

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~~enhancing plant performance ~~in~~under saline and nutrient-~~poor soils~~limited soil conditions, where plant establishment is strongly constrained. In this study, the plant growth-promoting potential of rhizobacteria isolated from the rhizosphere of *Lotus creticus* growing naturally ~~growing in~~along the Mediterranean ~~coastal regions~~coast of ~~northwestern~~ Morocco was evaluated. This is the first report on rhizosphere-associated bacteria linked to *L. creticus* in Mediterranean coastal zones. 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*~~*L. 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·L⁻¹), and notable cellulase and protease activities. It also demonstrated high tolerance to salinity (up to 13% NaCl) and ~~elevated temperature~~temperature (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~~*helleri* R125 and *Pseudomonas* ~~*helleri* R125~~*trivialis* R150 significantly enhanced the aerial dry biomass ~~and shoot length~~ of ~~*Lotus*~~*L. creticus* (300%), while *Pseudomonas protegens* P79 was more effective in promoting root elongation (8%) under controlled growth chamber conditions. This study highlights ~~five the potential of~~ *Pseudomonas* strains, ~~particularly *Pseudomonas protegens* P79~~, as promising biostimulants for sustainable agriculture and the rehabilitation of ~~saline coastal~~ marginal lands, ~~due to their diverse plant growth promoting traits~~soils under combined salinity and nutrient stress.

Keywords: *Lotus creticus* L., PGPR, *Pseudomonas* ~~spp~~spp, 16S rRNA, ~~marginal coastal~~ soils.

1 Introduction

Coastal ~~pastures~~lands are agroecosystems ~~located near marine environments and are found in many temperate regions around~~
35 ~~the world, including the Mediterranean (Romano et al., 2022). These unique ecosystems that support a variety of various~~ plant
species adapted to saline, nutrient-poor soils ~~and. They also~~ serve as valuable habitats for wildlife, providing food and breeding
grounds for ~~various~~ livestock ~~species~~ ~~(Romano et al., 2022; Younsi and Bouziane, 2023). However, they have undergone~~
~~significant decline globally due to~~ Despite their ecological importance, these coastal ecosystems are increasingly threatened by
climate change, overgrazing, and anthropogenic pressures, ~~resulting in leading to~~ habitat ~~loss, reduced degradation,~~ biodiversity
40 ~~loss, and declining soil degradation quality~~ (Younsi and Bouziane, 2023; ~~El Yemlahi~~). As in many regions worldwide,
Mediterranean coastal soils are generally sandy or weakly developed, exhibiting elevated salinity, low organic matter and
nutrient availability, and poor water-holding capacity, which together impose strong constraints on plant establishment and
growth (Ferreira et al., 2022; Sun et al., 2026).
Therefore, ~~et al., 2025~~ ~~To address these challenges, it is essential to implement sustainable management practices and~~
45 ~~ecological restoration strategies are required to preserve their ecological functions and maintain their role in supporting~~
biodiversity ecosystem functionality and support agricultural productivity. Legumes (Fabaceae) ~~have often been utilized as~~
~~eco-friendly solutions are widely recognized as effective biological tools~~ for sustainable revegetation ~~to address the challenges~~
~~of modern agriculture (El Yemlahi et al., 2024; and soil restoration (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
50 chemical fertilizers and promoting sustainable farming systems (~~Petrushin Abd-Alla et al., 2023). One such legume is. Among~~
them, *Lotus creticus* L., commonly known as Cretan trefoil. Native a perennial legume native to the Mediterranean region, this
pastoral and forage legume is particularly well-known for its ability adapted to thrive in saline and arid environments (~~Kew~~
~~Science, 2024). This remarkable adaptability makes *L. creticus* an excellent coastal environments (Belechheb et al., 2021). Its~~
high salinity and poor soil tolerance make it a promising candidate for soil stabilization and ~~reclamation efforts (ICARDA,~~
55 ~~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 rehabilitation of degraded coastal areas (Navarro et al., 2023). Like ~~other legumes, the root zone of many~~
leguminous plants, *L. creticus* can may harbor a variety of beneficial ~~microorganisms bacteria~~ known as plant growth-
promoting rhizobacteria (PGPR) in its rhizosphere. These bacteria have been extensively studied and demonstrated significant
60 potential to enhance plant growth and stress tolerance under saline and nutrient-limited conditions (Vejan et al., 2016; de
Andrade et al., 2023; Nisa et al., 2026). They promote plant performance through multiple mechanisms, including nutrient
acquisition, phytohormone production, and stress alleviation (Sharma et al., 2025). 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. For instance, phosphate solubilization improves phosphorus availability in calcareous soils, siderophore production enhances iron uptake under alkaline conditions, and ACC deaminase activity reduces ethylene-induced stress under salinity (Pattnaik et al., 2021; Khan et al., 2022; Yang et al., 2026). Additionally, these microorganisms can induce systemic tolerance and improve plant resilience to abiotic 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). Although most investigations have centered on cultivated species like wheat, rice, and maize, the rhizobacterial communities of wild coastal legumes, particularly *Lotus creticus*, remain insufficiently explored. Under coastal environmental conditions, rhizosphere processes, particularly microbial-mediated nutrient mobilization, play a key role in plant survival and growth (Adeleke et al., 2024; Chu et al., 2025). Those environments also represent environmentally challenging ecosystems that provide a relevant natural model for studying plant-microbe interactions under combined salinity and nutrient limitations (Marien et al., 2023).

In this context, assessing the functional diversity of rhizobacteria associated with wild coastal legumes is key to understanding how soil limitations shape microbially mediated plant adaptation in saline coastal soils. Therefore, this study aims to identify bacterial strains isolated from the rhizospheric soil of *L. creticus* and characterize rhizobacteria associated with *L. creticus* with strong, evaluate their plant growth-promoting (PGP) traits. These strains could serve as effective, and assess their potential as bioinoculants to support the growth-improve plant performance in Mediterranean coastal environments. Such results provide new insights into the role of rhizosphere bacteria in enhancing plant growth and productivity of *L. creticus* in saline, marginal soils adaptation under saline and nutrient-limited conditions.

2 Materials and methods

2.1 Plant and soil sampling

Bacteria were isolated from the rhizosphere of *L. creticus*, which grows naturally. Plant samples of *L. creticus* were collected in March 2024 from a natural population of *L. creticus* growing wild in the coastal zone region of northwestern Morocco, near Tahadart beach. Soil samples Beach (35°34'56.0"N, 5°59'23.5"W). The region is characterized by a Mediterranean climate, with approximately 525 mm of annual precipitation and an average temperature of 19.1 °C. Sampling was conducted at the late flowering stage on mobile dunes. Three independent biological replicate plots of 1 m² were randomly established from the surface layer were also area near the seashore to capture spatial variability. Each replicate corresponds to a composite sample collected from each plot. Samples (stems and leaves) were oven-dried at 60 °C to constant weight prior to analysis. The dried samples were analyzed for crude protein content using the Kjeldahl method (AOAC, 1997) and for organic matter by incineration at 550 °C for 12 h (AOAC, 1997).

For bacterial isolation, plant roots from each plot were carefully excavated, and loosely adhering soil was removed by gentle shaking and collected in sterile plastic bags and stored at 4 °C for analysis. The soil tightly attached to the roots was then recovered by brushing the roots into the same bags. Also, rhizosphere soil firmly adhering to the roots was sampled from the

0-20 cm surface layer. Electrical conductivity (EC) is measured by the saturated paste method using an ORION brand conductivity meter, model 162 (USSLS, 1954). The exchangeable sodium percentage (ESP) was calculated as $ESP = (Na^+ / CEC) \times 100$, where CEC represents the cation exchange capacity (FAO, 1970). Total soil nitrogen was measured using the Kjeldahl method (ISO, 1995). Total 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 $CaCO_3$ 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 μ L 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 ~~were streaked~~ 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 genomic DNA was extracted using the phenol/~~chloroform~~ ~~protocol established method described~~ by Chen and Kuo (1993). DNA concentration ~~was measured and purity were determined~~ using a NanoDrop spectrophotometer (Thermo Scientific™ NanoDrop 2000). ~~Two universal primers, rD1 and fD1, were used to amplify the~~ The nearly full-length 16S rRNA gene ~~sequence, following the methodology of~~ was amplified using the universal primers rD1 and fD1, as described by Weisburg et al. (1991). ~~The amplified DNA-PCR products were initially assessed via~~ verified by horizontal agarose gel electrophoresis (70 V for 1 hour) in Tris-acetate-EDTA (TAE) buffer. ~~Subsequently, The amplified fragments~~ were purified and subjected to cycle sequencing ~~analysis was performed~~ using the same primers for the 16S rRNA PCR amplification. The resulting primer set. The obtained sequences (≈ 1500 bp) were assembled and edited using BioEdit software (version 7.0.5.3), ~~and manually verified, and compared with sequences in GenBank~~ checked for quality. Sequence similarity searches were performed using the BLAST algorithm (<https://blast.ncbi.nlm.nih.gov/blast.cgi/>). ~~Finally, against the GenBank database. The closest type strains and their corresponding sequence similarities (%) were~~

130 recorded. The nucleotide sequences generated in this study were deposited in GenBank under accession numbers: OM403655, OM420233, OQ324690, OM317560, and OM403658. For phylogenetic analysis, sequences were aligned with reference sequences retrieved from GenBank using ClustalW implemented in MEGA version 11. A phylogenetic tree was constructed using the neighbor-joining method ~~with~~based on Kimura's two-parameter model ~~in MEGA software version 11.~~ The robustness of the tree topology was evaluated by bootstrap analysis with 1000 replicates.

135 **2.5 Assessment of PGP traits**

2.5.1 ~~2.5.1~~ **Phosphate solubilization**

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

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

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
145 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-

Through nitrogen enrichment and biocontrol in the rhizosphere, ammonia production improves nutrient availability and
150 contributes to plant growth under stress conditions (Yang et al., 2025). 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 to brown upon the addition of Nessler's reagent.

2.5.3 Production of hydrogen cyanide (HCN)

155 The isolates were evaluated for their ability to produce hydrogen cyanide (HCN), a key trait involved in the suppression of soil-borne pathogens and the maintenance of rhizosphere health (Schrawat et al., 2022). 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).

160 **2.5.4 Production of siderophores**

~~Bacterial~~ Siderophore secretion facilitates iron acquisition under limiting conditions by chelating Fe³⁺ and improving its bioavailability to plants (Timofeeva et al., 2022). The 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

ACC (1-aminocyclopropane-1-carboxylate) deaminase activity enables rhizobacteria to mitigate plant stress by lowering ethylene levels through the degradation of its precursor (Orozco-Mosqueda et al., 2020). 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 ((NH₄)₂SO₄).

2.5.6 Production of ~~Indole Acetic Acid (IAA)~~

IAA (Indole-3-acetic acid) is a phytohormone involved in the regulation of root development and the stimulation of cell elongation and division (Etesami and Glick, 2024). To evaluate the ability of the isolated bacteria to synthesize ~~indole 3-acetic acid (IAA)~~, 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% FeCl₃ [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. Through the degradation of cellulose-rich compounds, cellulase production supports carbon transformation and nutrient recycling in soil environments (Datta et al., 2024). 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 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. Amylase activity reflects the capacity of microorganisms to hydrolyze starch into assimilable sugars, supporting microbial metabolism and soil biochemical processes (Morbia et al., 2024). 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~~Chitinase production is linked to antifungal activity through the enzymatic degradation of chitin, a major structural component of fungal cell walls (Veliz et al., 2017). To confirm chitinase synthesis by the bacterial isolates, colloidal chitin (C₈H₁₃O₅N)_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.~~**Production of protease.** Protease activity contributes to nitrogen cycling by facilitating the degradation of proteinaceous substrates and may also play a role in suppressing pathogenic microorganisms (Adamczyk et al., 2021). 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~~Urease activity catalyzes the hydrolysis of urea into ammonia, contributing to nitrogen transformation in soils (Zhang et al., 2026). Urease production was detected by the alkalization 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. ~~By converting hydrogen peroxide into water and oxygen, catalase plays a key role in protecting cells from oxidative damage caused by reactive oxygen species~~ (Roy et al., 2022). 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

The antagonistic potential of rhizobacteria against phytopathogens represents a critical mechanism of biological control, limiting pathogen growth through direct or indirect interactions (Espinosa-Palomeque et al., 2025). The bacteria were assessed for their potential to inhibit the growth of four plant pathogenic fungi, *Fusarium oxysporum*, *Botrytis cinerea*, *Aspergillus ochraceus*, and *Aspergillus flavus*, chosen for their prevalence and significant impact on various important legumes in the Mediterranean climate, including *L. creticus* (Maciá-Vicente et al., 2008; Tsitsigiannis et al., 2012). 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 the diameter of the control culture and ‘r2’ is the diameter of the treated culture.

2.7 Stress effect

2.7.1 Effect of NaCl

~~To determine~~ Salt tolerance reflects the effectability of salinity on the growth microorganisms to survive and maintain metabolic activity under high osmotic pressure conditions typical of saline environments (Witzel et al., 2025). Salt tolerance of the selected strain, ~~was evaluated by streaking~~ a single colony ~~was streaked~~ onto Petri dishes containing King’s B (KB) medium supplemented with ~~different~~increasing NaCl concentrations of NaCl, ranging from 0% to 14% [(w/v)]. ~~The plates~~. Plates were incubated at ~~28-28~~ °C for ~~48 hours~~48 h in the dark. ~~Colony~~Bacterial growth was ~~monitored~~assessed qualitatively based on colony formation and compared ~~to salt free controls~~ with control plates without NaCl. All assays were performed in triplicate (Novitsky and Kushner, 1975)).

2.7.2 Effect of Temperature

~~Additionally, the isolates were examined for their~~ Temperature tolerance indicates the capacity of bacterial strains to withstand thermal stress and maintain functional stability across a range of environmental conditions (Sharma et al., 2025). The tolerance of the bacterial isolates to heat stress. ~~The bacteria were streaked~~ was assessed by streaking them onto King’s B (KB) agar medium and incubated for 24 hours in the dark plates and incubating at ~~different~~various temperatures (30, 37, 42, 45, and ~~50-50~~ °C). ~~Colony growth was monitored~~ for 24 h in the dark. Growth was evaluated based on colony formation and compared to ~~controls~~control plates incubated at ~~28-28~~ °C (Ahmad et al., 2008). All experiments were conducted in triplicate.

2.7.3 Effect of pH

~~Tolerance~~ The ability to tolerate pH variations determines microbial viability and function by modulating enzyme activity and cellular homeostasis in acidic and/or alkaline pH-soils (Msimbira and Smith, 2020). Tolerance of the isolates to acidic and alkaline conditions was ~~assessed~~evaluated by streaking ~~bacteria~~bacterial isolates onto buffered King’s B (KB) medium, ~~with the pH adjusted to pH values of 3, 5, 6, 6.5, 7.5, 8.5, 9, and 10. A positive result was indicated by bacterial~~ Bacterial growth, ~~showing reflected~~ tolerance to the tested pH-levels, while ~~the its absence of growth suggested indicated~~ 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 plastic pots (25 × 22 × 15 cm) filled with 1 kg of sterilized soil obtained from the same location where *L. creticus* associated

soil- plants were originally collected and directly inoculated directly with 1 mL per seed of bacterial culture (10⁸ CFU colony-forming units/mL) grown in TSB. Each treatment consisted of three pots. Uninoculated pots served as controls. All pots were maintained under greenhouse conditions maintained at 25°C under a 16:8 h light-dark photoperiod and irrigated weekly with distilled water and/or a mineral solution (Broughton and Dilworth, 1970).

2.9 Statistical analysis

All results are experiments were performed in three independent replicates and presented as means ± standard deviation (SD) and). Differences among means were compared/evaluated using analysis of variance (ANOVA) followed by the Fisher protected once normality and homogeneity of variances had been verified. Significant differences were assessed using Fisher's Protected Least Significant Difference (LSD) test; (P<0.05). Statistical analyses were conducted using STATISTICA 13.3 software.

3 Results

3.1 Soil analysis and symbiosis evaluation

The on field survey revealed results of the preseneesoil analysis (Table 1) indicated the occurrence of *L. creticus* on in a sandy Arenosol, characterized by a high proportion of sand and low clay and silt fractions. The soil was alkaline sandy soil, low in (pH=8.4), showed moderate salinity (EC = 4.2 mS/cm) and low exchangeable sodium percentage (ESP = 6.3%). It was also nutrient-poor, containing very low organic matter (0.48%) and limited amounts of available phosphorus (3 ppm), potassium (45.2 ppm), phosphorus (3.0 ppm), and nitrogen (0.08%) (Table 1).-%).

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

<u>WRB</u> <u>Class</u>	<u>C</u> <u>(%)</u>	<u>FSi</u> <u>(%)</u>	<u>CSi</u> <u>(%)</u>	<u>FS</u> <u>(%)</u>	<u>CS</u> <u>(%)</u>	<u>EC</u> <u>(mS/cm)</u>	<u>pH</u> <u>($\frac{Ca+H_2}{O}$)</u>	<u>pH</u> <u>(KCl 1N)</u>	<u>MO</u> <u>OM</u> <u>(%)</u>	<u>P₂O₅P₂</u> <u>O_s</u> <u>(ppm)</u>	<u>K₂OK₂</u> <u>O</u> <u>(ppm)</u>	<u>N</u> <u>(%)</u>	<u>ESP</u> <u>(%)</u>
<u>Arenosol</u>	<u>1.3</u>	<u>1.3</u>	<u>3.6</u>	<u>69.5</u>	<u>24.3</u>	<u>4.2</u>	8.4	9.1	<u>0.44</u> <u>8</u>	<u>3.0</u>	45.2	0.08	<u>6.3</u>

WRB: World Reference Base, C: Clay, FSi: Fine Silt, CSi: Coarse Silt, FS: Fine Sand, CS: Coarse Sand, EC: Electrical conductivity, OM: Organic matter, ESP: exchangeable Sodium Percentage.

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.

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

Nodulation¹	DM (Kg/ha)	OM (%DM)	CP (%DM)
Abundant	781.25	91.32	12.60

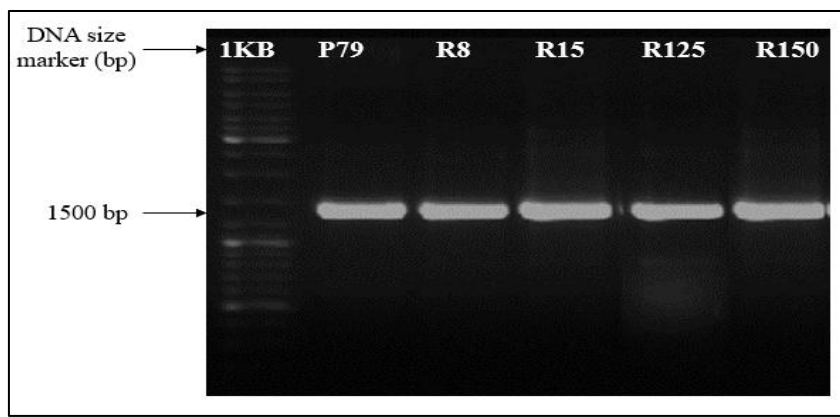
Values are presented as the mean of 3 replicates. DM: dry matter, OM: organic matter, CP: crude protein. ¹: using a nodule-scoring chart proposed by Howieson and Dilworth (2016).

3.2 Isolation and morphological characterization of rhizobacteria

285 Thirty bacteria were isolated from the rhizosphere of distinct *L. creticus* plants 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.

290 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).



295 **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.

300

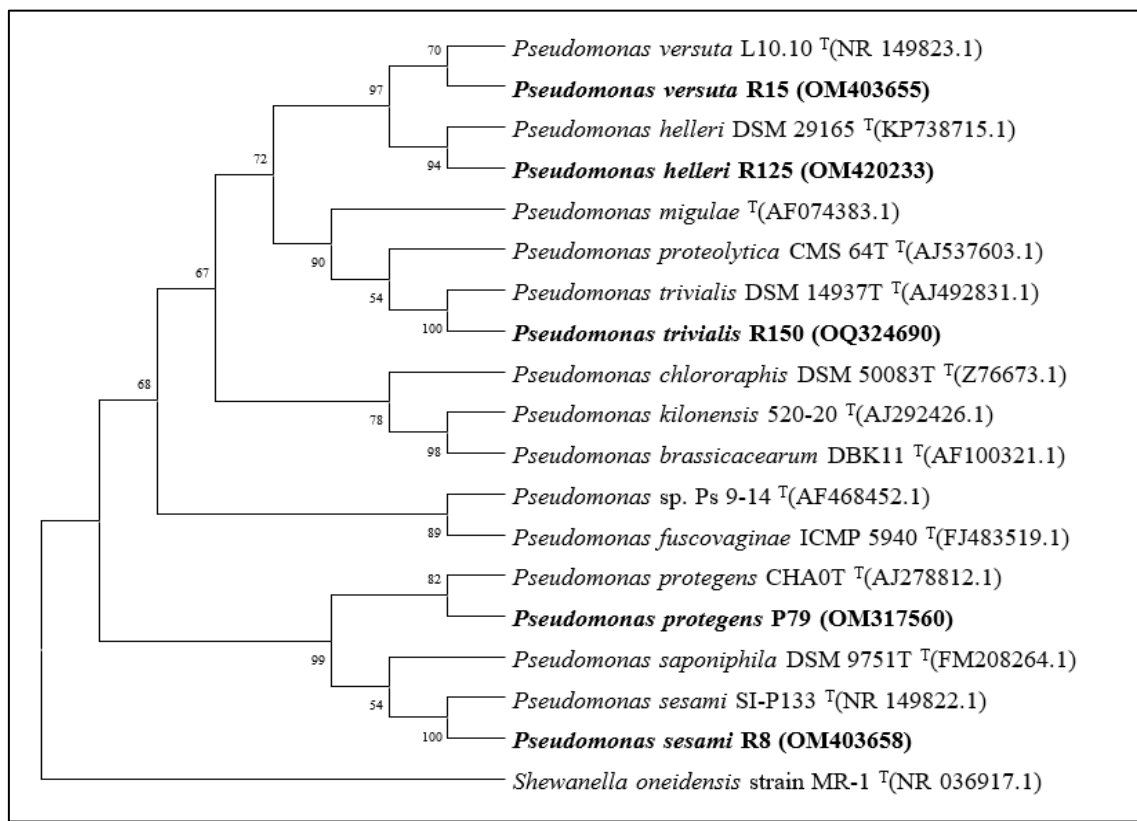


Figure 2: Phylogenetic tree based on the 16S rRNA gene sequences showing the relationship of the five *Pseudomonas* strains (**highlighted** in the tree) and other related members. The *Shewanella oneidensis* strain MR-1 acts as an outgroup to root the tree for comparison species. The tree was reconstructed by the Neighbor-Joining tree method. The bootstrap consensus tree inferred Bootstrap values are indicated as percentages derived from 1000 replicates. The tree is rooted with *Shewanella oneidensis* strain MR-1^T

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	0.770 ± 0.4	150.550 ± 0.2	5.16 ± 0.6	++	+	++	+++	+
R8	0.550 ± 0.1	59.70 ± 0.5	6.11 ± 0.4	+++	++	+++	+	+
R15	0.770 ± 0.5	76.09 ± 0.3	6.16 ± 0.1	+	-	++	+	+
R125	0.440 ± 0.2	91.25 ± 0.3	5.17 ± 0.5	-	++	+++	+	+
R150	0.440 ± 0.3	87.05 ± 0.1	4.65 ± 0.2	++	++	+++	+	+

Values are presented as the mean of 3 replicates. S.I: solubilization index, - IAA: Indole acetic acid, ACCD: ACC deaminase, P solubilization in $\text{mg}\cdot\text{L}^{-1}$, +, low activity; ++, moderate activity; +++, strong activity; -, no activity.

Pseudomonas protegens P79 exhibited the highest phosphate solubilization ($150.5 \text{ mg}\cdot\text{L}^{-1}$) along with moderate siderophore production. *Pseudomonas sesami* R8 stood out for its strong HCN production. However, it displayed the lowest phosphate solubilization capacity ($59.70 \text{ mg}\cdot\text{L}^{-1}$). *Pseudomonas helleri* R125 and *Pseudomonas trivialis* R150 showed similar profiles, both exhibiting moderate phosphate solubilization (91.25 and $87.05 \text{ mg}\cdot\text{L}^{-1}$, respectively) and ammonia production. *Pseudomonas versuta* R15 demonstrated relatively low phosphate solubilization ($76.09 \text{ mg}\cdot\text{L}^{-1}$) 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.

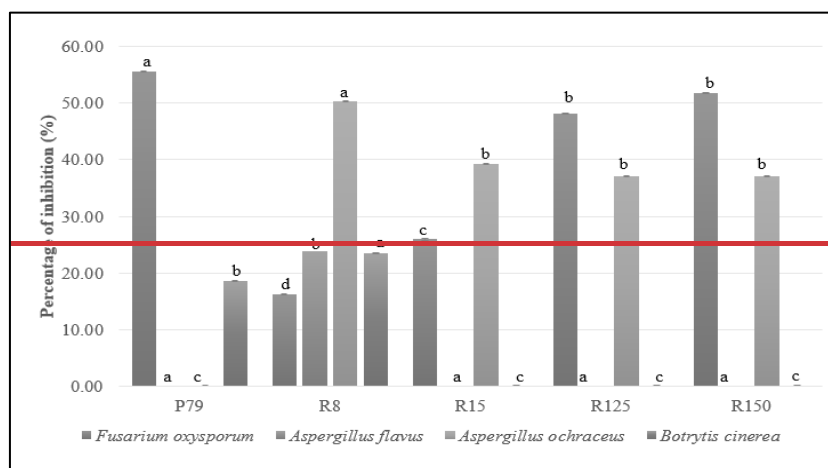


Figure 3: Percentage of growth inhibition of phytopathogenic fungi by *Pseudomonas* strains. Values are presented as means from 3 replicates. Bars with different letters are significantly different (ANOVA, $p < 0.05$)

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%.

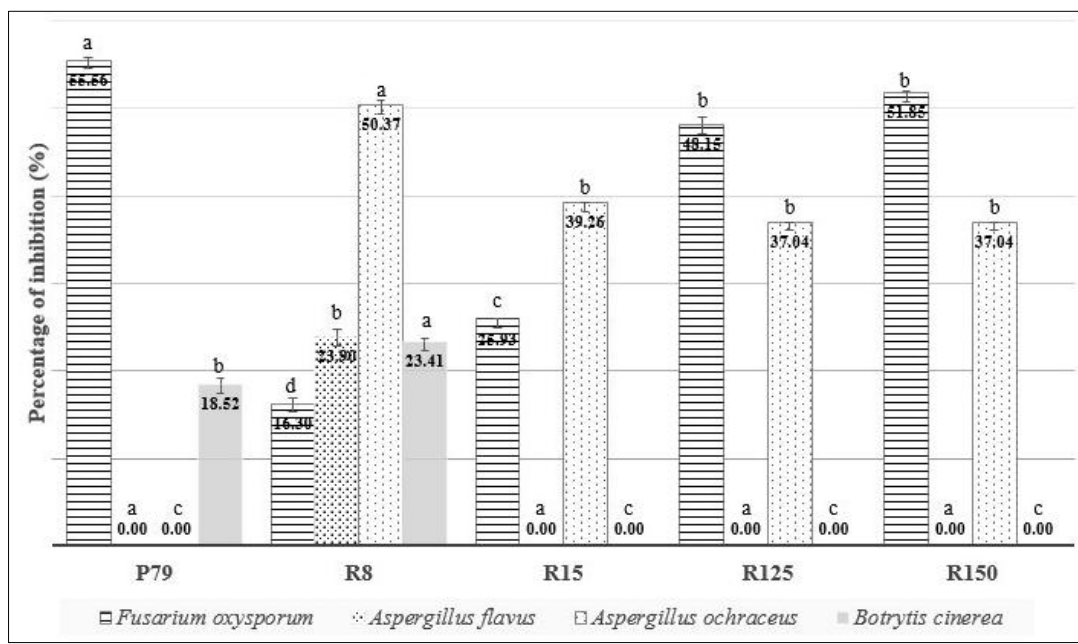


Figure 3: Percentage of growth inhibition of phytopathogenic fungi by *Pseudomonas* strains. Values are expressed as mean $\pm$ SD of three independent replicates ($n = 3$ pots). Different letters indicate significant differences (ANOVA, $p < 0.05$).

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 9% NaCl, pH 10, and a maximum temperature of 37 °C.

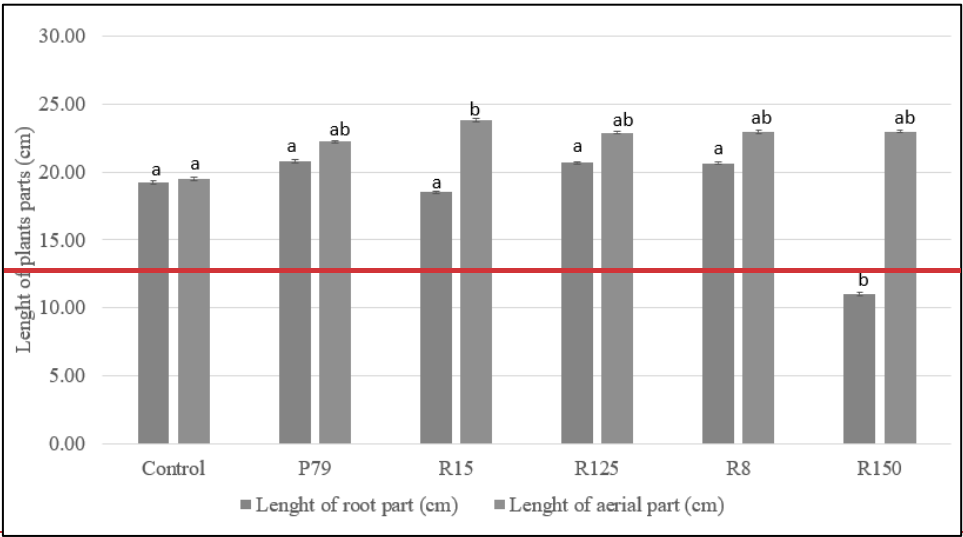
Table 5. Bacterial tolerance to stress conditions

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 *LotusL. 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 reduction~~ in root length ~~between inoculated plants and~~ was observed under R150 inoculation compared to the control- and most other treatments.



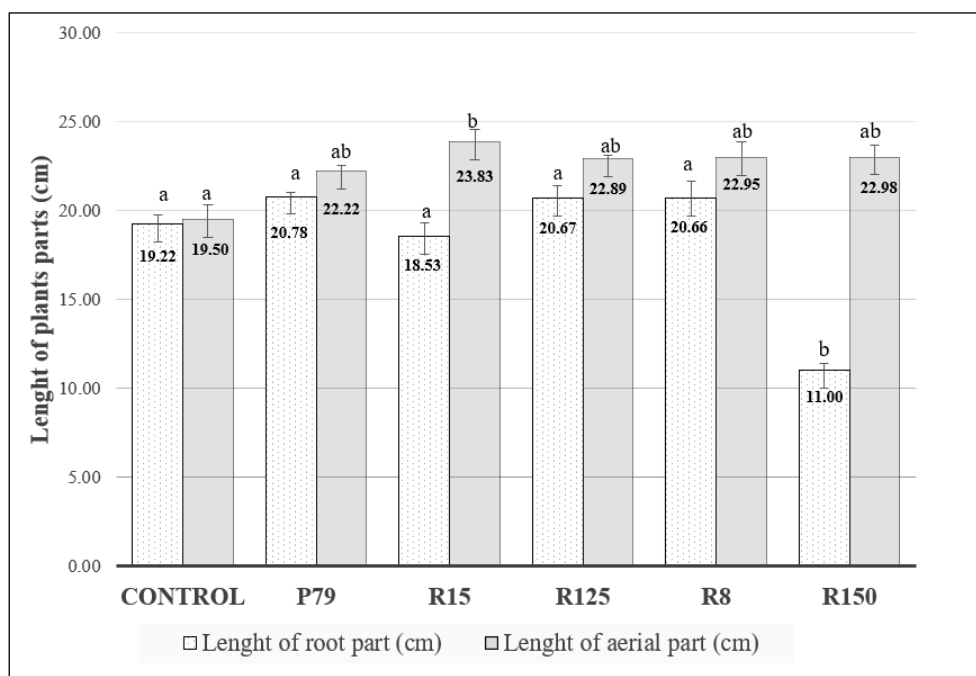


Figure 4: Effect of bacterial strains on the length of root and aerial parts compared to the control. Values are presented expressed as means from 3-mean $\pm$ SD of three independent replicates. Bars with different (n = 3 pots). Different letters are significantly different indicate significant differences (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 ($p \leq 0.05$) 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.

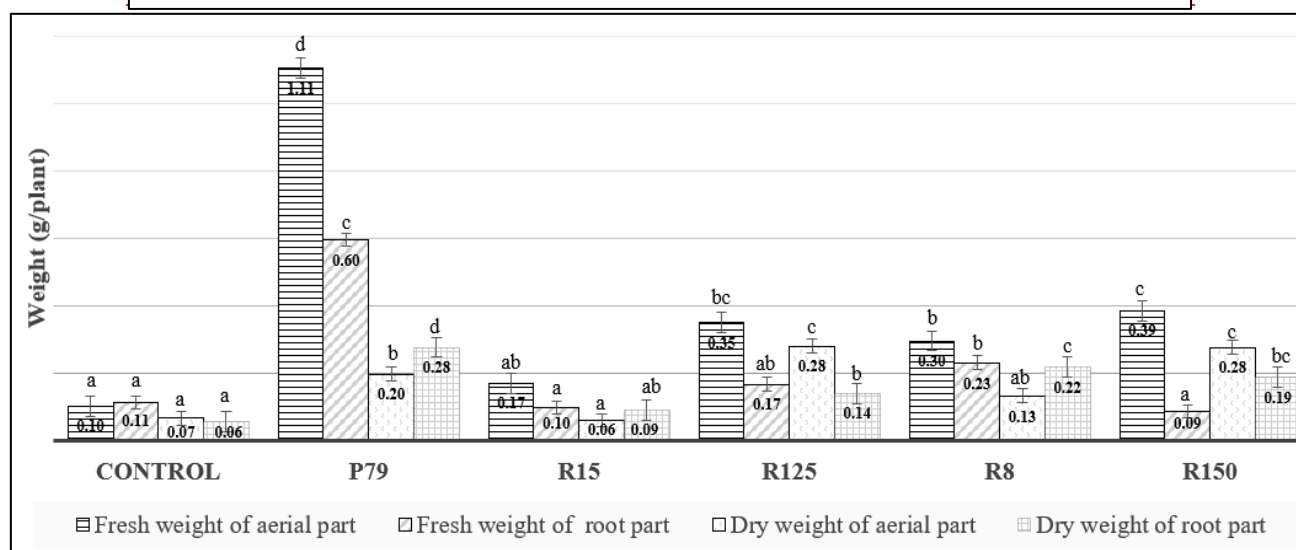
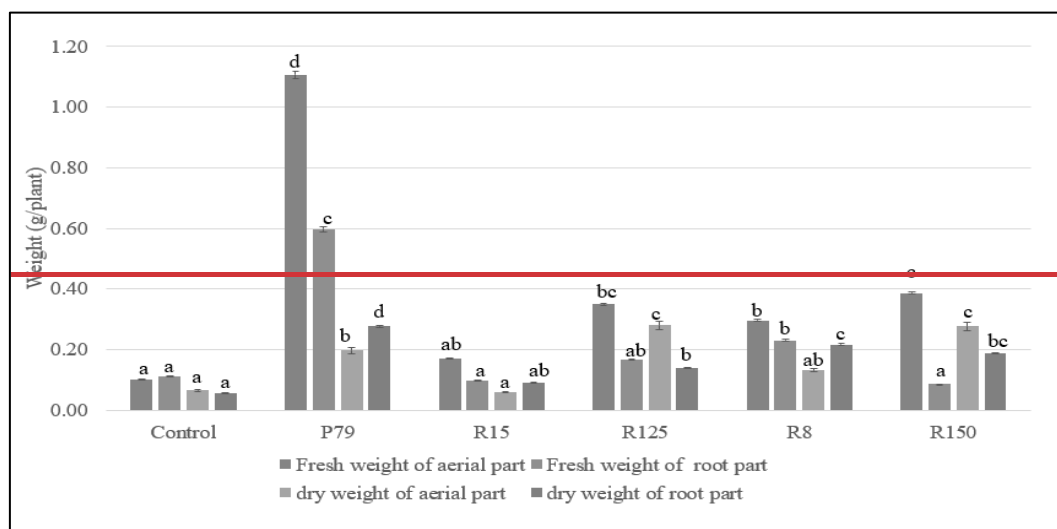


Figure 5: Effect of bacterial strains on the fresh and dry weights of aerial and root parts. Values are presented expressed as means from 3 mean $\pm$ SD of three independent replicates. Bars with different (n = 3). Different letters are significantly different indicate significant differences (ANOVA, $p < 0.05$).

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; Beleecheb 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 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, underseoring its crucial role in nutrient cycling (Khan et al., 2023).

In Mediterranean coastal regions, *L. creticus* is among the plant species that are well adapted to saline and nutrient-poor environments. Such adaptation is mainly attributed to the capacity of the species to make osmotic and transpiration adjustments, and produce bioactive compounds such as hydroxybenzoic acid and gallic acid, necessary for maintaining photosynthetic activity under stress conditions (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 mobilization and uptake as well as stress tolerance (Howieson et al., 2011; Rima et al., 2018).

In this study, the soil where *L. creticus* was identified was characterized as a sandy Arenosol with an alkaline pH, moderate salinity, and very low levels of organic matter. Such conditions are known to impose multiple constraints on plant growth, including water leaching due to sandy texture, nutrient immobilization and precipitation, under alkaline pH conditions, and osmotic stress associated with salinity (Zhu et al., 2024; Sun et al., 2026). In general, legumes are salt-sensitive, and symbiosis is often limited at high salinity (Bruning and Rozema, 2013). However, the *L. creticus* was able to maintain vigorous growth and high capacity of nodulation, indicating the functional importance of rhizosphere microorganisms. In this context, five rhizobacterial strains of the genus *Pseudomonas* were isolated from the rhizosphere of *L. creticus* growing wild in the coastal region of Morocco. These strains exhibited notable plant growth-promoting traits that support plant development in environments where nutrient availability is often limited by leaching and ionic imbalances (Sun et al., 2026). The relative contribution of these traits varied significantly among strains, reflecting a synergistic effect. At the strain level, distinct functional profiles indicate that the five *Pseudomonas* isolates may contribute to plant performance through different but complementary mechanisms that collectively support plant establishment and growth in constrained soil environments. Direct effects include plant-level physiological traits such as IAA production, known to stimulate root system development and improve the plant's capacity to explore the soil and nutrient uptake (Etesami and Glick, 2024). Also, ACC deaminase activity plays a key role in alleviating stress-induced ethylene accumulation and mitigating growth inhibition under adverse environmental conditions (Gamalero et al., 2023). In contrast, soil-mediated effects involve processes like phosphate solubilization and siderophore-based iron chelation. It could also include ammonia production, which affects rhizosphere pH and enhances nutrient solubility and availability (Yang et al., 2025).

Remarkably, all five *Pseudomonas* species, particularly *Pseudomonas protegens* P79, were able to solubilize tricalcium phosphate into soluble forms that can be readily absorbed by plants. Phosphorus is an essential element for plant growth; however, its uptake is often restricted due to its immobilization in soils, particularly sandy soils, where rapid leaching and low organic matter content reduce its uptake by plants (Gerke et al., 2024). In alkaline, calcareous coastal sandy soils, phosphate tends to precipitate by forming insoluble complexes with calcium ions, thereby decreasing its bioavailability (Johan et al., 2021). Increasing phosphate solubilization not only enhances plant growth but also increases microbial biomass P, as

microorganisms rapidly assimilate the released phosphate. This microbial pool acts as a temporary reservoir that is gradually made available to plants and contributes to phosphorus cycling at the soil-root interface. Additionally, the increase in dry weight observed with *Pseudomonas protegens* P79 may be attributed to its metabolic versatility and its ability to produce a wide array of extracellular enzymes, which facilitate organic matter decomposition and nutrient release under nutrient-limited conditions (Daunoras et al., 2024), but also to its ability to synthesize phytohormones such as IAA.

Likewise, the *in vitro* analysis revealed that all selected strains, except *Pseudomonas versuta* R15, produced siderophore compounds, which are known to enhance iron uptake (Kong et al., 2014). In sandy soils with low organic matter content, iron is commonly present as insoluble Fe³⁺ oxides, which limit its uptake by plants (Singh et al., 2022; Yamagata et al., 2022). Iron deficiency can affect various physiological processes (Singh et al., 2022). In response, many siderophore-producing rhizobacteria can chelate these Fe³⁺ forms and mobilize them into soluble complexes that enhance iron bioavailability (Yamagata et al., 2022). In this regard, results from the pot trial indicated that *Pseudomonas helleri* R125 and *Pseudomonas trivialis* R150 markedly improved the shoot and root dry biomass of *Lotus creticus*, likely due to their capacity to mobilize nutrients from the soil, particularly phosphorus and iron. In contrast, inoculation of *L. creticus* with *Pseudomonas versuta* R15 led to lower dry biomass but greater shoot length, suggesting that this strain promotes cell elongation, likely through hormonal effects rather than biomass accumulation (Kurepa et al., 2026).

Also, *Pseudomonas sesami* R8 was able to promote the root dry weight of *L. creticus*, underscoring its potential role in nutrient cycling (Kumar et al., 2021). However, the strain exhibited relatively low tolerance to abiotic stress *in vitro* compared with other *Pseudomonas* species. This suggests that plant growth promotion is primarily driven by functional traits, such as phytohormone production and nutrient solubilization, which can stimulate root development independently of stress tolerance (Glick, 2012). Although the strain shows limited stress tolerance under *in vitro* conditions, it can still exert beneficial effects *in vivo* through plant-associated protection and microbial interactions (Compant et al., 2010; Song et al., 2025). Additionally, secondary metabolites associated with biocontrol also contribute to improved rhizosphere competence and root colonization efficiency (Lugtenberg and Kamilova, 2009). Notably, *Pseudomonas sesami* R8 demonstrated strong antifungal activity against all tested fungi. A particular effect that could be attributed to the ability of the strain to produce secondary metabolites, such as ammonia and HCN, which restrict fungal growth, reduce their access to soil nutrients, and enhance rhizosphere health (Sehrawat et al., 2022). These findings suggest a functional bias of *Pseudomonas sesami* R8 toward biocontrol rather than abiotic stress tolerance (Sehrawat et al., 2022). In practice, the strain could be applied as a seed coating or soaking treatment to protect *L. creticus* seeds, suppress fungal colonization, and enhance early plant development. Alternatively, they could be introduced directly into the rhizosphere through soil inoculation (Paravar et al., 2023). For foliar pathogens such as *Botrytis cinerea*, spraying bacterial suspensions onto leaves may inhibit spore germination and lesion development (Roca-Couso et al., 2021).

Overall, these findings demonstrate the functional diversity of rhizosphere-associated *Pseudomonas* strains and their importance in supporting plant establishment and resilience under combined abiotic stresses of the coastal environment. Most importantly, the ability of strains to grow under high salinity conditions, often associated with arid and semi-arid coastal regions, indicates their capacity to remain metabolically active in saline soils (Wang et al., 2025).

5 Conclusion

This ~~research underscores the potential of study indicates that~~ five *Pseudomonas* strains, ~~most notably~~ particularly *Pseudomonas protegens* P79, ~~which exhibited strong IAA production, originating from Mediterranean coastal soils, can tolerate high phosphatesalinity and temperature while enhancing important soil functions such as phosphorus solubilization ability (150.5 mg·L⁻¹), and notable salinity tolerance as effective biostimulants. These strains were isolated from the~~, iron availability, and rhizosphere ~~of *Lotus creticus* naturally growing~~ enzymatic activity. Taken together, the findings emphasize the important role of diverse PGPR communities in northwestern Morocco and show promise for promoting sustainable agriculture ~~sustaining nutrient cycling and restoring Mediterranean coastal pastures~~, soil fertility in saline, nutrient-poor soils and in supporting plant adaptation to the physicochemical constraints of coastal ecosystems. Nevertheless, further field-based studies, ~~including single or co-inoculation trials~~, are required to ~~asses~~ evaluate their ~~practical~~ effectiveness in ~~improving~~ enhancing plant growth, nutrient acquisition, and resilience ~~to environmental stresses~~ under ~~harsh~~ natural conditions.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Imane Achkoui, Imane and Arakrak Abdelhay Arakrak. The first draft of the manuscript was written by Achkoui 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.

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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~~ analyze the data in this study.

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