https://doi.org/10.5194/egusphere-2026-298
Preprint. Discussion started: 11 February 2026

Unraveling the plant growth promotion potential of Pseudomonas species isolated from the rhizosphere of *Lotus creticus* grown in the Mediterranean coastal regions of Morocco

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Abstract. Rhizosphere-associated bacteria play a key role in improving plant performance in saline and nutrient-poor soils. In this study, the plant growth-promoting potential of rhizobacteria isolated from the rhizosphere of *Lotus creticus* naturally growing in Mediterranean coastal regions of northwestern Morocco was evaluated. Out of 30 isolates, five bacterial strains R125, P79, R8, R150, and R15 were selected based on their plant growth-promoting rhizobacteria (PGPR) traits. These strains were identified through 16S rRNA gene sequencing as *Pseudomonas protegens*, *Pseudomonas sesami*, *Pseudomonas versuta*, *Pseudomonas helleri*, and *Pseudomonas trivialis*, respectively. Phenotypic characteristics, including IAA production, phosphate solubilization capacity, cellulase and protease activities, and tolerance to salinity and temperature were evaluated. Additionally, a pot experiment was conducted to assess the impact of inoculation on the growth of *Lotus creticus*. Significant variation was observed among the selected strains. *Pseudomonas protegens* P79 stood out for its strong indole-3-acetic acid (IAA) production, high phosphate solubilization capacity (150.5 $mg \cdot L^{-1}$), and notable cellulase and protease activities. It also demonstrated high tolerance to salinity (up to 13% NaCl) and elevated temperatures (up to 45°C). Conversely, *Pseudomonas sesami* R8 exhibited a broad spectrum of antifungal activity, including strong inhibition of *Aspergillus ochraceus*. The pot experiment revealed that inoculation with *Pseudomonas versuta* R15 and *Pseudomonas helleri* R125 significantly enhanced the aerial dry biomass and shoot length of *Lotus creticus* under controlled chamber conditions. This study highlights five *Pseudomonas* strains, particularly *Pseudomonas protegens* P79, as promising biostimulants for sustainable agriculture and the rehabilitation of saline marginal lands, due to their diverse plant growth-promoting traits.

Keywords: *Lotus creticus* L., PGPR, *Pseudomonas* sp., 16S rRNA, marginal soils.

https://doi.org/10.5194/egusphere-2026-298
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1 Introduction

Coastal pastures are agroecosystems located near marine environments and are found in many temperate regions around the world, including the Mediterranean (Romano et al., 2022). These unique ecosystems support a variety of plant species adapted to saline, nutrient-poor soils and serve as valuable habitats for wildlife, providing food and breeding grounds for various livestock species (Younsi and Bouziane, 2023). However, they have undergone significant decline globally due to climate change, overgrazing, and anthropogenic pressures, resulting in habitat loss, reduced biodiversity, and soil degradation (Younsi and Bouziane, 2023; El Yemlahi et al., 2025). To address these challenges, it is essential to implement sustainable management practices and ecological restoration strategies to preserve their ecological functions and maintain their role in supporting biodiversity and agricultural productivity. Legumes (Fabaceae) have often been utilized as eco-friendly solutions for sustainable revegetation to address the challenges of modern agriculture (El Yemlahi et al., 2024; Ainane et al., 2021). Due to their capacity to fix atmospheric nitrogen in symbiosis with rhizobial bacteria, legumes enhance soil fertility, thereby reducing the need for chemical fertilizers and promoting sustainable farming systems (Petrushin et al., 2023). One such legume is *Lotus creticus* L., commonly known as Cretan trefoil. Native to the Mediterranean region, this pastoral and forage legume is well-known for its ability to thrive in saline and arid environments (Kew Science, 2024). This remarkable adaptability makes *L. creticus* an excellent candidate for soil stabilization and reclamation efforts (ICARDA, 2022). The importance of this species in ecological restoration and agricultural productivity is underscored by its capacity to grow in challenging coastal environments, where nutrient deficiencies and soil salinity often limit plant growth and vegetation establishment (Navarro et al., 2023). Like other legumes, the root zone of *L. creticus* can harbor a variety of beneficial microorganisms known as plant growth-promoting bacteria (PGPB), which play a vital role in plant growth and health. Several studies have highlighted the significance of plant-associated microorganisms in enhancing plant growth under nutrient-poor conditions (Srivastava et al., 2022) and in improving resistance to environmental stresses such as drought and salinity (Bashan et al., 2014). These beneficial microbes interact with plant roots to facilitate nutrient mobilization and uptake, particularly in soils prone to nutrient leaching (Javadzadeh et al., 2023). They also contribute to the synthesis of phytohormones (Mekureyaw et al., 2022) and enhance plant resilience by inducing systemic tolerance (Salwan et al., 2023). Therefore, this study aims to identify bacterial strains isolated from the rhizospheric soil of *L. creticus* with strong plant growth-promoting (PGP) traits. These strains could serve as effective bioinoculants to support the growth and productivity of *L. creticus* in saline, marginal soils.

2 Materials and methods

2.1 Plant and soil sampling

Bacteria were isolated from the rhizosphere of *L. creticus*, which grows naturally in the coastal zone of Morocco, near Tahadart beach. Soil samples from the surface layer were also collected in sterile plastic bags and stored at 4 °C for analysis. Total soil nitrogen was measured using the Kjeldahl method (ISO, 1995). Available phosphorus was assessed using the Olsen method (Olsen et al., 1954), and exchangeable potassium was measured by flame photometry following the method of Bower et al. (1952). Soil organic matter was evaluated using the Walkley and Black method (Walkley and Black, 1934), and total $CaCO_3$

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was measured using Bernard's calcimeter. Additionally, the nodulation ability of *Lotus creticus* was assessed based on the nodule-scoring chart suggested by Howieson and Dilworth (2016). Crude protein (CP) content was measured at the flowering stage according to the Kjeldahl method (AOAC, 1997). Finally, organic matter (OM) was determined by incinerating samples at 550 °C for 12 hours (AOAC, 1997).

2.2 Isolation of rhizobacteria

One gram of rhizospheric soil was mixed with 9 mL of sterile NaCl solution (0.9% w/v). The mixture was stirred at 200 rpm for 1 hour and subjected to serial dilutions ranging from 10^{-1} to 10^{-7}. Then, 100 µL from each dilution was plated onto Petri dishes containing King B (KB) medium and incubated at 28 °C for 3 days. After incubation, single colonies were selected and repeatedly streaked onto KB agar medium until pure colonies were obtained. Finally, pure isolates were preserved in 25% (v/v) sterile glycerol at −20 °C until analysis.

2.3 Morphological characterization and Gram staining

The morphological characteristics of each colony including shape, structure, and pigmentation were examined after streaking the bacteria onto KB agar plates. Additionally, Gram staining was performed on fixed smears of bacterial cultures following the method described by Cyrabree and Hindshill (1975).

2.4 Molecular characterization

Bacterial DNA was extracted using the phenol/chloroform protocol established by Chen and Kuo (1993). DNA concentration was measured using a NanoDrop spectrophotometer (Thermo Scientific™ NanoDrop 2000). Two universal primers, rD1 and fD1, were used to amplify the 16S rRNA gene sequence, following the methodology of Weisburg et al. (1991). The amplified DNA products were initially assessed via horizontal electrophoresis (70 V for 1 hour) in Tris-acetate-EDTA (TAE) buffer. Subsequently, cycle sequencing analysis was performed using the same primers for the 16S rRNA PCR amplification. The resulting sequences were assembled using BioEdit software (version 7.0.5.3), manually verified, and compared with sequences in GenBank using the BLAST algorithm (https://blast.ncbi.nlm.nih.gov/blast.cgi/). Finally, a phylogenetic tree was constructed using the neighbor-joining method with Kimura's two-parameter model in MEGA software version 11.

2.5 Assessment of PGP traits

2.5.1 Phosphate solubilization

The bacterial isolates were tested for their ability to solubilize tricalcium phosphate (TCP) using PVK agar medium as the sole phosphorus source (Pikovskaya, 1948). A 10 µL aliquot of fresh bacterial culture was inoculated onto PVK plates and incubated at 28 °C for 7 days. The plates were checked for halo formation, and the solubilization index (SI) was calculated using the formula described by Ed-Premono et al. (1996):

$$\mathrm{SI} = \frac{\text{Colony diameter} + \text{Halo diameter}}{\text{Colony diameter}}$$

The isolates were further evaluated for their ability to solubilize tricalcium phosphate (TCP) in liquid medium by inoculating 500 µL of bacterial culture into 50 mL of PVK liquid medium. Sterile, uninoculated media served as controls. Both inoculated

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and control media were incubated for 7 days at 28 °C on an orbital shaker at 180 rpm. After incubation, the cultures were centrifuged at 13.000 rpm for 20 minutes. The concentration of soluble phosphorus in the supernatant was determined using the colorimetric method developed by Ames (1966).

2.5.2 Production of ammonia.

Ammonia production was assessed following the method described by Cappuccino and Sherman (1992). Bacterial cultures were inoculated into 10 mL of peptone water and incubated at 36 ± 2 °C for 48 to 72 hours. Ammonia production was indicated by a color change from brown to yellow upon the addition of Nessler's reagent.

2.5.3 Production of hydrogen cyanide (HCN)

To estimate HCN production, 100 µL of bacterial culture was streaked onto KB agar plates supplemented with 4.4 g of glycine. Filter paper discs (9 cm in diameter), saturated with 2% sodium carbonate, were then placed inside each Petri plate. The plates were sealed with parafilm and incubated at 28 °C. A color change from yellow to orange or brown indicated HCN production (Bakker and Schippers, 1987).

2.5.4 Production of siderophores

Bacterial isolates were spot-inoculated onto KB agar medium to assess siderophore production and incubated for 3 days at 28 °C. Subsequently, a layer of Chrome Azurol S (CAS) medium was overlaid onto the surface of the plates, which were then incubated in the dark for 24 hours. A color change in the CAS medium from blue to orange indicated siderophore production (Schwyn and Neilands, 1987).

2.5.5 Production of ACC deaminase

The isolates were evaluated for their ability to synthesize ACC (1-aminocyclopropane-1-carboxylate) deaminase, using ACC as the sole nitrogen source, following the procedure described by Jacobson et al. (1994). Bacterial growth was assessed by measuring the optical density (OD) at 600 nm. Higher OD values indicated increased ACC deaminase activity. Results were compared to a control medium containing an alternative nitrogen source, such as ammonium sulfate ($(NH_4)_2SO_4$).

2.5.6 Production of Indole Acetic Acid (IAA)

To evaluate the ability of the isolated bacteria to synthesize indole-3-acetic acid (IAA), 0.05% tryptophan was added to the TSA medium. After solidification, a sterile nitrocellulose membrane was carefully placed on the surface of the medium and inoculated with 10 µL of bacterial culture. Following incubation at 28 °C, the membrane containing the colonies was transferred onto a Whatman filter paper impregnated with Salkowski reagent (2% $FeCl_3$ [0.5 M] in 35% perchloric acid). Bacteria capable of synthesizing IAA were identified by the appearance of red halos surrounding the colonies (Bric et al., 1991).

2.5.7 Production of extracellular enzymes

Production of cellulase. The strains were cultured on CMC (carboxymethyl cellulose) agar plates. The medium's pH was adjusted to 7, and the plates were incubated at 30 °C for 5 days. After incubation, the plates were soaked in an aqueous solution

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of Congo red (0.1%) and then rinsed with 1 M NaCl solution. The formation of a clear zone around the colonies indicated cellulose degradation (Miller, 1959).

Production of amylase. For the quantification of amylase production, 10 µL of fresh bacterial culture was inoculated onto starch agar medium (SAM) and incubated at 37 °C for 48 hours. The plates were then soaked in a 1% iodine solution for 5 minutes. The appearance of a clear zone around the bacterial colonies indicated amylase production (Collins et al., 2004).

Production of chitinase. To confirm chitinase production by the bacterial isolates, colloidal chitin $(C_8H_{13}O_5N)_n$ was used as the carbon source (Renwick et al., 1991). Petri dishes containing colloidal chitin agar medium were inoculated with freshly prepared bacterial cultures. After incubation at 30 °C for 4 days, positive colonies were surrounded by distinct clear halos, indicating chitin degradation.

Production of protease. Protease production is assessed using skim milk agar medium prepared as described by Vijayaraghavan and Vincent (2013). The plates were inoculated with 100 µL of fresh bacterial culture and incubated at 28°C for 48 hours. The appearance of a clear halo around the colony indicates protein degradation.

Production of urease. Urease activity was detected by the alkalinization of Christensen's urea agar medium, which contained urea as the sole nitrogen source and phenol red as the pH indicator. Plates were inoculated with the bacterial strains and incubated at 30 °C for 48 hours. After incubation, positive colonies were identified by a pink-purple coloration against a yellow background (Brink, 2010).

Production of catalase. The catalase test was performed by adding a drop of 3% hydrogen peroxide onto a freshly cultured bacterial isolate on a sterile slide using a sterile inoculation loop. The appearance of effervescence indicated catalase activity (Bumunang and Babalola, 2014).

2.6 Antagonism against phytopathogenic fungi

Antifungal activity was evaluated using potato dextrose agar (PDA) medium, as described by Rabindran and Vidhyasekaran (1996). The bacteria were assessed for their potential to inhibit the growth of plant pathogenic fungi, including *Fusarium oxysporum*, *Botrytis cinerea*, *Aspergillus ochraceus*, and *Aspergillus flavus*, which were isolated and characterized by El Aaraj et al. (2015). A 5 mm agar disk from a fresh fungal culture was placed at the center of Petri plates filled with PDA medium. Subsequently, 20 µL of each bacterial culture was inoculated as spots 3 cm away from the fungal strain. Control experiments were conducted without bacteria. The plates were incubated at 25 °C for 7 days and examined for fungal inhibition using the following formula:

$$\% \text{ Inhibition of fungal growth} = 100 \times \frac{(r1 - r2)}{r1}$$

With ''r1'' is diameter of the control culture and ''r2'' is the diameter of the treated culture.

2.7 Stress effect

2.7.1 Effect of NaCl.

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To determine the effect of salinity on the growth of the selected strain, a single colony was streaked onto Petri dishes containing KB medium supplemented with different concentrations of NaCl, ranging from 0% to 14% [w/v]. The plates were incubated at 28 °C for 48 hours in the dark. Colony growth was monitored and compared to salt-free controls (Novitsky and Kushner 1975)

2.7.2 Effect of Temperature

Additionally, the isolates were examined for their tolerance to heat stress. The bacteria were streaked onto KB agar medium and incubated for 24 hours in the dark at different temperatures (30, 37, 42, 45, and 50 °C). Colony growth was monitored and compared to controls incubated at 28 °C (Ahmad et al., 2008).

2.7.3 Effect of pH

Tolerance to acidic and alkaline pH was assessed by streaking bacteria onto KB medium, with the pH adjusted to 3, 5, 6, 6.5, 7.5, 8.5, 9, and 10. A positive result was indicated by bacterial growth, showing tolerance to the tested pH levels, while the absence of growth suggested sensitivity to the given pH value.

2.8 Inoculation of *Lotus creticus*

The seeds of *L. creticus* were disinfected by immersion in 95% ethanol for 1 minute, followed by treatment with 1.2% sodium hypochlorite for 20 minutes, and then thoroughly rinsed several times with sterile water. The seeds were placed on filter paper discs moistened with 10 mL of sterile water in a Petri dish and incubated at 28 °C. Three germinated seeds were then planted in pots filled with *L. creticus*-associated soil and inoculated directly with 1 mL of bacterial culture (10^8 CFU/mL) grown in TSB. Uninoculated pots served as controls. All pots were maintained under greenhouse conditions and irrigated weekly with distilled water and/or a mineral solution (Broughton and Dilworth, 1970).

2.9 Statistical analysis

All results are presented as means ± standard deviation (SD) and were compared using analysis of variance (ANOVA) followed by the Fisher protected LSD test, using STATISTICA 13.3 software.

3 Results

3.1 Soil analysis and symbiosis evaluation

The on-field survey revealed the presence of *L. creticus* on alkaline sandy soil, low in potassium (45.2 ppm), phosphorus (3.0 ppm), and nitrogen (0.08%) (Table 1).

Table 1. Soil chemical characteristics of the sampling site of *L. creticus*

PH (EAU)	PH (KCL 1N)	MO (%)	P_2O_5 (PPM)	K_2O (PPM)	N(%)
8.4	9.1	0.1	3.0	45.2	0.08

Additionally, plant examination showed a strong symbiotic ability of *L. creticus*, forming large, pink nodules as assessed using the nodule-scoring chart proposed by Howieson and Dilworth (2016). The results (Table 2) demonstrated the high productivity of *L. creticus*, yielding up to 781.25 kg of dry matter per hectare. The plant produced high organic matter (91.32% DM) and crude protein (12.60% DM) content at the flowering stage.

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Table 2. Nodulation and growth of *L. creticus*

Nodulation[1]	DM (Kg/ha)	OM (%DM)	CP (%DM)
Abundant	781.25	91.32	12.60

Values are presented as the mean of 3 replicates. DM: dry matter, OM: organic matter, CP: crude protein. [1]: using a nodule-scoring chart proposed by Howieson and Dilworth (2016).

3.2 Isolation and morphological characterization of rhizobacteria

Thirty bacteria were isolated from the rhizosphere of *L. creticus* and cultured in King B medium. Five isolates were selected for further examination based on their growth characteristics. Morphologically, three isolates (P8, P15, and P79) exhibited bacillus-shaped colonies, while two isolates (P115 and P125) displayed cocci-shaped colonies. All isolates were identified as Gram-negative bacteria and formed cream-colored colonies, except for P79, which produced green-colored colonies.

3.3 Molecular characterization

Electrophoretic analysis showed distinct amplicons of about 1500 bp in all bacterial isolates, corresponding to the expected size of the 16S rRNA gene (Figure 1).

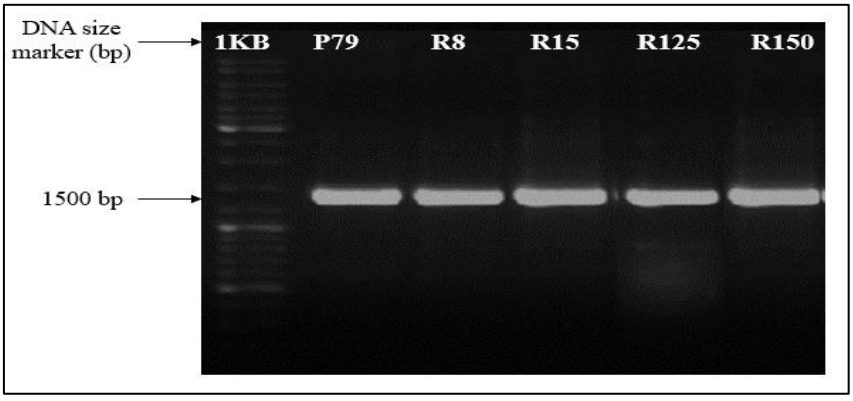

Figure 1: Agarose gel electrophoresis analysis of 16S rRNA genes amplified from 5 bacterial isolates.

The genetic characterization of the selected strains revealed a close relationship to the *Pseudomonas* genus based on nearly complete 16S rRNA gene sequences, with similarity values exceeding 98%. According to the phylogenetic tree (Figure 2) inferred from the 16S rRNA sequences, the five strains P79, R8, R15, R125, and R150 isolated from the rhizospheric soil of *L. creticus*, were closely related to *Pseudomonas protegens*, *Pseudomonas sesami*, *Pseudomonas versuta*, *Pseudomonas helleri*, and *Pseudomonas trivialis*, respectively. The 16S rRNA gene sequences of these five *Pseudomonas* strains have been submitted to NCBI GenBank, and their accession numbers are provided in Figure 2.

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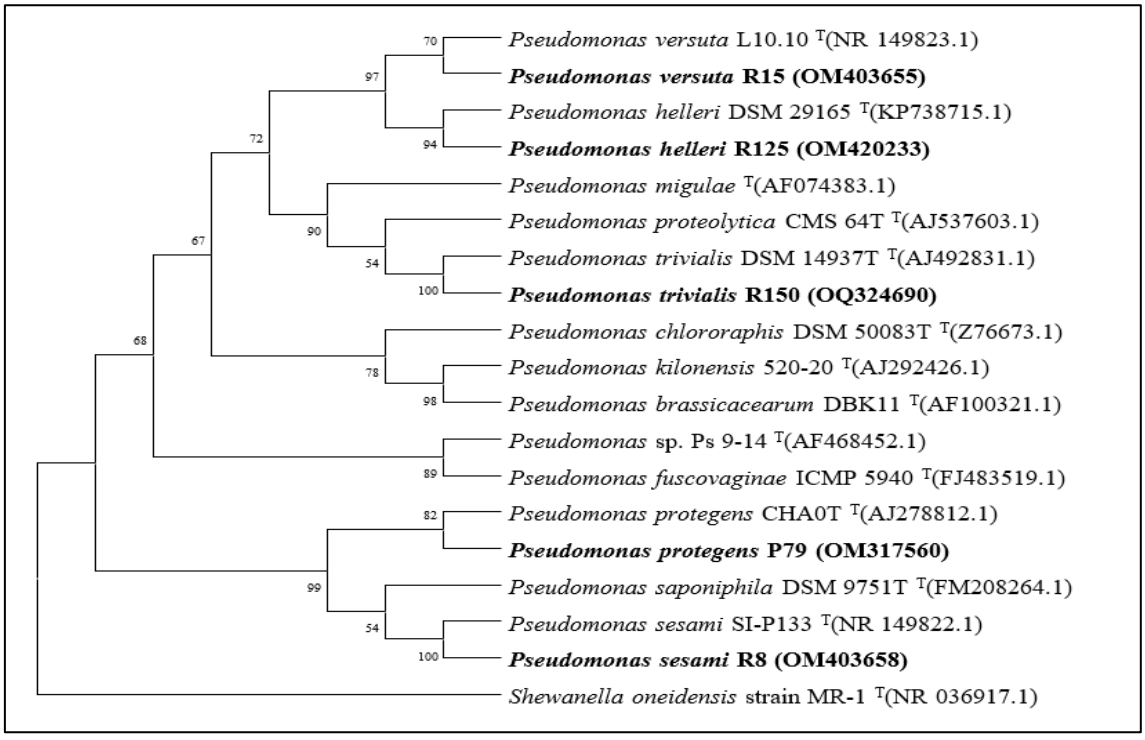

Figure 2: Phylogenetic tree based on the 16S rRNA gene sequences showing the relationship of the five Pseudomonas strains (highlited in the tree) and other related members. The *Shewanella oneidensis* strain MR-1 acts as an outgroup to root the tree for comparison. *The tree was reconstructed by the Neighbor-Joining tree method. The bootstrap consensus tree inferred from 1000 replicates*

3.4 Assessment of PGP traits

The plant growth-promoting properties of the five selected bacterial strains are summarized in Table 3. The results revealed significant variation in their ability to solubilize phosphate and acidify the culture medium.

Table 3. Plant growth promoting traits of the five selected rhizobacterial strains.

Strain	S. I	P solubilization	pH	HCN	Siderophores	Ammonia	IAA	ACCD
P79	0.7 ± 0.4	150.5 ± 0.2	5.16 ± 0.6	++	+	++	+++	+
R8	0.5 ± 0.1	59.70 ± 0.5	6.11± 0.4	+++	++	+++	+	+
R15	0.7 ± 0.5	76.09 ± 0.3	6.16 ± 0.1	+	-	++	+	+
R125	0.4 ± 0.2	91.25 ± 0.3	5.17 ± 0.5	-	++	+++	+	+
R150	0.4 ± 0.3	87.05 ± 0.1	4.65 ± 0.2	++	++	+++	+	+

Values are presented as the mean of 3 replicates. S.I: solubilization index, IAA: ACCD: ACC deaminase, P solubilization in mg·L⁻¹, +, low activity; ++, moderate activity; +++, strong activity; −, no activity.

Pseudomonas protegens P79 exhibited the highest phosphate solubilization (150.5 mg·L⁻¹) along with moderate siderophore production. *Pseudomonas sesami* R8 stood out for its strong HCN production. However, it displayed the lowest phosphate solubilization capacity (59.70 mg·L⁻¹). *Pseudomonas helleri* R125 and *Pseudomonas trivialis* R150 showed similar profiles,

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both exhibiting moderate phosphate solubilization (91.25 and 87.05 mg·L^{-1}, respectively) and ammonia production. *Pseudomonas versuta* R15 demonstrated relatively low phosphate solubilization (76.09 mg·L^{-1}) and weak HCN production, and it was unable to produce siderophores. All strains produced IAA and ACC deaminase, though only at low levels.

3.5 Production of extracellular enzymes

Table 4 highlights the ability of the isolates to produce extracellular enzymes, including cellulase, chitinase, amylase, protease, urease, and catalase. All bacterial strains exhibited minimal enzymatic activity, except *Pseudomonas protegens* P79, which showed significant cellulase and protease activities. This strain also exhibited moderate chitinase production, along with low levels of urease and catalase activity.

Table 4. Extracellular enzymes production by the bacterial isolates.

Strain	Cellulase	Chitinase	Amylase	Protease	Urease	Catalase
P79	+++	++	++	+++	+	+
R8	+	-	+	-	++	+
R15	+	+	+	+	++	+
R125	+	+	+	-	+	+
R150	+	+	+	+	-	+

(+) : low activity, (++) : moderate activity, (+++) : strong activity, (−) : no activity. *Values are presented as the mean of 3 replicates.*

3.6 Antagonism against phytopathogenic fungi

The antagonistic activity of the five rhizobacterial strains against four fungal pathogens, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus ochraceus*, and *Botrytis cinerea*, is presented in Figure 3.

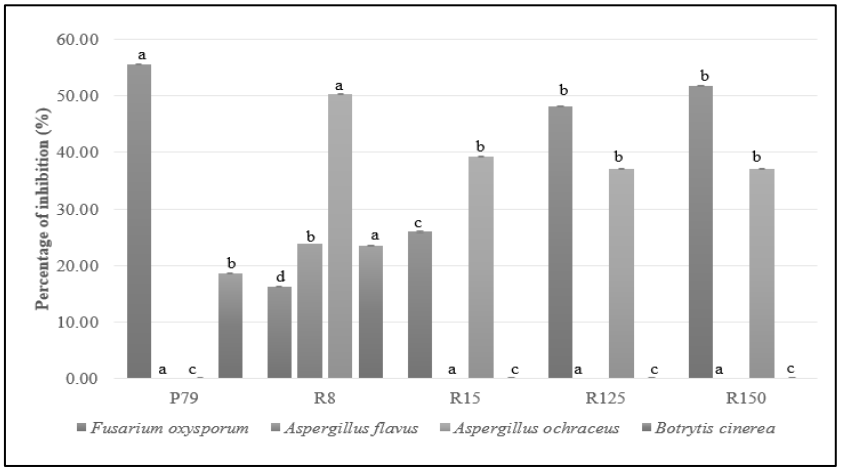

Figure 3: Percentage of growth inhibition of phytopathogenic fungi by Pseudomonas strains. ***Values are presented as means from 3 replicates. Bars with different letters are significantly different (ANOVA, p < 0.05)***

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Notably, *Pseudomonas sesami* R8 exhibited a broad spectrum of antifungal activity compared to the other strains, showing potent inhibition of *Aspergillus ochraceus* (50.37%). This strain also demonstrated moderate inhibitory effects against *Aspergillus flavus* (23.90%), *Botrytis cinerea* (23.41%), and *Fusarium oxysporum* (16.30%). Additionally, three strains i.e., *Pseudomonas versuta* R15, *Pseudomonas helleri* R125, and *Pseudomonas trivialis* R150, displayed a highly similar inhibition pattern, effectively suppressing *Fusarium oxysporum* and *Aspergillus ochraceus*, but showing no inhibitory activity against *Aspergillus flavus* and *Botrytis cinerea*. Finally, *Pseudomonas protegens* P79 exhibited vigorous antifungal activity against *Fusarium oxysporum*, with an inhibition rate of 55.56%.

3.7 Stress effect

The resistance of the isolates to salinity, high temperature, and alkaline pH was assessed and is summarized in Table 5. Among the five PGPR strains, *Pseudomonas protegens* P79 was the most tolerant, capable of growing at salinity levels up to 13% NaCl, which correlates with its native soil habitat. This strain also tolerated extreme temperatures of up to 45 °C and an alkaline pH of 11. In comparison, *Pseudomonas helleri* R125 and *Pseudomonas versuta* R15 exhibited moderate resistance, growing in the presence of 12% and 11% NaCl, respectively. Both strains also withstood temperatures of 42 °C and a pH of 10. *Pseudomonas sesami* R8 showed the lowest tolerance, with growth observed at 11% NaCl, pH 10, and a maximum temperature of 37 °C.

Table 5. Bacterial tolerance to stress conditions

Strain	Salinity	Temperature	pH
P79	13%	45°C	11
R8	9%	37°C	10
R15	11%	42°C	10
R125	12%	42°C	10
R150	9%	42°C	10

Values are presented as the means of 3 replicates.

3.8 Inoculation of *Lotus creticus*

The effects of the five rhizobacterial strains on the growth of *Lotus creticus* plants were investigated (Figures 4 and 5). Results from Figure 4 showed significant variation in the aerial part length among the inoculated plants, with the highest value recorded in plants treated with *Pseudomonas versuta* R15. However, no significant differences ($p > 0.05$) were observed in root length between inoculated plants and the control.

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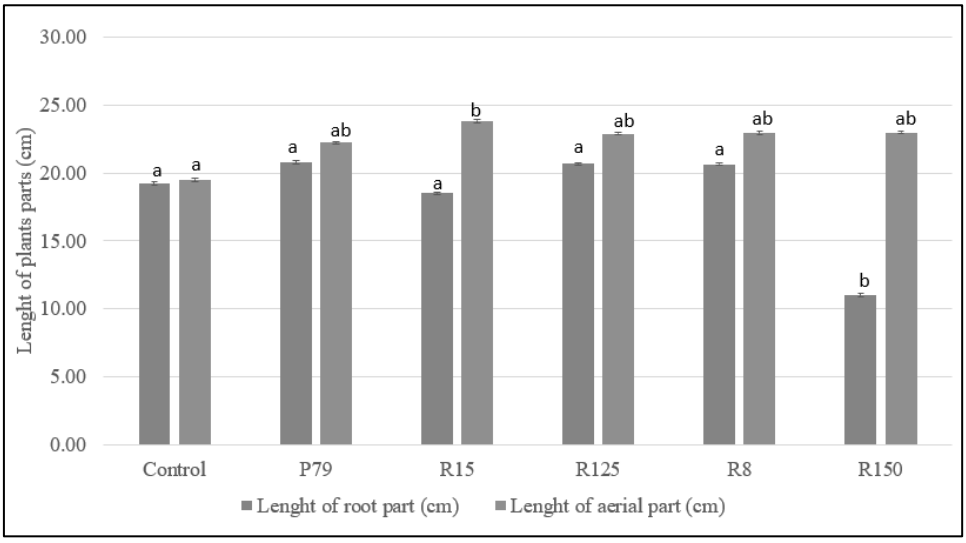

Figure 4: Effect of bacterial strains on the length of root and aerial parts compared to control. ***Values are presented as means from 3 replicates. Bars with different letters are significantly different (ANOVA, p < 0.05)***

Additionally, the impact of the five bacterial strains on the fresh and dry weights of both aerial and root parts of *L. creticus* was assessed (Figure 5). The highest dry weight values of aerial parts ($p < 0.05$) were observed in plants inoculated with *Pseudomonas helleri* R125 and *Pseudomonas trivialis* R150, followed by those treated with *Pseudomonas protegens* P79 and *Pseudomonas sesami* R8, though to a lesser extent. In contrast, *Pseudomonas protegens* P79 had the most pronounced effect on the root dry weight compared to the control.

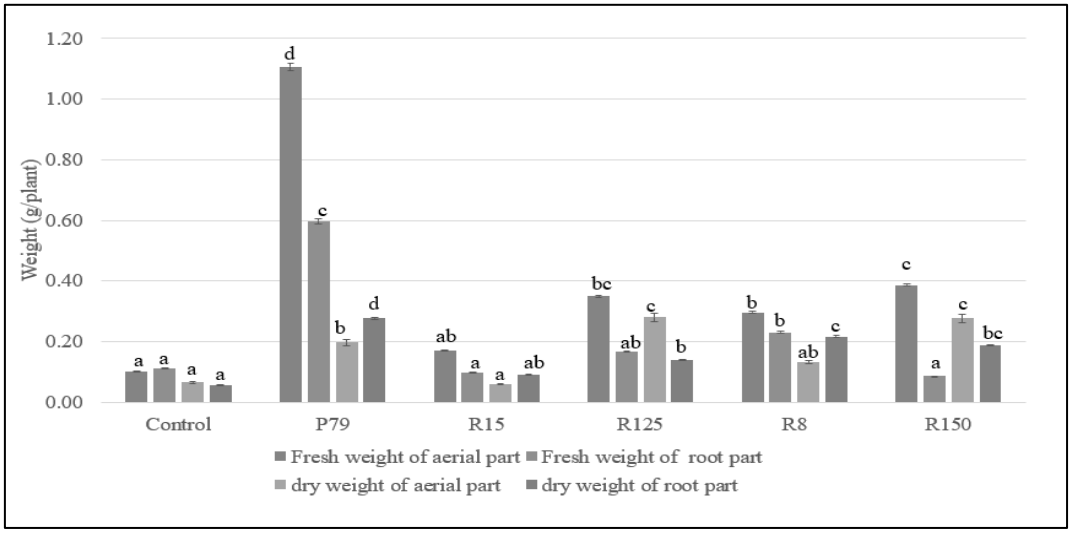

Figure 5: Effect of bacterial strains on the fresh and dry weights of aerial and root parts. ***Values are presented as means from 3 replicates. Bars with different letters are significantly different (ANOVA, p < 0.05)***

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4 Discussion

From an ecological perspective, *L. creticus* demonstrates remarkable adaptability to grow in harsh environments, including saline and nutrient-deficient soils. It produces a high biomass of dry material, comparable to that reported by Tlili and Louhaichi (2021). The species also exhibited a high nodulation rate, consistent with the findings of Belechheb et al. (2020), indicating a strong symbiotic relationship with rhizobial bacteria. This essential biological process aids in the conversion of atmospheric nitrogen into bioavailable forms, reducing reliance on synthetic fertilizers (Abd-Alla et al., 2023). The ability of *L. creticus* to thrive in such challenging environments can be largely attributed to its capacity to make osmotic and transpiration adjustments, as well as produce bioactive compounds such as hydroxybenzoic acid and gallic acid, which are necessary for maintaining photosynthetic activity under stress conditions (Rejili et al., 2008; Araniti et al., 2014; Barreira et al., 2017). On the other hand, soil microbiota has been extensively reported to play a critical role in enhancing nutrient uptake and stress tolerance (Howieson et al., 2011; Rima et al., 2018; Belechheb et al., 2020). In this study, five rhizobacterial strains within the *Pseudomonas* genus were isolated from the rhizosphere of *Lotus creticus* growing wild in the coastal region of Morocco. Some of these species exhibit promising characteristics for sustainable agriculture and ecological restoration. These bacteria are known for their ability to solubilize phosphate, promote plant vigor by producing organic acids, such as indole-3-acetic acid (IAA), a major phytohormone involved in root initiation and cell division, which can lower pH values and increase phosphorus availability (Tan et al., 2023; Janati et al., 2023). Additionally, Bakki et al. (2024) and Fitriatin et al. (2022) reported that *Pseudomonas* species utilize various organic acids, including gluconic, oxalic, and citric acids, for phosphate solubilization. *Pseudomonas protegens* has also been reported for its plant protection and biocontrol properties, including the induction of systemic resistance and the production of antimicrobial substances such as pyoluteorin and 2,4-diacetylphloroglucinol, which target specific fungal taxa (Balthazar et al., 2022; Garrido-Sanz et al., 2023). Among the tested *Pseudomonas* species, *Pseudomonas protegens* P79 stands out for its enzymatic activities, such as cellulase and protease production, which play a key role in the decomposition of organic matter into plant-available nutrients (Daunoras et al., 2024). It also exhibits significant chitinase production, highlighting its biocontrol potential, as chitinase can degrade fungal cell walls and prevent the proliferation of pathogens such as *Fusarium oxysporum* and *Botrytis cinerea* (Malik et al., 2023). Additionally, the strain demonstrated great tolerance to abiotic stresses such as salinity and alkaline pH, which are often associated with arid and semi-arid regions. This suggests its potential application in biotechnological processes under extreme conditions, including saline agriculture, industrial bioprocesses, and phytoremediation of degraded soils. On the other hand, *Pseudomonas helleri* R125 was able to grow at high salinity levels, indicating special adaptations to its habitat and demonstrating its potential for the bioremediation of saline soils. The strain significantly promoted shoot development, which aligns with the findings of Mehmood et al. (2023), who reported that *Pseudomonas* species can enhance plant growth, particularly in saline and stressful environments, due to their ability to produce phytohormones, solubilize phosphate, and assimilate a wide range of carbon sources (Von Neubeck et al., 2016). Similarly, *Pseudomonas trivialis* R150 significantly enhanced the growth of both the shoot and root parts of *L. creticus*, owing to its production of indole-3-acetic acid, ACC deaminase activity, and phosphate solubilization capacity. In comparison, the inoculation of *L. creticus* with *Pseudomonas versuta* R15 resulted in a low dry

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yield, suggesting that the role of the siderophore-producing strain in plant growth and nutrient uptake may be significant, as *Pseudomonas versuta* R15 was unable to produce siderophore compounds. Additionally, its ability to degrade organic pollutants makes it a promising candidate for environmental bioremediation (See-Too et al., 2017; Shabayev et al., 2020). Finally, *Pseudomonas sesami* R8 demonstrated a broad spectrum of antifungal activity, inhibiting the growth of *Aspergillus ochraceus*, *Aspergillus flavus*, *Botrytis cinerea*, and *Fusarium oxysporum*. This finding is consistent with Sehrawat et al. (2022), who reported that HCN-producing *Pseudomonas* strains are effective in inhibiting phytopathogens. Initially isolated from the rhizosphere of *Sesamum indicum* L. (Madhaiyan et al., 2017), *Pseudomonas sesami* was also able to promote root dry weight, underscoring its crucial role in nutrient cycling (Khan et al., 2023).

5 Conclusion

This research underscores the potential of five *Pseudomonas* strains, most notably *Pseudomonas protegens* P79, which exhibited strong IAA production, high phosphate solubilization ability (150.5 $mg \cdot L^{-1}$), and notable salinity tolerance as effective biostimulants. These strains were isolated from the rhizosphere of *Lotus creticus* naturally growing in northwestern Morocco and show promise for promoting sustainable agriculture and restoring Mediterranean coastal pastures. Nevertheless, further field-based studies are required to assess their practical effectiveness in improving plant growth, nutrient acquisition, and resilience to environmental stresses under harsh conditions.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Achkouk Imane and Arakrak Abdelhay. The first draft of the manuscript was written by Achkouk Imane and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Financial support

No funding was received to assist with the preparation of this manuscript.

Acknowledgements

The authors would like to thank the National Centre for Scientific and Technical Research (CNRST) for gene sequencing analysis.

Data availability

The data used in this study are available from the corresponding author upon reasonable request.

Code availability

No code was used to generate or analyse the data in this study.

References

Abd-Alla, M. H., Al-Amri, S. M., and El-Enany, A.-W. E.: Enhancing rhizobium–legume symbiosis and reducing nitrogen fertilizer use are potential options for mitigating climate change, *Agriculture*, 13, 2092, https://doi.org/10.3390/agriculture13112092, 2023.

https://doi.org/10.5194/egusphere-2026-298
Preprint. Discussion started: 11 February 2026

Ahmad, F., Ahmad, I., and Khan, M. S.: Screening of free-living rhizospheric bacteria for their multiple plant growth-promoting activities, *Microbiol. Res.*, 163, 173–181, https://doi.org/10.1016/j.micres.2006.04.001, 2008.

Ainane, A., Abdoul-Latif, F. M., and Ainane, T.: *Culture des plantes légumineuses en Afrique: un système agricole durable*, Éditions Universitaires Européennes, ISBN 978-620-341-419-6, 2021.

Ames, B. N.: Assay of inorganic phosphate, total phosphate and phosphatases, *Methods Enzymol.*, 8, 115–118, https://doi.org/10.1016/0076-6879(66)08014-5, 1966.

AOAC: *Official Methods of Analysis*, 16th edn., Association of Official Analytical Chemists, Gaithersburg, MD, 1997.

Araniti, F., Sunseri, F., and Abenavoli, M. R.: Phytotoxic activity and phytochemical characterization of *Lotus ornithopodioides* L., a spontaneous species of the Mediterranean area, *Phytochem. Lett.*, 8, 179–183, 2014.

Bakker, A. W. and Schippers, B.: Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth stimulation, *Soil Biol. Biochem.*, 19, 451–457, https://doi.org/10.1016/0038-0717(87)90037-X, 1987.

Bakki, M., Banane, B., Marhane, O., Esmaeel, Q., Hatimi, A., Barka, E. A., Azim, K., and Bouizgarne, B.: Phosphate-solubilizing *Pseudomonas* and *Bacillus* combined with rock phosphates promoting tomato growth and reducing bacterial canker disease, *Front. Microbiol.*, 15, 1289466, https://doi.org/10.3389/fmicb.2024.1289466, 2024.

Balthazar, C., St-Onge, R., Léger, G., Lamarre, S. G., Joly, D. L., and Filion, M.: Pyoluteorin and 2,4-diacetylphloroglucinol are major contributors to *Pseudomonas protegens* Pf-5 biocontrol against *Botrytis cinerea* in cannabis, *Front. Microbiol.*, 13, 945498, https://doi.org/10.3389/fmicb.2022.945498, 2022.

Barreira, J. C., Visnevschi-Necrasov, T., Pereira, G., Nunes, E., and Oliveira, M. B. P.: Phytochemical profiling of underexploited Fabaceae species: insights on ontogenic and phylogenetic effects over isoflavone levels, *Food Res. Int.*, 100, 517–523, 2017.

Bashan, Y., de-Bashan, L. E., Prabhu, S. R., and Hernandez, J.-P.: Advances in plant growth-promoting bacterial inoculant technology: formulations and practical perspectives (1998–2013), *Plant Soil*, 378, 1–33, https://doi.org/10.1007/s11104-013-1956-x, 2014.

Belechheb, T., Bakkali, M., Laglaoui, A., and Arakrak, A.: Characterization and efficiency of rhizobial isolates nodulating *Cytisus monspessulanus* L. in the northwest of Morocco in relation to environmental stresses, Springer, https://doi.org/10.1007/978-981-15-2576-6_3, 2020.

Bower, C. A., Reitemeier, R. F., and Fireman, M.: Exchangeable cation analysis of saline and alkali soils, *Soil Sci.*, 73, 251–262, https://doi.org/10.1097/00010694-195204000-00001, 1952.

Bric, J. M., Bostock, R. M., and Silverstone, S. E.: Rapid in situ assay for indoleacetic acid production by bacteria immobilized on nitrocellulose membrane, *Appl. Environ. Microbiol.*, 57, 535–538, 1991.

Brink, B.: Urease test protocol, American Society for Microbiology, 2010.

Broughton, W. J. and Dilworth, M. J.: Methods in legume–rhizobium technology: plant nutrient solutions, in: *Handbook of Rhizobia*, 245–249, 1970.

Cappuccino, J. C. and Sherman, N.: *Microbiology: A Laboratory Manual*, 3rd edn., Benjamin/Cummings, New York, 1992.

Chen, W. P. and Kuo, T. T.: A simple and rapid method for the preparation of Gram-negative bacterial genomic DNA, *Nucleic Acids Res.*, 21, 2260, https://doi.org/10.1093/nar/21.9.2260, 1993.

Daunoras, J., Kačergius, A., and Gudiukaitė, R.: Role of soil microbiota enzymes in soil health and activity changes depending on climate change and the type of soil ecosystem, *Biology*, 13, 85, https://doi.org/10.3390/biology13020085, 2024.

Fitriatin, B. N., Fauziah, D. A., Hindersah, R., and Simarmata, T.: Influence of different acidic conditions on the plant growth-promoting rhizobacteria activity of phosphate-solubilizing bacteria, *KnE Life Sci.*, 7, 72–78, 2022.

Garrido-Sanz, D., Martínez-García, P. M., Redondo-Nieto, M., Rivilla, R., and Martín, M.: Comparative genomics of *Pseudomonas protegens* highlights its biocontrol potential, *Microb. Genom.*, 9, e001234, https://doi.org/10.1099/mgen.0.001234, 2023.

https://doi.org/10.5194/egusphere-2026-298
Preprint. Discussion started: 11 February 2026

Howieson, J. G., Ballard, R. A., Yates, R. J., et al.: Selecting improved *Lotus*-nodulating rhizobia to expedite the development of new forage species, *Plant Soil*, 348, 231–243, https://doi.org/10.1007/s11104-011-0921-9, 2011.

Howieson, J. G. and Dilworth, M. J.: *Working with Rhizobia*, ACIAR Monograph No. 173, Australian Centre for International Agricultural Research, Canberra, Australia, 2016.

Jacobson, C. B., Pasternak, J. J., and Glick, B. R.: Partial purification and characterization of 1-aminocyclopropane-1-carboxylate deaminase from the plant growth-promoting rhizobacterium *Pseudomonas putida* GR12-2, *Can. J. Microbiol.*, 40, 1019–1025, 1994.

Janati, W., Bouabid, R., Mikou, K., Ghadraoui, L. E., and Errachidi, F.: Phosphate-solubilizing bacteria from soils with varying environmental conditions: occurrence and function, *PLoS ONE*, 18, e0289127, https://doi.org/10.1371/journal.pone.0289127, 2023.

Javadzadeh, M., Khavazi, K., Ghanavati, N., Jafarnejadi, A. R., and Jahandideh Mahjenabadi, V. A.: Utilizing indigenous plant growth-promoting rhizobacteria and sulfur to improve wheat yield and nutrient uptake in saline–sodic soils, *Eurasian Soil Sci.*, 56, 1101–1113, https://doi.org/10.1134/S106422932360015X, 2023.

Madhaiyan, M., Poonguzhali, S., Saravanan, V. S., et al.: *Pseudomonas sesami* sp. nov., a plant growth-promoting Gammaproteobacterium isolated from the rhizosphere of *Sesamum indicum* L., *Antonie van Leeuwenhoek*, 110, 843–852, https://doi.org/10.1007/s10482-017-0859-x, 2017.

Miller, G. L.: Use of dinitrosalicylic acid reagent for determination of reducing sugar, *Anal. Chem.*, 31, 426–428, https://doi.org/10.1021/ac60147a030, 1959.

Novitsky, T. J. and Kushner, D. J.: Influence of temperature and salt concentration on the growth of a facultatively halophilic *Micrococcus* sp., *Can. J. Microbiol.*, 21, 107–110, https://doi.org/10.1139/m75-017, 1975.

Pikovskaya, R. I.: Mobilization of phosphorus in soil in connection with the vital activity of some microbial species, *Mikrobiologiya*, 17, 362–370, 1948.

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

Sehrawat, A., Sindhu, S. S., and Glick, B. R.: Hydrogen cyanide production by soil bacteria: biological control of pests and promotion of plant growth in sustainable agriculture, *Pedosphere*, 32, 15–38, https://doi.org/10.1016/S1002-0160(21)60058-9, 2022.

Von Neubeck, M., Huptas, C., Glück, C., et al.: *Pseudomonas helleri* sp. nov. and *Pseudomonas weihenstephanensis* sp. nov., isolated from raw cow's milk, *Int. J. Syst. Evol. Microbiol.*, 66, 1163–1173, https://doi.org/10.1099/ijsem.0.000852, 2016.

Walkley, A. and Black, I. A.: An examination of the method for determining soil organic matter and a proposed modification of the chromic acid titration method, *Soil Sci.*, 37, 29–38, https://doi.org/10.1097/00010694-193401000-00003, 1934.

Weisburg, W. G., Barns, S. M., Pelletier, D. A., and Lane, D. J.: 16S ribosomal DNA amplification for phylogenetic study, *J. Bacteriol.*, 173, 697–703, https://doi.org/10.1128/jb.173.2.697-703.1991, 1991.

Younsi, S. E. and Bouziane, Z.: Plant diversity in Mediterranean coastal dune systems subjected to anthropogenic disturbances, *Biodivers. Res. Conserv.*, 72, 25–38, https://doi.org/10.14746/biorc.2023.72.4, 2023.