Isolation and characterization of endophytic bacteria associated with roots of jojoba (*Simmondsia chinensis* (Link) Schneid)

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In this communication, the diversity and beneficial characteristics of endophytic bacteria have been studied in *Simmondsia chinensis* that has industrial importance because of the quality of its seed oil. Endophytes were isolated ($N = 101$) from roots of the jojoba plants collected, of which eight were identified by partial sequencing of the 16S rDNA gene. The isolated bacteria were *Bacillus* sp., *Methylobacterium aminovorans*, *Oceanobacillus kimchi*, *Rhodococcus pyridinivorans* and *Streptomyces* sp. All isolates had at least one positive feature, characterizing them as potential plant growth promoting bacteria. In this study, *R. pyridinivorans* and *O. kimchi* are reported as plant growth promoters.

Keywords: Endophytic bacteria, plant growth promoters, *Simmondsia chinensis*, seed oil.

IN recent years, there is increasing interest regarding plant–microbe association, particularly in composition, structure and function of endophytic bacteria[1] that colonize plants without apparently causing any damage[2]. Endophytes establish a mutualistic symbiosis with their hosts because they are selectively favoured[3,4]. They are classified according to their colonization strategy. Obligatory endophytes are unable to proliferate outside the plant. Facultative endophytes live in the soil and are able to colonize the plant when the opportunity arises. Passive endophytes colonize the plant through open wounds in the root hairs[3,5].

Endophyte community structure and composition are closely related to plant phenology, soil type, pH and bacterial density in the rhizosphere[4]. Endophytes stimulate plant growth through several mechanisms such as nitrogen fixation, inorganic phosphate solubilization, siderophore production, micronutrient supply[6], photosynthetic activity and phytostimulation[5]. Endophytic bacteria can also contribute to soil pathogen control with the production of antibiotics and secondary metabolites and induction of the plant defence system[3], besides heavy-metal biotransformation, biodegradation of organic pollutants[2], osmotic adjustment, stomatal regulation, modification of the root morphology and metabolism[1].

Jojoba (*Simmondsia chinensis*) is a shrub native to northwestern Mexico and southwestern United States. The cultivation of this plant can be used as an alternative for soil conservation and desertification[7]. Jojoba seed has high commercial value due to the production of liquid wax with various applications, including cosmetics and pharmaceuticals; and it is also used in the production of bio-fuels[7]. Plants in semiarid and arid regions are affected by biotic and abiotic factors[8]; so the partnerships established with endophytic bacteria are crucial for their growth and development[9]. Despite the importance of the endophytic communities in desert plants, few studies have characterized the bacterial communities with plant growth promotion. The objective of this study was to isolate, characterize and identify bacterial root endophytes with the ability to promote growth in jojoba root.

Samples were taken from 27 sites of wild jojoba populations in the state of Baja California Sur, Mexico (Figure 1). From each site, four apparently healthy jojoba plants were collected. Three secondary roots (~10 cm in length) collected from each plant were placed in sterile bags, stored at 5°C and processed within 48 h after collection[2].

At each site, a soil sample (~500 g) was collected and stored at 5°C to be analysed subsequently; pH was determined by a pH meter (soil : water ratio 1 : 1), and organic matter by the chromic acid filtration method. Available phosphorus was determined according to the methodology described by Jackson[10]. Texture was determined using a particle autoanalyzer (Horiba LA-950V2, Kyoto, Japan)[11].

The roots were washed with tap water to remove adhering soil. Then they were superficially sterilized with sodium hypochlorite (30%) for 30 min and washed with sterile distilled water five times to remove any debris. The disinfection process was tested by placing portions of sterilized roots in nutrient agar plates, which were incubated at 28°C for three days. Each root sample (10 g) was macerated in a 90 ml aliquot of sterile distilled water. Serial dilutions were made and plated on nutrient agar for total bacteria and on Czapeck medium for actinomycetes[12]. The samples were incubated at 28°C for seven days and checked every 24 h for counting. Colonies exhibiting a different morphology were purified by the cross groove method. The strains were characterized by colonial and cellular morphology by Gram stain[2].

The strains ($N = 8$) with the highest number of mechanisms to promote plant growth were selected for molecular identification. For each strain, total genomic DNA was extracted, following the DNeasy (QIAGEN Inc., Valencia, CA, USA) protocol and modified as follows: three colonies of strains were added to a lysis buffer and incubated at 37°C for 24 h. After proteinase K was added, the sample was kept at 70°C for 1 h. Genomic DNA was

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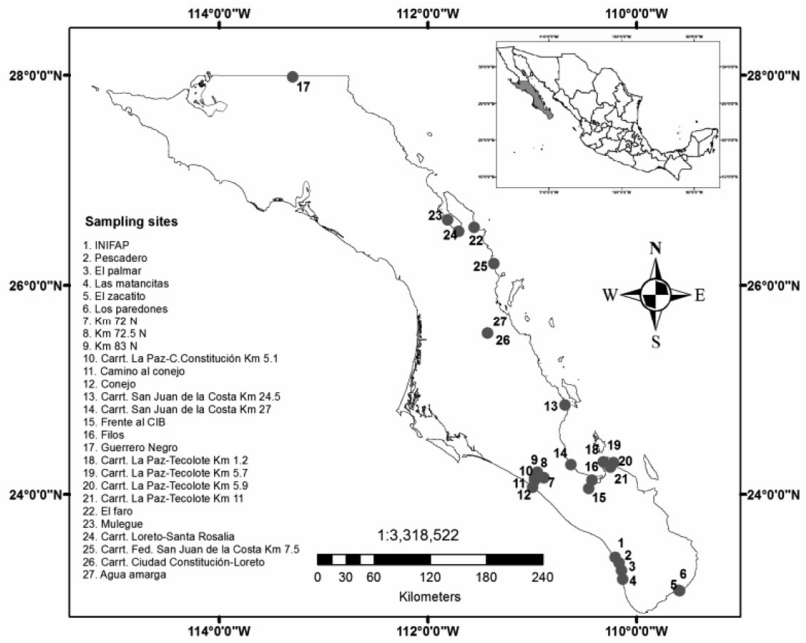

Figure 1. Location of sampling sites of *Simmondsia chinensis*. For sampling site description, refer to Table 1.

eluted in a column and then centrifuged with 100 µl of elution buffer for immediate use; 16S rDNA genes were amplified from genomic DNA by PCR using universal primers 27f (5′-GAGTTTGATCCTGGCTCA-3′) and 1385 (5′-CGGTGTGT (A/G) CAAGGCCC-3′)[13]. The reaction contained DNA (1 µl), 1 µl of each oligonucleotide, 2.5 µl of 10 × PCR buffer, 0.2 mM dNTPs, 25 mM MgCl (Promega, Pharmacia Biotech), 1.25 U *Taq* polymerase (Promega), 0.13 µl of each oligonucleotide and Milli-Q water to give a final volume of 12.5 µl. The reaction started with a 2 min denaturation at 95°C and followed by 30 cycles of denaturation at 95°C for 1 min; the first alignment (50–54°C depending on the strain for 1 min) and an extension of 72°C for 1 min were followed by a final alignment at 72°C (from 5 to 10 min depending on the strain). The PCR product was examined by agarose gel electrophoresis and purified using Wizard SV Gel kit and PCR Clean-Up System (Promega); sequencing was performed on GENEWIZ, Inc. (South Plainfield, NJ, USA).

The partial sequences were compared with those available from DNA databases, and also compared with those in GenBank using the NCBI BLASTN program. Sequence alignment and construction of the phylogenetic tree were performed using MEGA version 5.2.

The phosphorus solubilizing activity was determined for each of the isolated strains by measuring the size of the halo at insoluble phosphorus solubilizing medium agar Pikovskaya plates[14].

Bacterial isolates were inoculated in vials containing 5 ml of semisolid nitrogen-free (JNFb) medium. After seven days of incubation at 28°C, the isolates forming a film characteristic of free-living diazotrophis were seeded in JNFb medium supplemented with 20 mg l^{-1} yeast extract. Isolates growing on solid medium were re-inoculated in the semisolid JNFb medium. Strains forming the film again were considered positive for nitrogen fixation[11].

Each of the strains was grown in minimal medium supplemented with tryptophan (0.5 mg ml^{-1})[14]. The cultures were incubated at 28°C in the dark under agitation for four days. After incubation, cell suspension was centrifuged at 5000 *g* for 10 min. Next, 1 ml of supernatant was transferred to a tube containing 4 ml of Salkowski's reagent. The mix was incubated at room temperature for 20 min and kept in the dark. Sample absorbance was recorded at 530 nm. The concentration of each sample was calculated from a standard plot ranging from 0.01 to 40.0 µg ml^{-1} pure indole acetic acid (IAA).

Bacteria were plated on minimal medium Dworkin Foster (DF) supplemented with 3 mM 1-aminocyclopropane-1-carboxylase (ACC). Medium without ACC was used as negative control and plates of DF medium supplemented with $(NH_4)_2SO_4$ (0.2%) as a nitrogen source were used as positive control[6]. The strains that grew were considered as positive result.

To promote siderophore secretion, strains were grown in nutrient broth under constant stirring at 28°C for 48 h. A 50 µl aliquot of the homogenized cell suspension was taken and placed at three equidistant points of a plate containing chrome azurol sulphonate (CAS)[11]. It was then incubated at 28°C for seven days. The strains that developed a coloured halo were taken as indicators of positive siderophore secretion.

All bacteria isolated were screened for their ability to suppress the mycelial growth of *Alternaria* sp. (isolated from *in vitro* jojoba strains) to determine their antifungal

Table 1. Physical–chemical properties of soil sampling sites of jojoba (*Simmondsia chinensis*) and endophytic bacterial population density (cfu g^{-1} FW)

Site	pH	% Organic matter	Phosphorus (mg/kg)	Silt (%)	Sand (%)	Bacterial population density (CFU g^{-1} FW)
1	6.96	0.3	23.3	25.82	56.27	$8.3 \times 10^3 \pm 0.24$
2	6.15	0.3	5.6	23.33	56.51	$2.94 \times 10^3 \pm 0.09$
3	6.92	0.3	19.9	16.46	50.02	$2.7 \times 10^3 \pm 0.18$
4	6.25	1	9.1	37.86	37.59	$8.6 \times 10^3 \pm 0.45$
5	9.11	0.3	0.8	31.45	34.5	$1.8 \times 10^4 \pm 0.07$
6	8.45	2	7.8	45.7	37.06	$5.25 \times 10^3 \pm 0.5$
7	7.97	1.5	2	41.58	48.23	$9.3 \times 10^3 \pm 0.51$
8	8.3	1.2	4.4	19.93	54.34	$1.47 \times 10^3 \pm 0.05$
9	7.75	2.9	8.7	54.03	25.33	$3.38 \times 10^3 \pm 0.04$
10	6.18	1.2	11.9	32.23	51.76	$5.19 \times 10^3 \pm 0.9$
11	6.96	2.2	14	29.9	37.24	$1.04 \times 10^4 \pm 0.03$
12	8.42	0.2	11.7	14.85	78.21	$2.3 \times 10^4 \pm 0.43$
13	8.51	0.8	9.3	24.11	57.63	$1.65 \times 10^5 \pm 0.01$
14	8.12	2.4	9.9	60.99	36.71	$1.42 \times 10^4 \pm 0.29$
15	7.52	0.7	13.8	29.87	57.5	$3.64 \times 10^3 \pm 0.13$
16	8.43	2.1	3.2	11.44	34.23	$6.76 \times 10^3 \pm 0.27$
17	6.85	0.7	7.8	11.19	33.6	$1.98 \times 10^3 \pm 0.36$
18	8.09	1	9.9	42.08	55.31	$1.68 \times 10^4 \pm 0.17$
19	7.63	3.4	10.3	42.68	40.93	$2.16 \times 10^5 \pm 0.14$
20	9.19	0.7	2.7	10.61	37.87	$1.09 \times 10^3 \pm 0.08$
21	8.81	1.9	5.9	11.05	27.29	$7.94 \times 10^3 \pm 0.81$
22	8.47	1.9	7.1	18.4	26.49	$1.48 \times 10^4 \pm 0.15$
23	8.2	4.8	3.6	57.2	29.73	$7.2 \times 10^3 \pm 0.01$
24	8.58	0.4	6.1	28.47	64.65	$7.04 \times 10^3 \pm 0.19$
25	7.07	4.3	11.2	50.42	30.13	$9.5 \times 10^3 \pm 0.45$
26	7.65	8.1	11.1	56.88	33.47	$6.05 \times 10^3 \pm 0.14$
27	8.72	1	4.6	83.7	16.3	$7 \times 10^3 \pm 0.48$

behaviour by dual culture technique, which involves placing a 4 mm agar disc with *Alternaria* sp., at the centre of a plaque of potato dextrose agar (PDA). The fungus was incubated at 28°C for 48 h, placing three drops of 50 µl of inoculum of each of the bacterial strains at equidistant points for the fungus after this period. Subsequently, the cultures were incubated at 28°C for seven days. The control followed the same procedure, but without bacterial inoculum. Percentage inhibition (PI) was calculated using the formula PI = (DC – DT)/DC × 100%, where DC and DT represent the colony diameters of *Alternaria* sp. on the control and treatments respectively[15].

Jojoba is a shrub found in arid zones with rainfall of 80–120 mm/year, temperature ranging from –5°C to 54°C and soils with pH 5–8 (ref. 8). However, our study shows that this plant also grows in places where pH ranges from 6.1 to 9.19 (Table 1). Concentration of soluble phosphorus in the soil ranges from 0.8 to 23.3 mg kg^{-1} and organic matter from 0.2% to 8.1% (Table 1). Texture in 24 of the 27 collection sites has been characterized by greater percentage of sandy soils, whereas it is predominantly silty in the remaining three sites (Table 1). No correlation is found between these variables; so jojoba can be grown in a wide range of soil conditions.

In desert environments, plants partner with endophytes, as their relationship enhances growth and development whereas reducing stress caused by biotic and abiotic factors. In turn, the plants provide nutrients and protection for these bacteria[1,16]. However, studies of beneficial endophytic bacteria for desert plants are still scarce. The population density of endophytes in plants from desert areas is lower (1.1×10^2–1.5×10^2) compared to those growing in other environmental conditions (5.2×10^6)[2]. Our study has recorded population of endophytic bacteria in the range 1.09×10^3 (site 20) to 2.16×10^5 (site 19) cfu g^{-1} FW (Table 1), higher than that recorded by El-Deeb *et al.*[2]. This difference may be due to very different environmental conditions, even if they are desert plants[11,17]. In our study, the abundance of endophytes decreased with soil alkalinity in site 20 where pH was 9.19 and had the lowest population density (Table 1). However, bacteria yielding the maximum number of mechanisms to promote plant growth were collected at the sampling sites where soil pH was both more acid (6.18) and alkaline (9.19) (Table 1), which could be related to the production of root exudates acting as chemical signals to attract endophytic bacteria. It has been reported that there is increased production of root exudates when soil pH is acidic (pH 6) or alkaline (pH 8)[17].

Only eight representative isolates were chosen for having multiple plant-growth promoting properties for further analysis. For accurate identification, 16S rDNA sequence analysis was performed using BLAST (National Center for Biotechnology Information; http://www.ncbi.

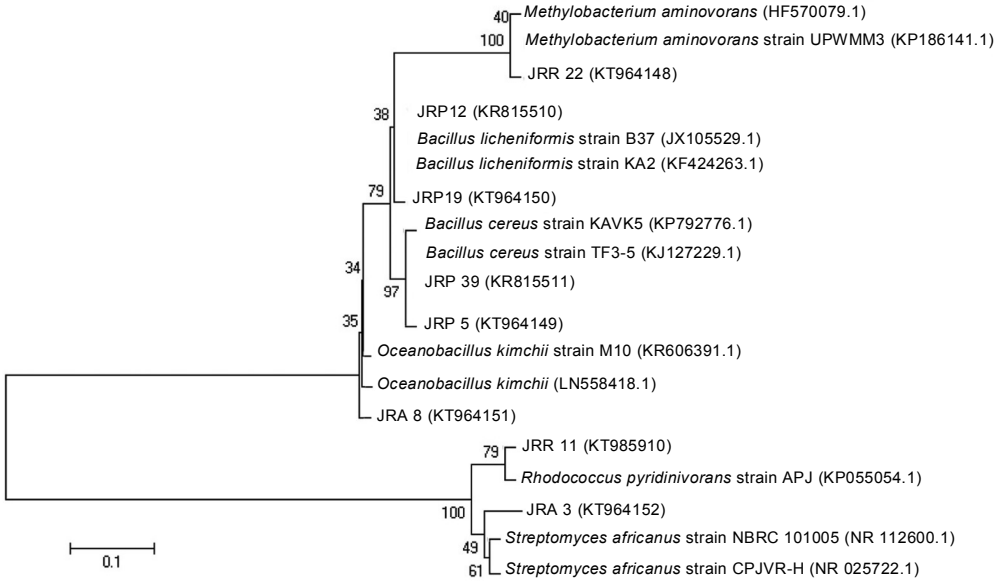

Figure 2. Phylogeny of endophytic bacteria from *S. chinensis* by 16S rDNA sequences. The numbers at the nodes indicate the level of bootstrap support, based on neighbour-joining analysis of 1000 re-sampled datasets. Scale bar indicates 0.05 changes/site.

Table 2. Identification of eight bacterial strains isolated from jojoba roots. Partial sequencing of 16S rDNA region was used (see text for details)

Strain	Order	Identification	Similarity (%) of 16S rDNA gene	GenBank accession no.
JRP5	Bacillales	*Bacillus* sp.	99	KT964149
JRP12	Bacillales	*Bacillus* sp.	99	KR815510
JRP19	Bacillales	*Bacillus* sp.	99	KT964150
JRP39	Bacillales	*Bacillus* sp.	99	KR815511
JRR22	Rhizobiales	*Methylobacterium aminovorans*	99	KT964148
JRR11	Actinimycetales	*Rhodococcus pyridinivorans*	99	KT985910
JRA3	Actinimycetales	*Streptomyces* sp.	99	KT964152
JRA8	Bacillales	*Oceanobacillus kimchii*	99	KT964151

nlm.nih.gov). Multiple sequence alignment was done using the program Mega. Bacterial identifications were based on the 16S rDNA gene sequence similarity. Neighbour-joining phylogenetic trees were generated using sequence data from GenBank for strains that showed a high percentage of similarity with those in the present study (Figure 2). The phylogenetic tree indicated that each bacterial strain from jojoba clustered to its corresponding strain from GenBank with 100% bootstrap factor. The 16S rDNA sequences of endophytic bacteria reported here have been deposited in GenBank under accession numbers: KT964149 (*Bacillus* sp.), KR815510 (*Bacillus* sp.), KT964150 (*Bacillus* sp.), KR15511 (*Bacillus* sp.), KT964148 (*Methylobacterium aminovorans*), KT985910 (*Rhodococcus pyridinivorans*), KT964152 (*Streptomyces* sp.) and KT964151 (*Oceanobacillus kimchii*) (Table 2). Previously reported endophytic bacteria genus *Bacillus* and *Pseudomonas* have been isolated from plants in the arid regions[2,8]. *Methylobacterium* spp., a common endophyte bacterium, can form biofilms and shows resistance to stress and heavy metals[3]. Likewise, *Streptomyces* is an endophytic actinobacterium of special interest, since it possesses many properties that could benefit plant growth. It has been isolated from various plant species, and many of the species have positive effects on host plants[16]. *R. pyridinivorans* and *O. kimchii* have not been reported as endophytic bacteria in plants. However, *R. pyridinivorans* is regarded as a biodegrader as it can transform a wide range of xenobiotic compounds commonly present in contaminated soil or water[18].

Endophytic bacteria secrete secondary metabolites that influence plant growth and development[2]. In our study, a total of 101 bacterial strains have been isolated and characterized based on their colonial morphology and observation of cellular morphology by microscopic and Gram stain. Preliminary characterization of the strains yielded 76.23% Gram-negative and 23.77% Gram-positive bacteria, among which 24 strains were identified presumptively as actinomycetes, given their in-plate growth characteristics and cell morphology.

The bacterial isolates in this study showed several desirable features for plant growth promotion; one of these

Table 3. Evaluation of direct and indirect mechanisms of growth promotion in bacterial strains isolated from jojoba roots

Strain	Site	Phosphate solubilization[a]	N-fixing[b]	IAA production (μg ml^{-1})	ACC deaminase activity[c]	Siderophores production[d]	Antagonism to *Alternaria* sp. % inhibition
JRP5	4	+	+	5.4	–	+	36.2
JRP12	1	+	+	0.3	+	+	
JRP19	9	+	–	4.25	–	+	22.05
JRP39	20	++	+	0.1	+	+	15.23
JRR22	20	+	+	0.3	+	+	56.14
JRR11	9	+	+	0.28	+	–	35.82
JRA3	1	+	+	0.3	+	–	43.93
JRA8	10	+	+	0.21	+	–	0

[a]Production of phosphate solubilization: (+) halo up to 1 cm in diameter, (++) halo greater than 1 cm in diameter. [b](+) N-fixing, (–) no N-fixing. [c](+) ACC deaminase activity; (–), no activity. [d](+) Siderophores detected, (−), Siderophores not detected.

beneficial characteristics is the capacity to solubilize phosphorus. In our study, 56 strains of bacteria and 10 strains of actinomycetes were grown in Pikovskaya medium plates forming the characteristic halo resultant of the production of organic acids[5] such as lactic, itaconic, isovaleric, isobutyric, acetic, formic, gluconic, succinic and propionic acids. The isolated *Bacillus* sp. (JRP 39) showed the highest phosphorus solubilization capacity compared to the other strains tested (Table 3), as it is known that this genus can solubilize tricalcium phosphate[19]. Our results suggest the possibility of using this bacterium as a plant growth promoter in arid and semiarid regions, as it increases the availability of inorganic phosphorus in the soil.

Isolation and identification of bacteria capable of fixing nitrogen were performed to find alternatives for synthetic fertilizers. In our study, 51 bacterial strains and 4 actinomycete strains were able to grow and form a film in JNFb, suggesting that they have the ability to reduce atmospheric nitrogen through nitrogenase reductase enzyme. We also found species of the genera *Bacillus*, *Methylobacterium*, *Rhodococcus*, *Oceanobacillus* and *Streptomyces*, which have the ability to grow in JNFb, indicating their ability to fix nitrogen (Table 3). Recent studies have recorded species of the genus *Bacillus*[2,20] and *Methylobacterium*[3] that are capable of fixing atmospheric nitrogen; however, little is known about the actinomycetes with the capacity to fix nitrogen[11]. The results of our study suggest that the strains of both *Streptomyces* sp. (JRA3) and *R. pyridinivorans* (JRR11) are capable of fixing nitrogen.

IAA induces physiological activities, such as plant cell division and root initiation[11]. It can also act as a signalling molecule in the initial steps of the plant–bacterium symbiosis[4]. Of the total 101 strains isolated in this study, 37 bacterial and 19 actinomycete strains showed the ability to produce IAA using tryptophan as substrate. Isolates synthesized IAA in the 0.1–5.4 μg ml^{-1} range (Table 3) compared to that reported by Dias *et al.*[21], where the endophytic bacteria produced 20 μg IAA ml, suggesting that the synthesis of this enzyme depends on the species and strains, culture conditions, plant growth stage and substrate availability, although low levels of IAA synthesis (2.01 μg ml^{-1}) can promote elongation of the primary root[2].

Bacteria that have ACC deaminase activity help plants withstand stress (biotic and abiotic) by reducing the level of ethylene stress[14,22]. In this study, 31 of the 101 endophyte bacterial strains associated with jojoba roots and seven actinomycetes were able to grow on DF-modified agar medium with ACC, which suggests they show ACC deaminase activity. This study reveals that enzyme activity in jojoba plant increases under environmental stress. It has been reported that plants inoculated with bacteria that synthesize this enzyme are more resistant to stress such as drought, heavy metals, pathogens and high salinity[7,22]. Six isolates showed ACC deaminase activity and were also screened for multiple plant growth promoting traits, including IAA production, phosphate solubilization and nitrogen fixation. Table 3 shows that some of them exhibit the capacity to grow in nitrogen-free conditions and produce IAA.

Of the total 101 isolates, 38 bacterial strains and 5 actinomycetes formed a halo around the colonies on this medium. This result is considered as positive for siderophore production, showing the capacity to improve iron nutrition in plants, because siderophores are responsible of chelating and transport[3], and the ability to fix nitrogen[11]. Similarly, four strains that produced siderophores also showed the ability to inhibit the growth of fungi which was likely due to sequestration of iron by bacteria limiting growth of pathogenic fungus[15].

Isolation of microorganisms that have the ability to inhibit growth of pathogenic fungi could be an alternative to reduce economic losses in agriculture[11]. A dual culture test was used for preliminary detection of agents with potential use as biological control, but the results depend on culture medium and secondary metabolite production[15]. In this study 39 bacterial and 9 actinomycete strains were able to inhibit the growth of *Alternaria* sp. *M. aminovorans* (JRR22) showed the highest antagonism against *Alternaria* sp. with an inhibition of 56.14%. It is

frequently found as an endophyte, is characterized by its biofilm formation capacity for signalling the induction of the plant defence system and inhibiting the growth of *Pectobacterium atrosepticum*, a bacteria causing black foot disease in potatoes[3].

Similarly, we found that JRP12, JRP19 and JRP39 strains from the genus *Bacillus* inhibited fungal growth; this genus has been described as efficient for biological control of fungal diseases[20]. The JRA3 (*Streptomyces* sp.) strains inhibited fungal growth (43.93%) likely due to the production of peptides that have been reported to inhibit the growth of pathogenic fungi in plants[3,21]. Finally, *R. pyridinivorans* (JRR11) had only been reported with mycotoxin degrading capability, but in this study it also inhibited fungus growth (Table 3)[18].

Thus this study suggests that the jojoba plant is naturally associated with different endophytic bacteria that have different physiological and biochemical capabilities. Eight isolates from the genera *Bacillus*, *Methylobacterium*, *Rhodococcus*, *Oceanobacillus* and *Streptomyces* show plant growth promoting features, which could benefit the plant. Nonetheless, further studies are required at molecular and biochemical level to ensure plant growth promoting properties of the isolated strains and to exploit their potential as biological inoculants in jojoba cultivation.

1. Sun, H., He, Y., Xiao, Q., Ye, R. and Tian, Y., Isolation, characterization, and antimicrobial activity of endophytic bacteria from *Polygonum cuspidatum*. *Afr. J. Microbiol. Res*., 2013, **7**, 1496–1504.
2. El-Deeb, B., Fayez, K. and Gherbawy, Y., Isolation and characterization of endophytic bacteria from *Plectranthus tenuiflorus* medicinal plant in Saudi Arabia desert and their antimicrobial activities. *J. Plant Interact*., 2013, **8**, 56–64.
3. Ardanov, P., Sessitsch, A., Haggman, H., Kozyrovska, N. and Pirttila, A. M., *Methylobacterium*-induced endophyte community changes correspond with protection of plants against pathogen attack. *PLOS ONE*, 2012, **7**, 1–7.
4. Hardoim, P. R., Hardoim, C. P., van-Overbeek, L. S. and van-Elsas, J. D., Dynamics of seed-borne rice endophytes on early plant growth stages. *PLoS ONE*, 2012, **7**(2), 1–13.
5. Gaiero, J. R., McCall, C. A., Thompson, K. A., Day, N. J., Best, A. S. and Dunfield, K. E., Inside the root microbiome: bacterial root endophytes and plant growth promotion. *Am. J. Bot*., 2013, **100**, 1738–1750.
6. Peronse, D. M. and Glick, B. R., Methods for isolating and characterizing ACC deaminase-containing plant growth-promoting rhizobacteria. *Physiol. Plant*., 2003, **88**, 10–15.
7. Kumar, S., Singh, N., Mangal, M. and Dhawan, A. K., Biotechnological advances in jojoba [*Simmondsia chinensis* (Link) Schneider]: recent developments and prospects for further research. *Plant Biotechnol. Rep*., 2012, **6**, 97–106.
8. El-Deeb, B., Bazaid, S., Gherbawy, Y. and Elhariry, H., Characterization of endophytic bacteria associated with rose plant (Rosa *damascena trigintipeta*) during flowering stage and their plant growth promoting traits. *J. Plant Interact*., 2012, **7**, 248–253.
9. Krishnan, P., Bhat, R., Kush, A. and Ravikumar, P., Isolation and functional characterization of bacterial endophytes from *Carica papaya* fruits. *J. Appl. Microbiol*., 2012, **113**, 308–317.
10. Jackson, M. L., *Soil Chemical Analysis*, Prentice Hall of India Pvt Ltd, New Delhi, 1973, pp. 1–498.
11. Valdés, M., Pérez, N. O., Estrada de los Santos, P., Caballero-Mellado, J., Peña-Cabriales, J. J., Normand, P. and Hirsch, A. M., Non-Frankia actinomycetes isolated from surface-sterilized roots of *Casuarina equisetifolia* fix nitrogen. *Appl. Environ. Microbiol*., 2005, **71**, 460–466.
12. Hentschel, U., Schmid, M., Wagner, M., Fieseler, L., Gernert, C. and Hacker, J., Isolation and phylogenetic analysis of bacteria with antimicrobial activities from the Mediterranean sponges *Aplysina aerophoba* and *Aplysina cavernicola*. *FEMS Microbiol. Ecol*., 2001, **35**, 303–312.
13. Faria, D. C., Melo, I. S., Franco, A. C. and de Carvalho, E. F., Endophytic bacteria isolated from orchid and their potential to promote plant growth. *World J. Microbiol. Biotechnol*., 2013, **29**, 217–221.
14. Ribeiro, C. M. and Cardoso, E. J. B. N., Isolation, selection and characterization of root-associated growth promoting bacteria in Brazil Pine (*Araucariaan gustifolia*). *Microbiol. Res*., 2012, **167**, 69–78.
15. Ma, L. *et al.*, Phylogenetic diversity of bacterial endophytes of *Panax notoginseng* with antagonistic characteristics towards pathogens of root-rot disease complex. *Antonie van Leeuwenhoek J. Microbiol*., 2013, **103**, 299–312.
16. Qin, S., Zhang, Y. J., Yuan, B., Xu, P., Xing, K., Wang, J. and Jiang, J., Isolation of ACC deaminase-producing habitat-adapted symbiotic bacteria associated with halophyte *Limonium sinense* (Girard) Kuntze and evaluating their plant growth-promoting activity under salt stress. *Plant Soil*, 2014, **374**, 753–766.
17. Knoth, J. L., Kim, S. H., Ettl, G. and Doty, S., Effects of cross host species inoculation of nitrogen-fixing endophyte on growth and leaf physiology of maize. *GCB Bioenergy*, 2013, **5**, 408–418.
18. Cserháti, M. *et al.*, Mycotoxin-degradation profile of *Rhodococcus* strains. *Int. J. Food Microbiol.*, 2013, **166**, 176–185.
19. Anzuay, M. S., Frola, O., Angelini, J. G., Ludueña, L. M., Fabra, A. and Taurian, T., Genetic diversity of phosphate-solubilizing peanut (*Arachis hypogaea* L.) associated bacteria and mechanisms involved in this ability. *Symbiosis*, 2013, **60**, 143–154.
20. Ikeda, A. C. *et al.*, Morphological and genetic characterization of endophytic bacteria isolated from roots of different maize genotypes. *Microb. Ecol*., 2013, **65**, 154–160.
21. Dias, A. C. F. *et al.*, Isolation of micropropagated strawberry endophytic bacteria and assessment of their potential for plant growth promotion. *World J. Microbiol. Biotechnol*., 2009, **25**, 189–195.
22. Biswas, R. B., Jourand, P., Chaintreuil, C., Dreyfus, B., Singh, A., Narayan, S. and Mukhopadhyay, N., Indole acetic acid and ACC deaminase-producing *Rhizobium leguminosarum* bv. Trifolli SN 10 promote rice growth and in the process undergo colonization and chemotaxis. *Biol. Fertil. Soils*, 2012, **48**, 173–182.

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