

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 enhancing plant performance under saline and nutrient-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 along the Mediterranean coast of 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 *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 temperature (up to 45°C). Conversely, *Pseudomonas sesami* R8 exhibited broad-spectrum antifungal activity, including strong inhibition of *Aspergillus ochraceus*. The pot experiment revealed that inoculation with *Pseudomonas helleri* R125 and *Pseudomonas trivialis* R150 significantly enhanced the aerial dry biomass of *L. creticus* (300%), while *Pseudomonas protegens* P79 was more effective in promoting root elongation (8%) under growth chamber conditions. This study highlights the potential of *Pseudomonas* strains as promising biostimulants for sustainable agriculture and the rehabilitation of coastal marginal soils under combined salinity and nutrient stress.

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

1 Introduction

Coastal lands are agroecosystems that support various plant species adapted to saline, nutrient-poor soils. They also serve as valuable habitats for wildlife, providing food and breeding grounds for livestock (Romano et al., 2022; Younsi and Bouziane, 2023). Despite their ecological importance, these coastal ecosystems are increasingly threatened by climate change, overgrazing, and anthropogenic pressures, leading to habitat degradation, biodiversity loss, and declining soil quality (Younsi and Bouziane, 2023). 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, sustainable management and ecological restoration strategies are required to preserve ecosystem functionality and support agricultural productivity. Legumes (Fabaceae) are widely recognized as effective biological tools for sustainable revegetation 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 chemical fertilizers and promoting sustainable farming systems (Abd-Alla et al., 2023). Among them, *Lotus creticus* L., a perennial legume native to the Mediterranean region, is particularly well adapted to saline and arid coastal environments (Belechheb et al., 2021). Its high salinity and poor soil tolerance make it a promising candidate for soil stabilization and rehabilitation of degraded coastal areas (Navarro et al., 2023). Like many leguminous plants, *L. creticus* may harbor beneficial bacteria known as plant growth-promoting rhizobacteria (PGPR) in its rhizosphere. These bacteria have been extensively studied and demonstrated significant 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). 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; Mekureyaw et al., 2022; 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 isolate and characterize rhizobacteria associated with *L. creticus*, evaluate their plant growth-promoting traits, and assess their potential as bioinoculants to improve plant performance in Mediterranean coastal environments. Such results provide new

insights into the role of rhizosphere bacteria in enhancing plant growth and adaptation under saline and nutrient-limited conditions.

2 Materials and methods

2.1 Plant and soil sampling

Plant samples of *L. creticus* were collected in March 2024 from a natural population of *L. creticus* growing wild in the coastal region of northwestern Morocco, near Tahadart 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 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 bags. 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).

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 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 method described by Chen and Kuo (1993). DNA concentration and purity were determined using a NanoDrop spectrophotometer (Thermo Scientific™ NanoDrop 2000). The

nearly full-length 16S rRNA gene was amplified using the universal primers rD1 and fD1, as described by Weisburg et al. (1991). PCR products were verified by horizontal agarose gel electrophoresis (70 V for 1 h) in Tris-acetate-EDTA (TAE) buffer.

100 The amplified fragments were purified and subjected to cycle sequencing using the same primer set. The obtained sequences (≈ 1500 bp) were assembled and edited using BioEdit software (version 7.0.5.3) and manually checked for quality. Sequence similarity searches were performed using the BLAST algorithm against the GenBank database. The closest type strains and their corresponding sequence similarities (%) were recorded. The nucleotide sequences generated in this study were deposited in GenBank under accession numbers: OM403655, OM420233, OQ324690, OM317560, and OM403658. For phylogenetic

105 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 based on Kimura's two-parameter model. The robustness of the tree topology was evaluated by bootstrap analysis with 1000 replicates.

2.5 Assessment of PGP traits

2.5.1 Phosphate solubilization

110 The bacterial isolates were tested for their ability to solubilize tricalcium phosphate (TCP), which increases the pool of plant-available phosphorus in soils. A 10 μ L aliquot of fresh bacterial culture was inoculated onto PVK agar medium, served as the sole phosphorus source, and incubated at 28 $^{\circ}$ C for 7 days (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):

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

115 The isolates were further evaluated for their ability to solubilize tricalcium phosphate (TCP) in liquid medium by inoculating 500 μ L of bacterial culture into 50 mL of PVK liquid medium. Sterile, uninoculated media served as controls. Both inoculated and control media were incubated for 7 days at 28 $^{\circ}$ 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).

120 2.5.2 Production of ammonia

Through nitrogen enrichment and biocontrol in the rhizosphere, ammonia production improves nutrient availability and 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 $^{\circ}$ C for 48 to 72 hours. Ammonia production was indicated by a color change from yellow to brown upon the addition

125 of Nessler's reagent.

2.5.3 Production of hydrogen cyanide (HCN)

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 (Sehrawat et al., 2022). To estimate HCN production, 100 μ L of bacterial culture was streaked onto KB agar plates supplemented with 4.4 g of glycine. Filter paper discs (9 cm in diameter),

130 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

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
135 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
140 ethylene levels through the degradation of its precursor (Orozco-Mosqueda et al., 2020). The isolates were evaluated for their ability to synthesize ACC 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).

145 2.5.6 Production of 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 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
150 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
155 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,
160 supporting microbial metabolism and soil biochemical processes (Morbía 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. 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. 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 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

200 Salt tolerance reflects the ability of 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 onto Petri dishes containing King’s B (KB) medium supplemented with increasing NaCl concentrations ranging from 0 to 14% (w/v). Plates were incubated at 28 °C for 48 h in the dark. Bacterial growth was assessed qualitatively based on colony formation and compared with control plates without NaCl. All assays were performed in triplicate (Novitsky and
205 Kushner, 1975).

2.7.2 Effect of Temperature

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 was assessed by streaking them onto King’s B (KB) agar plates and incubating at various temperatures (30, 37, 42, 45, and 50 °C)
210 for 24 h in the dark. Growth was evaluated based on colony formation and compared to control plates incubated at 28 °C. All experiments were conducted in triplicate.

2.7.3 Effect of pH

The ability to tolerate pH variations determines microbial viability and function by modulating enzyme activity and cellular homeostasis in acidic or alkaline soils (Msimbira and Smith, 2020). Tolerance of the isolates to acidic and alkaline conditions
215 was evaluated by streaking bacterial isolates onto buffered King’s B (KB) medium adjusted to pH values of 3, 5, 6, 6.5, 7.5, 8.5, 9, and 10. Bacterial growth reflected tolerance to the tested pH, while its absence indicated sensitivity.

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
220 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* plants were originally collected and directly inoculated with 1 mL per seed of bacterial culture (10⁸ 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
225 and/or a mineral solution (Broughton and Dilworth, 1970).

2.9 Statistical analysis

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

3 **Results**

3.1 **Soil analysis and symbiosis evaluation**

235 The results of the soil analysis (Table 1) indicated the occurrence of *L. creticus* in a sandy Arenosol, characterized by a high proportion of sand and low clay and silt fractions. The soil was alkaline (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), and nitrogen (0.08%).

Table 1. Soil physicochemical properties of the sampling site

WRB Class	C (%)	FSi (%)	CSi (%)	FS (%)	CS (%)	EC (mS/cm)	pH (H₂O)	pH (KCl 1N)	OM (%)	P₂O₅ (ppm)	K₂O (ppm)	N (%)	ESP (%)
Arenosol	1.3	1.3	3.6	69.5	24.3	4.2	8.4	9.1	0.48	3	45.2	0.08	6.3

240 *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.*

245 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).

250 **3.2 Isolation and morphological characterization of rhizobacteria**

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.

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).

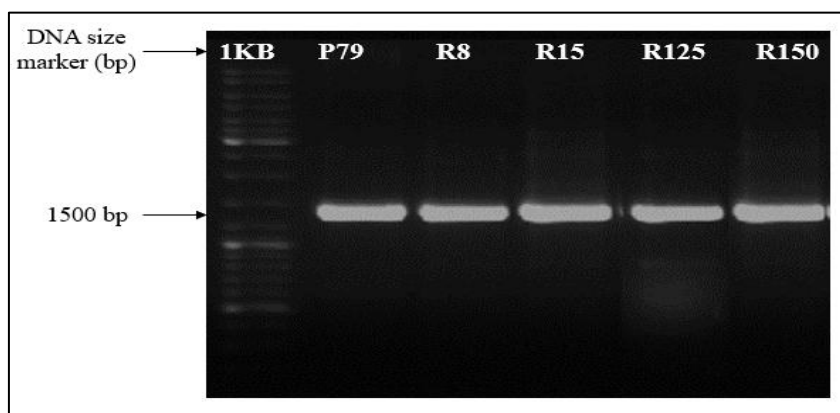


Figure 1: Agarose gel electrophoresis analysis of 16S rRNA genes amplified from 5 bacterial isolates.

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.

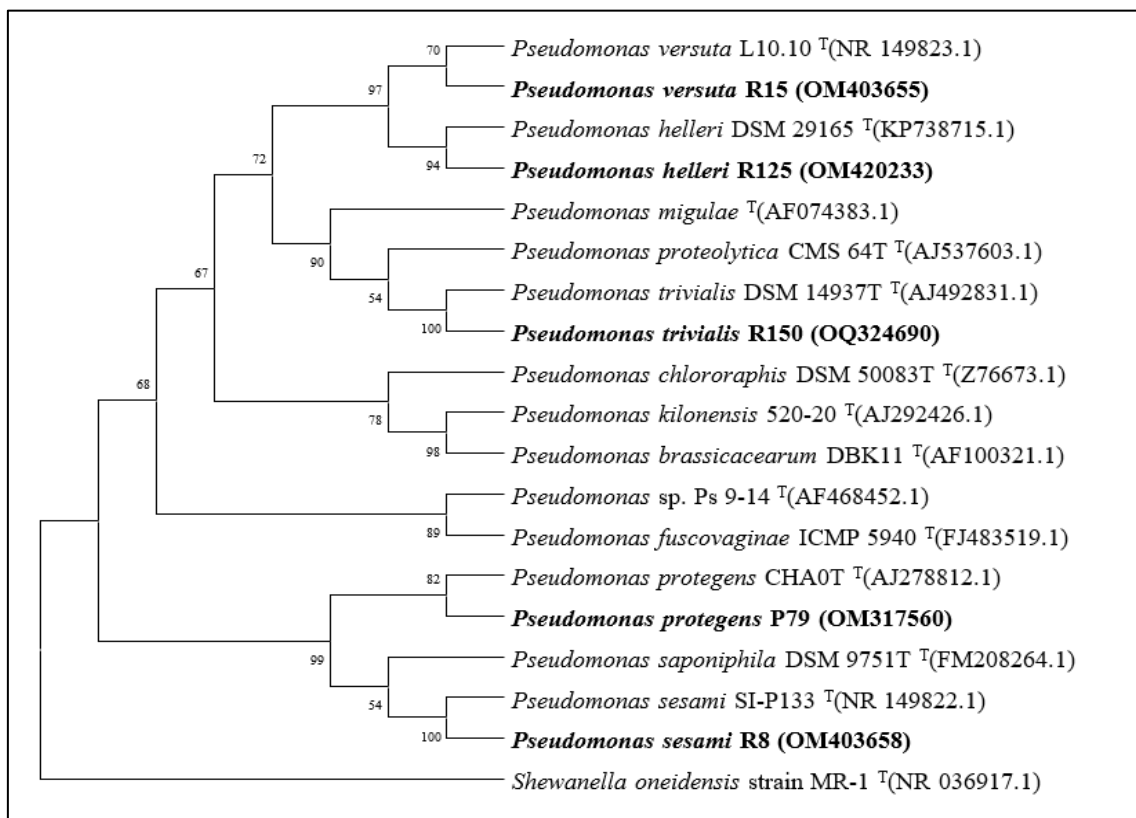


Figure 2: Phylogenetic tree based on the 16S rRNA gene sequences showing the relationship of the five *Pseudomonas* strains (highlighted in the tree) and other related species. The tree was reconstructed by the Neighbor-Joining tree method. Bootstrap values are indicated as percentages derived from 1000 replications. 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	0.70 ± 0.4	150.50 ± 0.2	5.16 ± 0.6	++	+	++	+++	+
R8	0.50 ± 0.1	59.70 ± 0.5	6.11 ± 0.4	+++	++	+++	+	+
R15	0.70 ± 0.5	76.09 ± 0.3	6.16 ± 0.1	+	-	++	+	+
R125	0.40 ± 0.2	91.25 ± 0.3	5.17 ± 0.5	-	++	+++	+	+
R150	0.40 ± 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 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, 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. 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%.

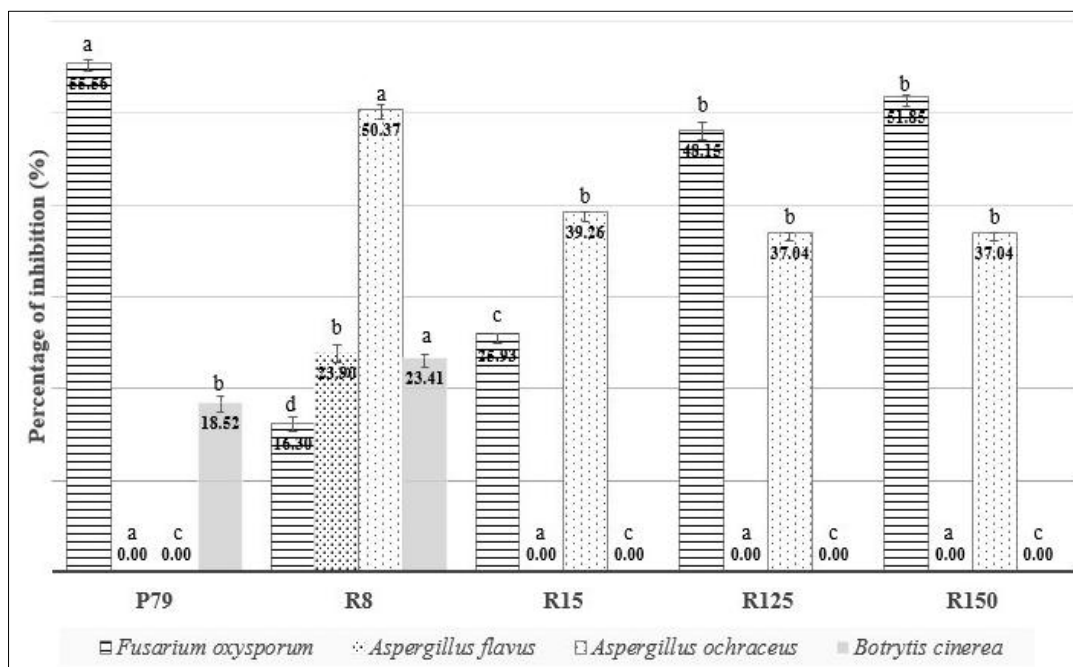


Figure 3: Percentage of growth inhibition of phytopathogenic fungi by *Pseudomonas* strains. Values are expressed as mean $\pm$ SD of three independent replicates ($n = 3$ pots). Different letters indicate significant differences (ANOVA, $p < 0.05$).

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.

320 **3.8 Inoculation of *Lotus creticus***

The effects of the five rhizobacterial strains on the growth of *L. creticus* plants were investigated (Figures 4 and 5). Results (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. A significant reduction in root length was observed under R150 inoculation compared to the control and most other treatments.

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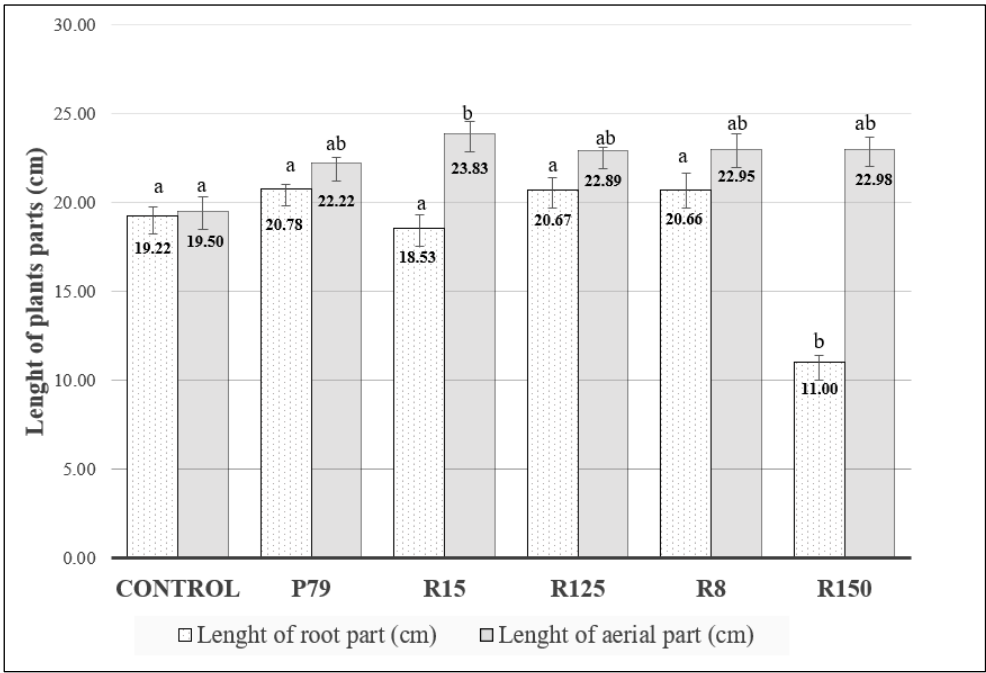


Figure 4: Effect of bacterial strains on the length of root and aerial parts compared to the control. Values are expressed as mean $\pm$ SD of three independent replicates ($n = 3$ pots). Different letters indicate significant differences (ANOVA, $p < 0.05$).

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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.

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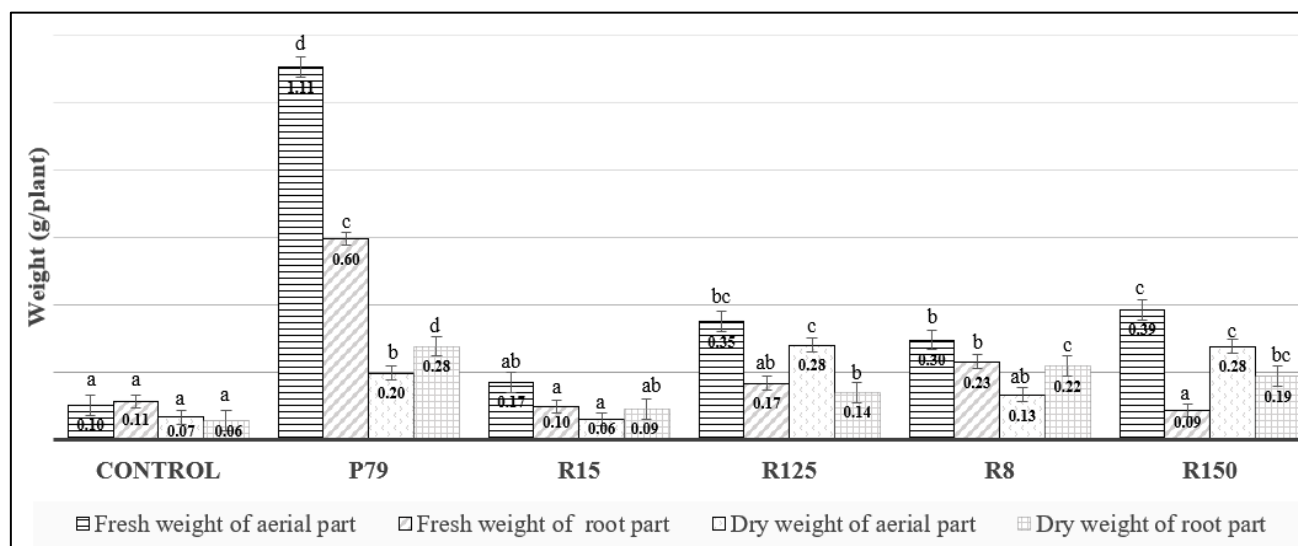


Figure 5: Effect of bacterial strains on the fresh and dry weights of aerial and root parts. Values are expressed as mean $\pm$ SD of three independent replicates ($n = 3$). Different letters indicate significant differences (ANOVA, $p < 0.05$).

4 Discussion

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 Fe^{3+} 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 Fe^{3+} 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

395 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
400 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
405 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
410 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 study indicates that five *Pseudomonas* strains, particularly *Pseudomonas protegens* P79, originating from Mediterranean coastal soils, can tolerate high salinity and temperature while enhancing important soil functions such as phosphorus
415 solubilization, iron availability, and rhizosphere enzymatic activity. Taken together, the findings emphasize the important role of diverse PGPR communities in sustaining nutrient cycling and 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 evaluate their effectiveness in enhancing plant growth, nutrient acquisition, and resilience under natural conditions.

420 Author contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Imane Achkouk and Abdelhay Arakrak. 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

425 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 analyze the data in this study.

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