44 from the ectorizosphere, using culture media Lb, KB, Jensen and NFb and Ramos-Acosta et al. (2015) in mesquite rhizosphere, 64 bacterial strains were isolated (16 from the endorizosphere and 28 from the ectorizosphere and 19 from the mesquite rhizoplane with the culture media (CRYEMA, KB and LB).

An evident in vitro phyto-stimulation was observed in A. thaliana seedlings after 12 days of inoculation. The root architecture was modified through changes in the growth of the main root, as well as the increased formation of lateral roots and root hairs, this could translate into a benefit of great importance for the acquisition of water resources and nutrients for the plant. In a study conducted by Estrada-González et al. (2017) seeds of A. thaliana were inoculated with PGPR to see their response, and a root growth promotion was obtained between 48% and 82% compared to the control without inoculation.

In our study, five rhizobacteria (LbEcto8, CASEcto4, CASEcto13, CASEndo10, CASEndo7) belonging to the moderately halophilic group were identified since they can tolerate a wide range of salt concentrations, evidenced by their growth in different concentrations of NaCl. In accordance with the above and knowing the classification of halophilic microorganisms with respect to their salt requirement, it is possible to confirm that the strains isolated in this study belong to the group of moderately halophilic bacteria, since they can tolerate a wide range of salt concentrations.

It is possible to use this type of halophilic bacteria, isolated from saline environments, as a possible option for the bioremediation of salinized soils for agricultural production. Palacio-Rodriguez et al. (2017) isolated
PGPR from the rhizosphere of *D. spicata* from hypersaline rhizospheric soils (EC of 10.12 mS cm⁻¹), these rhizobacteria were subjected to a salinity tolerance and had a resistance to a concentration of 5%, 10% and 15% NaCl, a same salt concentration used in our study, where LbEcto8, CASEcto4 and CASEcto13 rhizobacteria were isolated. One of the most obvious effects of rhizobacteria in our study was observed in the increase in root mass, mainly in the number of secondary roots, which provides plants with a larger area for the absorption of water and nutrients. This is because PGPR can enhance plant growth by a wide variety of mechanisms like phosphate solubilization, siderophore production, biological nitrogen fixation, rhizosphere engineering, production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), phytohormone production, exhibiting antifungal activity, production of volatile organic compounds (VOCs) and induction of systemic resistance, etc. (Bhattacharyya and Jha, 2012). As in this investigation, Ramos-Acosta *et al.* (2015) determined the solubilization of phosphates, it was observed that the rhizobacteria isolated from the mesquite root of La Poza Salada; EctoLB15 and RizoNFB2, showed growth potential in solid Pikovskaya medium, determined by the formation of a clear halo around the bacterial growth.

Angulo *et al.* (2014) reported PGPR with the ability to solubilize phosphates with a halo diameter between 2 ± 0.2 and 1.07 ± 0.1 mm. At the same study, a test of indoleacetic acid (IAA) production of rhizobacteria isolated from the root of *Eucalyptus nitens* reported the presence of this phytohormone with values from 4.60 μg mL⁻¹ to 28.09 μg mL⁻¹. The evaluation by Gonzalez-Mancilla *et al.* (2017) indicated that the isolated strains of PGPR produced between 21 μg mL⁻¹ and 26 μg mL⁻¹ of IAA, where it is also mentioned that the production of auxins by the strains is conditioned by the type and age of the associated culture, in addition to the bacterium species and genus.

Indoleacetic acid produced by PGPR plays an important role in the plant-microorganism interaction, benefiting the promotion of root and foliar growth of plants as can be seen in the results obtained in the growth promotion test of this research; in this variable was standing out that isolate grown in NFB medium and that was obtained from the candelilla rhizosphere from Viesca region. In second position are the microorganisms named CASEndo4 and CASEcto2. The least producers were CASEndo17 and the NFBECTO8.

The results reported by Ramos-Acosta *et al.* (2015) in the trial to demonstrate the production of siderophores in CAS culture medium, six PGPR isolated from the rhizosphere of mesquite, showed the formation of a translucent orange halo around their growth, which allows confirming that these bacterial strains have the ability to produce siderophores. It has been shown that siderophores can, by themselves, act as efficient activators of induced systemic resistance systems in plants (Ran *et al.*., 2005; Meziane *et al.*, 2005; Bakker *et al.*, 2007). The ability of siderophores to act as pathogen suppressors depends on the plant, the phytopathogen to be controlled and the composition of the soil, the bacteria and the affinity of the siderophore for iron (Glick, 1995; Dellagi *et al.*, 2009).

Recent studies have shown that PGPRs containing ACC deaminase induce the production of longer roots, increasing the absorption of water (Zahir *et al.*, 2008; Esquivel-Cote *et al.*, 2013). Ribaudor *et al.* (2013) mentions that from the study of the relationship between the presence of ACC deaminase activity in PGPR and the inhibitory effect of ethylene on it, a possible role of the enzyme in the PGPR/PGPR interaction mechanism has been proposed plant. At the same time, Palacio-Rodriguez *et al.* (2017) report an activity of the ACC deaminase enzyme of 91 μmol α-KB / h / mg Pr of a PGPR isolated from the rhizosphere of *D. spicata*.

Regarding the rhizobacteria identified, the *Bacillus* has stood out for the promoting effect it exerts on plant growth. *Bacillus megaterium* and *Bacillus subtilis* are the most studied to determine the solubilization of phosphates, which is one of the mechanisms that PGPR use to promote growth (Tejera *et al.*, 2011) In the research carried out by Nawangsih *et al.* (2010) they were able to isolate *Staphylococcus epidermidis* from healthy tomato stems and when testing it as a control agent against the *Ralstonia solanacearum* its effect was positive, they mention that *Staphylococcus epidermidis* had not been reported as a bacterium endophyte tomato. Few reports of *Staphylococcus epidermidis* mention its ability to promote plant growth.
Conclusions

The rhizosphere of candelilla (*Euphorbia antisyphilitica*) that develops naturally in the Coahuila desert, specifically in the Sierra Mojada and Sierra Viesca, has a large reservoir of PGPR; in the present study 165 bacterial strains were isolated, from which 21 excelled in growth promotion, which was evident in the increase in root mass, mainly in the number of roots secondary in *A. thaliana*.

The rhizobacteria LbEcto8, CASEcto4, CASEcto13, CASEndo10 and CASEndo7 showed the ability to resist high concentrations of salt (15%) for which it is possible to confirm that the strains isolated in this study belong to the group of moderately halophilic bacteria.

The solubilization capacities of phosphates, production of IAA and siderophores that the isolated rhizobacteria presented, indicate that they can be used as growth promoters and for biological control in a crop of agronomic interest in future investigations.

The molecular identification of the most efficient rhizobacteria in plant growth promotion was achieved, finding bacteria of the genera *Bacillus* sp., *Staphylococcus* sp., *Acinetobacter* sp., *Cronobacter* sp. and *Siccibacter* sp. This is a first indication to proceed to carry out tests in different phenological stages in crops of agricultural importance.

Authors’ Contributions

Conceptualization: MTSR, JAOV, JSM; Methodology: MTSR, JSM, EORP; Validation: JAOV, JSM, EORP; Formal analysis: MFH, PPR; Investigation: MTSR; Data curation: EORP, PYC, PPR; Funding acquisition: JAOV, JSM; Project administration: MTSR; Writing: MTSR, EORP; Review and editing: JAOV, JSM, EORP. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Aguilar X, Valle G, González G, Murillo B (2013). Guía de cultivo de orégano. [Oregano grow guide]. In: La Paz SC (Ed). Centro de Investigaciones Biológicas del Noreste. Baja California Sur, México pp 106.

Altomare C, Tringovska I (2011). Beneficial soil microorganisms, an ecological alternative for soil fertility management. Genetics, biofuels and local farming systems. In: Lichtfouse E (Ed). Sustainable Agriculture Reviews. Springer 7:161-214. https://doi.org/10.1007/978-94-007-1521-9

Angulo VC, Sanfuentes EA, Rodríguez F, Sossa KE (2014). Caracterización de rizobacterias promotoras de crecimiento en plántulas de *Eucalyptus nitens*. [Characterization of growth-promoting rhizobacteria in seedlings of *Eucalyptus nitens*]. Revista Argentina de Microbiología 46(4):338-347. https://doi.org/10.1016/S0325-7541(14)70093-8
Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. Annual Review Plant Biology 57:233-66. https://doi.org/10.1146/annurev.arplant.57.032905.105159

Bakker PAHM, Pieterse CMJ, Loon LC (2007). Induced systemic resistance by fluorescent Pseudomonas spp. Phytopathology 97:239-243. http://doi.org/10.1094/PHYTO-97-2-0239

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR); emergence in agriculture. World J Microbiology Biotechnology 28:1327-1350. https://doi.org/10.1007/s11274-011-0979-9

Bric JM, Bostock RM, Silverstone SE (1991). Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Applied and Environmental Microbiology 57:535-538. https://doi.org/10.1128/AEM.57.2.535-538.1991

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World J Microbiology Biotechnology 28:1327-1350. https://doi.org/10.1007/s11274-011-0979-9

Bakker PAHM, Pieterse CMJ, Loon LC (2007). Induced systemic resistance by fluorescent Pseudomonas spp. Phytopathology 97:239-243. http://doi.org/10.1094/PHYTO-97-2-0239.

Bhattacharyya PN, Jha DK (2012) Plant growth-promoting rhizobacteria (PGPR); emergence in agriculture. World J Microbiology Biotechnology 28:1327-1350. https://doi.org/10.1007/s11274-011-0979-9

Bric JM, Bostock RM, Silverstone SE (1991). Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Applied and Environmental Microbiology 57:535-538. https://doi.org/10.1128/AEM.57.2.535-538.1991

Carrillo A, Puente M, Castellanos T, Bashan Y (1998). Aplicaciones biotecnológicas de ecología microbiana. Manual de laboratorio. [Biotechnological applications of microbial ecology. Laboratory manual]. Pontifica Universidad Javeriana, Santa Fe de Bogotá Colombia. Investigaciones Biológicas del Noreste. Baja California Sur, México pp 51.

Comisión Nacional Forestal (CONAFOR) (2009). Gerencia de plantaciones forestales comerciales. [National Forestry Commission. Management of the Forest Plantations] http://www.conafor.gob.mx/portal/index.php/temas-forestales/competitividad/estudio-2009

De-Bashan LE, Holguin G, Glick BR, Bashan Y (2007). Bacterias promotoras de crecimiento en plantas para propósitos agrícolas y ambientales. [Growth-promoting bacteria in plants for agricultural and environmental purposes]. Microbiología agrícola. Editorial Trillas, México, D.F., México.

Dellagi A, Rigault M, Segond D, Roux C, Kraepiel Y, Cellier F, ... Expert D (2005). Siderophore-mediated upregulation of Arabidopsis ferritin expression in response to Erwinia chrysanthemi infection. The Plant Journal 43:262-272. https://doi.org/10.1111/j.1365-313X.2005.02451.x

Dias ACF, Costa FEC, Andreote FD, Lacava PT, Teixeira MA, Assumpcao LC, ... Melo IS (2009). Isolation of micropropagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion. World Journal of Microbiology and Biotechnology 25(2):189-195. https://doi.org/10.1007/s11274-008-9878-0

Dijkstra FA, Carrillo Y, Pendall E, Morgan JA (2014). Rhizosphere priming: a nutrient perspective. The Microbial Regulation of Global Biogeochemical Cycles 183. https://doi.org/10.3389/finch.2013.00216

Doyle JJ, Doyle JL (1990). Isolation of plant DNA from fresh tissue. Focus 12:13-15.

Esquivel-Cote R, Ramírez-Gama RM, Tsuzuki-Reyes G, Orozco-Segovia A, Huante P (2010). Azospirillum lipoferum containing 1-aminocyclopropane-1-carboxylic acid deaminase improves early growth of tomato seedlings under nitrogen deficiency. Plant and Soil 337:65-75. https://doi.org/10.1007/s11104-010-0499-7

Estrada-González ÁDJ (2017). Aislamiento, caracterización e identificación de bacterias asociadas a Opuntia spp. y su efecto en la germinación y crecimiento de Arabidopsis thaliana L. [Isolation, characterization and identification of bacteria associated with Opuntia spp. and its effect on the germination and growth of Arabidopsis thaliana L.]. Master tesis, Instituto Potosino De Investigación Científica Y Tecnológica, A. C. https://ipicty.repositorioinstitucional.mx

Faraj-Edbeib M, Abdul-Wahab R, Huyop F (2016). Halophiles: biology, adaptation, and their role in decontamination of hypersaline environments. World Journal Microbiology Biotechnology 32(135):1-24. https://doi.org/10.1007/s11274-016-2081-9

Frommel MI, Nowak J, Lazarovits G (1991). Growth enhancement and development modification of in vitro grown potato (Solanum tuberosum spp. tuberosum) as affected by a non-fluorescent Pseudomonas sp. Plant Physiology 96:928-936. https://doi.org/10.1104/pp.96.3.928

Glick BR (1995). The enhancement of plant growth by free-living bacteria. Canadian Journal of Microbiology 41:109-117. https://doi.org/10.6064/2012/963401

Goldstein AH (1986). Bacterial solubilization of mineral phosphates: historical perspectives and future prospects. American Journal of Alternative Agriculture 1:51-57. http://www.jstor.org/stable/44506926

González-Mancilla A, Almaraz-Suárez JJ, Ferrera-Cerrato R, Rodríguez-Guzmán MDP, Gaytán T, Rey O, Arteaga-Garibay RI (2017). Characterización y selección de rizobacterias promotoras de crecimiento en plántulas de chile Poblano (Capsicum annuum L.). [Characterization and selection of growth-promoting rhizobacteria in Poblano pepper seedlings]. Revista Internacional de Contaminación Ambiental 33(3):463-474. https://doi.org/10.20937/RICA.2017.33.03.09

Salazar-Ramírez MA et al. (2021). Not Bot Horti Agrobo 49(2):12294
Hariprasad P, Niranjana SR (2009). Isolation and characterization of phosphate solubilizing rhizobacteria to improve plant health of tomato. Plant and Soil 316(1):13-24. https://doi.org/10.1007/s11104-008-9754-6

Jha B, Gontia I, Hartmann A (2011). The roots of the halophyte Salicornia brachiata are a source of new halotolerant diazotrophic bacteria with plant growth-promoting potential. Plant and Soil 356:265-277. https://doi.org/10.1007/s11104-011-0877-9

Johansson JF, Paul LR, Finlay RD (2004). Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microbiology Ecology 48:1-13. https://doi.org/10.1016/j.femsec.2003.11.012

Kanekar P, Kanekar S, Kelkar A, Dhakephalkar P (2011). Halophiles - taxonomy, diversity, physiology and applications. Satyanarayana NT, Nairn B, Prakash JA (Eds). Microorganisms in Environmental Management 1-34. https://doi.org/10.1007/978-94-007-2229-3_1

Kloeper JW, Lifshitz R, Schroth MN (1988). Pseudomonas inoculants to benefit plant production. ISI Atlas of Science. Animal and Plant Sciences 1:60-64.

López-Bucio J, Campos-Cuevas C, Hernández-Calderón E, Velásquez-Becerra C, Farias-Rodríguez R, Macías-Rodríguez I, Valencia-Cantero E (2007). Bacillus megaterium rhizobacteria promote growth and alter root-system architecture through an auxin- and ethylene-independent signaling mechanism in Arabidopsis thaliana. Molecular Plant-Microbe Interactions 20:207-217. https://doi.org/10.1094/MPMI-20-2-0207

MacFaddin J (2000). Biochemical tests for identification of medical bacteria. Lippincott Williams & Wilkins, Philadelphia pp 912.

Oren A (2008). Microbial life at high salt concentrations: phylogenetic and metabolic diversity in saline systems. Saline Systems 4(2):1-13. https://doi.org/10.1186/1746-1448-4-2

Penrose DM, Glick BR (2003). Methods for isolating and characterizing ACC deaminase containing plant growth-promoting rhizobacteria. Physiologia Plantarum 118:10-15. https://doi.org/10.1046/j.1399-3054.2003.00865.x

Ramos-Acosta BP (2015). Aislamiento y caracterización de PGPR’s de mezquite (Prosopis spp.). [Isolation and characterization of PGPR of mesquite]. Master Thesis, Universidad Autónoma Agraria Antonio Narro. Unidad Laguna. http://repositorio.uaaan.mx:8080/xmlui/handle/123456789/42377

Ran LX, Li ZN, Wu GJ, Van Loon LC, Bakker PAHM (2005). Induction of systemic resistance against bacterial wilt in Eucalyptus urophylla by fluorescent Pseudomonas spp. European Journal of Plant Pathology 113:59-70. https://doi.org/10.1007/s10658-004-0623-3

Reinold B, Hurek T, Fendrik I, Pot B, Gillis M, Kersters K, Thielmans S, De Ley J (1987). Azospirillum halopraeferens sp. nov. A nitrogen-fixing organism associated whit roots of Kallar grass (Leptochloa fusca (L.) Kunth). International Journal of Systematic Bacteriology 37:43-51. https://doi.org/10.1099/00207733-37-1-43

Ribaudo CM (2013). Mecanismos bioquímicos y moleculares desencadenados en la interacción bacterias promotoras de crecimiento vegetal y plantas de interés agronómico [Biochemical and molecular mechanisms triggered in the interaction of plant growth promoting bacteria and plants of agronomic interest]. Ph. D. Thesis Universidad de
Salazar-Ramírez MA et al. (2021). Not Bot Horti Agrobo 49(2):12294

Buenos Aires. Facultad de Ciencias Exactas y Naturales.

https://bibliotecadigital.exactas.uba.ar/collection/tesis/document/tesis_n5372_Ribaudo

Sandhya V, Ali SZ, Venkateswarlu B, Reddy G, Grover M (2010). Effect of osmotic stress on plant growth promoting Pseudomonas spp. Archives of Microbiology 192(10):867-876. https://doi.org/10.1007/s00203-010-0613-5

Saraf M, Kumar JC, Patel D (2011). The role of ACC deaminase producing PGPR in sustainable agriculture. In: Maheshwari DK (Ed). Plant Growth and Health Promoting Bacteria. Springer Berlin Heidelberg, Microbiology Monographs 18:365-385. https://doi.org/10.1007/978-3-642-13612-2_16

Schwyn B, Neilands JB (1987). Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry 160:47-56. https://doi.org/10.1016/0003-2697(87)90612-9

Sharma A, Pathak A, Sahgal M, Meyer JM, Wray V, Johri BN (2007). Molecular characterization of plant growth promoting rhizobacteria that enhance peroxidase and phenylalanine ammonia-lyase activities in chili (Capsicum annuum L.) and tomato (Lycopersicon esculentum Mill.). Archives of Microbiology 188(5):483-494. https://doi.org/10.1007/s00203-007-0270-5

Singh G, Mukerji KG (2006). Root exudates as determinant of rhizospheric microbial biodiversity. In: Microbial Activity in the Rhizosphere. Springer Berlin Heidelberg, pp 39-53. https://doi.org/10.1007/3-540-29420-1_3

Stafford WH, Baker GC, Brown SA, Burton SG, Cowan DA (2005). Bacterial diversity in the rhizosphere of Proteaceae species. Environmental Microbiology 7:1755-1768. https://doi.org/10.1111/j.1462-2920.2005.00929.x

Tejera-Hernández B, Rojas-Badia M, Heydrich-Pérez M (2011). Potencialidades del género Bacillus en la promoción del crecimiento vegetal y el control biológico de hongos fitopatógenos. [Potentialities of the genus Bacillus in the promotion of plant growth and the biological control of phytopathogenic fungi]. Revista CENIC Ciencias Biológicas Redalyc 42:131-138. https://www.redalyc.org/articulo.oa?id=181222321004

Weisburg W, Barns S, Pelletier D, David P, Gene L (1991). 16s ribosomal DNA amplification for phylogenetic study. Journal of Bacteriology 173(2):697-703. https://doi.org/10.1128/jb.173.2.697-703.1991

Zahir ZA, Arshad M, Frankenberger WT Jr (2004). Plant growth promoting rhizobacteria: applications and perspectives in agriculture. Advances in Agronomy 81:97-168. https://doi.org/10.1016/S0065-2113(03)81003-9

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