Eurasian J Soil Sci 2018, 7 (4) 284 - 291

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

Screening for *Lotus creticus* growth promoting rhizobacteria under greenhouse conditions

Imane Achkouk [a], Saida Aarab [a,b], Amin Laglaoui [a], Mohammed Bakkali [a], Abdelhay Arakrak [a,*]

[a] Equipe de Recherche de Biotechnologies et Génie des Biomolécules (ERBGB), Faculté des Sciences et Techniques de Tanger, Tanger, Morocco

[b] Faculté des Sciences et Techniques d'Al Hoceima, Ajdir Al Hoceima, Morocco

Abstract

Utilization of plant growth promoting rhizobacteria (PGPR) is now gradually increasing in agriculture and offers an attractive way to replace chemical fertilizers, pesticides, and supplements. This study was conducted with a view to isolate bacteria from the rhizosphere of the legume *Lotus creticus* (*L. creticus*) and to assess their plant growth promoting functional potentialities. A total of 113 rhizobacteria was isolated from the rhizosphere of *L. creticus* and were tested for their capacity of solubilizing tricalcium phosphate (TCP) on Pikovskaya (PVK) solid medium. Out of 29 phosphate solubilizing bacteria (PSB), 5 isolates were selected for their solubilization diameters (between 0.6 and 1.5 cm). These isolates were characterized for plant growth promoting (PGP) traits. The results showed that the highest concentration of indole acetic acid (IAA) was produced by LCR33 (19.08 ± 0.96 mg L^{-1}). All 5 isolates could produce hydrogen cyanide (HCN), siderophores, ammonia and amino-cyclopropane carboxylate (ACC) deaminase. The isolates were evaluated for TCP solubilizing quantitative assay in PVK liquid medium. The concentrations of solubilized P were between 43.34±0.18 mg L^{-1} and 173.57±0.77 mg L^{-1}. This solubilization was accompanied by a pH decrease of the culture media from 7 to 4.06. Furthermore, the 5 selected PSB were tested in vitro for antagonism against phytopathogenic fungus *Fusarium oxysporum*. In fact, all the PSB, were capable of inhibiting its growth and the highest percentages of inhibition were obtained for LCP27 and LCR33 (48.15±0.99% and 40.74±0.45%). Also, the effect of these 2 PSB on growth of *L. creticus* plants was investigated under greenhouse conditions. Significant increases were obtained for shoot and root length and dry and fresh matter production of plants as compared to the uninoculated control. These PSB could be recommended as biofertilizers for contributing to the rehabilitation of degraded soils.

Keywords: Farmyard manure, compost, soil acidity, incubation.

Article Info

Received : 20.03.2018
Accepted : 21.06.2018

Introduction

It is estimated that about 15% of the total land area in the world is facing serious problems caused by physical or chemical factors including salinization, erosion, low availability of nutrients and the absence of fertility (Wild, 2003). The main challenges faced in the reclamation of severely degraded lands is the management of the systems and finding plant species that will grow under the harsh conditions common in degraded soils.

Concerning these reasons, introducing legumes to improve soil fertility is considered as a sustainable management practice, due to their capacity of establishing symbiotic interactions with soil living microorganisms; almost all legumes are known for their ability to establish symbiotic interactions with soil

* Corresponding author.
Equipe de Recherche de Biotechnologies et Génie des Biomolécules (ERBGB), Faculté des Sciences et Techniques de Tanger B.P.: 416 – Tanger, Morocco
Tel.: +212 539 39 39 54
E-mail address: arakrak_abdelhay@yahoo.fr
e-ISSN: 2147-4249
DOI: 10.18393/ejss.436181

living bacteria, this increases their competitiveness in nutrient deficient soils, so they are usually regarded as "pioneer" plants (Hirsch et al., 2001).

Leguminosae family comprises 800 genera and 20.000 species (Lewis et al., 2005). They have a cosmopolitan distribution, representing important ecological constituents in almost all biomes across the globe and occur in even the most extreme habitats (Schrire et al., 2005). Legumes constitute significant elements in terms of both species diversity and abundance, in lowland wet tropical forests in Africa, South America, and Asia (Yahara et al., 2013). Lotus is a large cosmopolitan genus (150 spp.) that occupies two major centers of diversity, the Mediterranean region (including portions of Europe, Africa, and western Asia) and Western North America (Allan et al., 2004). The adaptive characteristics shown by several Lotus species make them good candidates for restoration and phytoremediation of degraded environments, and the species with the higher potentials are *L. creticus, L. tenuis, L. uliginosus* and *L. corniculatus* (Escaray et al., 2012).

Free soil living bacteria that enhance plant growth are collectively known as plant growth promoting rhizobacteria (PGPR). They can be found in the rhizosphere and are capable of promoting plant growth by colonizing their roots and can play an essential role in helping plants to establish and grow in nutrient deficient conditions. Their use in crop production can reduce the agro-chemical use (chemical fertilizers and pesticides) and support ecofriendly sustainable agriculture. PGPR helps plants by various mechanisms to increase plant growth-promoting attributes such as increase in seedling emergence, effective nodulation as well as nodule functioning, increase in indigenous plant hormones, root hair proliferation, root hair deformation and branching, early mineral and water uptake, accumulation of carbohydrates and increasing the yield (Podile and Kishore, 2006). The exact mechanism by which PGPR promote plant growth are not fully understood. However, studies carried out by different researchers suggest some of these as follows (i) the ability to produce or change the concentration of plant hormones as the indole acetic acid (IAA), gibberellic acid, cytokinins and ethylene (ii) asymbiotic N2 fixation (iii) antagonism against phytopathogens by production of siderophores, 1-3- Glucanase (EC 3.2.1.6), chitinase (EC 3.2.1.14) and cyanide (iv) solubilization of mineral phosphate and other minerals (Singh, 2015).

The advantages of legumes stimulated their adoption in the ancient agriculture and then became an important part of sustainable agricultural systems (Singh et al., 2007). The present study was designed to evaluate the capacity of plant growth promoting rhizobacteria to enhance the growth of *L. creticus* so that they can be exploited in the rehabilitation of degraded soils.

Material and Methods

Isolation of rhizobacteria

The rhizobacteria were isolated from the rhizosphere soil of *L. creticus* that was collected from in Northwest of Morocco (35.79339°N, 5.937434°W, 23m above sea level). One gram of rhizospheric soil was suspended in 9 mL of sterile physiologic water. After 1h of agitation aliquots of 100 µL of each dilution (10-1 to 10-7) were plated on Tryptic Soy Agar (TSA) medium. Plates were incubated at 30 °C for 24 to 48 h. Colonies were isolated and purified on the same medium.

Selection of phosphate solubilizing bacteria

The isolates were screened for phosphate solubilization; the purified isolates were transferred on PVK medium (Pikovskaya, 1948), and then incubated at 28 °C. The plates were examined after 7 days of incubation and data were recorded. The phosphate solubilizing ability of bacteria is possible by plate screening methods that show clear zone around the colonies in media containing insoluble mineral phosphate (TCP) as P source. The diameter of solubilization was calculated by subtracting colony diameter from the total diameter.

Determination of indole acetic acid (IAA) production

The tested bacterial strains were cultured for 2 days in sucrose-minimal salts (SMS) medium (sucrose 1%; $(NH_4)_2SO_4$ 0.1%; K_2HPO_4 0.2%; $MgSO_4$ 0.05%; NaCl 0.01%; yeast extract 0.05%; $CaCO_3$ 0.05%; pH 7.2) supplemented with 0.05% of L-tryptophan. After incubation, 1mL of supernatant was mixed with 2 mL of Salkowski reagent and the development of a pinkish color indicated the production of IAA (Gordon and Weber, 1951). The absorbance of pink color developed after 25 minutes of incubation at room temperature was read at 535 nm.

Production of hydrogen cyanide (HCN)

To estimate HCN production, 100 µL of bacterial culture were streaked on TSA supplemented with 4.4 g L-1glycine. Filter paper discs (9 cm diameter) soaked in 2% sodium carbonate in 0.5% picric acid solution, and were placed in the lid of each Petri dish (Bakker and Schippers, 1987). The plates were sealed with parafilm

and incubated at 28 °C. Change in color from yellow to orange or brown indicated the synthesis of HCN production.

Production of siderophores

The bacteria were spot inoculated on TSA medium and the plates were incubated for 3 days at 28 °C. A layer of chrome azurol S medium (CAS) (Schwyn and Neilands, 1987) was poured on the surface of these plates. After 24 h in the dark, change in color of CAS medium from blue to orange indicated the production of siderophores.

Production of ammonia

All the bacterial isolates were tested for the production of ammonia as described by Cappuccino and Sherman (1992). Twelve hours old bacterial cultures were inoculated in peptone water (10 mL) and incubated for 48-72 h at 36 ± 2 °C. Development of brown to yellow color after addition of Nesseler's reagent indicates the production of ammonia, no color change indicates negative test.

Testing ability of rhizobacteria to use 1-aminocyclopropane-1-carboxylate (ACC) as sole source of nitrogen

The existence of ACC deaminase (EC 3.5.99.7) is determined by the ability of bacterial strains to use ACC as the sole source of nitrogen. According to the method described by Jacobson et al. (1994), bacterial strains cultured in the presence of two sources of nitrogen, ACC and ammonium sulfate $(NH_4)_2SO_4$ and a mineral source magnesium sulfate ($MgSO_47H_2O$) are compared according to their growth rate.

A volume of 122 µL of the minimum DF salt medium (Dworkin and Foster, 1958) was distributed per well in a 96-well microplate. A volume of 15 µL of a solution of $MgSO_47H_2O$ (0.1 M) is added in lines 3, 6, 9 and 12 of the microplate, 15 µL of $(NH_4)_2SO_4$ (0.1 M) in lines 2, 5, 8, and 11 and 15 µL of the ACC solution (3.0 mM) in lines 1, 4, 7, and 10. The bacterial cultures were inoculated in TSB (Tryptic soy broth) and incubated for 24h at 30°C. These cultures are diluted 1:10 in MgSO4 and 22 µL of each dilution is used to inoculate a well of each successive line. The negative control is inoculated with 22 µL of $MgSO_47H_2O$. The optical density is measured at 600 nm after 0, 24, 48, 72, and 96 hours. Optical densities (OD) values are compared. The isolates with OD higher than that of the $MgSO_47H_2O$ solution are considered to be positive for the production of ACC.

Qualitative phosphate solubilization assay

Isolates were tested for their ability to solubilize the TCP in the liquid medium; this is realized by inoculating 50 mL of PVK liquid medium by 500µL of bacterial culture. Autoclaved and not inoculated media were used as controls. The inoculated media and controls were incubated for 7 days at 28 °C on shaker (180 rpm). The media were centrifuged at 13.000 rpm for 20 min. The concentration of soluble P of the supernatant was determined by the colorimetric method of Ames (1966) and the pH of the medium was also determined.

Antagonism against *Fusarium oxysporum*

The antifungal activity was tested using potato dextrose agar (PDA) (Rabindran and Vidhyasekaran, 1996). Bacterial isolates were tested for their ability to inhibit the growth of the plant pathogenic fungus *Fusarium oxysporum* isolated and characterized by El Aaraj et al. (2015). A 5 mm agar disk of the fungus was deposited in the center of the PDA Petri plates. A volume of 20µL of each bacterial culture was seeded in 3 cm spot of the fungal strain. A negative control of the fungal strain is tested in the absence of bacteria. Plates were incubated for 7 days at 25ºC and examined for evidence of fungal growth inhibition. The zone of inhibition was determined using the following formula: % Inhibition of radial growth = 100 ×((r1-r2)÷r1)

With r1 is the radial growth of the mycelium in control and r2 is the radial growth of the mycelium in treatment. The results represent the average of three replicates.

Inoculation of *Lotus creticus*

The seeds of *L. creticus* were surface sterilized by agitation in 95% ethanol for 1 min then in 1.2% sodium hypochlorite for 20 min, followed by several washings with sterile water. Seeds were then placed on filter paper discs (9 cm diameter) soaked in 10 mL of sterile water on petri dish. The plates were then incubated for 3-4 days at 28± 2 °C. After germination, each pot filled with *L. creticus* soil was sowed by 5 germinated seeds and each seed was inoculated directly with 1 mL of bacterial culture (108 CFU mL^{-1}) grown in TSB. Uninoculated pots were used as controls. All pots were maintained under greenhouse conditions. Three replications were maintained for each treatment. Plants were harvested after 90 days. To evaluate the response of the selected PSB, growth parameters (shoot and root length, dry and fresh weight of shoot and root) were measured.

Statistical analysis

The data are reported as means ± SD (standard deviation) for three replicates. The results were compared by analysis of variance (ANOVA) according to Fisher protected LSD test ($p < 0.05$).

Results and Discussion

Isolation and selection of phosphate solubilizing bacteria

A total of 113 rhizobacteria were isolated from the rhizosphere of *L. creticus*, of which 25.66% (29 PSB) were able to solubilize TCP on solid PVK medium. The solubilization is indicated by the formation of a clear halo around the bacterial colony. The P-solubilizing potential varied amongst these isolates as evidenced by the size of halo on Pikovskaya's agar plates. Five PSB were selected based on their halo diameters ⩾ 0.6 cm (Table 1). The most important halo diameter was formed by the isolate LCP27 (1.5 cm) followed by LCP28 (1.3 cm). This phosphate solubilization mechanism is generally correlated with the production of organic acids (OA) via the direct oxidation pathway that occurs on the outer face of the cytoplasmic membrane, with concomitant drop in pH value (Khan et al., 2009). The OA(s) released chelate mineral ions or drop the pH to bring P into solution (Maliha et al. 2004). The OA produced by bacteria leads to acidification of microbial cells and their surroundings and, consequently, the release of P-ions from the P-mineral by H+ substitution for Ca2+ (Goldstein, 1986).

Table 1.Halo diameters and plant growth promoting activities of the selected rhizobacteria.

Isolates	HD	IAA mg L-1	HCN	SID	NH_3	ACCD	P mg L-1	pH
LCP26	0.8	7.00 ± 0.99 c	+++	+	+	+	59.57 ± 0.6 b	5.95 ± 0.04 b
LCP27	1.5	6.88 ± 0.74 d	++	+	+	+	43.34 ± 0.18 b	5.37 ± 0.4 b
LCP28	1.3	5.64 ± 0.91 e	++	+	+	+	173.57 ± 0.77 a	4.49 ± 0.63 a
LCR14	0.9	8.76 ± 0.55 b	++	+	+	+	145.53 ± 0.13 a	5.39 ± 0.89 b
LCR33	0.6	19.08 ± 0.96 a	+++	+	+++	+	58.54 ± 0.32 b	4 .06 ± 0.09 a

+ HD: halo diameter, IAA: Concentration of indole acetic acid, HCN: Hydrogen cyanide, SID: Siderophores, NH_3: Ammonia, ACCD: 1-aminocyclopropane-1-carboxylate deaminase, P: Concentration of solubilized P. The data presented are the mean of 3 replicates. Means in the same column followed by the same letter are not significantly different $P < 0.05$ (Fisher's least significant difference (LSD) test; ± values indicate standard errors of the means).

Screening of plant growth promoting traits

Screening results of PGP activities of the selected bacteria are shown in the Table 1. IAA production was detected in all the 5 isolates with different amounts that were significantly different. The highest concentration was produced by LCR33 (19.08 ± 0.96 mg L^{-1}) while the lowest value was presented by LCP28 (5.64 ± 0.91 mg L-1). The production of IAA is one of the most common mechanisms of action implicated in PGPR (Vessey, 2003). It affects the division, extension, and differentiation of plant cells. Besides, IAA stimulates seeds and tuber germination, increases the rate of xylem and root development, controls processes of vegetative growth; initiates lateral and adventitious root formation; mediates responses to light and gravity; affects photosynthesis, pigment formation, biosynthesis of various metabolites and resistance to stressful conditions (Gupta et al., 2015). The potential for IAA synthesis varies with different species and strains as well as cultural condition, growth stage and availability of substrate (Mirza et al., 2001).

All 5 PSB were able to produce hydrogen cyanide, siderophores, ammonia and ACC deaminase. This could make our selected rhizobacteria, powerful biofertlizing and biocontrol agents. In fact, according to Singh (2015), the presence or absence and intensity of HCN production can play a significant role in antagonistic potential of soil bacteria against phytopathogens. Moreover, the production of siderophores that scavenge iron by formation of soluble Fe3+ complexes is a very important mechanism of biofertilization and biocontrol; they improve iron availability to plants (Barness et al., 1992; Sharma et al., 2003) and deprive other pathogenic bacteria from iron (Miethke and Marahiel, 2007). The production of ammonia is also considered to be an important mechanism, it fulfils several biological roles. In addition to its important metabolic role in many organisms, ammonia's toxicity is well known. One prerequisite for toxic functionality appears to be its rapid diffusion through the majority of biological membranes (Kleiner et al., 1998). Deamination of ACC is another direct phytostimulator feature that may influence plant growth by reduction of ethylene levels (Glick et al., 2007). By producing ACC deaminase, our PSB could utilize ammonia as N source thereby restricting the ethylene accumulation consequently rescuing the plant growth from the stress (Khan et al., 2009). This could be of great interest to use our PSB as inoculants for rehabilitation of degraded soils due to their capacity of resisting stress conditions.

The quantitative test of phosphate solubilization was also checked for selected bacteria. The concentration of solubilized P was between 43.34±0.18 and 173.57±0.77 mg L^{-1}. This solubilization was accompanied by a significant decrease in pH of the media from 7 to 4.06. Solubilized P values were significantly negatively correlated ($r=-0.51$, $p<0.01$) with the final pH of the culture medium. Several studies demonstrated the existence of negative correlation between pH of the culture and the release of P (Wani et al., 2008; Bhatt and Vyas, 2014). This consolidates the hypothesis of OA involvement in the solubilization of insoluble P (Maliha et al., 2004). Yet, acidification could not be presumed the sole mechanism of inorganic P solubilization (Yang et al., 2012). Altomare et al. (1999) demonstrated that Trichoderma harzianum Rifai has not produced any known organic acid but solubilized P by chelating and reducing molecules. Additionally, it has been demonstrated that siderophores and exopolysaccharides synthesized by PSB bring out locked P into soluble form probably by charge related interactions (Yi et al., 2008; Sharma et al., 2013).

Antagonism against *Fusarium oxysporum*

The selected isolates were evaluated for their antagonistic effect against Fusarium sp. (Figure 1 and 2). All the tested PSB were able to inhibit its growth. Statistical analysis showed that LCP27 had maximum inhibition (48.15±0.99%), whereas LCP28 showed the lowest inhibitory effect (25.93±1.03 %). The percentages of inhibition registered by LCP26, LCP27 and LCR33 were significantly different from LCP28. All 5 isolates were positive for the production of HCN, siderophores and ammonia. So, the production of these compounds can result in fungal growth inhibition (Dubey and Gupta, 2012; Prashar et al., 2013). However, Kumar et al. (2011) showed that toxins and enzymes may also play a role in the growth inhibition process of the phytopathogen fungus *Fusarium oxysporum*.

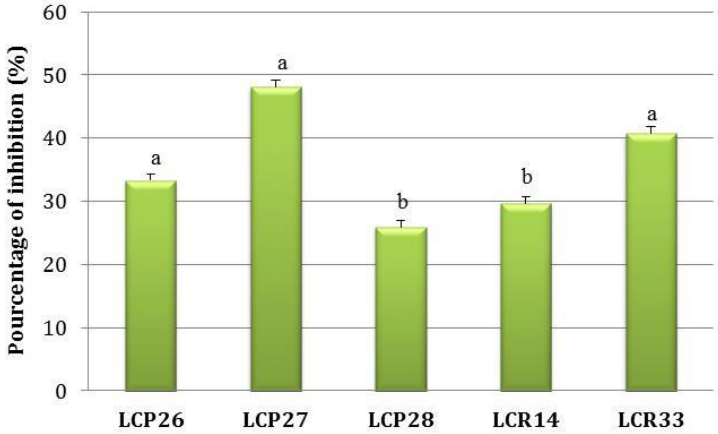

Figure 1. Pourcentage of inhibition of the selected isolates against Fusarium oxysporum. The data presented are the mean of 3 replicates. Means in the same column followed by the same letter are not significantly different $P < 0.05$ (Fisher's least significant difference (LSD) test).

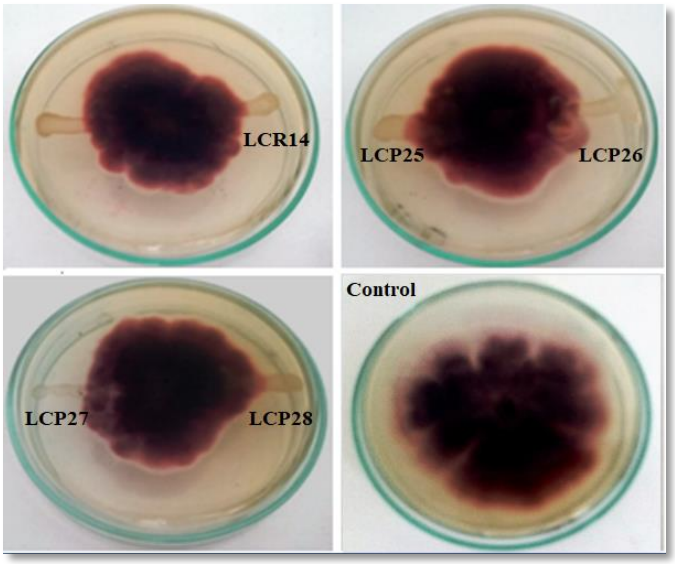

Figure 2. Antagonistic activity of the selected isolates against *Fusarium oxysporum* on potato dextrose agar medium

Inoculation of *Lotus creticus*

The 2 bacterial isolates that presented the highest antagonistic activity against *Fusarium oxysporum* (LCP27 and LCR33) were selected to test their effect on *L. creticus* growth under greenhouse conditions. Results regarding fresh and dry matter and shoot and root length were presented in Figures 3 and 4. Bacterial inoculation of these two rhizobacterial isolates showed positive influence on *L. creticus* growth significantly in comparison to un-inoculated control. In fact, results showed a significant increase in shoot fresh matter in presence of LCR33 and LCP27 compared to the un-inoculated control, while fresh matter yield was significantly increased by LCR33 compared to LCP27 and the control. Concerning the dry matter yield, a significant stimulation was registered by inoculation with LCR33 and LCP27. Inoculation test showed also a significant increase of shoot and root length (Figure 4) with the application of LCR33 compared to LCP27 and the un-inoculated control. The most significant positive effect on *L. creticus* growth was obtained by LCR33 inoculation. Increase in fresh and dry biomass, shoot and root length by inoculation with the selected PSB could be linked to the production of phytohoromones, siderophores, hydrogen cyanide (HCN), ACC deaminase and solubilization of phosphates (Sessitsch et al., 2002; Glick et al., 2007; Sachdev et al., 2009; Mia and Shamsuddin, 2010).

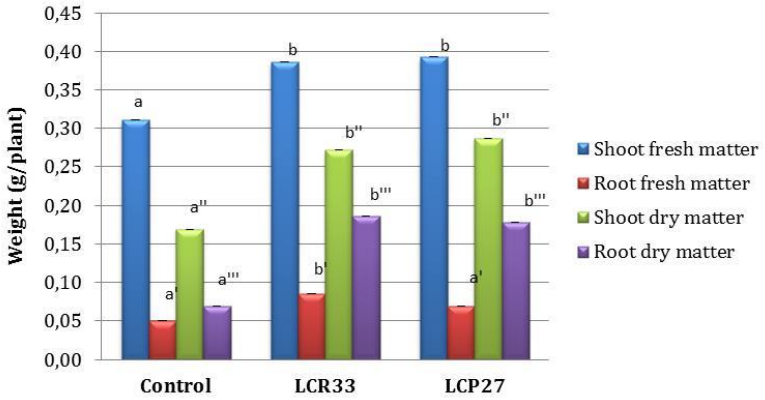

Figure 3. The dry and fresh matter of shoot and root of plants after 90 days of growth in pots. The data presented are the mean of 3 replicates. Means in the same column followed by the same letter are not significantly different P < 0.05 (Fisher's least significant difference (LSD) test).

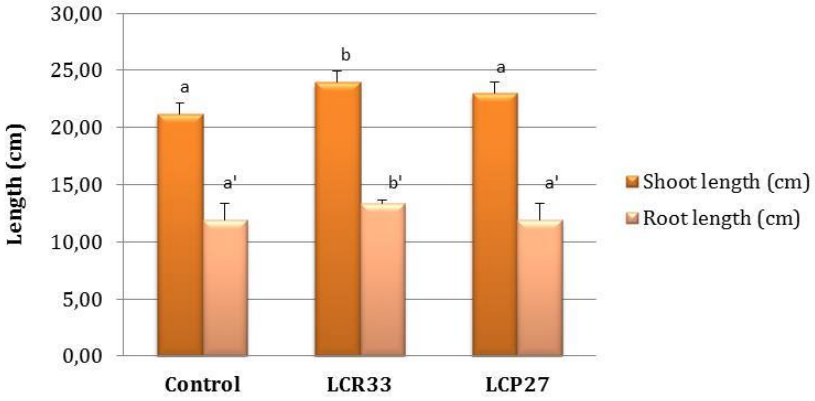

Figure 4. The length of shoot and root of plants after 90 days of growth in pots. The data presented are the mean of 3 replicates. Means in the same column followed by the same letter are not significantly different P < 0.05 (Fisher's least significant difference (LSD) test).

Conclusion

The present study emphasizes the positive effects of PSB isolated from the rhizosphere of *L. creticus* on the growth of this legume. LCP27 and LCR33 can stimulate plant's nutritional intake capacity and growth as well; they could be used as biofertilizers and biocontrol agents based on their positive effect on growth under greenhouse conditions. The use of these rhizobacteria as inoculants may present an efficient alternative to inorganic phosphate fertilizers and could contribute to the rehabilitation of degraded soils. However, tests in the field are necessary to complete this work, in order to evaluate the effect of biotic and abiotic factors on these isolates efficiency.

References

Allan, G.J., Francisco-Ortega, J., Santos-Guerra, A., Boerner, E., Zimmer, E.A., 2004. Molecular phylogenetic evidence for the geographic origin and classification of Canary Island Lotus (Fabaceae: Loteae). *Molecular Phylogenetics and Evolution* 32(1): 123–138.

Altomare, C., Norvell, W.A., Björkman, T., Harman, G.E., 1999. Solubilization of phosphates and micronutrients by the plant growth-promoting and biocontrol fungus *Trichoderma harzianum Rifai* 1295-22. *Applied and Environmental Microbiology* 65(7): 2926-2933.

Ames, B.N., 1966. Assay of inorganic phosphate, total phosphate and phosphatases. *Methods in Enzymology* 8: 115-118.

Bakker, A.W., Schippers, B., 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp.-mediated plant growth stimulation. *Soil Biology and Biochemistry* 19(4) : 451-457.

Barness, E., Hadar, Y., Chen, Y., Romheld, V., Marschner, H., 1992. Short-term effects of rhizosphere microorganisms on Fe uptake from microbial siderophores by maize and oat. *Plant Physiology* 100(1): 451-456.

Bhatt, P.V., Vyas, R.M.B., 2014. Screening and characterization of plant growth and health promoting rhizobacteria. *International Journal of Current Microbiology and Applied Sciences* 3(6): 139-155.

Cappuccino, J.C., Sherman, N., 1992. Microbiology : A Laboratory Manual. 3th Edition, Benjamin-Cummings Publishing Company, New York, USA.

Dubey, P., Gupta, G.P., 2012. Synergistic and anagonistic interactions among endophytic bacterial isolates of Vigna mungo (L .) Hepper. *Journal of Current Perspectives in Applied Microbiology* 1: 1–12.

Dworkin, M., Foster, J., 1958. Experiments with some microorganisms which utilize ethane and hydrogen. *Journal of Bacteriology* 75(5): 592-601.

El Aaraj, C., Bakkali, M., Infantino, A., Arakrak, A., Laglaoui, A., 2015. Mycotoxigenic fungi in cereals grains and coffee from the North of Morocco. *American Journal of Research Communication* 3(2): 130-142.

Escaray, F.J., Menendez, A.B., Gárriz, A., Pieckenstain, F.L., Estrella, M.J., Castagno, L.N., Carrasco, P., Sanjuán, J., Ruiz, O.A., 2012. Ecological and agronomic importance of the plant genus Lotus. Its application in grassland sustainability and the amelioration of constrained and contaminated soils. *Plant Science* 182: 121–133.

Glick, B.R., Cheng, Z., Czarny, J., Duan, J., 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. *European Journal of Plant Pathology* 119(3): 329-339.

Goldstein, A.H., 1986. Bacterial solubilization of mineral phosphates: historical perspectives and future prospects. *American Journal of Agricultural Economics* 1(2): 57–65.

Gordon, S.A., Weber, R.P., 1951.Colorimetric estimation of indole-acetic acid. *Plant Physiology* 26: 192-195.

Gupta, G., Parihar, S.S., Ahirwar, N.K., Snehi, S.K., Singh, V., 2015. Plant growth promoting rhizobacteria (PGPR): Current and future prospects for development of sustainable agriculture. *Journal of Microbial and Biochemical Technology* 7: 96-102.

Hirsch, A.M., Lum, M.R., Downie, J.A., 2001. What makes the rhizobia-legume symbiosis so special?. *Plant Physiology* 127 (4): 1484–1492.

Jacobson, C.B., Pasternak, J.J., Glick, B.R., 1994. Partial purification and characterization of ACC deaminase from the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12–2. *Canadian Journal of Microbiology* 40(12): 1019-1025.

Khan, M.S., Zaidi, A., Vani, P., Oves, M. 2009. Role of plant growth promoting rhizobacteria in the remediation of contaminated soils. *Environmental Chemistry Letters* 7(1): 1–19.

Kleiner, D., Traglauer, A., Domm, S., 1998. Does ammonia production by *Klebsiella* contribute to pathogenesis?. *Bulletin de l'Institut Pasteur* 96(4): 257–265.

Kumar, H., Dubey, R.C., Maheshwari, D.K., 2011. Effect of plan growth promoting rhizobia on seed germination, growth promotion and suppression of *Fusarium wilt* of fenugreek (Trigonella foenum-graecum L.). *Crop Protection* 30(11): 1396–1403.

Lewis, G., Schrire, B., Mackinder, B., Lock, M., 2005. Legumes of the world. Royal Botanic Gardens, Kew Publishing. UK 592 p.

Maliha, R., Samina, K., Najma, A., Sadia, A., Farooq, L., 2004. Organic acids production and phosphate solubilization by phosphate solubilizing microorganisms (PSM) under in vitro conditions. *Pakistan Journal of Biological Sciences* 7(2): 187–196.

Mia, M.A.B., Shamsuddin, Z.H., 2010. *Rhizobium* as a crop enhancer and biofertilizer for increased cereal production. *African Journal of Biotechnology* 9(37): 6001-6009.

Miethke, M., Marahiel, M., 2007. Siderophore-based iron acquisition and pathogen control. *Microbiology and Molecular Biology Reviews* 71(3): 413–451.

Mirza, M.S., Ahmad, W., Latif, F., Haurat, J., Bally, R., Normand, P., Malik, K.A., 2001. Isolation, partial characterization, and the effect of plant growth-promoting bacteria (PGPB) on micro propagated sugarcane in vitro. *Plant and Soil* 237(1): 47-54.

Pikovskaya, R.I., 1948. Mobilization of phosphorous in soil in connection with vital activity of some microbial species. *Microbiologiya* 17: 362-370.

Podile, A.R., Kishore, G.K., 2006. Plant growth-promoting rhizobacteria. In: Plant associated bacteria. Gnanamanickam, S.S. (Ed.). Springer, Dordrecht, The Netherlands. pp.195–230.

Prashar, P., Kapoor, N., Sachdeva, S., 2013. Isolation and characterization of *Bacillus* sp with in vitro antagonistic activity against *Fusarium oxysporum* from rhizosphere of Tomato. *Journal of Agricultural Science and Technology* 15: 1501-1512.

Rabindran, R., Vidhyasekaran, P., 1996. Developpment of powder formulation of Pseudomonas Pf ALR 2 for the management of rice sheath blight. *Crop Protection* 15(8): 715-721.

Sachdev, D.P., Chaudhari,H.G., Kasture, V.M., Dhavale, D.D., Chopade, B.A., 2009. Isolation and characterization of Indole acetic acid (IAA) producing *Klebsiella pneumoniae* strains from rhizosphere of wheat (*Triticum aestivum*) and their effect on plant growth. *Indian Journal of Experimental Biology* 47(12): 993-1000.

Schrire, B.D., Lavin, M., Lewis, G.P., 2005. Global distribution patterns of the Leguminosae: Insights from recent phylogenies. *Biologiske Skrifter - Det Kongelige Danske Videnskabernes Selskab* 55: 375–422.

Schwyn, B., Neilands, J.B., 1987. Universal chemical assay for the detection and determination of siderophores. *Analytical Biochemisty* 160(1): 47-56.

Sessitsch, A., Howieson, J.G., Perret, X., Antoun, H., Martínez-Romero, E., 2002. Advances in *Rhizobium* research. *Critical Reviews in Plant Sciences* 21(4): 323-387.

Sharma, A., Johri, B.N., Sharma, A.K., Glick, B.R., 2003. Plant growth-promoting bacterium *Pseudomonas* sp. strain GRP3 influences iron acquisition in mung bean (Vigna radiata L. Wilzeck). *Soil Biology and Biochemistry* 35(7): 887-894.

Sharma, S.B., Sayyed, R.Z., Trivedi, M.H., Gobi, T.A., 2013. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springer Plus* 2: 587.

Singh, B.P., 2015. Screening and characterization of plant growth promoting rhizobacteria (PGPR): An overview. *Bulletin of Environmental and Scientific Research* 4(1-2): 1-14.

Singh, R.J., Chung, G.H., Nelson, R.L., 2007. Landmark research in legumes. *Genome* 50(6): 525–537.

Vessey, J. K., 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* 255(2): 571–586.

Wani, P.A., Khan, M.S., Zaidi, A., 2008. Chromium-reducing and plant growth-promoting *Mesorhizobium* improves chickpea growth in chromium-amended soil. *Biotechnology Letters* 30(1): 159–163.

Wild, A., 2003. Soils Land food: Managing the land during the twenty-first century. Cambridge University Press, Cambridge, UK. 258p.

Yahara, T., Javadi, F., Onoda, Y., Queiroz, L.P., Faith, D., Prado, D.E., Akasaka, M., Kadoya, T., Ishihama, F., Davies, S., Slik, J.W.F., Yi, T., Ma, K., Bin, C., Darnaedi, D., Pennington, R.T., Tuda, M., Shimada, M., Ito, M., Egan, A.N., Buerki, S., Raes, N., Kajita, T., Vatanparast, M., Mimura, M., Tachida, H., Iwasa, Y., Smith, G.F., Victor, J.E., Nkonki, T., 2013. Global legume diversity assessment: Concepts, key indicators, and strategies. *Taxon* 62 (2): 249–266.

Yang, P.X., Ma, L., Chen, M.H., Xi, J.Q., He, F., Duan, C.Q., Mo, M.H., Fang, D.H., Duan, Y.Q., Yang, F.X., 2012. Phosphate solubilizing ability and phylogenetic diversity of bacteria from p-rich soils around Dianchi Lake drainage area of China. *Pedosphere* 22: 707-716.

Yi, Y., Huang, W., Ge, Y., 2008. Exopolysaccharide: a novel important factor in the microbial dissolution of tricalcium phosphate. *World Journal of Microbiology and Biotechnology* 24(7): 1059-1065.