

# ISOLATION AND CHARACTERIZATION OF TWO PLANT GROWTH-PROMOTING BACTERIA FROM THE RHIZOPLANE OF A LEGUME (*Lupinus albus*) IN SANDY SOIL<sup>(1)</sup>

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## SUMMARY

Two bacterial strains that amplified part of the *nifH* gene, RP1p and RP2p, belonging to the genus *Enterobacter* and *Serratia*, were isolated from the rhizoplane of *Lupinus albus*. These bacteria are Gram-negative, rod-shaped, motile, facultative anaerobic, and fast-growing; the colonies reach diameters of 3–4 mm within 24 h of incubation at 28 °C. The bacteria were also able to grow at temperatures as high as 40 °C, in the presence of high (2–3 % w/v) NaCl concentrations and pH 4–10. Strain RP1p was able to utilize 10 of 14 C sources, while RP2p utilized nine. The isolates produced siderophores and indolic compounds, but none of them was able to solubilize phosphate. Inoculation of *L. albus* with RP1p and RP2p strains resulted in a significant increase in plant dry matter, indicating the plant-growth-promoting abilities of these bacteria.

**Index terms:** indolic compounds, siderophores, inoculation experiment.

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**RESUMO:** *ISOLAMENTO E CARACTERIZAÇÃO DE DUAS BACTÉRIAS PROMOTORAS DE CRESCIMENTO VEGETAL DO RIZOPLANO DE UMA LEGUMINOSA (Lupinus albus) DE SOLO ARENOSO*

*Duas linhagens bacterianas que apresentaram amplificação de parte do gene nifH, RP1p e RP2p, pertencentes aos gêneros Enterobacter e Serratia, foram isoladas do rizoplane de Lupinus albus. Essas bactérias são Gram-negativas, com formato de bastonete, móveis, anaeróbias facultativas e apresentam multiplicação rápida, com colônias alcançando diâmetros de 3–4 mm em 24 h de incubação a 28 °C. RP1p e RP2p também foram capazes de multiplicação em temperaturas elevadas, como 40 °C, na presença de alta concentração de NaCl (2–3 % v/v) e em valores de pH que variaram de 4 a 10. A linhagem RP1p foi capaz de utilizar 10 das 14 fontes de carbono avaliadas, enquanto a linhagem RP2p utilizou nove. Os isolados produziram sideróforos e compostos indólicos, mas foram incapazes de solubilizar fosfatos. A inoculação de L. albus com as linhagens RP1p e RP2p resultou em aumento significativo do peso das plantas secas, o que demonstra que essas bactérias apresentam propriedades que favorecem o crescimento vegetal.*

*Termos de indexação: compostos indólicos, sideróforos, experimentos de inoculação.*

## INTRODUCTION

It is widely accepted that rhizosphere and rhizoplane microorganisms can influence plant growth and development. The term plant growth-promoting rhizobacteria (PGPR) was coined for the bacterial biocontrol agents of the rhizosphere (Kloepper et al., 1980). Some years later, the term plant growth-promoting bacteria (PGPB) was proposed to designate rhizobacteria that enhance plant growth by other ways (Bashan & Holguin, 1998). Plant growth-promoting (PGP) activities have been reported for a series of bacterial species including *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthobacter*, *Burkholderia*, *Bacillus* and *Serratia* (Versalovic et al., 1994; Glick, 1995; Han et al., 2005). Although the mechanisms by which PGPB promote plant growth are not fully understood (Glick, 1995; Ahmad et al., 2008), these bacteria can improve plant growth by N<sub>2</sub> fixation, phytohormone and siderophore production, phosphorus solubilization, and disease control (Bashan & Holguin, 1998; Barea et al., 2005). Moreover, the root-surrounding environment, the rhizosphere, is a dynamic soil site of nutrient turnover. Bacteria around plant roots perform a wide range of metabolic activities and are able to make use of a wide range of low molecular mass organic compounds and of some more complex compounds as C and energy sources (Misko & Germida, 2002). These microorganisms play major roles in nutrient transformation and element cycling and influence the availability of these nutrients for plant uptake (Dunfield & Germida, 2001).

The family Leguminosae comprises about 750 genera and more than 18,000 species, from which only

8 % are cultivated plants. Little information is available on wild legumes and their root-associated bacteria. These interactions are particularly important when they result in tolerance to extreme environmental conditions such as severe drought, elevated temperature and salinity (Moschetti et al., 2005). High tolerance to extreme abiotic situations is very important in some regions of Rio Grande do Sul (RS), Brazil, where about 5.2 % of the soils are sandy and acid, which is characteristic of subtropical arid climates (Schumacher, 2000). *Lupinus albus* H. et Arn, an indigenous leguminous species, has been used in the recovery of these sandy soils due to its high rusticity, strong adaptability to sandy regions, ability to reduce the eolic erosion process, upgrade dry matter, improve the soil quality and cover, and the capacity of biological N<sub>2</sub> fixation when establishing symbiosis with some bacteria belonging to the genus *Bradyrhizobium*, as identified by sequencing of the ITS region of strains obtained by Stroschein (2007).

So far, no study has described or selected PGPB associated to *L. albus* plants. Moreover, a better knowledge of these bacteria and their implications on plant physiology could change traditional crop management practices regarding plant nutrition and defense mechanisms. For a maximum exploitation of the plant-bacteria association, effective bacteria must be selected in plant studies that take specific ecological conditions into consideration, e.g., crop management, soil, temperature, etc. The primary aim of this study was to isolate and characterize rhizoplane bacteria associated with native *Lupinus albus* H. et Arn. grown in sandy soils of RS. These bacteria may help their host plant to support adverse ecological conditions due to their PGP characteristics.

## MATERIALS AND METHODS

### Collection site and soil sampling

Small root pieces from *Lupinus albus* H. et Arn. were collected from an experimental field (29° 39' 58" S, 55° 23' 43" W), in Alegrete, Rio Grande do Sul, Brazil. The regional climate is classified as Cfa according to Köppen, humid subtropical, no dry season, with an annual rainfall of about 1,400 mm (Souto, 1984). The area under study had never been inoculated with commercial bacterial inoculants but underwent some degradation processes, as huge sandy spots indicate.

### Bacteria isolation and reference strains

Bacteria from the rhizoplane of *L. albus* were isolated in yeast-mannitol agar (YMA) medium by standard procedures (Fred & Waksman, 1928). The bacteria were purified by repeated streaking and the pure cultures stored at -10 °C, in 30 % glycerol-yeast mannitol broth. Two rhizobial reference strains (*Bradyrhizobium* sp. SEMIA 928 and SEMIA 938), from the Biological Nitrogen Fixation Center, Fepagro, Brazil, and three other bacteria (*Enterobacter* sp., *Pseudomonas* sp., and *Klebsiella* sp.) available in the laboratory were used as control. To confirm their purity, all isolates and control strains were streaked on YMA medium plus 25 mg mL<sup>-1</sup> Congo red dye and on glucose-peptone agar (GPA) medium plus 100 mg mL<sup>-1</sup> bromothymol blue (Fred & Waksman, 1928), as pH indicator.

### Phenotypic characterization

#### Morphology, motility and electron microscopy

The cell morphology was examined by phase-contrast microscopy and the spore types under a scanning electron microscope in cells from 48 h cultures in YMA medium. Cells were fixed according to Borges et al. (2004) and the grids examined by a scanning electron microscope (model XL-30, Philips). Gram staining was performed (Doetsch, 1981) and motility verified by the SIM (Hydrogen-Sulfide, Indole, Motility; Mac Faddin, 2000) test.

#### Growth rates

The bacterial colony size was determined on YMA after 48 h at 28 °C. The strains were grown in YMA plates and categorized as very fast (colonies visible in less than one day), fast (colonies visible in 2–3 days) and slow-growing (colonies visible in 3–5 days) (Somasegaran & Hoben, 1992).

#### Carbohydrate utilization

Different groups of C sources were evaluated: monosaccharides (L-arabinose, D-fructose, D-

galactose, D-glucose, D-mannose, rhamnose, and D-xylose), disaccharides (lactose, maltose, and sucrose) and sugar alcohol (glycerol, inositol, mannitol, and sorbitol). The basal medium used for C assimilation was a minimum salt medium (MSM, Brown & Dilworth, 1975). Carbon sources were sterilized by filtration (pore size 0.45 µm, Millipore) and added aseptically to the autoclaved MSM at a final concentration of 1 g L<sup>-1</sup>. The medium contained 2 mL of 0.5 % phenol red per liter as pH indicator. About 50 µL of culture of each bacterium grown in YEM broth for 48 h at 28 °C was filled into an individual culture tube containing MSM and the designed carbon source and left to grow at 128 rpm at 28 °C. After 72 h, the bacteria that were able to grow in a given C source modified the pH of the medium, turning it from red to yellow. This behavior was recorded as growth (+) or no growth (-) in comparison with controls. All experiments were performed in triplicate.

#### Salt, pH and temperature tolerance

Salt tolerance was evaluated using 0.5, 1, 2, 3, or 4 % (w/v) NaCl in YEM broth. The pH tolerance was evaluated using YEM broth with pH ranging between 4 and 10 (Solano et al., 2006). About 50 µL of culture of each isolate grown in YEM broth for 48 h at 28 °C were filled into individual tubes containing the different pH or NaCl concentration and left to grow at 128 rpm. The growth (+) or no growth (-) in comparison with the controls after 72 h was recorded. The temperature tolerance of isolates was tested at 28 (control), 37 and 40 °C on YMA for two days. All tests were carried out in triplicate.

#### Siderophore production

Bacterial samples were evaluated for their capacity of siderophore production in Petri dishes containing King B medium supplemented with a complex chrome azurol S [CAS/iron(III)/ hexadecyl trimethyl ammonium bromide] as described by Schwyn & Neilands (1987). Bacteria that were able to produce siderophores grew and formed a yellow halo in the blue-green media.

#### Phosphate solubilization

The method described by Sylvester-Bradley et al. (1982) was used to identify isolates able to solubilize phosphates. Bacteria were grown in glucose yeast medium (GL) containing 10 g glucose, 2 g yeast extract and 15 g agar per liter. Two other solutions were prepared separately, one containing 5 g K<sub>2</sub>HPO<sub>4</sub> in 50 mL distilled water, and the other containing 10 g CaCl<sub>2</sub> in 100 mL distilled water. These solutions were added to one liter of glucose yeast (GY) medium immediately before pouring into Petri dishes, forming insoluble calcium phosphate. The isolates that formed visible clear halos around their colonies were considered phosphate solubilizers.

### ***In vitro* indolic compound production**

All isolates were grown in YEM broth. Optical density was used to control inoculum size ( $10^5$ – $10^6$  cfu mL<sup>-1</sup>). Inocula were transferred (100 mL) to King B medium, which is the medium used to quantify indolic compound production, according to Glickmann & Dessaux (1995). This method makes use of the Salkowski reagent ( $12 \text{ g L}^{-1} \text{ FeCl}_3 + 7.9 \text{ mol L}^{-1} \text{ H}_2\text{SO}_4$ ), and was used to quantify indole 3 acetic acid (IAA), indolepyruvic acid (IPyA), and indoleacetamide (IAM) production, which will be cited in the text collectively as indolic compounds.

### **Anaerobiosis**

All isolates were tested for their growth capacity in an anaerobiosis chamber. The isolates were streaked on two media, YMA and Tiamine-biotine agar (TBA, Seldin et al., 1983) and incubated in anaerobiosis jars for seven days at 28°C.

### **Genotype characterization**

#### **Genotypic characterization by rep-PCR and RFLP of the *nifH* gene**

Bacterial total DNA was extracted as described by Giongo et al. (2007). Rep-PCR reactions were performed using enterobacterial repetitive intergenic consensus primers ERIC1-R (ATGTAAGCTCCTGGGGATTAC) and ERIC-2 (AAGTAAGTGACTGGGGTGAGCG) (De Bruijn, 1992) and enterobacterial repetitive sequences BOX A1 primer (CTACGGCAAGGCGACGCTGACG, Versalovic et al., 1994). Reactions were performed in a PCR Express Temperature Cycling System (Thermo Hybaid) and the fragments visualized after electrophoresis on 1.0 % UltraPure agarose gels (Invitrogen Life Technologies). A molecular size marker (1 Kb Plus DNA ladder, Gibco BRL) was run on all gels.

A template of 100 ng DNA was used in RFLP procedures. Selected primers PolF and PolR [TGCGAYCCSAARGCBGACTC and ATSGCCATCATYTCRCCGGA, respectively (Poly et al., 2001)] were used to amplify a 360-bp region of *nifH* gene. PCR amplifications were carried out as described by Poly et al. (2001). For restriction enzyme cleavage, 10 µL of each PCR product was directly used. The reaction enzyme mixture contained 1 X restriction enzyme buffer and 1.25 U of restriction endonuclease. *TaqI* and *HaeIII* were selected for their specificity for the amplified *nifH* region. The PCR products were digested overnight. Digested DNA samples were analyzed by electrophoresis in a 30 % polyacrylamide gel. The electrophoresis conditions were 5 h at 150 V in 1 X Tris-borate-EDTA buffer at room temperature, followed by 30 min of silver nitrate staining. All fingerprint patterns were converted into a two-dimensional binary matrix (1, presence of a band; 0, absence of a band) and analyzed using the Jaccard (J) coefficient, which does not consider the negative

similarities. The matrix was analyzed using the NTSYS-PC package. UPGMA (Unweighted Pair Group Method with Arithmetic mean) algorithm was used to perform hierarchical cluster analysis and construct a dendrogram.

### **Partial sequencing of the 16S rRNA gene**

PCR fragments obtained by the amplification of a 434 bp DNA fragment corresponding to a region of the 16S rDNA gene of each isolate were sequenced. This region was amplified using primers U968 (AACGCGAAGAACCCTTAC) and L1401 (CGGTGTGTACAAGACCC) (Felske et al., 1997). PCR reactions were performed in a 25 µL volume, containing 50 ng of DNA template, 1 U of *Taq* DNA polymerase (Life Technologies), 1 X *Taq* DNA polymerase reaction buffer,  $1.5 \text{ mmol L}^{-1} \text{ MgCl}_2$ ,  $200 \text{ mmol L}^{-1}$  dNTPs (Life Technologies) and 10 pmoles of each primer. Amplification was carried out for 32 cycles as follows: one initial denaturation cycle at 95 °C for 5 min, 30 denaturation cycles for 45 s at 94 °C, annealing for 45 s at 52 °C, and extension for 45 s at 72 °C, and one final extension cycle for 5 min at 72 °C. Reactions were performed in a PCR Express Temperature Cycling System (Thermo Hybaid) and the fragments were visualized after electrophoresis at 80 V for 1 h on 1.5 % agarose gel stained with ethidium bromide. A molecular size marker (1 Kb plus DNA ladder, Gibco BRL) was run on all gels. Sequences of partial 16S rDNA genes were determined in both the forward and reverse directions with U986 and L1401 primers in the ACTGene Laboratory (Centro de Biotecnologia, UFRGS, RS, Brazil) using the automatic sequencer ABI-PRISM 3100 Genetic Analyzer (Applied Biosystems). Sequences were submitted to the GenBank database, using the program BLASTN (National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/BLAST/>). The nucleotide sequences of the two partial 16S rDNA segments (434 bp for both sequences) determined in this study were deposited in the GenBank database (Rp1p isolate labeled EF585402 and Rp2p isolate labeled EF585401).

### **Plant growth promotion experiment**

Pure bacterial cultures were grown in TY medium at 28 °C until they reached a final concentration of  $10^9$  cfu mL<sup>-1</sup>. Seeds of *L. albescens* were surface-sterilized in 70 % ethanol for 2 min and 1.2 % Na hypochlorite for 10 min, and rinsed 10 times in sterile tap water. Pots (500 mL) filled with a sterile mixture of vermiculite and sand (1:1) were seeded with three seeds. After emergence, seedlings were thinned to one per pot and were grown in a growth chamber with 12 h of light at 25 °C. The experiment was conducted in a completely randomized design, with three replicates. Treatments consisted of inoculation with 1 mL of RP1p or RP2p bacterial culture or one mL of sterile TY medium, in the uninoculated control. Besides, each pot received 20 mg of N as NH<sub>4</sub>NO<sub>3</sub>

and was irrigated with distilled water without micro or macronutrients. Forty days after sprouting, plants were harvested and dried to constant weight at 65 °C. Next, the dry weight was determined and the data subjected to ANOVA and Turkey's test ( $p = 0.05$ ).

## RESULTS AND DISCUSSION

### Genotypic characterization of the isolates by rep-PCR and RFLP of the *nifH* gene

Aiming to identify some of the plant growth-promoting bacteria (PGPB) that inhabit the rhizoplane of *L. albescens*, small root pieces of plants cultivated in a sandy area of an experimental field in Alegrete, Rio Grande do Sul, Brazil, were collected. Five bacterial strains were randomly selected from this PGPB collection. These strains were genotypically characterized in electrophoretic profiles of amplification products using rep-PCR and RFLP methodologies. The RFLP analysis was based on a 360 bp fragment of the *nifH* gene, which is one of the most important genes in the biological N<sub>2</sub> fixation system and encodes the nitrogenase reductase protein (Mortenson, 1979). Although the existence of the *nifH* gene does not necessarily represent nitrogenase activity, as this enzyme is regulated at both pre- and posttranslational levels (Dean & Jacobson, 1992), its presence in the genome is a good evidence that the bacterium is a nitrogen-fixer. The genotypic results obtained with the isolates (RP1p, RP1g, RP2p, RP2g, and RP3p) and the reference strains (*Bradyrhizobium* sp. SEMIA 928 and 938, *Klebsiella* sp., *Enterobacter* sp., *Pseudomonas* sp.) were used to construct a dendrogram (Figure 1). The dendrogram obtained indicated that in fact there were only two different isolates, RP1p and RP2p, and that both contained a *nifH* gene. The similarity of these bacteria with the

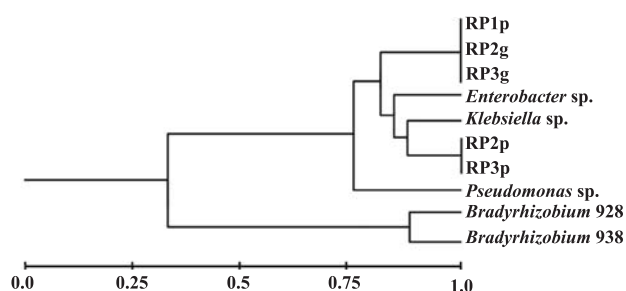
*Bradyrhizobium* reference strains SEMIA 928 and 938 was about 30 % of *nifH*, differing about 30 % from each other (Figure 1). For this reason, all following experiments were carried out with these two strains together with the reference strains.

### Identification of PGPB strains

According to the sequence analysis of the 16S rDNA partial sequences (434 bp) RP1p strain showed highly genetic similarity (95 % homology) with *Enterobacter* sp. PW4 (GenBank number DQ985288.1), and RP2p strain showed 95.2 % of homology with *Serratia* sp. TCR (GenBank number EF070125.1). These results indicated that the strains RP1p and RP2p belong to the Enterobacteriaceae family that contains several N<sub>2</sub>-fixing organisms (Reinhardt et al., 2008). Although rhizosphere bacteria are largely represented by species of *Pseudomonas* and *Bacillus* genera, some authors have found bacteria belonging to the Enterobacteriaceae family including *Serratia*, *Pantoea* and *Enterobacter* strains in the rhizoplane of leguminous plants (Bent, 2006).

### Phenotypic characterization of the isolates

The capacity to use different carbon sources of strains RP1p and RP2p was evaluated (Table 1) since these data can permit a greater insight into the ecology and metabolism of microbial species in the rhizosphere. Strain RP1p was able to utilize 10 out of 14 C sources evaluated while RP2p utilized nine C sources. Both strains were unable to utilize either L-arabinose or lactose as sole C source. Although glucose is the preferred C source for diazotrophic strains of Enterobacteriaceae, bacteria from this family vary a lot in terms of C source utilization (Cannon et al., 1974). Strains belonging to *Pseudomonas*, one of the most well-known PGPB genera, are especially well-suited to the rhizosphere because they are able to use a wide variety of C sources as nutrients (Misko & Germida, 2002). PGPBs that are capable of a wide range of metabolic activities and able to utilize a wide range of C and energy sources may be well-suited in the rhizosphere (Dunfield & Germida, 2001; Misko & Germida, 2002). This characteristic can be useful not only to observe if isolates can utilize a particular C source, but whether the strains are metabolically similar. Understanding the metabolic profile of strains could help to understand how bacteria can compete and survive in the root environment.



**Figure 1.** Dendrogram based on UPGMA cluster analysis with the NTSYS-PC program using rep-PCR and *nifH*-PCR-RFLP data obtained from *L. albescens* isolates (RP1p, RP2g, RP3g, RP2p, and RP3p) and strains used as control (*Bradyrhizobium* sp. SEMIA 928, *Bradyrhizobium* sp. SEMIA 938, *Enterobacter* sp., *Pseudomonas* sp., and *Klebsiella* sp.).

Based on the experiments of phenotypic characterization, the two isolates correspond to Gram-negative, rod-shaped, motile, and facultative anaerobic bacteria. Under electron microscopy cells are often observed in pairs when grown on YMA (Figure 2). The two isolates were very fast-growing, forming convex, white, single, circular and mucoid colonies, (diameter of 3–4 mm) within 24 h of incubation, at 28 °C on YMA. These findings corroborate with the results

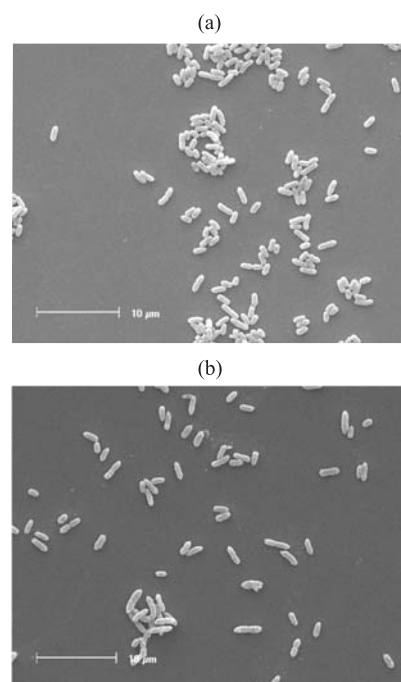
**Table 1. Morphological, physiological and biochemical characteristics of the strains isolated from the rhizoplane of *L. albescens* plants**

Characteristic	Strain	
	RP1p	RP2p
Gram staining	-	-
Congo red utilization	-	-
Monosaccharides	L-arabinose	-
	D-fructose	+
	D-galactose	+
	D-glucose	+
	D-mannose	+
	Rhamnose	+
	D-xylose	+
Disaccharides	Lactose	-
	Maltose	+
	Sucrose	+
Sugar alcohols	Glycerol	+
	Inositol	-
	Mannitol	+
	Sorbitol	-
0.5 % NaCl	+	
1 % NaCl	+	
2 % NaCl	+	
3 % NaCl	+	
4 % NaCl	-	
Siderophore production	+	
Indolic compound production	+	
Phosphate solubilization	-	
Anaerobiosis	+	
Growth at 40 °C	+	
Growth at pH 4	+	
Growth at pH10	+	
Growth rate	very fast	very fast

obtained by the partial sequencing of 16S rRNA that classified the bacteria as belonging to the genera *Enterobacter* and *Serratia*.

The sensitivity of the isolates to extreme pH, high salinity and high temperatures was also analyzed (Table 1). While 28 °C is the optimum temperature for the growth of many soil strains, the two isolates were able to grow at temperatures as high as 40 °C. Although *in vitro* temperature selection is not considered a promising approach for field applications (Hungria & Vargas, 2000), this high temperature tolerance can be useful for the purpose of isolating competitive PGPR in oscillating temperature fields. Both isolates were also able to grow in the presence of high (2–3 % w/v) NaCl concentrations. This result indicates that these bacteria could be potential candidates for applications in high-salinity soils.

Extreme pH values may be one of the major limiting factors for the presence of microorganisms in soils (Brockwell et al., 1991). RP1p and RP2p strains were able to grow in pH ranging from 4 to 10. This indicates that these isolates could also be strong candidates for the improvement of highly alkaline or



**Figure 2. Scanning electron microscopy of vegetative cells of strain RP1p (a) and RP2p (b) shows frequent occurrence in pairs. Bars = 10 µm.**

acidic soil, as is the soil in Alegrete, where the pH ranges from 4.5 to 5.0. The two bacterial isolates were therefore capable of producing a huge amount of mucous and exopolysaccharides (data not shown). These compounds are probably a protection against desiccation and favor growth at extreme temperatures, salinity and pH values.

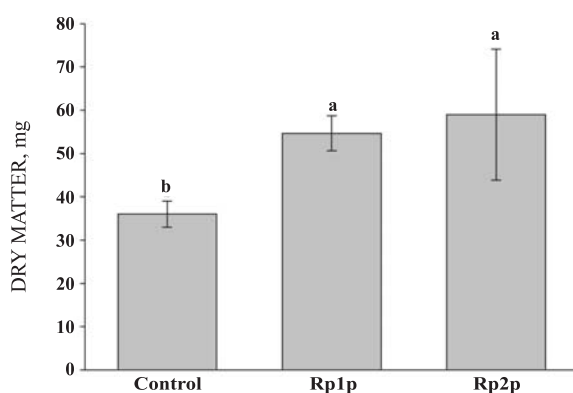
Various soil microorganisms live in the plant rhizosphere and rhizoplane due to the rich nutrient availability. Although bacteria such as *Rhizobium*, *Azotobacter* and *Azospirillum* enhance plant growth as a result of their ability to fix-N<sub>2</sub>, bacteria with mechanisms such as phytohormone production in the rhizosphere and other PGP activities can contribute to improve the ability of the host plant to survive in extreme environments (Glick, 1995; Bashan & Holgin, 1998). RP1p and RP2p strains produced 85.0 and 53.0 µg mL<sup>-1</sup> of indolic compounds *in vitro*, respectively. Mirza et al. (2001) showed that indolic compound production can vary among different species and strains and is also influenced by culture condition, growth stage and availability of the substrate. Barazani & Friedman (1999) reported PGPR that were able to secrete up to 13.5 µg mL<sup>-1</sup> of indolic compounds. Strains of *Enterobacter* sp. isolated from the rhizosphere of sugarcane produced about 2.21 µg mL<sup>-1</sup> of indolic compounds *in vitro* when tryptophan was added to the medium (Mirza et al., 2001). The comparison with the above results allowed us to conclude that the two PGPR isolated in this study are very efficient indolic compound producers.

Another PGP feature is the production of siderophores that may influence plant growth by binding to the available form of iron ( $\text{Fe}^{3+}$ ) in the rhizosphere. Through this process, Fe is made unavailable to the phytopathogens. Consequently, siderophores protect the plant health (Siddiqui, 2005). As observed with indolic compounds, both isolates produced a large amount of siderophores on medium with CAS. Press et al. (2001) reported that the catechol siderophore biosynthesis genes of *Serratia marcescens* are associated with resistance of cucumber against anthracnose. The only characteristic of all PGPB features tested in this study that was not observed in the two isolated strains was phosphate solubilization. This characteristic was absent in the reference strains as well.

### Growth-promoting effect of bacterial treatment of *Lupinus* plants

In order to test the effective contribution to plant growth, the two isolates were used for *in vivo* experiment. A significant increase in dry matter weight was recorded in *L. albescens* plants inoculated with RP1p and RP2p. Inoculation with these two bacteria increased 75 and 81 %, respectively, the dry matter plant contents when compared with the uninoculated control (Figure 3). The observed growth promotion and increase in dry matter contents in response to PGPR inoculation could be explained on the basis of the abilities identified in these two isolates, including the production of plant growth regulators, such as indolic compounds.

Since the benefits from the plant growth-promoting bacteria could be synergistic (Ahmad et al., 2008), further studies on the effects of RP1p and RP2p isolates on plant growth in sandy areas will be necessary to evaluate the utility of this extra tool to recover such degraded fields.



**Figure 3. Effect of inoculation of plant-growth-promoting bacteria on *L. albescens* plants, 40 days after emergence. Different letters indicate significant values at  $p = 0.05$  in the Tukey test.**

## CONCLUSION

Two bacteria with plant growth-promoting abilities and carrying the *nifH* gene were isolated from the rhizoplane of *L. albescens* where a significant increase in the dry matter production of the analyzed plants was observed in comparison with non-inoculated plants.

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