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Research Article

Plant Growth-Promoting Rhizobacteria as a Sustainable Tool for Enhancing Growth and Germination of *Chrysopogon aucheri* in Degraded Arid Rangelands of Pothwar, Pakistan

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ABSTRACT

Plant growth-promoting rhizobacteria (PGPR) play a crucial role in enhancing plant productivity and sustainability by improving nutrient availability and promoting growth. This study aimed to isolate, characterize, and evaluate rhizosphere bacteria from *Chrysopogon aucheri* for their potential as PGPR. A total of 85 bacterial isolates were obtained and subjected to morphological, biochemical, and molecular characterization. Based on colony morphology, Gram reaction, cell shape, and biochemical tests, ten distinct isolates were selected. These isolates were further screened for plant growth-promoting traits, including indole-3-acetic acid (IAA) production, phosphate solubilization, siderophore, and ammonia production. Among them, strains UUHH-1, UUHH-5, and UUHH-8 exhibited the highest PGP potential, with UUHH-1 (*Bacillus subtilis*) producing 80.78 ± 1.5 µg ml^{-1} IAA and the largest phosphate solubilization halo (70.25 ± 5.50 mm^2). Molecular identification using 16S rRNA gene sequencing confirmed UUHH-1 as *B. subtilis*, UUHH-5 as *B. cereus*, and UUHH-8 as *B. mojavensis*. *In vivo* evaluation of *C. aucheri* seedlings demonstrated that bacterial inoculation significantly enhanced growth parameters compared with uninoculated controls. Strain UUHH-1 showed the greatest enhancement in shoot length (38.33 ± 3.51 cm), root length (46.73 ± 1.53 cm), and biomass accumulation, followed by UUHH-5 and UUHH-8. These findings highlight the effectiveness of selected *Bacillus* strains in promoting plant growth and suggest their potential as sustainable biofertilizers. Integration of PGPR with organic amendments like vermicompost further supports nutrient availability and bacterial survival. Overall, this study identifies UUHH-1, UUHH-5, and UUHH-8 as promising PGPR candidates for sustainable enhancement of *C. aucheri* growth.

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Introduction

In the 21st century, climate change is recognized as one of the most critical global issues facing the world today, and various environmental stresses also pose serious threats to global food security (Singh et al., 2020). These include both biotic and abiotic factors that severely damage

rangeland ecosystems. Moreover, these factors adversely affect rangeland productivity, nutrient availability, and soil fertility, while also causing serious erosion problems and depleting water resources (Gemechu and Dalle, 2023).

Approximately 50% of the Earth's land surface is covered by rangelands that are predominantly distributed in arid and semi-arid regions (Friedel et al., 2000; Reeves et al., 2015). The Pothwar rangelands of Pakistan have also been depleting due to these environmental stressors over the past few decades (Ali et al., 2022). Climate change-induced abiotic stressors not only disturb rangeland productivity but also exacerbate biotic stresses, leading to reduced plant survival and, in severe cases, plant death. Such impacts also reduce soil microbial diversity and limit nutrient uptake due to intensified competition, thereby decreasing overall rangeland productivity (Pereira et al., 2020).

The application of plant growth-promoting bacteria (PGPB) represents a promising alternative to mitigate environmental stress and enhance plant growth (Glick, 2020). It is an effective biological approach that contributes to the rehabilitation of degraded rangelands (Kumar and Verma, 2018). Existing literature has shown that PGPB influence plant survival and growth through both direct and indirect mechanisms, ultimately supporting long-term rangeland productivity and ecosystem stability (Oleńska et al., 2020). These PGPB produce various mechanisms that collectively promote plant growth and resilience. They facilitate nitrogen fixation, solubilize essential nutrients, and synthesize plant hormones (Karimzadeh et al., 2021; Lata et al., 2024). Furthermore, they suppress phytopathogens, produce antimicrobial compounds, antibiotics, and exhibit antifungal properties, thereby contributing to improved plant health and vigor (Ferreira et al., 2019; Seitz et al., 2022).

Moreover, PGPB have emerged as effective substitutes for chemical fertilizers. Incorporating these microbes supports sustainable, eco-friendly practices and offers a long-term solution for overall plant growth (Albdaiwi et al., 2019; Pappalettere et al., 2024). Different genera, including *Bacillus*, *Azotobacter*, and *Pseudomonas*, are well-known plant growth promoters (El-Sawah et al., 2021). The Pothwar rangelands of Pakistan have also been degrading over the past few decades due to these environmental stressors.

Chrysopogon aucheri is a grass species belonging to the Poaceae family (tribe Andropogoneae). It is a perennial bunchgrass widely distributed in tropical and subtropical regions. This species occurs in North America, the Middle East, Eastern Africa, and South Asia, including Pakistan, India, and Iran (Ahmad et al., 2009). It is extensively used in rangelands worldwide and can be utilized for rangeland restoration projects due to its strong adaptability and relatively high nutritional protein value compared to other grass species. It provides high-quality forage for livestock, contributes to carbon sequestration, and protects land from soil erosion through its dense fibrous root system, which binds soil and reduces losses caused by wind and water.

Although a few studies on PGPR bacterial seed inoculation have been reported, their application for the rehabilitation of degraded Pothwar rangelands remains limited. This gap highlights the novelty of the present study in addressing rangeland restoration using PGPR. The goal of this study was to characterize PGPR strains and investigate their effects on the germination and early growth of *C. aucheri*. The main objectives were to isolate bacteria from the rhizospheric soil of *C. aucheri*; to characterize plant growth-promoting traits, including morphological and biochemical attributes, and to perform molecular identification using 16S rRNA; and to evaluate the effects of PGPR on *C. aucheri* under controlled conditions. It was hypothesized that the potential strains would exert growth-promoting effects on *C. aucheri* due to the plant growth-promoting traits produced by PGPR.

Materials and methods

PGPR isolation from rhizospheric soil of *C. aucheri*

Soil samples were collected from the rhizospheric region of *C. aucheri* in the Pothwar rangelands of Pakistan (32º 10′ to 34º 9′ N and 71º 10′ to 73º 55′ E), including Chakwal, Jhelum, and Attock. The details of soil sample collection, the total number of bacterial isolates, and the sampling districts of the Pothwar rangelands are presented in Table 1. These samples were aseptically collected at a depth of 0-30 cm, placed into sterile bags, transported to the laboratory, and preserved at 4°C for further use. The serial dilution plate technique was used for the isolation of rhizobacteria; 1 g of soil was suspended in sterile distilled water (9 ml) and further serially diluted up to 10^{-9}. Approximately 0.1 ml aliquots of each dilution were spread onto nutrient agar (NA) plates and incubated at 28 ± 2°C for 48 h (Afzal, 2021). Based on morphological differences, distinct colonies were selected, purified, and re-streaked on NA plates to obtain pure cultures, and were preserved in glycerol

stocks (-80°C) for further studies (Etminani and Harighi, 2018). Samples were aseptically collected from a depth of 0-30 cm, transferred to sterile bags, transported to the laboratory, and stored at 4°C until further analysis.

Table 1. Soil sample collection, total number of bacterial isolates, and sampling districts of Pothwar rangelands from the rhizosphere of *C. aucheri*.

District	Name of Rangeland	Coordinates	Total No. of isolates
Attock	Kala-Chitta	33°38'0"N, 72°31'0"E	10
	Kharimurat	33°30'50"N, 74°35'18"E	08
Jhelum	Sohawan	33°8'55.50"N, 73°24'5.18"E	12
	Lehri	32°49′31.05″N, 73°50′22″E	12
	Pabbi Hills	32°50′31″N, 72°51′22″E	07
	Salt Range	33°11′0.58″N, 73°14′42.60″E	08
	Ghazi Barotha	33°45′48″N, 72°17′35″E	05
	Fath-e-Jhang	33°48′48.25″N, 72°18′35″E	08
Chakwal	Neela Dulha	33°11′6″N, 72°38′45″E	06
	Gulial	33°13′54.25″N, 72°13′41.25″E	09

Biochemical and morphological characterization of PGPR

Isolates were initially characterized based on morphological characters, including colony color, shape, elevation, margin, Gram reaction, and cell shape. Biochemical tests, including oxidase and catalase tests, were also conducted following standard protocols. Isolates were initially characterized on the basis of colony morphology, including color, shape, elevation, margin, Gram reaction, and cell shape.

PGPR screening for plant growth-promoting (PGP) traits

Phosphate solubilization

Bacterial isolates were inoculated on Pikovskaya's agar plates and incubated at 28°C for about 1 week to evaluate phosphate-solubilizing ability. Colonies showing clear halo zones were considered positive for phosphate solubilization. The solubilization index (SI) was measured using the following formula (Pikovskaya, 1948):

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

Indole-3-Acetic Acid (IAA) production test

Bacterial isolates were inoculated in nutrient broth supplemented with 0.1% L-tryptophan and incubated at 28 ± 2°C for IAA production. After 48 h, cultures were centrifuged at 10,000 rpm for 10 min. A 2 ml aliquot of the supernatant was mixed with 4 ml of Salkowski's reagent, prepared from 50 ml of 35% perchloric acid and 1 ml of 0.5% $FeCl_3$. The development of a pink color acted as an indicator of IAA production. Absorbance was measured at 530 nm using a spectrophotometer. The concentration of IAA was also determined using a standard curve (Abdelwahed et al., 2023).

Siderophore production test

For siderophore production, bacterial isolates were assessed using Chrome Azurol S (CAS) agar plates. Under aseptic conditions, isolates were spot-inoculated onto CAS plates and incubated at 28°C for about 48 h. The formation of yellow or orange halo zones surrounding the colonies indicated positive siderophore production due to iron chelation by the bacterial isolates (Alexander and Zuberer, 1991).

Hydrogen cyanide (HCN) test

Isolates were cultured on plate count agar medium supplemented with 4.4 g/L glycine. A Whatman filter paper No. 1 dipped in 2% sodium carbonate and 0.5% picric acid was placed in the lid of the Petri plate, followed by incubation at 28 ± 2°C for 7 days (Kumar et al., 2015).

KOH solubility test

A bacterial colony was inoculated onto a glass slide, and a drop of 3% KOH was added and mixed until a uniform suspension formed. Gram-negative strains formed a viscous thread, whereas the absence of thread formation indicated a Gram-positive reaction (Hardiansyah et al., 2020).

Ammonia production

The ability of bacterial isolates to produce ammonia was investigated by inoculating a pure colony into 10 ml peptone water. After incubation at 28°C for 48 h, the cultures were centrifuged at 10,000 rpm for 10 min, and 0.5 ml of Nessler's reagent was added. The development of brown or yellow color indicated a positive result for ammonia production (Mir et al., 2022).

Identification of isolates by 16S rRNA

Bacterial genomic DNA was obtained using Prep-Man Ultra™ isolation solution (Life Technologies). A single colony of each isolate was picked and gently mixed with the Prep-Man Ultra™ solution. The isolates were heated at 100°C for 10 min to induce cell lysis. Subsequently, the lysates were centrifuged for 2 min to eliminate cellular debris, and the resulting DNA product was stored at -20°C for subsequent PCR analysis. Five µl of genomic DNA was used for PCR amplification. The reaction mixture (25 µl) was prepared containing 12 µl Taq Green Master Mix (Promega GmbH, Germany), 12 µl sterile deionized water, and 0.5 µl each of universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Nardi et al., 2004). PCR amplification was performed in a thermal cycler using the following protocol: initial denaturation at 95°C for 1 min, followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 73°C for 1 min, and a final extension at 72°C for 10 min.

The amplified PCR products were resolved on a 1% agarose gel prepared in 0.5× TE buffer containing 1 µl of ethidium bromide (20 µg/ml). A DNA marker (λ HindIII) was used to determine the size of PCR-amplified DNA fragments, and the gel was visualized under UV light using a gel documentation system. Subsequently, PCR purification was performed, and the final product was sent to Macrogen, Korea, for sequence analysis. The 16S rRNA gene sequences were aligned using BLAST, and a phylogenetic tree was constructed using the neighbor-joining method with 1000 bootstrap replications in MEGA software version 10 (Kumar et al., 2018). The aligned sequences were also submitted to NCBI to obtain accession numbers for each isolate.

Plant growth-promoting effect of PGPR on seeds of *C. aucheri*

The most promising strains were selected on the basis of PGPR traits for the evaluation of their growth-promoting potential in *C. aucheri*. These strains were inoculated into nutrient broth and incubated at 28 ± 2°C and 180 rpm in an incubator shaker for approximately 24 h. Subsequently, cells of each strain were collected by centrifugation at 8000 rpm for 15 min at 4°C. The obtained pellet was washed three times with sterile distilled water and then resuspended in sterile distilled water to obtain a bacterial density of 10^5 to 10^7 CFU ml^{-1}. Seeds of the respective species were surface-sterilized using 70% ethanol for 2 min, followed by immersion in 2% sodium hypochlorite solution for 3 min. The seeds were then rinsed 3-5 times with sterile distilled water prior to use. Thereafter, the seeds were coated with 1% carboxymethyl cellulose as an adhesive and soaked in the prepared bacterial suspension in a flask. The seeds were further placed on an orbital shaker at 120 rpm for 30 min to ensure uniform agitation. Afterwards, the seeds were sown in autoclaved vermicompost (121°C; 15 psi; 20 min) in plastic pots.

The experiment consisted of four treatments, detailed below:

Treatment 1 = Control (sterile vermicompost),
Treatment 2 = Sterile vermicompost + UUHH-1 bacteria,
Treatment 3 = Sterile vermicompost + UUHH-5 bacteria,
Treatment 4 = Sterile vermicompost + UUHH-8 bacteria

The control treatment consisted of sterile vermicompost only, whereas the other treatments contained sterile vermicompost inoculated with PGPR strains, with three replicates per treatment under controlled growth conditions. After 90 days, plants were carefully washed with distilled water to measure various growth parameters, including root length, shoot length, fresh root weight, fresh shoot weight, dry root weight, and dry shoot weight.

Statistical analysis

In the current study, the germination of *C. aucheri* was evaluated using potential PGPR bacterial strains under a completely randomized design. Analysis of variance (ANOVA) was performed with three replicates for each treatment. The least significant difference (LSD) test at $P \leq 0.05$ was used to compare treatment means.

Results

Isolation and characterization of isolates

Bacterial isolates were collected from the rhizosphere soils of *C. aucheri*, and various PGPR tests were subsequently conducted to identify the most potential strains. A total of approximately 85 isolates were obtained, and morphological characterization was performed, including Gram reaction, colony color, margin, texture, elevation, cell shape, and consistency. Based on morphological and biochemical analyses, ten distinct isolates were selected. These isolates exhibited rod-shaped cells and formed colonies that were irregular, round, smooth, and raised, as detailed in Table 2. Furthermore, several biochemical tests were conducted for each isolate (Table 3). Bacterial isolates were collected from the rhizosphere of *C. aucheri* and screened through multiple PGPR assays to identify the most promising strains.

Table 2. Morphological characterization of selected bacterial strains.

SR. No.	Strain I.D	Colony morphology							
		Shape	Color	Elevation	Cell Shape	Margin	Gram reaction	Endospore	Consistency
1	UUHH-1	Circular/ Rod	off-white	Raised	Rod	Undulate	+	+	Dry and rough
2	UUHH -2	Circular/ Rod	White	Flat	Rod	Irregular	+	+	Dry and rough
3	UUHH -3	Filamentous/ thread	Pale yellow	Convex	Rod	Smooth	+	+	Smooth and dry
4	UUHH -4	Circular/ Rod	Cream	Convex	Rod	Smooth	-	+	Smooth and moist
5	UUHH -5	Circular/ Rod	Cream	Raised	Rod	Irregular	+	+	Slightly mucoid
6	UUHH -6	Filamentous/ thread	Cream	Raised	Rod	Smooth	+	+	Smooth and dry
7	UUHH -7	Circular/ Rod	White	Raised	Rod	Entire	+	+	Smooth
8	UUHH -8	Circular/ Rod	Off-white	Convex	Rod	Slightly undulate	+	+	Rough
9	UUHH -9	Irregular/ Uneven	White	Raised	Rod	Entire	+	+	Slightly mucoid
10	UUHH -10	Circular/ Rod	Off-white	Convex	Rod	Slightly undulate	+	+	Rough

Table 3. Biochemical characteristics of the selected strains.

SR. No.	Strain I. D	Biochemical test									
		Oxidas	Catala	KOH	H_2S	Glucos	Sucros	Lactos	Manni	Urase	Citrate
1	UUHH-1	-	+	-	-	+	-	-	-	-	+
2	UUHH -2	-	+	+	-	-	-	+	+	-	+
3	UUHH -3	+	-	-	-	-	+	-	-	+	-
4	UUHH -4	+	-	+	+	+	+	-	-	-	-
5	UUHH -5	-	+	-	-	+	-	-	-	-	+
6	UUHH -6	+	+	-	+	+	+	-	+	-	+
7	UUHH -7	-	+	+	-	-	-	+	-	-	-
8	UUHH -8	-	+	-	-	+	-	-	-	-	+
9	UUHH -9	+	+	-	-	+	-	-	+	-	+
10	UUHH -10	-	+	-	-	+	-	-	+	-	+

+ Sign showed Positive result and – showed for negative results.

Screening for plant growth-promoting properties

The results for IAA production, both with and without the addition of tryptophan in LB broth, showed positive outcomes for most of the strains compared to the control (Table 4). *Bacillus subtilis* (UUHH-1), with 80.78 ± 1.5 µg ml^{-1}, exhibited the highest production compared to the control (1.95 µg ml^{-1}). Similarly, UUHH-5 and UUHH-8 produced 20.85 ± 0.5 µg ml^{-1} and 32.02 ± 0.5 µg ml^{-1} indole-3-acetic acid, respectively, in the presence of tryptophan.

Phosphate solubilization results showed that the bacterial isolates were capable of solubilizing phosphorus, and *B. subtilis* (UUHH-1) showed the best performance, forming the largest halo zone (70.25 ± 5.50 mm^2) around the colony compared to the control. Similarly, strains UUHH-5 and UUHH-8 formed halo zones of 52.25 ± 2.20 mm^2 and 30 ± 2.50 mm^2, respectively.

The HCN test showed negative results for *B. subtilis* (UUHH-1), whereas most of the other strains showed positive results. Similarly, four bacterial isolates produced siderophores, with *B. subtilis* (UUHH-1) showing the most effective performance. The KOH solubility test was negative for *B. subtilis* (UUHH-1). Moreover, three strains, including *B. subtilis* (UUHH-1), showed positive results for ammonia production.

Overall, these findings highlight the potential role of this strain in plant growth promotion and nutrient cycling.

Table 4. Plant growth-promoting traits of potential bacterial isolates.

SR. No.	Strain I.D	Plant growth promoting traits					
		IAA (μg mL^{-1})	Phosphorus solubilization (mm^2)	HCN	KOH solubility	Ammonia production	Siderophore production*
1	UUHH-1	80.78±1.5	70.25 ± 5.50	-	-	+	+++
2	UUHH -2	15.30±3.15	25.02 ± 1.50	+	-	-	-
3	UUHH -3	18.45±1.5	12 ± 6.25	+	-	-	-
4	UUHH -4	8.90±2.25	20 ± 3.50	+	+	+	-
5	UUHH -5	20.85±0.5	52.25 ± 2.20	-	-	+	++
6	UUHH -6	5.05±2.5	22 ± 2.72	-	+	+	-
7	UUHH -7	10.38±2.52	10.08 ± 6.50	+	-	-	+
8	UUHH -8	32.02±0.5	30 ± 2.50	-	-	+	+
9	UUHH -9	10.05±1.5	9.36 ± 0.50	-	-	-	-
10	UUHH -10	12.82±1.5	8.42 ± 4.30	-	-	+	-

(+) showed positive and (−) showed negative result. *Halo zone diameter ≥ 10 mm (+++), 6–10 mm (++), 1–5 mm (+).

Molecular characterization of PGPR strains using 16S rRNA gene sequencing

Gel electrophoresis results are presented in Figure 1. The selected isolates were sequenced using the 16S rRNA gene, and the selected strains belonged to the genus *Bacillus*. The sequence of strain UUHH-1, obtained through BLAST analysis, showed 100% similarity with *B. subtilis*. Similarly, strains UUHH-5 and UUHH-8 showed 100% similarity with *B. cereus* and *B. mojavensis*, respectively. Phylogenetic tree of the potential bacterial strains based on 16S rRNA gene sequences, constructed using the neighbor-joining method, as shown in Figure 2. The sequence data were further submitted to GenBank, and accession numbers were obtained for the closely related isolates (Table 5). The selected isolates were identified by 16S rRNA gene sequencing, and all selected strains belonged to the genus *Bacillus*. The resulting sequences were submitted to GenBank, and accession numbers were obtained for the closely related isolates.

PGPR effect on the growth promotion of *C. aucheri*

Ten most potential strains were selected on the basis of morphological and biochemical tests and were further evaluated for PGPR traits. Overall, the results revealed that strains UUHH-1, UUHH-5, and UUHH-8 showed the best performance in terms of PGP traits and were the most promising strains. Furthermore, the strains were identified through 16S rRNA gene sequencing as *B. subtilis* (UUHH-1), *B. cereus* (UUHH-5), and *B. mojavensis* (UUHH-8). Plant growth of *C. aucheri* was evaluated using three different bacterial strains in comparison with the control. Among the treatments, strain UUHH-1 showed maximum potential to enhance plant growth, as demonstrated by root and shoot length, fresh and dry shoot weight, and root weight, followed by strains UUHH-5 and UUHH-8 under *in vivo* conditions.

A significant difference among treatments was observed in shoot length of *C. aucheri*. The maximum shoot length was recorded in strain UUHH-1 (38.33 ± 3.51 cm), while other strains, UUHH-5 and UUHH-8, showed comparatively lower values of 28.67 ± 1.15 cm and 24.67 ± 0.58 cm, respectively. In contrast, the control treatment showed the lowest shoot length of 19.67 ± 1.53 cm. Significant differences in root length were also observed among treatments. Strain UUHH-1 produced the highest root length (46.73 ± 1.53 cm), whereas UUHH-5 (33.8 ± 1.08 cm) and UUHH-8 (27.67 ± 0.58 cm) showed moderate increases, and the control treatment recorded 26.33 ± 1.53 cm.

Similarly, fresh root weight showed the highest value in UUHH-1 (12.37 ± 0.32 g), followed by UUHH-5 (10.28 ± 0.04 g) and UUHH-8 (9.20 ± 0.30 g). The uninoculated

control treatment recorded the minimum value of 7.77 ± 0.25 g, thus indicating that PGPR inoculation can increase different growth parameters. The highest dry root weight was noted in UUHH-1 (7.17 ± 0.23 g), followed by UUHH-5 (6.27 ± 0.17 g) and UUHH-8 (3.53 ± 0.28 g), compared to the control (3.17 ± 0.23 g).

Similarly, the maximum fresh shoot weight was recorded in UUHH-1 (12.20 ± 0.25 g), followed by UUHH-5 (10.65 ± 0.17 g) and UUHH-8 (8.43 ± 0.21 g), whereas the control (8.27 ± 0.25 g) represented the lowest value. Moreover, the highest dry shoot weight was measured in UUHH-1 (6.27 ± 0.19 g), followed by UUHH-5 (5.47 ± 0.26 g) and UUHH-8 (4.70 ± 0.33 g). The control treatment showed the lowest value of 4.53 ± 0.25 g.

All the potential strains showed a significant increase in growth compared to the control, and the results were also statistically proven, indicating the effectiveness of the applied PGPR treatments as mentioned in Figure 3. Moreover, vermicompost also effectively enhances bacterial survival and nutrient availability. These findings highlight the importance of PGPR as sustainable tools for enhancing *C. aucheri* productivity while reducing dependency on chemical fertilizers. Overall, the results showed that strains UUHH-1, UUHH-5, and UUHH-8 performed best for the evaluated PGP traits and were considered the most promising isolates. All selected strains significantly improved plant growth compared with the control, and statistical analysis confirmed the effectiveness of the PGPR treatments.

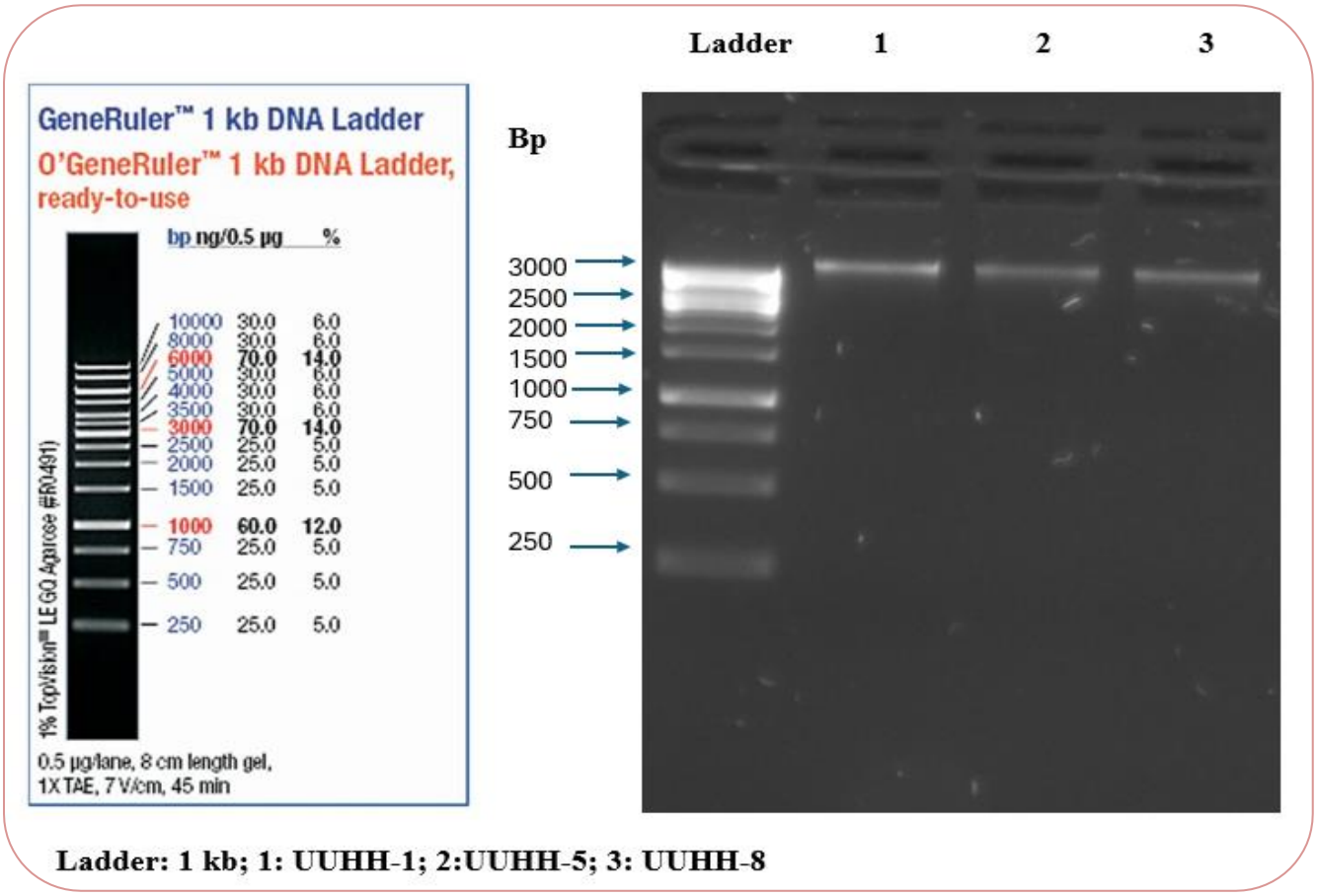


Figure 1. PCR amplification of selected isolates.

Table 5. Molecular identification of closely related isolates, accession numbers, and similarity index of the potential strains.

Strain identity	Accession number	Closet related isolates	% Similarity index
UUHH-1	PQ653540	*Bacillus subtilis*	100
UUHH-8	PQ653545.1	*Bacillus mojavensis*	100
UUHH-5	PQ653541	*Bacillus cereus*	100

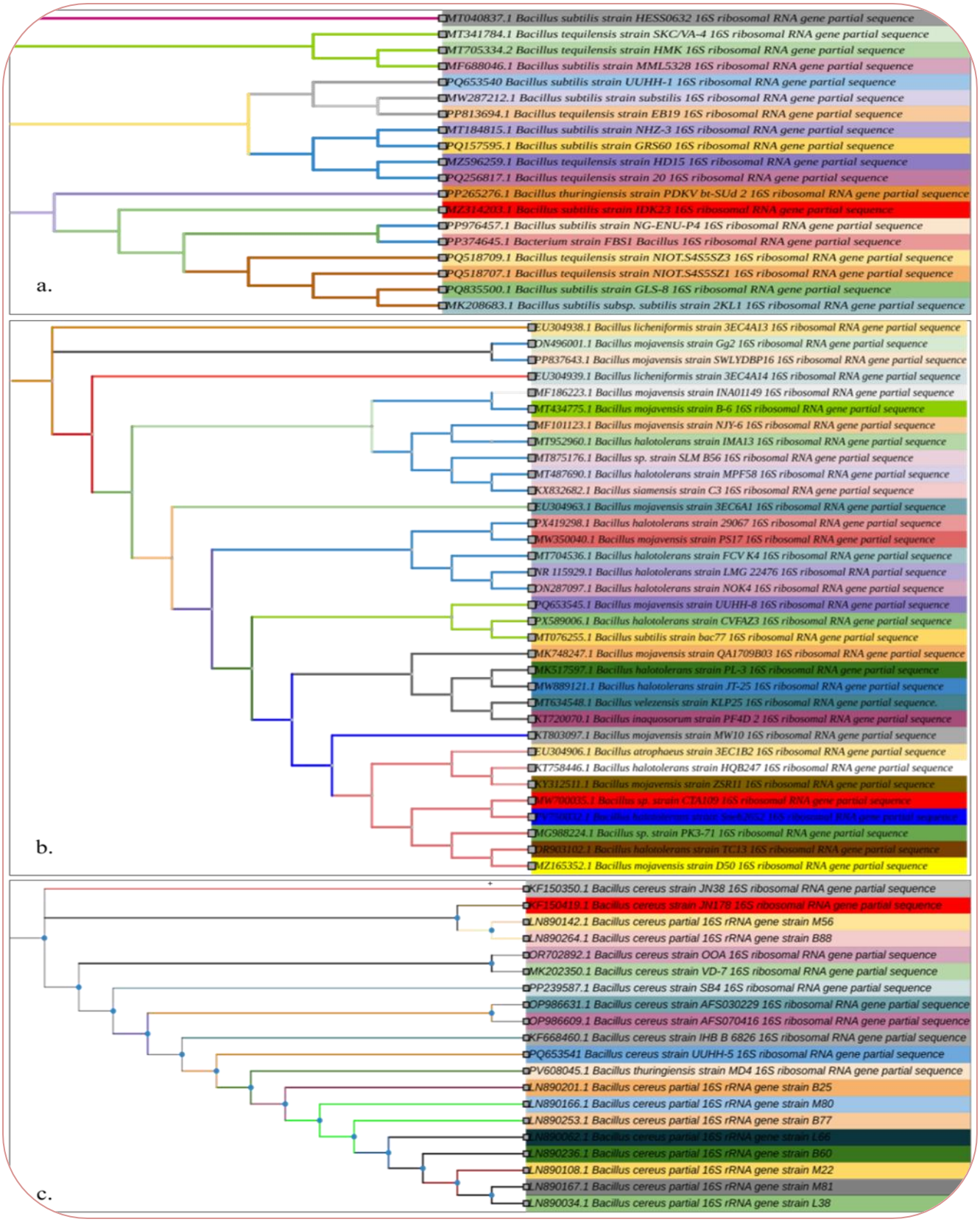


Figure 2. Phylogenetic tree of bacterial strains based on 16S rRNA gene sequences constructed using the neighbor-joining method: (a) UUHH-1 (*B. subtilis*), (b) UUHH-8 (*B. mojavensis*), and (c) UUHH-5 (*B. cereus*).

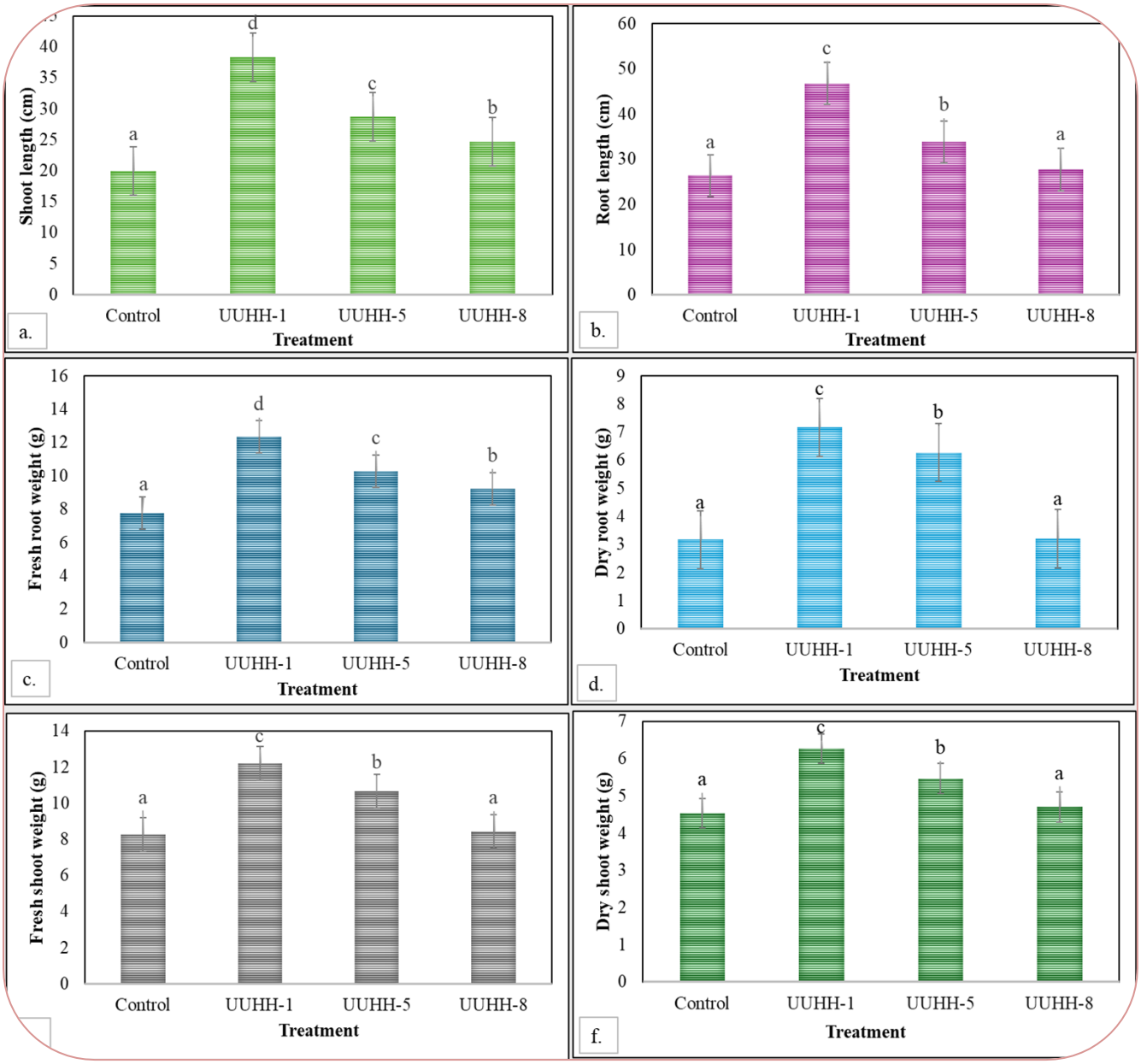


Figure 3. Growth response of *C. aucheri* seedlings to bacterial inoculation. (a) Shoot length; (b) Fresh shoot weight; (c) Dry shoot weight; (d) Root length; (e) Fresh root weight; and (f) Dry root weight. Data are presented as means ± SE of three replicates. Bars with the same letter indicate no significant difference, while bars with different letters indicate significant differences between treatments.

Discussion

In the present investigation, a total of 85 isolates were screened and morphologically characterized. Based on biochemical and morphological traits, different isolates were selected, and ten potential PGPR isolates were further evaluated for multiple plant growth-promoting traits. These potential strains were applied to seeds of *C. aucheri* to assess their growth performance under controlled conditions. PGPR employ multiple mechanisms of action, including phosphate solubilization, which is a critical trait that enhances phosphorus availability to plants. Various *Bacillus* strains formed halo zones around colonies, indicating positive phosphate solubilization, including UUHH-1, UUHH-5, and UUHH-8. Phosphate-solubilizing bacteria also showed beneficial effects on *C. aucheri* due to their multifunctional abilities, including

phosphate solubilization, IAA production, HCN, and siderophore synthesis. These traits directly promote plant growth (Zaheer et al., 2019).

The results indicate that the genus *Bacillus* is a significant contributor to phosphate-solubilizing bacteria. This finding aligns with previous reports (Zaheer et al., 2019; Yanez-Ocampo et al., 2020; Aliyat et al., 2022). The growth-promoting effect is largely attributed to *B. subtilis*, which enhances phosphorus solubilization, stimulates root growth, and promotes overall plant development. Physiological analyses revealed that *B. subtilis* increased both shoot and root length and biomass. Several phosphate-solubilizing, IAA-, ammonia-, and siderophore-producing bacterial strains were further confirmed through 16S rRNA sequencing, including *B. subtilis* UUHH-1 (accession # PQ653540), *B. mojavensis* UUHH-8 (accession # PQ653545.1), and *B. cereus* UUHH-5 (accession # PQ653541). Similar findings have been reported by Ahmad et al. (2021) and Gashash et al. (2022); highlighting the potential of PGPR strains as inoculants for improving plant growth.

PGPR promote plant growth through multiple mechanisms, with phosphate solubilization being a key pathway that improves phosphorus availability. Our physiological results showed that *B. subtilis* enhanced both shoot and root biomass and length. Enhancing the success of rangeland restoration is critical given ongoing losses due to climate change and environmental stress. Under these conditions, ecological restoration efforts should prioritize maximizing plant growth in the shortest possible time using PGPB (Delshadi et al., 2017; Shah et al., 2021). Short-term interactions between plants and beneficial bacteria can also facilitate long-term restoration outcomes.

Overall, the present study demonstrated that PGPR significantly increased the fresh and dry weights of roots and shoots, as well as roots and shoot length of *C. aucheri*. PGPR-inoculated treatments performed better than uninoculated controls under controlled conditions. Strains UUHH-1, UUHH-5, and UUHH-8 positively influenced physiological traits, confirming the ability of PGPR to enhance overall plant growth and survival. These results are consistent with previous studies reporting positive PGPR effects on plant growth promotion and nutrient uptake (Rahnama et al., 2023; Hosseini-Moghaddam et al., 2024). The increase in fresh weight, dry weight, and seedling length indicates that PGPR act directly on plant biomass (Liu et al., 2023). Among the tested strains, UUHH-1 exhibited the most significant increases in root and shoot length, as well as fresh and dry weights, compared to control and other treatments (UUHH-5 and UUHH-8). This strong growth-promoting effect is attributed to the versatile mechanisms of *B. subtilis*, including phosphate solubilization, IAA production, and ammonia synthesis, which collectively enhance *C. aucheri* growth.

Other studies have also shown that PGPR act as growth regulators by producing phytohormones such as IAA, which improve plant vigor and development (Ahmad et al., 2016; Pérez-Montaño et al., 2025). PGPR create a favorable environment for plant growth through direct and indirect mechanisms (Salimi et al., 2025). Inoculation with different *Bacillus* strains significantly increased fresh and dry biomass compared to controls, with *B. subtilis* UUHH-1 showing the maximum enhancement in plant height, shoot and root length, and biomass. Similar effects of PGPR on root and shoot growth and biomass accumulation have been reported in numerous studies (Chandran et al., 2021; Gashash et al., 2022; Najafi Vafa et al., 2022). The positive impact on plant physiological status is closely linked to phytohormone production by PGPR, which stimulates cell division and elongation (Hosseini-Moghaddam et al., 2024).

PGPR also enhance nutrient availability by promoting root growth, thereby improving nutrient uptake and various plant growth traits (Etesami and Maheshwari, 2018). The superior growth promotion by *B. subtilis* in this study is likely due to combined effects of IAA production, phosphate solubilization, ammonia, and siderophore synthesis, which directly stimulate plant growth and improve soil water retention. In contrast, *B. mojavensis* and *B. cereus* exhibited comparatively lower growth promotion. Other PGPR strains, including *Rhizobium*, *Pseudomonas*, and *Azotobacter*, exhibit multifunctional PGP activities and environmental resilience, effectively improving plant growth and soil health in rangeland ecosystems (Hosseini-Moghaddam et al., 2024). These differences may reflect variation in nutrient solubilization capabilities. Overall, the findings highlight *B. subtilis* as a promising PGPR strain for the restoration of degraded rangelands. Future studies should include field-based validation to confirm the effectiveness of these *Bacillus* strains under natural environmental conditions. Even short-term associations between plants and beneficial bacteria may contribute to long-term restoration outcomes.

Conclusion

This study demonstrated that PGPR inoculation, particularly with *B. subtilis* UUHH-1, significantly increased fresh and dry weight of *C. aucheri* seedlings, improving seedling vigor and nutrient uptake compared to uninoculated controls. PGPR treatments consistently enhanced plant growth and biomass accumulation. The effectiveness of PGPB seed priming depends on the bacterial species, plant genotype, and growth conditions. These findings suggest that PGPR can play a crucial role in addressing global food security and climate change challenges while supporting rangeland restoration. Consequently, these bacteria represent a promising approach for promoting *C. aucheri* growth. Furthermore, PGPR offer an eco-friendly alternative to synthetic fertilizers and growth regulators, providing a sustainable strategy for enhancing plant growth in degraded rangelands and reducing pressure on natural resources.

Author's Contributions

HT conducted the experiments, performed data analysis, and prepared the initial draft of the manuscript. HT, LA, AS, RH, FN, and AT conceived and supervised the research project, guided the experimental design, and critically reviewed and revised the manuscript for intellectual content. HT and AT also provided critical evaluation of the study, contributed to manuscript editing and refinement, and assisted in improving the scientific clarity and overall presentation of the work. All authors read and approved the final version of the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

Sustainable Development Goals Targeted

SDG 2: Zero Hunger
SDG 13: Climate Action
SDG 15: Life on Land

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