



Exploring plant growth-promoting bacteria from peanut (*Arachis hypogaea* L.) rhizosphere in Morocco's sandy soils: Potential for soil fertility improvement

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Abstract

Peanut (*Arachis hypogaea* L.) cultivation in Morocco's sandy soils is limited by poor soil fertility and high permeability, which restricts nutrient and water retention. This study aims to isolate, identify, and investigate native plant growth-promoting rhizobacteria to improve the growth and resilience of peanut. Fifteen bacterial isolates were recovered from the peanut rhizosphere collected from the surface layer of the sandy soils in northwestern Morocco, characterized by low phosphorus and organic matter contents. Two isolates, JR62 and JR86, exhibiting high phosphate-solubilizing activity (238.651 and 196.111 mg/L, respectively), were chosen and identified based on 16S rRNA gene sequencing analysis as being closely related to *Microbacterium oxydans* (PQ758594) and *Enterobacter asburiae* (OM101034), respectively. These strains were initially characterized for soil-related biochemical activities phosphate solubilization, siderophore production, and lytic enzyme activities given their pivotal roles in driving soil nutrient cycling and improving nutrient bioavailability. Subsequently, they were examined for additional plant growth-promoting attributes, including IAA production, ACC deaminase activity, HCN and ammonia synthesis, antifungal activity, and tolerance to multiple abiotic stresses (salinity, pH and temperature variability, and PEG-induced drought). Collectively, these functional traits underscore the strains' potential to substantially influence soil nutrient dynamics while promoting plant health. Inoculation of peanut plants with *E. asburiae* JR86 and *M. oxydans* JR62 significantly enhanced growth parameters. The JR86 strain demonstrated the highest increases in shoot (42%) lengths and biomass (180%), whereas JR62 resulted in increases of 24% and 62%, respectively. Such results are attributed to soil-mediated processes, including phosphate solubilization and iron chelation, which together enhance nutrient availability, promote soil nutrient cycling, and improve the fertility of sandy soils.

Keywords: *Arachis hypogaea* L., *Microbacterium oxydans*, *Enterobacter asburiae*, PGPR, inoculation, sandy soil fertility.

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Introduction

The peanut (*Arachis hypogaea* L.), known as groundnut, is an annual herbaceous legume from the *Fabaceae* family, widely cultivated for its 1) nutritional value, rich in oil, protein and essential mineral, 2) forage feeding, and 3) its economic income derived from its seeds and various by-products, including peanut oil, and butter (Khan et al., 2013; Davis and Dean, 2016).

Furthermore, peanut plants contribute to soil fertility improvement through their ability to fix atmospheric nitrogen via symbiosis with rhizobia, making it beneficial in sustainable agricultural systems (Akram et al., 2018). Reported rates of biological nitrogen fixation in peanut systems vary widely depending on genotype,



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soil conditions, and management practices, ranging from 20 to 200 kg N ha⁻¹ year⁻¹ (Toomsan et al., 1995). Under well-managed conditions, average fixation values of approximately 140 kg N ha⁻¹ year⁻¹ have been recorded (Dakora and Keya, 1997; Peoples et al., 2009). On the other hand, rhizobia inoculation increased the amount of nitrogen fixation by up to 46% (Yakubu et al. 2010), and by 43–60% according to Jovino et al. (2022). Such funding supports the role of peanut-rhizobia symbiosis as an important mechanism for maintaining or enhancing soil nitrogen balance and overall fertility, particularly in nutrient-poor sandy soils. However, peanut productivity remains strongly dependent on the biochemical functioning of the soil. As matter of fact, many peanut-producing sandy soils exhibit low organic matter, limited available phosphorus and reduced microbial biomass and enzymatic activity, factors known to constrain nutrient cycling and root nutrient acquisition (Bouhraoua et al., 2015; Bünemann et al., 2018; Mohamed et al., 2024; Wu et al., 2024). In such environments, soil biochemical processes, particularly microbial-driven P solubilization, organic-acid production, siderophore-mediated Fe mobilization, and enzymatic turnover of organic matter, play a disproportionately important role in nutrient cycling compared to more fertile soils (Richardson et al., 2009; Hinsinger et al., 2011; Richardson and Simpson, 2011). Recent studies confirm that microbial respiration, phosphatase and urease activities, and rhizosphere-mediated solubilization are key drivers of nutrient availability and peanut performance (Bouhraoua et al., 2015; Bhattacharyya and Furtak, 2023; Mohamed et al., 2024; Pradhan et al., 2025).

To overcome the obstacles posed by this challenging environment, researchers have increasingly turned to ecological, sustainable, and innovative strategies aimed at improving yield growth and production while supporting soil fertility. As a part of these strategies, plant growth-promoting bacteria (PGPR) have emerged as a highly effective solution, playing a key role in enhancing plant growth while supporting soil health (Achkouk et al., 2018; Prasanna et al., 2022). These beneficial microorganisms interact with plant roots and contribute to nutrient mobilization and acquisition from the soils, particularly those susceptible to nutrient leaching, such as sandy soils (Etesami and Adl, 2020). These microorganisms directly modulate soil biochemical processes including P solubilization, enzyme-mediated nutrient release, organic-acid exudation, and carbon-nitrogen transformation pathways that collectively enhance nutrient cycling (Richardson et al., 2009; Richardson and Simpson, 2011; Lurthy et al., 2020). They also contribute to phytohormone synthesis (Grossi et al., 2024) and antagonism against pathogens (Wei et al., 2014).

In Morocco, peanuts (*Arachis hypogaea* L.) are one of the principal leguminous crops, primarily cultivated along the Atlantic coastal regions, taking advantage of the favorable agroclimatic conditions. The plant is commonly cultivated in soils that typically display a sandy texture (80-85% sand) and range from neutral to alkaline in pH. They are also characterized by low total organic matter (0.5–1.1%) and phosphorus content (P₂O₅ 62-97 ppm), which together limit nutrient availability and crop productivity (El-Akhal et al., 2009; Bouhraoua et al., 2015). Due to their high permeability and limited organic matter, these soils are prone to rapid nutrient leaching, hindering crop growth and reducing long-term soil fertility. Equally important, significant economic losses are associated with reduced peanut yields caused by pathogenic fungi (Gonzalez et al., 2008; Wei et al., 2014). *Fusarium* spp. are the predominant fungus in the soil, stated as the most prevalent fungus causing *Fusarium* wilt of *Arachis hypogaea* L. from sowing to harvest (Gonzalez et al., 2008). The *Fusarium* species associated with peanut wilt are soil-borne pathogens that can persist for extended periods in the rhizosphere and soil environment. Their survival is primarily due to the formation of resistant chlamydospores, which enable them to remain viable in soil for several years even in the absence of a host plant (Gordon and Martyn, 1997). In sandy soils, characterized by low organic matter and high porosity, the pathogens can still persist as saprophytes on crop residues, contributing to recurrent infection cycles.

Moreover, peanut cultivation in Morocco's presents a unique soil-microbe interaction context due to the predominance of sandy soils, which are characterized by extremely low organic matter, weak cation exchange capacity, and poor nutrient retention. These constraints create a markedly different rhizosphere environment compared with typical agricultural soils, making nutrient acquisition highly dependent on microbial processes rather than soil reserves. Despite the agronomic importance of peanuts in these regions, very few studies have examined how native rhizobacteria contribute to nutrient mobilization and soil fertility under such nutrient-poor, rapidly draining substrates. Understanding PGPR functionality in these coastal sandy soils is therefore of particular scientific interest, as it provides insight into microbial-driven nutrient cycling under low-input, low-buffering conditions, and may reveal microbial traits specifically adapted to these harsh edaphic environments. This regional context underscores the novelty of investigating peanut-associated PGPR in Moroccan sandy soils, where microbial contributions are likely to play a disproportionately important role in sustaining crop productivity. However, the relationships between PGPR functional traits and nutrient cycling in Moroccan sandy soils remain poorly characterized.

In this context, the screening of native plant growth-promoting rhizobacteria (PGPR) must consider their ability to survive and function under such challenging soil conditions. Therefore, this research sought to isolate effective PGPR strains from the rhizosphere of *Arachis hypogaea* L. and evaluate their plant growth-promoting characteristics, such as phosphate solubilization, siderophore production, and IAA synthesis, as well as their tolerance to salinity, pH variations, and drought stress. These assessments were supplemented by physicochemical analyses of the rhizospheric soils, including texture, pH, electrical conductivity, organic matter content, and available phosphorus, which provided a baseline for interpreting microbial activity and potential soil-mediated effects. By linking microbial functional traits to soil characteristics, this study aims to identify strains that are both effective in nutrient mobilization and adapted to the environmental constraints of Moroccan sandy soils.

Material and Methods

Soil sampling and bacteria isolation

Soil samples were taken from the topsoil layer (0–20 cm) of peanut-growing fields in the coastal area of Larache, northwestern Morocco (31°47'52.2"N 8°42'39.6"W; 53 m a.s.l.). Total soil nitrogen was determined using the Kjeldahl method (ISO 11261:1995). Available phosphorus was analyzed according to the Olsen procedure (Olsen et al., 1954), whereas exchangeable potassium was quantified by flame photometry following the method of Bower et al. (1952). Soil organic matter content was determined using the Walkley and Black method (Walkley and Black, 1934), and total calcium carbonate (CaCO₃) content was determined with Bernard's calcimeter. The soil particle-size distribution was evaluated by the densimetric method described by Bouyoucos (1962).

For bacterial isolation, the rhizosphere of *Arachis hypogaea* L. was collected by gently brushing the adherent soil into sterile plastic bags. For each soil sample, three replicates were processed independently. Soil suspensions were prepared at a 1:10 (w/v) ratio using sterile physiological water and maintained at 28 °C for 1 hour under constant shaking (200 rpm). Afterwards, serial dilutions (10⁻¹ to 10⁻⁷) were prepared, and 100 µL from each dilution was distributed onto Tryptic Soy Agar (TSA) medium (Atlas, 2010) and incubated at 30 °C for 48 hours. Sterile controls (uninoculated media) were included throughout the isolation process to ensure contamination-free cultures. Colonies with distinct morphological characteristics were then selected and preserved at –20 °C in 25% (v/v) sterile glycerol.

Plant Growth Promoting (PGP) Attributes

The functional traits evaluated in this study are related to key soil fertility mechanisms. These include inorganic phosphate solubilization, indole-3-acetic acid (IAA) synthesis, hydrogen cyanide production, siderophore production ammonia production, and ACC deaminase activity. Hydrolytic enzymes such as amylase, chitinase, and protease and antagonistic activities against *Fusarium oxysporum*, *Aspergillus niger*, and *Aspergillus ochraceus* were also assessed. In addition, the isolates were tested for their tolerance to abiotic stress factors, for instance, salinity, drought, pH variation, and temperature fluctuation.

Inorganic Phosphate Solubilization

The isolates were first evaluated for their potential to tri-calcium phosphate (TCP), which increases the pool of plant-available phosphorus in sandy soils. The isolates were spread on Petri dishes containing Pikovskaya medium (PVK) (Pikovskaya, 1948) and maintained for 7 days at 28 ± 2°C. Phosphate-solubilizing activity was verified by the development of a clear halo (mm) zone around the colonies. Phosphorus solubilization was quantitatively evaluated by adding 500 µL of bacterial culture to 50 mL of PVK liquid medium, followed by incubation at 28 °C with constant agitation for 7 days. After incubation, the cultures were centrifuged at 13,000 rpm for 20 min, and the concentration of soluble phosphorus was quantified following the colorimetric method of Ames (1966). Uninoculated broth was also included as a control. Colony and halo diameters were measured in millimeters (mm), and the Phosphate Solubilization Index (SI) was calculated using the formula (Edi Premono et al., 1996):

$$\text{Phosphate solubilization index} = \frac{\text{Colony diameter} + \text{Halozone diameter}}{\text{Colony diameter (mm)}}$$

Hydrogen cyanide production

The isolates were tested for hydrogen cyanide (HCN) production, an important trait for suppressing soil-borne pathogens and maintaining rhizosphere health. Thus, the capacity of the isolates to synthesize (HCN) was evaluated using the method of Bakker and Schippers (1987). 100µL aliquot of bacterial culture spread onto TSA medium appended with 4.4 g L⁻¹ glycine. Subsequently, Whatman filter paper discs impregnated with a solution of 2% Na₂CO₃ and 0.5% picric acid were affixed to the inner surface of each plate lid and incubated at

28 °C for 96 h. HCN production was indicated by the development of an orange-brown coloration, allowing the isolates to be ranked as low (+), moderate (++), or high (+++) producers according to the color intensity.

Quantitative estimation of siderophore production

The isolates were evaluated for siderophore production, a key functional trait that enhances iron cycling by chelating Fe^{3+} and increasing its availability to plants and soil microorganisms. To assess siderophore production by the selected bacteria, the Chrome Azurol S (CAS) assay was performed according to [Schwyn and Neilands \(1987\)](#). The bacterial isolate grown in Tryptic Soy Broth (TSB) ([Atlas, 2010](#)) was treated with the CAS reagent, followed by a 20-minute incubation in the dark. Finally, the absorbance at 630 nm was measured, and the siderophore production efficiency was calculated using the formula described by [Pal and Gokarn \(2010\)](#).

$$\text{Siderophore \%} = \frac{(\text{Optical density of reference (CAS)} - \text{Optical density of the sample}) \times 100}{\text{Optical density of the sample}}$$

Ammonia production

The isolates were tested for ammonia production, an important trait for enhancing soil nitrogen availability. Ammonia (NH_3) production was evaluated by inoculating 10 mL of peptone water with 10^6 CFU mL^{-1} of bacterial culture, followed by incubation at 28°C for three days. After incubation, Nessler's reagent was then added, and the emergence of an orange-brown coloration confirmed a positive test for NH_3 production ([Cappuccino and Sherman, 1996](#)).

ACC deaminase production

ACC deaminase activity was evaluated, as it plays a role in enhancing plant stress tolerance through ethylene regulation. The selected isolates were evaluated for their ability to utilize ACC (1-aminocyclopropane-1-carboxylate) as the sole nitrogen source, following the procedure established by [Jacobson et al. \(1994\)](#). Bacteria were inoculated into Tryptic Soy Broth (TSB) and incubated for 48 hours at 30 °C under continuous shaking. The cultures were then diluted, and 15 μL of each dilution was added to 120 μL of DF minimal salt medium ([Dworkin and Foster, 1958](#)) complemented with 15 μL of thawed ACC (3 mM) solution in a 96-well plate format. Optical density was measured after 48 h at OD_{600} (in absorbance units, AU). Additionally, DF medium supplemented with $(\text{NH}_4)_2\text{SO}_4$ (0.1 M) (DF + $(\text{NH}_4)_2\text{SO}_4$) served as the positive growth control and and DF – ACC as a negative control. The strains exhibiting greater growth than the controls were considered ACC deaminase-positive.

Estimation of indole acetic acid production

Indole acetic acid (IAA) production was evaluated, as a plant growth-promoting trait that stimulates root growth, expanding the rhizosphere and facilitating microbial-mediated nutrient mobilization. The isolates were cultivated in sucrose-minimal salts medium (SMS) ([Vincent, 1970](#)), supplemented with 0.05% L-tryptophan, followed by incubation at 28°C for 2 days. After centrifugation at 13,000 rpm for 10 minutes, 1 mL of the supernatant was mixed with 2 mL of Salkowski reagent. The mixture was incubated in the dark at room temperature for 20 minutes, and absorbance was measured at 535 nm ([Gordon and Weber, 1951](#)). Results were expressed as milligrams of IAA per liter of bacterial culture basis.

Evaluation of hydrolytic enzymes

The isolates were evaluated for hydrolytic enzyme activity such as amylases, chitinase, and protease, which contributes to the decomposition of complex organic matter into simpler compounds, increasing soil nutrient availability and organic matter turnover. To assess the hydrolytic enzymatic production, the isolates were cultured in the nutrient broth for 24 hours at 28°C. Urea hydrolysis was tested using a urea agar medium as described by [Maheshwari et al. \(2010\)](#). The development of a pink or red coloration around the colonies, due to ammonia release, indicated a positive results. Amylase production was evaluated on starch-yeast extract (SYE) medium according to the procedure of [Tsai et al. \(2007\)](#). The presence of a clear halo (mm) around the colony following iodine staining signaled as a positive result. Chitinase activity was assessed on colloidal chitin agar ([Chaiharn et al., 2008](#)), while cellulase activity was determined on CMC agar using Congo red staining ([Gupta et al., 2012](#)). A clear halo (mm) surrounding the colony indicated chitinase activity, whereas a yellow halo (mm) signified cellulase activity. Protease production was tested using skim milk agar as described by [Kuddus and Ahmad \(2013\)](#). Positive results were recorded by the appearance of a clear halo (mm) resulting from casein degradation. Catalase production was tested following the method of [Dacre and Sharpe \(1956\)](#). Enzyme activity was confirmed by the rapid release of oxygen bubbles after adding 3% hydrogen peroxide. Lipase activity was detected on nutrient agar supplemented with egg yolk, and the appearance of a greenish-

blue or opaque precipitation zone (mm) around the colonies was taken as evidence of lipase production (Wai et al., 2016).

Antagonism activity

The isolated bacteria were further assessed, in triplicate, for their potential to inhibit *Aspergillus niger*, *Aspergillus ochraceus*, and *Fusarium oxysporum*, which helps maintain microbial community balance and promote healthy soil function. Those pathogenic fungi were chosen for their prevalent and significant impact on various economically important crops in the Mediterranean agroecosystems including *Arachis hypogaea* L. (Tsitsigiannis et al., 2012). Thus, a 5mm disk containing a pure fungal culture was placed in Petri dishes filled with PDA medium (Atlas, 2010). Subsequently, bacterial suspensions were inoculated 30 mm away from the fungal culture. Additionally, a negative control without bacteria was also included. All tests plates were maintained at 25 °C for 7 days. The inhibitory effect was assessed based on the formula (Kumar, 2002):

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

r1: Mycelial radial growth in the control test (mm); r2: Mycelial radial growth of the treated plates (mm).

Response to abiotic stress factors

To ensure their continued activity and contribution to nutrient cycling in challenging environments, such as those encountered in sandy soils, the isolates were tested for their ability to tolerate various abiotic stress conditions. Tolerance to acidic and alkaline pH was assessed by culturing the isolates on TSA medium (Phosphate dipotassique 2.5 g L⁻¹, glucose 2.5 g L⁻¹, casein peptone 17 g L⁻¹, soy peptone 5 g L⁻¹, sodium chloride 5 g L⁻¹ and agar 15 g L⁻¹) adjusted to pH values from 4 up to 10 and incubated at 28°C for 48 h. Heat tolerance was tested by incubating the strains for 48 h at 28°C, 37°C, 40°C, and 42°C, and salt tolerance was tested by cultivating the bacterial isolates on TSA plates supplemented with NaCl (0.5%, 1%, and 2% w/v) at 28°C for 48h. To assess drought stress tolerance, the isolates were incubated in TBS broth medium (Phosphate dipotassique 2.5 g L⁻¹, glucose 2.5 g L⁻¹, casein peptone 17 g L⁻¹, soy peptone 5 g L⁻¹, and sodium chloride 5 g L⁻¹) supplemented with different levels (–0.2 MPa to –1.5 MPa) of polyethylene glycol (PEG-6000). Cultures were incubated for 48 h at 28 °C, and their optical density at 600 nm (OD₆₀₀) was measured and compared with the control (TBS broth without PEG).

Molecular characterization

The total genomic DNA was extracted according to the phenol/chloroform protocol as outlined by Chen and Kuo (1993). Two bacterial universal primers, fD1 and rD1, were employed to amplify the bacterial 16S rRNA gene sequence (Weisburg et al., 1991). Amplified fragments were confirmed by electrophoresis in 1% (w/v) TAE-agarose gel for 1h at 70V, then purified using Qiagen's purification system. The purified products were subjected to cycle sequencing using the Sanger method with the same primers as those used in the PCR amplification. The obtained sequences were compared with those from the NCBI GenBank database using the BLAST algorithm and then corrected manually using MEGA 7 software. Finally, the phylogenetic tree was generated using the neighbor-joining method, with the distance estimations calculated according to Kimura's 2-parameter model, using MEGA 7.

Pot assay

Peanut seeds (KT-22 variety) were surface-sterilized with 0.5% sodium hypochlorite for 5 minutes, rinsed thoroughly with sterile distilled water, and then immersed in sterile water for 6 hours. The sterilized seeds were aseptically transferred to Petri dishes containing Whatman No. 2 paper moistened with sterile water and incubated in the dark for 48 hours at 28°C. Three germinated seeds were sown in plastic pots (25 cm x 22 cm x 15 cm) filled with 1kg of sterilized soil, at 80-100% field capacity, collected from the same location where the peanut plants were originally collected (Table 1).

Table 1. Physical and chemical soil analysis of the sampling site

pH	EC, μS/cm	Min. N, ppm	P ₂ O ₅ , ppm	K ₂ O, ppm	OM, %	CaCO ₃ , %	Clay, %	Fine Silt, %	Coarse Silt, %	Fine Sand, %	Coarse Sand, %
6.1	169	50	97.5	103	1.1	3.40	10.10	5.05	0.61	52.22	28.59

Each pot received a single inoculation with 2 mL of the bacterial suspension (approx. 10⁸ cells mL⁻¹ seed⁻¹) and was placed in a greenhouse chamber maintained at 25°C under a 16:8 h light–dark photoperiod. Non-inoculated pots were also used and served as controls. All pots were irrigated three times a week with 150 mL of sterile distilled water. After a 30-day growth period, plants were collected, partitioned into shoots and roots, and dried at 55 °C for 72 hours. Shoot and root length and dry weights were determined, and the results were

expressed on a dry-weight basis. Total chlorophyll content was determined from five central leaves per plant using a portable chlorophyll meter (SPAD-502 Plus). Each treatment was conducted in triplicate per pot.

Statistical analysis

All experiments were conducted in triplicate, and results are presented as mean $\pm$ standard deviation. Analysis of Variance (ANOVA) was conducted using Statgraphics Plus version 4.0, and Significant differences were assessed using ANOVA and Fisher's Protected Least Significant Difference (LSD) test ($P < 0.05$).

Results

Plant Growth Promoting (PGP) Attributes

Fifteen bacteria were recovered from the rhizosphere of *Arachis hypogaea* L. The isolates were first assessed for their ability to solubilize inorganic phosphate as phosphate mobilization was the initial target trait of this study, given the naturally low phosphorus availability in coastal sandy soils. To ensure the selection of the most efficient phosphate-solubilizing strains, two bacteria (JR62 and JR86) were selected based on their halo diameters (≥ 1 cm) compared to other isolates, and investigated for various growth-promoting (PGP) properties. The results of the selected strains are listed in Table 2. The results reveal significant differences among the isolated bacterial strains in their ability to solubilize inorganic tricalcium phosphate (TCP). JR62 exhibited the highest solubilization capacity, reaching 238.6 mg L⁻¹ after seven days of incubation, in comparison with JR86 (196 mg L⁻¹). Moreover, both isolates displayed a significant capacity for siderophore production, with JR86 showing a notably higher production rate of 51.69%, whereas JR62 exhibited a comparatively lower percentage (12.33%). Both isolates exhibited the ability to produce IAA when supplemented with L-tryptophan as a precursor. However, the production levels varied significantly ($P < 0.05$), with JR62 exhibiting a higher concentration of IAA (33.26 mg L⁻¹), whereas JR86 produced 15.32 mg L⁻¹. Additionally, the selected strains displayed a strong capacity for HCN and ammonia production, as well as the ability to synthesize ACC deaminase.

Table 2. Plant growth-promoting traits of the selected rhizobacterial strains

	PSI *	Phosphate solubilisation (mg.L ⁻¹)	pH	IAA production (mg.L ⁻¹)	HCN production	Siderophores production (%)	Ammonia production
JR62	5.66 $\pm$ 0.07 ^b	238.651 $\pm$ 2.5 ^b	4.76 $\pm$ 0.08 ^a	33.26 $\pm$ 0.06 ^b	++	12.33 ^a	+++
JR86	4.33 $\pm$ 0.18 ^a	196.111 $\pm$ 5.07 ^a	5.66 $\pm$ 0.84 ^b	15.32 $\pm$ 0.07 ^a	+	51.69 ^b	+++

The data are the mean of three replicates. Means in the same column followed by the same letter are not significantly different ($P < 0.05$). Low (+), moderate (++), and high (+++) production.

* Phosphate solubilisation index calculated using the formula of [Edi-Premono et al. \(1996\)](#).

Hydrolytic enzymes analysis

Both bacterial isolates (JR62 and JR86) showed almost identical extracellular enzyme profiles, with the exception of protease and catalase activities (Table 3). Protease production was observed exclusively in JR86 (1.3 $\pm$ 0.14 mm), while catalase activity was detected only in JR62. Amylase activity did not differ significantly between the isolates ($P > 0.05$), with halo diameters of 2.7 $\pm$ 0.25 mm for JR62 and 2.87 $\pm$ 0.21 mm for JR86. However, neither isolate demonstrated the ability to produce chitinase, lipase, or cellulase.

Table 3. Extracellular enzyme production by the bacterial isolates.

	Cellulase (mm)	Chitinase (mm)	Amylase (mm)	Protease (mm)	Urease	Catalase	Lipase	ACC Deaminase
JR62	-	-	2.7($\pm$ 0.25) ^a	-	+	+	-	+
JR86	-	-	2.87($\pm$ 0.21) ^a	1.3($\pm$ 0.14)	+	-	-	+

Values are the means of three replicates $\pm$ standard deviation. Different letters within the same column indicate significant differences ($P < 0.05$, Fisher's protected LSD test). (+): positive reaction; (-): negative reaction.

Antagonistic Activity

Evaluation of the antagonistic activity of JR62 and JR86 against *Fusarium oxysporum* and *Aspergillus ochraceus* showed that JR62 was the only strain exhibiting meaningful inhibition, reducing the growth of *F. oxysporum* by 55.46 $\pm$ 0.8% and *A. ochraceus* by 22.5 $\pm$ 0.5%. Nevertheless, JR62 remained highly susceptible to *Aspergillus niger*. In contrast, JR86 showed no detectable antagonistic effect and instead displayed strong sensitivity toward all tested fungal strains (Table 4).

Table 4. Inhibition rates (%) of the isolates against three phytopathogenic fungi

	<i>Fusarium oxysporum</i>	<i>Aspergillus niger</i>	<i>Aspergillus ochraceus</i>
JR62	55.46 (± 0.8) *	-	22.5 (± 0.5) *
JR86	-	-	-

The data are the mean of three replicates (-): no inhibition

* Inhibition rates calculated using Tiru's formula (Tiru et al., 2013).

Tolerance to abiotic stress conditions

The aptitude of the two isolates (JR62 and JR86) to grow under different abiotic stresses was evaluated (Table 5). Both isolates were highly sensitive to increasing NaCl concentrations, although JR62 was able to grow up to 2% NaCl, suggests a moderate tolerance that may still be relevant in coastal zones where salt accumulation can occur due to irrigation practices. The ability of JR62 to grow at pH 10, despite field soils being slightly acidic, indicates physiological robustness that may allow the strain to remain active in alkaline-calcareous soils. Both strains grew best at 28 °C, but failed to grow at 37 °C, reflecting their susceptibility to high temperatures. Furthermore, PEG-induced drought stress assays revealed that both strains were highly tolerant to water deficit, especially JR62, which maintained significant growth at -1 MPa. Such tolerance aligns well with the characteristics of sandy soils, which have low water-holding capacity and frequently expose microorganisms to drought-like conditions. However, neither strain managed to grow at -1.5 MPa, marking the limit of their drought tolerance. These results indicate that JR62, and to a lesser extent JR86, possess stress-adaptive traits enabling them to maintain activity and contribute to soil nutrient cycling under the fluctuating and challenging conditions typical of coastal sandy environments.

Table 5. The growth of the isolates in different abiotic stress conditions

	NaCl (%)				PEG (MPa)				Temperature (°C)				pH			
	0.5	1	1.5	2	Control	-0.2	-0.5	-1	-1.5	4	28	37	4	6	8	10
JR62	+	+	+	+	0.862 (±0.02) ^b	0.839 (±0.05) ^b	0.697 (±0.04) ^a	0.519 (±0.05) ^b	0	+	+	-	-	+	+	+
JR86	+	+	-	-	0.781 (±0.03) ^a	0.713 (±0.02) ^a	0.662 (±0.01) ^a	0.285 (±0.01) ^a	0	-	+	-	-	+	+	-

The data are the mean of three replicates. Means in the same column followed by the same letter are not significantly different ($P < 0.05$).

(+): positive growth; (-): negative growth

Analysis of the 16S rRNA gene

The genetic analysis of the selected strains showed they are closely related to the *Enterobacter* and *Microbacterium* genera, with similarity values up to 100%. Based on the phylogenetic tree derived from the 16S rRNA gene sequences, strains JR62 and JR86, isolated from the peanut rhizosphere, showed 99.86% and 99.93% similarity to *Enterobacter asburiae* JCM6051^T and *Microbacterium oxydans* DSM20578^T, respectively (Figure 1). The sequences of *Enterobacter asburiae* strain JR86 and *Microbacterium oxydans* strain JR62 have been deposited in GenBank under the accession numbers OM101034 and PQ758594.1, respectively.

Plant inoculation

Inoculation of peanut plants with the two indigenous PGPR strains, *Microbacterium oxydans* JR62 and *Enterobacter asburiae* JR86, significantly ($P < 0.05$) enhanced growth parameters after one month of growth in a greenhouse chamber compared to uninoculated controls. Notably, the JR86 strain demonstrated the highest enrichment in shoot ($P < 0.01$; Cohen's $d = 6.79$) and root ($P < 0.01$; Cohen's $d = 6.35$) lengths compared to the JR62 strain (Figure 2). Such results can be attributed to the strain's capacity to synthesize siderophores (Table 2). Additionally, the JR86 strain shows considerable improvement in shoot ($P < 0.01$; Cohen's $d = 8.94$) and root ($P < 0.01$; Cohen's $d = 4.13$) biomass yield compared to the control (Figure 3). Finally, the results of measured chlorophyll content revealed a significant gain for the plant inoculated with both strains (Figure 4).

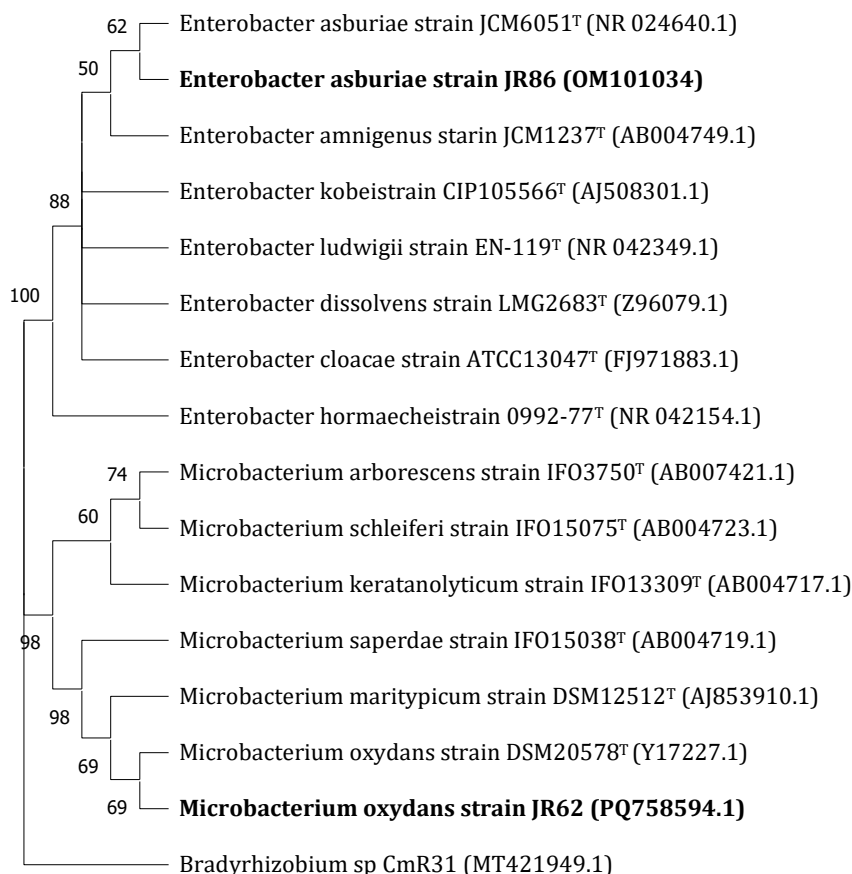


Figure 1. Neighbor-joining phylogeny of selected isolates (in bold) and their phylogenetically related species based on 16S rRNA sequences. Bootstrap values are indicated as percentages derived from 1000 replications. The tree is rooted on *Bradyrhizobium* sp. Strain CmR31.

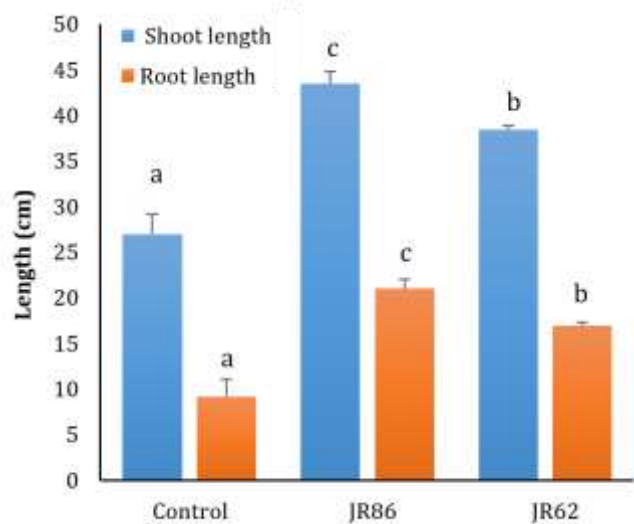


Figure 2. Effect of inoculation with *Microbacterium oxydans* JR62 and *Enterobacter asburiae* JR86 on the root and the shoot length of *Arachis hypogaea* L. Values are expressed as the mean of three replicates. Means followed by the same letter (a, b and c) are not significantly different ($P < 0.05$).

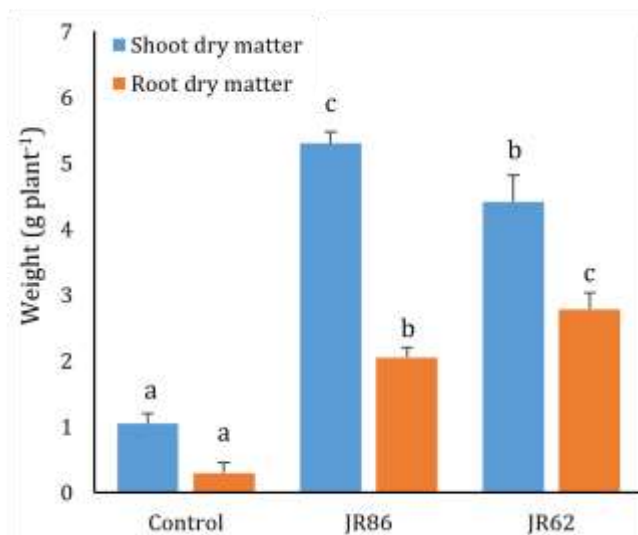


Figure 3. Inoculation effect with *Microbacterium oxydans* JR62 and *Enterobacter asburiae* JR86 on the shoot and the root dry weight of *Arachis hypogaea* L. Values are expressed as the mean of three replicates. Means followed by the same letter (a, b and c) are not significantly different ($P < 0.05$).

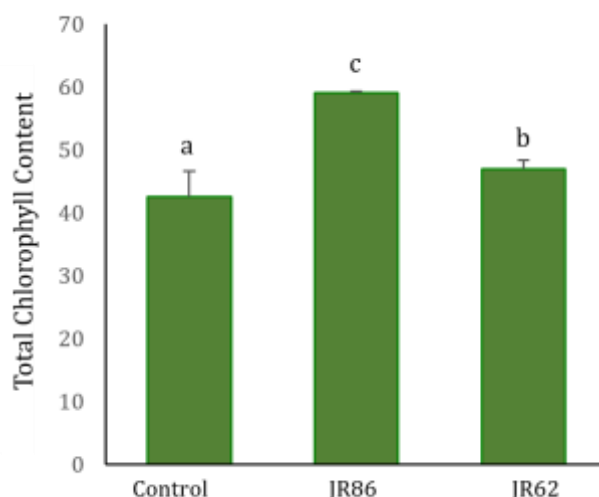


Figure 4. Effect of inoculation with *Microbacterium oxydans* JR62 and *Enterobacter asburiae* JR86 on the total chlorophyll content of *Arachis hypogaea* L. Values are expressed as the mean of three replicates. Means followed by the same letter (a, b and c) are not significantly different ($P < 0.05$).

Discussion

In this study, we evaluated two PSB strains, i.e., *Microbacterium oxydans* JR62 and *Enterobacter asburiae* JR86, isolated from the rhizosphere of *Arachis hypogaea* L. in Northeastern Morocco. Both strains demonstrated significant plant growth-promoting abilities, which is consistent with some previous findings on the impact of inoculation of indigenous PGPR bacteria on peanut growth and mineral acquisition (Anzuay et al., 2015; Bouhraoua et al., 2015). Direct effects include traits that act at the plant physiological level, such as IAA production, which promotes root elongation. Conversely, the soil-mediated effects involve mechanisms like phosphate-solubilizing capacity, siderophore-mediated iron chelation, a key process in low-organic, highly permeable sandy soils where P and Fe availability is constrained.

Results revealed that both strains JR62 and JR86 are classified as phosphate-solubilizing bacteria (PSB) as they demonstrated a strong capacity to convert insoluble inorganic phosphate (tricalcium phosphate, TCP) into soluble forms that can be readily absorbed by plants. Phosphorus serves as a crucial element necessary for plant growth, however, its uptake is often limited due to its immobilization in soil, particularly in sandy soil, where factors such as rapid leaching and low organic matter reduce its availability (Weaver et al., 1988). Quantitatively, JR62 and JR86 solubilized 238.6 and 196 mg L⁻¹ of phosphorus, respectively, over seven days of incubation in PVK medium, with solubilization indices of 5.66 and 4.33. These values fall within the typical range reported for highly efficient phosphate-solubilizing bacteria (Mohamed et al., 2019). Additionally, the phosphate solubilization capacity displayed by JR62 and JR86 is associated with a marked decrease in medium pH, indicating their ability to produce organic acids and chelating compounds capable of mobilizing mineral-bound phosphorus (Alori et al., 2017; Zuluaga et al., 2023). Under field conditions, such microbial activity directly increases the pool of readily plant-available P by releasing phosphate ions from Ca-bound complexes, which dominate in sandy soils (Penn et al., 2019). Additionally, enhanced P solubilization may stimulate microbial biomass P, as microorganisms rapidly assimilate the mobilized phosphate, acting as a temporary P reservoir that can be gradually released back to plants. Consequently, inoculation with JR62 or JR86 is expected to enhance P cycling at the soil-root interface, contributing to improved soil fertility and supporting peanut growth under nutrient-deficient conditions relevant in Moroccan sandy soil (Bouhraoua et al., 2015).

Accordingly, a significant enhancement of peanut growth was observed following inoculation with the selected strains compared to the non-treated ones, with *Enterobacter asburiae* JR86 being more effective, demonstrated the highest enrichment in shoot (42%) and root (99%) lengths, as well as in shoot and root biomass (180% and 233%, respectively). Similar results were stated by Saikia et al. (2023) for *Enterobacter asburiae* EBRJ12, isolated from the rhizosphere of *Phaseolus vulgaris* L. growing in water-limited agricultural soils. Likewise, Ning et al (2024) demonstrated that *Enterobacter asburiae* D2 enhanced rice growth under salinity, whereas Singh et al (2021) showed that *Enterobacter asburiae* BY4 enhanced chlorophyll content and photosynthesis activity.

Our findings showed that *E. asburiae* JR86 and *M. oxydans* JR62 produced high amounts of siderophore compounds, which reported to improve iron uptake under sandy soil conditions (Kong et al., 2014). In sandy soils with low organic matter, iron is often present in the form of insoluble Fe^{3+} oxides that plants cannot readily access. However, recent studies highlight that microbial siderophores can chelate these Fe^{3+} forms and mobilize iron into soluble complexes that enhance iron bioavailability, thereby improving plant iron uptake and affecting iron dynamics in the rhizosphere (Lurthy et al., 2020 ; Molnár et al., 2023; Zhang et al., 2023). In our experiment, the control plants occasionally exhibited pale, interveinal chlorosis consistent with mild Fe-deficiency, suggesting limited Fe bioavailability. By contrast, inoculation with the high-siderophore-producing strain *E. asburiae* JR86 likely enhanced iron mobilization in the rhizosphere, helping to alleviate Fe-limitation and contributing to improved plant growth. According to Jetiyanon (2015), *E. asburiae* possesses several bacterial determinants involved with plant growth promotion, including siderophore production. Nithyapriya et al. (2024) reported that the siderophore-producing bacteria enhanced peanut growth and overall crop yield. Iron deficiency in peanuts can affect various physiological processes (Kong et al., 2014). As a response, many PGPR produce siderophore iron chelators that improve plant iron nutrition and reduce pathogen growth by limiting their access to iron (Ellermann et al., 2017). Additionally, low concentrations of IAA as those produced by *E. asburiae* JR86 were inferred to contribute to the improvement of root development and mineral uptake (Spaepen et al., 2007).

On the other hand, *Microbacterium oxydans* JR62 showed strong inhibitory activity, particularly against *Fusarium oxysporum* and *Aspergillus ochraceus*, which may help regulate the soil microbiome by reducing fungal propagules and enhancing rhizosphere health. Similar findings were documented by Attab et al. (2019), who demonstrated that some rhizobial strains isolated from *Arachis hypogaea* L. growing in Algerian Sahara soils exhibited effective biocontrol activity against *Fusarium oxysporum*. In their study, Syed et al. (2020) demonstrated successful pathogen suppression by a PGPR consortium during the early stages of the disease of *Arachis hypogaea* L. The inhibition of pathogens was mediated by antagonistic bacteria through the release of either hydrolytic enzymes that degrade pathogen-derived enzymes or antifungal compounds (Ramamoorthy and Samiyappan, 2001; Reddy et al., 2022). In this study, *Microbacterium oxydans* JR62 exhibited significant amylase activity, which contributes to the breakdown of starch and other polysaccharides in the soil, promoting the degradation of organic matter, enhancing nutrient availability and supporting microbial growth and overall soil fertility. In comparison, Sun et al. (2022), demonstrated that an increased abundance of siderophore-producing bacteria such as *Microbacterium oxydans* JR62, enhanced pathogen suppression and improved resistance against *Fusarium oxysporum* and *Aspergillus ochraceus*. Likewise, Suárez-Estrella et al. (2023) revealed that *Microbacterium* spp. producing volatile compounds like NH_3 and HCN can inhibit the growth of pathogenic fungi (Blom et al., 2011). Practically, the bacteria could be applied directly into the soil via soil inoculation, or used as a seed-applied or soaking treatment to protect *Arachis hypogaea* L. seeds, suppress fungal colonization, and enhance early plant development.

From an ecological perspective, the isolates, particularly *Microbacterium oxydans* JR62, showed high tolerance and were able to growth across various abiotic stress conditions. The stress conditions used in this study were chosen to reflect the abiotic constraints commonly encountered in Moroccan sandy soils. The pH range (4-10) covers the slightly alkaline conditions generally reported in semi-arid Moroccan soils (Oubdil et al. 2025), allowing detection of highly tolerant isolates. Although the soil used in this study has a near-neutral pH (6.1), assessing pH tolerance remains relevant for field application since soil pH can vary spatially and temporally in sandy coastal environments. The aptitude of the isolates, particularly JR62, to grow at pH 10 reflects their physiological robustness and potential to withstand alkaline microenvironments that may develop around decomposing organic matter, irrigation water, or localized fertilization events. Similarly, salt treatments (0.5–2% NaCl) simulate moderate to severe salinity, relevant to irrigated Moroccan soils where salinization reduces soil fertility and microbial diversity (Ondrasek et al., 2022; Ouzemou et al., 2025).

Remarkably, *Microbacterium oxydans* JR62 was able to grow at high salinity levels, indicating its ability to survive in soils with elevated osmotic potential, such as those expected in saline-sandy soils. Comparable results were observed by Khan et al. (2019), who indicates the potential *Microbacterium oxydans* for application in saline soil environments as a salt-tolerant plant growth-promoting bacterium (Shukla et al., 2012; Siraj et al., 2022). In the studied sandy soil, which is characterized by low organic matter (1.1 %) and moderate EC ($169 \mu\text{S cm}^{-1}$), osmotic constraints can limit microbial activity and nutrient availability. The demonstrated salinity tolerance of JR62 suggests that it could maintain metabolic activity and support plant growth under conditions of increased soil salinity. Future field experiments or greenhouse trials simulating salt stress ($\text{EC} > 4 \text{ dS m}^{-1}$) would be necessary to validate its effectiveness *in situ*.

Likewise, the PEG-induced drought assay showed that isolate JR62 displayed strong tolerance, maintaining growth even at high osmotic potential of -1 MPa. In the present study, the drought conditions tested (-0.2 to -1.5 MPa induced by PEG-6000) simulate plausible soil moisture levels, where -1.5 MPa reflects an extreme dry state known to substantially inhibit microbial proliferation and enzymatic activity (Siebielec et al., 2020; Bogati and Walczak, 2022). Although simplified compared to complex soil environments, these controlled stress levels provide a realistic model for evaluating bacterial tolerance and identifying strains capable of functioning under field-relevant stresses.

Overall, the inoculation with *Microbacterium oxydans* JR62 and *Enterobacter asburiae* JR86 not only enhances nutrient cycling in nutrient-limited soils but also has the potential to improve soil physicochemical properties. Specifically, such inoculation may increase microbial biomass, enhance nutrient retention, and contribute to better soil structure.

Conclusion

This study demonstrates that *Microbacterium oxydans* JR62 and *Enterobacter asburiae* JR86 can enhance key soil functions, including phosphorus solubilization, iron availability, and rhizosphere enzymatic activity, which together improve nutrient cycling, reduce nutrient leaching, and enhance soil fertility in sandy, nutrient-poor soils. Future field trials should monitor key soil parameters, including soil organic carbon, available phosphorus, microbial biomass C and N, soil pH stability, and enzyme activities (e.g., phosphatase, dehydrogenase, urease), to evaluate changes in nutrient cycling and overall soil health. Assessing these indicators over time will help clarify the long-term impact of these PGPR on soil fertility, ecosystem functioning, and crop performance across diverse environments.

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