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RESEARCH ARTICLE

Unveiling *Pseudochrobactrum asaccharolyticum* and associated rhizobacteria with *Lepidium meyenii* W.: PGPR potential and interaction with high Andean soil properties

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ABSTRACT

Intensive cultivation of maca (*Lepidium meyenii* Walp.) causes significant physicochemical degradation and a loss of microbiological resilience in High Andean soils. Native rhizobacteria-based biofertilizers emerge as a sustainable alternative to restore soil quality. This study explored the microbial diversity of the maca rhizosphere at extreme altitudes (> 4000 m a.s.l.) and evaluated the potential of native isolates as plant growth-promoting rhizobacteria (PGPR). A total of 133 isolates were obtained using selective methodologies. Six elite strains were molecularly identified (16S rRNA gene) as *Peribacillus, Pseudomonas, Pantoea*, and notably, *Pseudochrobactrum asaccharolyticum*. Low microbial density was observed, likely linked to degradation from intensive monoculture. Microbial functionality was associated with soil management, enabling the identification of strains with high viability (> 10^{10} CFU mL^{-1}) adapted to extreme conditions. In this study, the presence of *P. asaccharolyticum* was identified in association with maca crops. This species is of particular interest due to its previously recognized potential for soil bioremediation. These findings suggest functional specialization of the native microbiota and a high biotechnological potential for developing specific bio-inputs. These results contribute to mitigating monoculture impacts, restoring degraded soils, and promoting sustainable High-Andean agriculture.

Key words: Andean agriculture, extreme environments, microbial characterization, soil degradation.

INTRODUCTION

Maca (*Lepidium meyenii* Walp.) is a high-altitude tuberous root of global economic significance, widely recognized as a "superfood" due to its rich composition of essential amino acids, fatty acids, and nutrients such as vitamin C, Fe, Ca, and K (da Silva et al., 2020). Beyond its nutritional value, maca exhibits significant pharmacological properties, including neuroprotective, antinociceptive (Quispe-Díaz et al., 2025), memory enhancement, regulation of sexual function, and antidepressant properties, among others (da Silva et al., 2020).

Cultivation predominantly occurs between 3700 and 4500 m a.s.l., with the Bombón Plateau in Junín serving as the primary production hub (Gonzales, 2011). However, maca is primarily cultivated as a monoculture (Dostert et al., 2009). The ecological drawbacks of such systems – ranging from inefficient resource utilization to the disruption of soil homeostasis – have been extensively documented (Salaheen and Biswas, 2019; Belete and Yadete, 2023). In these High Andean ecosystems, continuous monoculture has

triggered a sharp decline in soil fertility and nutrient depletion, forcing a heavy reliance on synthetic fertilizers and pesticides (Belete and Yadete, 2023). The intensive use and excessive application of chemical fertilizers, such as N, have been shown to drastically alter the structure, abundance, and physiological profiles of rhizospheric microbial communities (Xu et al., 2025). This cycle has severely compromised the resilience of maca-producing lands, where natural soil restoration is increasingly hampered by anthropogenic pressure (De la Cruz, 2021).

To address this, the development of bio-inputs - formulated from beneficial microorganisms and bioactive molecules - has emerged as a sustainable alternative to enhance crop quality and productivity (da Silva et al., 2025). Integrating bio-inputs into agricultural practices not only presents technical advantages but also a paradigm shift and the adoption of new technologies that prioritize the sustainability and regeneration of natural resources (Mattos et al., 2025). Under this approach, plant growth-promoting rhizobacteria (PGPR) are pivotal due to their functional versatility. These microorganisms facilitate nutrient uptake through biological N fixation, phosphate solubilization and siderophores production (Ahmad et al., 2018). They also play a crucial role in soil structural stabilization by improving physical properties such as particle aggregation, reducing compaction, increasing spore space, and enhancing water infiltration (Alori et al., 2017).

Considering that the selection of microorganisms and the characterization of their functional attributes represent a critical step in the formulation of microbial inoculants (Valente et al., 2025), this study aimed to isolate and characterize rhizospheric microorganisms from different maca fields in Junín. This research establishes the scientific foundation for developing biotechnological tools that not only promote maca yield but also mitigate the degradation of fragile High Andean soils.

MATERIALS AND METHODS

Soil sampling

Soil samples (M1-M5) were collected in five maca (*Lepidium meyenii* Walp.) production fields located at over 4000 m a.s.l. in the district of Junín (11°01’12’’ S, 76°06’08’’ W), province of Junín, department of Junín, Peru (Figure 1, Table 1).

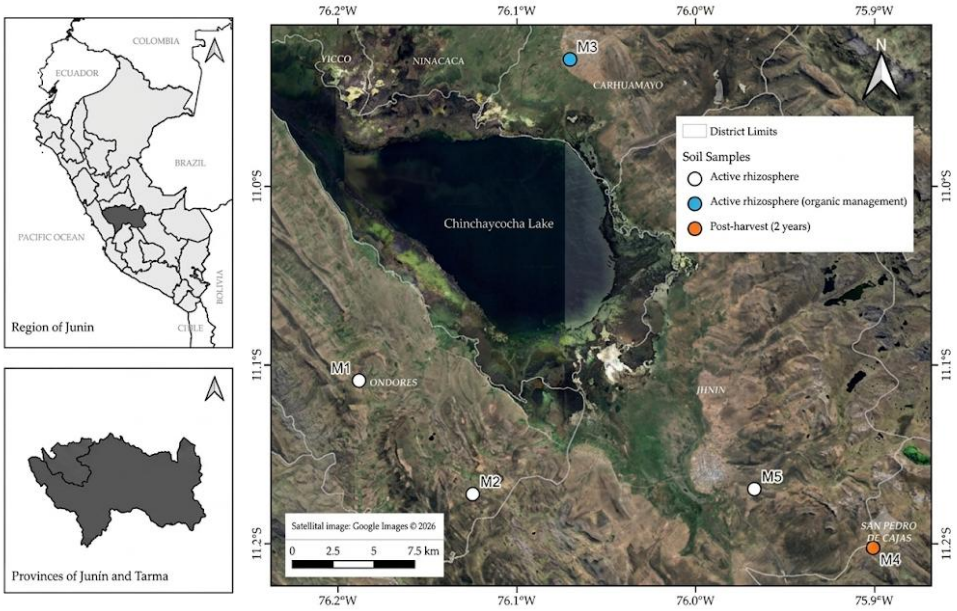

Figure 1. Soil sampling area in Junín, Peru.

Table 1. Passport data of soil sampling points. M1: Active rhizosphere; M2: active rhizosphere; M3: active rhizosphere and organic management; M4: postharvest; M5: active rhizosphere.

Sample ID	Community	Latitude (S)	Longitude (W)	Altitude (m a.s.l.)	Field condition
M1	Pishqa Puquio	11°06'31''	76°11'18''	4320	Active rhizosphere
M2	Ondores	11°10'20''	76°07'29''	4439	Active rhizosphere
M3	Carhuamayo	10°55'44''	76°04'13''	4101	Active rhizosphere (organic management)
M4	Chacamarca	11°12'08''	75°54'03''	4370	Post-harvest (2 yr)
M5	Cerro San Cristóbal	11°10'10''	75°58'02''	4257	Active rhizosphere

Sampling conditions included active rhizosphere stages under organic and conventional management, as well as post-harvest soils with different periods of agricultural use (Table 1). In fields with crops, rhizosphere soil adhering to the roots was extracted, while in fields without active cultivation, bulk soil was collected at a depth of 15-20 cm. Two hundred grams were taken from each sampling point and transported and stored at 4-8 °C in the Technological Research Laboratory on Climate Change for the Agricultural Sector of the National Institute of Agricultural Innovation (INIA) until processing. The physicochemical properties of each sample were also analyzed in the Soil, Water, and Foliar Analysis Laboratory (LABSAF, for its Spanish acronym) of the INIA Santa Ana Experimental Station.

Microbial isolation

Microbial isolation was performed using the serial dilution technique. Briefly, 10 g soil sample were suspended in 90 mL sterile saline solution (0.85% w/v NaCl) and shaken at 120 rpm for 3 h. After allowing the sediment to settle for 1 h at room temperature, this initial suspension (10^{-1} dilution) was used to prepare a dilution series up to 10^{-7} in 0.85% NaCl.

To target the genera *Bacillus* and *Pseudomonas*, two selective approaches were employed using nutrient agar (NA) and Pseudomonas agar for fluorescein (PAF), respectively. For each medium, 100 µL each dilution were spread in triplicate using Drigalski loop and incubated at 28 °C for 72 h. After incubation, colonies exhibiting distinct macromorphological features consistent with each target genus were selected and purified through successive subculturing.

To specifically select endospore-forming microorganisms - a distinctive trait of the Bacillaceae family and related genera - a heat shock treatment was applied. Pure isolates were inoculated into Luria-Bertani (LB) broth and subjected to 80 °C for 10 min (Rodríguez et al., 2017). Post-treatment viability was confirmed by inoculation on NA medium; subsequent growth was used as a presumptive criterion for the identifying belonging to the *Bacillus* group and related spore-formers.

For isolates obtained on PAF, priority was given to those exhibiting fluorescence under UV light, a hallmark of the fluorescent *Pseudomonas* group.

Finally, isolate purity and preliminary biochemical characteristics were verified through Gram staining and the catalase test (using 30% v/v H_2O_2).

Microbial functional characterization

The functional characterization of the selected isolates involved the evaluation of indole-3-acetic acid (IAA) production, tricalcium phosphate solubilization, and siderophore production.

The IAA production was determined following the methodology of Asghar et al. (2002) with adaptations for microtubes. Each isolate was inoculated into yeast-mannitol broth (YMB) supplemented with 50 mg L^{-1} L-tryptophan. After 4 d of incubation at 28 °C, a 2 mL aliquot was centrifuged at 10 000 rpm for 5 min. For the colorimetric reaction, 1.5 mL resulting supernatant were mixed with 1 mL Salkowski reagent and incubated in the dark for 30 min.

Tricalcium phosphate solubilization was assessed by inoculating each isolate onto NBRIP agar (Nautiyal, 1999) and incubated at 28 °C for 5 d; a positive result was indicated by the presence of a translucent halo around the colony. Similarly, siderophore production was evaluated on diluted King B agar supplemented with chrome-azurol S (CAS) (Louden et al., 2011). Following 5 d incubation at 28 °C, a color shift from blue to yellow/orange was recorded as positive for Fe chelating activity.

For all plant growth-promotion tests, activity was recorded semi-quantitatively using a scale of 0 to 3, defined as follows: (0) No activity, (1) low activity, (2) moderate activity, and (3) high activity.

Finally, growth kinetics were evaluated for the top-performing isolates by determining colony-forming units (CFU) at 24 and 48 h incubation. This parameter allowed for the differentiation of colonization rates among the most promising candidates – a critical factor for their future implementation as robust biological inoculants.

Multivariate analysis

To evaluate the relationship between the origin of the isolates and their biofertilizer potential, a principal component analysis (PCA) was performed. This analysis integrated physicochemical properties (e.g., pH, organic matter, available P) with microbial functional data. Additionally, a heatmap with hierarchical clustering was generated to visualize the functional profiles and establish groups based on isolate-origin interactions. All analyses were conducted in RStudio software (version 2025.05.0+496; Posit PBC, Boston, Massachusetts, USA).

Molecular identification

Genomic DNA was extracted from pure bacterial cultures using the phenol-chloroform-isoamyl alcohol (25:24:1) method, as described by Ausubel (2003). The taxonomic identity of the isolates was determined by amplifying and sequencing the 16S rRNA gene. The PCR amplification was performed using the universal primers 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-GGTTACCTTGTTACGACTT-3′) (Reysenbach et al., 1992). The reaction mixture contained 1x DreamTaq Green PCR Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts, USA) and 0.4 µM each primer. The thermal cycling conditions consisted of an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 1 min; with a final extension step at 72 °C for 10 min. The PCR products were subsequently sequenced using the Sanger dideoxy method.

The resulting sequences were edited using Chromas Lite 2.0 software (Technelysium Pty. Ltd., Tewantin, Queensland, Australia). Chromatogram parameters, including peak symmetry and base spacing, were rigorously inspected; irregular or low confidence nucleotides were discarded to ensure sequence integrity (Al-Shuhaib and Hashim, 2023). Finally, homology searches were conducted using the Basic Local Alignment Search Tool (BLAST) against the National Center for Biotechnology Information (NCBI) database to determinate the taxonomic affiliation of each isolated based on 16S rRNA gene similarity.

RESULTS

Soil physicochemical analysis

The physicochemical analysis of the sampled soils revealed a gradient of acidity and fertility (Table 2). Soils located at higher altitudes (M1, M2, and M5) exhibited the lowest pH values (5.3-5.4), indicating a moderately acidic nature, coupled with the highest organic matter (OM) concentrations (8.5%-10.3%). In contrast, sample M3 - originating from an organically managed plot in Carhuamayo - showed the least acidic pH (6.2) but the lowest levels of OM (4.7%) and total N (0.24%).

Table 2. Physicochemical analysis of the soils collected in Junín. M1: Pishqa Puquio, active rhizosphere; M2: Ondores, active rhizosphere; M3: Carhuamayo, active rhizosphere and organic management; M4: Chacamarca, postharvest; M5: Cerro San Cristóbal, active rhizosphere.

Soil sample	pH	Organic matter	Electrical conductivity	Available P	Available K	N	Texture
		%	mS m^{-1}	mg kg^{-1}	mg kg^{-1}	%	
M1	5.3	9.2	5.2	137.7	124.89	0.46	Sandy loam
M2	5.4	8.5	3.4	229.8	186.49	0.43	Sandy loam
M3	6.2	4.7	6.1	59.4	71.13	0.24	Sandy loam
M4	5.6	10.3	3.3	219.3	152.26	0.52	Sandy loam
M5	5.3	6	4.5	300.8	108.8	0.3	Sandy loam

Across all sampling points, electrical conductivity remained at extremely low levels (3.3 to 6.1 mS m^{-1}), categorizing these soils as non-saline. Finally, exceptionally high P availability was recorded at all points, with sample M5 reaching a peak concentration of 300.8 mg kg^{-1}.

Microbial isolation

A higher population density was observed on Pseudomonas Agar F (PAF) compared to nutrient agar (NA), particularly within the lower dilution (10^{-1} and 10^{-2}). However, the detection threshold in both media was reached at the 10^{-4} dilution, beyond which no viable colonies were recovered (Figure 2). This relatively low total culturable microbial load is consistent with the extreme environmental stressors of High Andean soils, such as low temperatures and high UV radiation. The total microbial concentrations were calculated for each sample. The results showed that the highest bacterial density in both media was recorded in sample M3 (*p*-value = < 0.01), originating from a field under organic management, while M4 obtained the lowest value, from a post-harvest field (Figure 3).

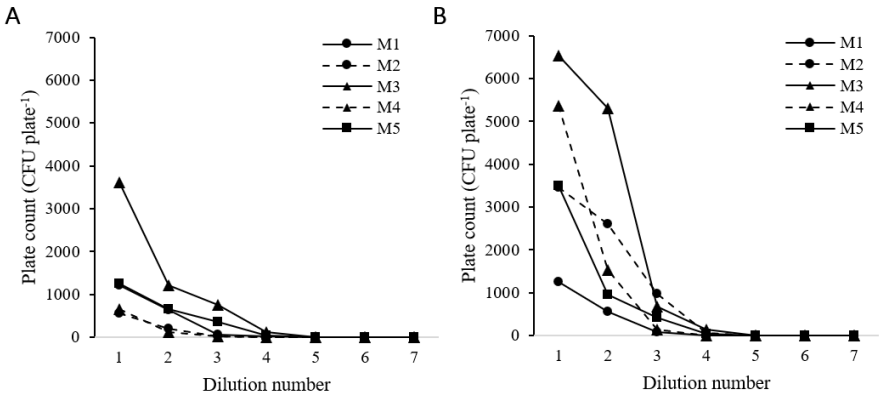

Figure 2. Colony density on (A) nutrient agar and (B) Pseudomonas agar F observed at all successive dilutions of soil samples (M1-M5). M1: Pishqa Puquio, active rhizosphere; M2: Ondores, active rhizosphere; M3: Carhuamayo, active rhizosphere and organic management; M4: Chacamarca, postharvest; M5: Cerro San Cristóbal, active rhizosphere. CFU: Colony-forming units.

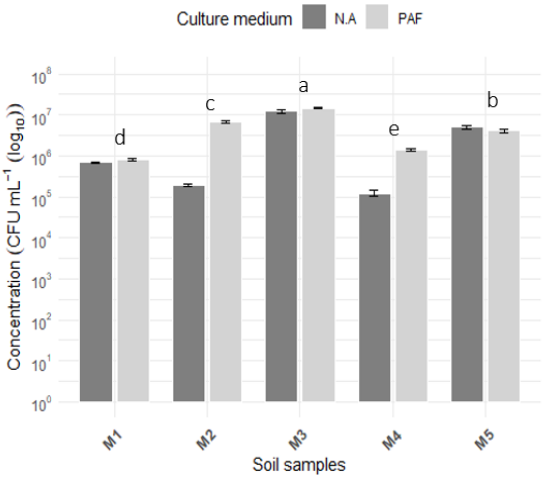

Figure 3. Concentration of total microorganisms in nutrient agar (NA) and Pseudomonas Agar F (PAF) for the collected samples. Data are means ± SD (*n* = 3). Different letters indicate significant difference according to Tukey's test (α = 0.05). M1: Pishqa Puquio, active rhizosphere; M2: Ondores, active rhizosphere; M3: Carhuamayo, active rhizosphere and organic management; M4: Chacamarca, postharvest; M5: Cerro San Cristóbal, active rhizosphere.

A total of 133 bacterial isolates were recovered from the five soil samples: 43 from NA and 90 from PAF. In the case of NA isolates, the heat shock treatment served as a rigorous selective pressure, resulting in a significant reduction, with only 9 isolates (20.9%) surviving the procedure. Whitin this subgroup, 7 isolates (16.3% of the initial NA pool) were confirmed as catalase-positive and Gram-positive bacilli, consistent with the morphotype of Bacillaceae family. In contrast, the selection process on PAF demonstrated high specificity. Of the 90 initial isolates, 28 (31.1%) exhibited fluorescence under UV light, a phenotypic trait typically associated with the fluorescent *Pseudomonas* group.

Functional characterization

The functional characterization was performed on the 35 pre-selected isolates (7 presumptive *Bacillus* spp. and 28 presumptive *Pseudomonas* spp.) Overall, the *Pseudomonas* group exhibited significantly superior performance in plant growth-promoting (PGP) traits compared to the *Bacillus* isolates. To better categorize this functional diversity, a hierarchical cluster analysis was performed, identifying three distinct groups based on their biofertilizer potential (Figure 4): Cluster A (incipient activity) comprised isolates with null or marginal PGP traits, representing the baseline functional group; Cluster B (elite PGPR potential) identified as the most promising group, consisting of strains with the highest scores across all evaluated traits (IAA, P solubilization and siderophores); Cluster C (intermediate activity) included isolates with moderate or specialized performance in only one or two functional categories.

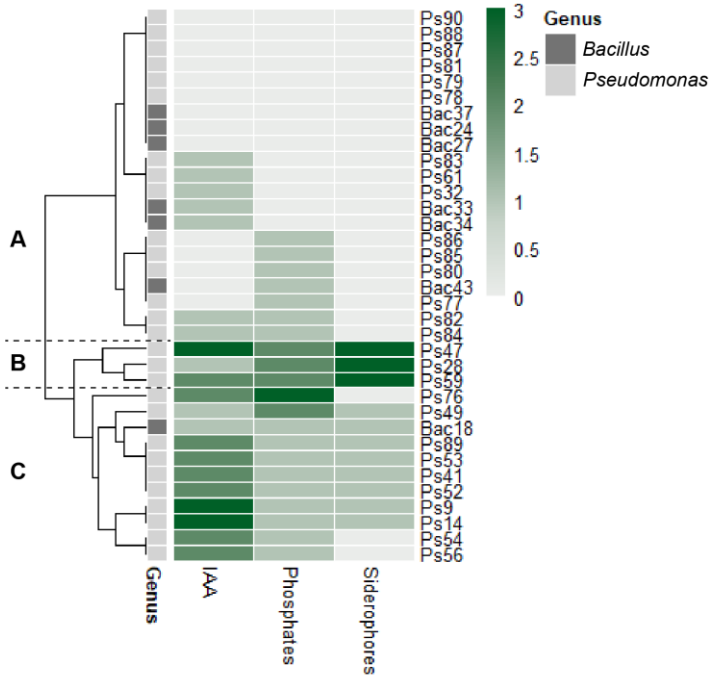

Figure 4. Functional characterization of plant growth promoting traits for the 35 isolates evaluated. Genus: Presumptive genus; IAA: production of indole-3-acetic acid; Phosphates: solubilization of phosphates; Siderophores: production of siderophores.

Molecular identification

Based on the hierarchical clustering and functional representativeness, six high-performing candidates were prioritized for molecular identification via 16S rRNA gene sequencing. The selection included the top three strains from Cluster B: Ps47, Ps28, and Ps59. Additionally, isolates with specialized outstanding traits were incorporated to ensure functional diversity: Ps76 (exhibiting high dual-activity in PGP assays) and Ps9 (high IAA production). From the *Bacillus* group, isolate Bac18 was selected as the most robust representative.

The taxonomic affiliation and sequence homology for these strains are summarized in Table 3. The identification revealed a diverse group of rhizobacteria, including members of the genera *Pseudochrobactrum*, *Pantoea* and *Pseudomonas*, as well as spore-forming bacilli.

Table 3. Molecular identification of the six isolates selected for their functional representativeness. QC: Query cover; PI: per identity. *Accession numbers of the closest phylogenetic relatives identified via BLASTn.

Strain ID	Accession*	Scientific name	QC (%)	PI (%)	Key feature	Cluster	Soil sample
Ps47	PX022456.1	*Pseudochrobactrum asaccharolyticum*	99	99.7	High PGPR potential with adaptation to altitude	B	Active rhizosphere
Ps28	PV563462.1	*Pseudomonas* sp.	97	99.9	High performance cluster	B	Active rhizosphere (organic management)
Ps59	LK391522.1	*Pseudomonas koreensis*	100	99.5	High performance cluster	B	Active rhizosphere
Ps9	PV563462.1	*Pseudomonas* sp.	100	99.3	High production of IAA	C	Active rhizosphere
Ps76	OQ708967.1	*Pantoea agglomerans*	99	99.4	High phosphates solubilization	C	Active rhizosphere (organic management)
Bac18	OR652418.1	*Peribacillus frigoritolerans*	98	99.8	The best activity of the *Bacillus* group	C	Active rhizosphere

Multivariate analysis

The interaction between soil physicochemical properties and the functional profiles of the isolates was analyzed using principal component analysis (PCA) (Figure 5). The first two dimensions explained 70.5% of the total variance, with PC1 (49.9%) primarily associated with soil variables and PC2 (20.6%) linked to PGP and metabolic activities, such as catalase and fluorescence.

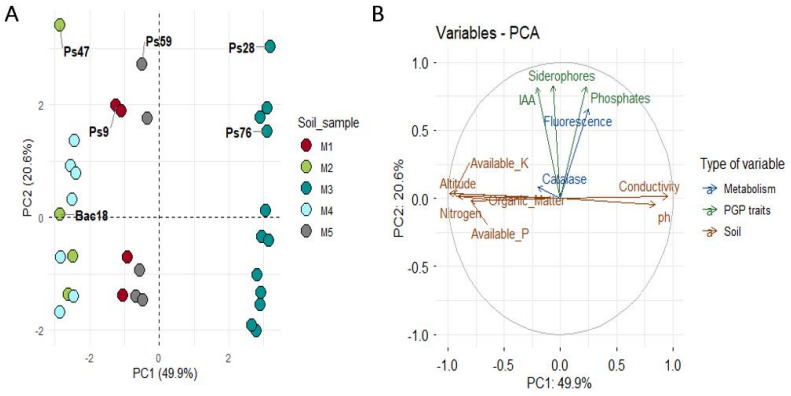

Figure 5. Principal component analysis (PCA) for the 35 characterized isolates. (A) Distribution of the isolates in the principal components according to their origin samples (M1-M5). (B) Distribution of the variables in the principal components. M1: Pishqa Puquio, active rhizosphere; M2: Ondores, active rhizosphere; M3: Carhuamayo, active rhizosphere and organic management; M4: Chacamarca, postharvest; M5: Cerro San Cristóbal, active rhizosphere; Bac18: *Peribacillus frigoritolerans*; Ps9: *Pseudomonas* sp.; Ps28: *Pseudomonas* sp.; Ps47: *Pseudochrobactrum asaccharolyticum*; Ps59: *Pseudomonas koreensis*; Ps76: *Pantoea agglomerans*; IAA: production of indole-3-acetic acid; Phosphates: solubilization of phosphates; Siderophores: production of siderophores; PGP traits: plant growth promoting traits.

In the rhizosphere samples (M1, M2, M3, M5), a marked functional heterogeneity was observed. In the case of the organically managed sample (M3), two distinct groups were identified: One associated with fluorescence and phosphate solubilization, and another characterized by a different metabolic profile (Figure 5A). In contrast, isolates from sample M4 (post-harvest) clustered near the PC1, indicating basal metabolism without prominent activity in the PGP assays performed. This justifying their exclusion from the molecular identification stages.

The PCA biplot also showed an orthogonal relationship between the vector representing soil properties and those corresponding to PGP functionality (Figure 5B). Specifically, the direction of the vector for phosphate solubilization, IAA production and siderophore synthesis was nearly perpendicular to the vectors for soil pH, organic matter and P availability.

Cell viability

The cell viability of the six selected top isolates reached high concentrations starting from 24 h of incubation, with values ranging between 10^8 and 10^{10} CFU mL^{-1} (Figure 6) These concentrations exceed the minimum industrial thresholds required for the formulation of agricultural bio-inputs. Strains Ps28, Ps47, and Ps76 were particularly noteworthy, consistently reaching densities on the order of 10^{10}. This performance reflects high efficiency in nutrient assimilation and a rapid doubling rate under the evaluated conditions.

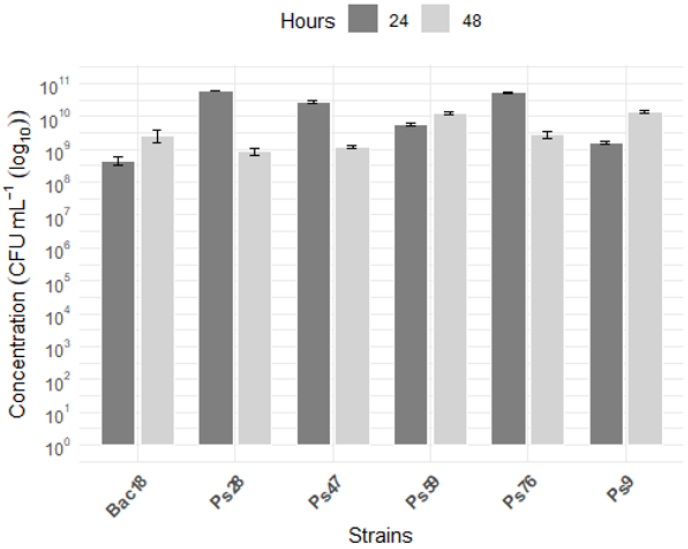

Figure 6. Concentrations of microorganisms at 24 and 48 h of the six selected isolates. Data are means ± SD (n = 3). Bac18: *Peribacillus frigoritolerans*; Ps28: *Pseudomonas* sp.; Ps47: *Pseudochrobactrum asaccharolyticum*; Ps59: *Pseudomonas koreensis*; Ps76: *Pantoea agglomerans*; Ps9: *Pseudomonas* sp.

DISCUSSION

High Andean soils in Peru are characterized by elevated organic matter (OM) content, as soil organic C (SOC) storage capacity is strongly modulated by altitude and temperature. In these ecosystems, SOC typically increases with altitude, while low temperatures suppress mineralization rates, favoring C sequestration (Huamán-Carrión et al., 2021). Consistent with recent studies in the Junín region, the evaluated soils exhibited an acidic pH and a non-saline condition, as evidenced by their low electrical conductivity (Zúñiga-Dávila et al., 2011; Arias-Arredondo et al., 2026).

Despite the high concentration of available P recorded (300.8 mg kg^{-1}), the acidic nature of theses soils suggests that a significant fraction of this element may be chemically immobilized or present in forms with low

bioavailability for plants. This geochemical context is further complicated by the presence of As in the region (Pizarro et al., 2025), which often competes for the same soil adsorption sites as P.

The physicochemical analysis corroborated these trends, reporting OM values up to 10.3%, pH levels between 5.3-6.2, and electrical conductivity of 3.3 mS m^{-1} (Table 2). Notably, organically managed, lower altitude sample (M3), exhibited the highest pH value (6.2, close to neutral) but the lowest OM content (4.7%). This apparent paradox can be explained by microbial population dynamics: A less acidic pH enhances nutrient availability, which in turn stimulates the proliferation of microorganisms, particularly bacteria (Rousk et al., 2009). This is reflected in the higher culturable bacterial density recorded in this soil (Figure 3). Consequently, the lower OM content in M3 does not necessarily indicate a fertility deficiency, but rather a higher mineralization rate driven by an active and dense microbial community.

Unlike observations in rhizospheres from conventional lowland crops, where population densities typically allow for bacterial isolation at dilutions 10^{-7} or higher (Yasin et al., 2022), the recovery in this study was only viable up to a 10^{-4} dilution (Figure 2). This significantly reduced microbial load may serve as a biological indicator of the severe ecological degradation affecting High Andean soils, likely exacerbated by the intensive monoculture of *Lepidium meyenii* (Custodio et al., 2021). Such conditions suggest a depletion of available ecological niches, where extreme environmental pressure and soil exhaustion limit the expansion of culturable populations.

Conversely, the isolates showed the highest plant growth-promoting rhizobacteria (PGPR) potential originated exclusively from active rhizosphere samples (Table 3). This reinforces the "rhizosphere effect", confirming the central role of root exudates in the selection of functional communities (Chen and Liu, 2024). Furthermore, the functional superiority of the *Pseudomonas* group over the *Bacillus* group (Figure 4) is consistent with international reports highlighting the remarkable psychrotolerant adaptation of *Pseudomonas* spp. These bacteria maintain high metabolic efficiency in high altitude ecosystems characterized by low temperatures and fluctuating UV radiation (Adhikari et al., 2021).

In Peru, research on rhizobacteria associated with *L. meyenii* remains limited, with notable exceptions such as the work of Zúñiga-Dávila et al. (2011), who reported classical genera like *Pseudomonas* and *Bacillus*, and the metagenomic insights provided by Custodio et al. (2021). Our study expands this spectrum by identifying genera previously underreported for this crop, revealing a broader microbial diversity and unexplored biotechnological potential in high-Andean soils. Molecular identification confirmed that strain Ps47 was closely related to *Pseudochrobactrum asaccharolyticum*, while Ps59 was identified as *Pseudomonas koreensis*, Ps76 as *Pantoea agglomerans*, and Bac18 as *Peribacillus frigoritolerans*. Additionally, isolates Ps28 and Ps9 were confirmed as members of the *Pseudomonas* genus. While 16S rRNA sequencing provides a robust preliminary identification, further polyphasic taxonomic studies or whole-genome sequencing (WGS) will be essential to definitively confirm the species-level assignment.

The principal component analysis (PCA) allowed for a spatial visualization of how these strains are functionally grouped in relation to soil properties (Figure 5). The orthogonal distribution of plant growth promoting (PGP) traits and physicochemical characteristics suggests that the expression of growth-promoting capabilities is not directly depend on bulk soil conditions, but rather reflects the adaptation of bacteria to specific niches. In this context, isolates from sample M3 clustered on the right from the biplot. Correspondingly, strains Ps47 (*P. asaccharolyticum*), Ps59 (*P. koreensis*), and Ps28 (*Pseudomonas* sp.) were located in the upper quadrant, associated with the strongest PGPR activities, confirming their membership in cluster B (Figure 4), which represents the highest performing isolates.

A key observation was that catalase activity correlated more closely with bacterial resistance markers than with growth promotion (Figure 5B). This suggests a metabolic trade-off where microorganisms in high-altitude ecosystems (such as M2 at 4339 m a.s.l.) may prioritize enzymatic defense mechanisms to mitigate oxidative stress induced by intense UV radiation (Sartorio et al., 2020) over PGP functions. Remarkably, isolate Ps47 (*P. asaccharolyticum*) maintained high PGP activity despite these extreme conditions, highlighting its exceptional functional resilience.

Furthermore, a close correlation was identified between phosphate solubilization capacity and fluorescence emission (Figure 5B). This synergy is likely driven by the secretion of organic acids, that act as chelating agents for metal cations (Al^{+3}, Fe^{+3}, and Ca^{+2}), thereby increasing nutrient bioavailability (Rawat et al., 2020). In genera such as *Pseudomonas*, this activity is often coupled with the synthesis of fluorescent siderophores, suggesting an integrated resource-acquisition mechanism essential for surviving the restrictive environment of high-Andean soils.

The isolate Ps47, identified as *P. asaccharolyticum*, represents a finding of particular biotechnological relevance. This species has recently been described internationally for its ability to mitigate oxidative stress in crops under heavy metals toxicity by activating antioxidant defense systems (Waheed et al., 2024). To our knowledge, this study provides the first evidence of an association between the genus *Pseudochrobactrum* and *L. meyenii* cultivation. The identification of the Ps47 not only expands the taxonomic catalog of Andean rhizobacteria but also suggests an unexplored ecological niche where this microorganism may play a crucial role in plant adaptation to the extreme conditions of the High Andean.

On the other hand, *P. koreensis* (Ps59) is recognized as a potent biological control agent, with reported antagonistic activity against phytopathogens such as *Cephalosporium maydis* and *Fusarium* spp. (Rafikova et al., 2016; Ghazy and El-Nahrawy, 2020). This biocontrol potential is consistent with the high siderophore production observed in our functional characterization (Figure 4). In addition to its PGP traits (Kang et al., 2019), recent genomic studies have identified genes in *P. koreensis* that are critical for adapting to oxidative, osmotic, and thermal stress (Ayangbenro et al., 2024).

The isolate Ps76, identified as *P. agglomerans*, serves as a multifunctional PGPR. Genomic analyses have confirmed that this species harbors key sequences for phosphate solubilization, siderophore synthesis, N metabolism, and indole-3-acetic acid production (Kumar et al., 2022). Furthermore, *P. agglomerans* possesses genetic markers for heavy metal tolerance and adaptation to adverse environmental conditions (Luziatelli et al., 2020). This metabolic versatility explains why sample M3 maintained a high bacterial population density despite its lower OM content, as *P. agglomerans* efficiently colonizes and thrives in niches with high nutrient turnover.

The functional diversity of the identified isolates reveals an adaptive specialization geared towards mitigating the extreme environmental stressors of the Puna region. Notably, genera such as *Pseudochrobactrum*, *Pseudomonas* and *Pantoea* not only exhibit PGP traits but also possess robust genetic and metabolic mechanisms to counteract oxidative stress. This remarkable metabolic plasticity aligns with reports identifying As as a predominant anthropogenic contaminant in Junín's agricultural soils, where concentrations often exceed background levels (Pizarro et al., 2025). Such localized microbial adaptation is consistent with evidence demonstrating that native rhizobacteria play a critical role in enhancing plant biochemical resilience and growth under challenging environmental conditions (Chompa et al., 2024). Under this scenario, the persistence of these genera and their ability to reach high population densities suggests coevolution with metalloids and other soil stressors. Thus, the presence of these bacteria would not only benefit maca nutrition but also act as a biological barrier against heavy metal phytotoxicity, a critical factor for food security in the central Andes.

Furthermore, the cell viability maintained for up to 48 h is a key trait for rhizosphere competition, allowing the inoculum to establish itself effectively in limiting and degraded ecological niches, such as post-maca cultivation soils. The observation of sustained growth confirms the potential of these isolates for the development of high-cell-density biofertilizers.

In conclusion, our results suggest that isolates Ps47 (*P. asaccharolyticum*), Ps59 (*P. koreensis*), Ps28 (*Pseudomonas* sp.), and Ps76 (*P. agglomerans*) are high-value biotechnological candidates for the development of bioinoculants in agroecosystems with extreme climates. Their ability to integrate plant growth promotion with oxidative stress response mechanisms positions the native microbiota as a strategic pillar for the climate resilience of *L. meyenii*. These findings underscore the potential of these microorganisms for the design of biofertilizers specifically formulated for the critical conditions of the central Andes, offering a sustainable alternative for soil conservation and agricultural productivity in high-altitude areas.

CONCLUSIONS

This study successfully identified and characterized a diverse rhizosphere microbiota associated with *Lepidium meyenii* in high-Andean environments, encompassing both established genera like *Pseudomonas* and less frequently reported genera. A particularly noteworthy finding is the presence of *Pseudochrobactrum asaccharolyticum* (Ps47) associated with this crop, a strain that exhibited exceptional functional resilience. Multivariate analysis demonstrated that soil physicochemical properties and plant growth-promoting traits are independently distributed. Collectively, these findings confirm that the maca rhizosphere serves as a reservoir

for microorganisms with significant biotechnological potential. These isolates are prime candidates for the development of inoculants specifically adapted to extreme altitudes, expanding the know spectrum of plant growth-promoting rhizobacteria genera and providing a sustainable strategy for enhancing agricultural productivity and resilience in High Andean ecosystems.

Author contribution

Conceptualization: L.H., J.C. Methodology: L.H., J.C. Validation: L.H. Formal analysis: L.H., J.C. Investigation: L.H., J.C., L.L.S., S.S.S. Data curation: J.C. Writing-original draft: L.H. Writing-review & editing: I.C. Supervision: E.M. Funding acquisition: E.M. All co-authors reviewed the final version and approved the manuscript before submission.

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References

Adhikari, P., Jain, R., Sharma, A., Pandey, A. 2021. Plant growth promotion at low temperature by phosphate-solubilizing *Pseudomonas* spp. isolated from high-altitude Himalayan soil. Microbial Ecology 82:677-687. doi:10.1007/s00248-021-01702-1.

Ahmad, M., Pataczek, L., Hilger, T.H., Ahmad, Z., Hussain, A., Rasche, F., et al. 2018. Perspectives of microbial inoculation for sustainable development and environmental management. Frontiers in Microbiology 9:2992. doi:10.3389/fmicb.2018.02992.

Alori, E.T., Dare, M.O., Babalola, O.O. 2017. Microbials inoculants for soil quality and plant health. p. 281-307. In Lichtfouse, E. (ed.) Sustainable agriculture reviews. Volume 22. Springer Natura, Cham, Switzerland. doi:10.1007/978-3-319-48006-0_9.

Al-Shuhaib, M.B.S. Hashim, H.O. 2023. Mastering DNA chromatogram analysis in Sanger sequencing for reliable clinical analysis. Journal of genetic Engineering and Biotechnology 21:115. doi:10.1186/s43141-023-00587-6.

Arias-Arredondo, A., Pérez, W.E., Lastra, S., Cruz, J., Solórzano-Acosta, R., Turín, C. 2026. Assessing soil and native high Andean grassland quality under grazing: A case study from the wet Puna of Peru. Grassland Science 72(3):163-174. doi:10.1111/grs.70029.

Asghar, H.N., Zahir, Z.A., Arshad, M., Khaliq, A. 2002. Relationship between in vitro production of auxins by rhizobacteria and their growth-promoting activities in *Brassica juncea* L. Biology and Fertility of Soils 35:231-237. doi:10.1007/s00374-002-0462-8.

Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., et al. (eds.) 2003. Current protocols in molecular biology: Supplement 59. John Wiley & Sons, Hoboken, New Jersey, USA.

Ayangbenro, A.S., Olanrewaju, O.S., Amoo, A.E., Babalola, O.O. 2024. *Pseudomonas koreensis* AB36 from a gold mine: Genomic insights into adaptation to extreme conditions and potential plant growth promotion. Biologia 79:3677-3694. doi:10.1007/s11756-024-01799-y.

Belete, T., Yadete, E. 2023. Effect of mono cropping of soil health and fertility management for sustainable agriculture practices: A review. Journal of Plant Sciences 11(6):192-197. doi:10.11648/j.jps.20231106.13.

Chen, L., Liu, Y. 2024. The function of root exudates in the root colonization by beneficial soil rhizobacteria. Biology 13:95. doi:10.3390/biology13020095.

Chompa, S.S., Zuan, A.T.K., Amin, A.M., Hun, T.G., Ghazali, A.H.A., Sadeq, B.M., et al. 2024. Molecular identification and effect of salt-tolerant plant growth-promoting rhizobacteria on the biochemical aspect and growth of rice. Chilean Journal of Agricultural Research 84:606-619. doi:10.4067/S0718-58392024000500606.

Custodio, M., Huaraca-Meza, F., Peñaloza, R., Alvarado-Ibañez, J.C., de la Cruz-Solano, H. 2021. Bacterial composition in maca (*Lepidium meyenii* Walp.) crop soils analyzed by metagenomics: A study in the central Andes of Peru. Scientia Agropecuaria 12(2):175-183. doi:10.17268/sci.agropecu.2021.020.

da Silva, J.M., Araújo, V., Cardoso, A., Beatriz, C., Carvalho, T.M., Lino, M.E., et al. 2025. Bioinputs in sustainable agriculture: Benefits, challenges, and pathways for resilient farming systems. Asian Journal of Research in Crop Science 10(2):85-97. doi:10.9734/ajrcs/2025/v10i2354.

da Silva, N., Cabrera, L., Medeiros, L.L., Formigoni, M., Hernandez, R., Aparecida, A., et al. 2020. Medicinal effects of Peruvian maca (*Lepidium meyenii*): a review. Food & Function 11(1):83-92. doi:10.1039/C9FO02732G.

de la Cruz, H.M. 2021. Influencia del cultivo de maca en la calidad físico-química de suelos en diferentes tiempos de descanso en la meseta de Bombón, Junín. Tesis para optar al grado de Maestra en Gestión Ambiental y Desarrollo Sostenible. Universidad Nacional del Centro del Perú, Huancayo, Perú.

Dostert, N., Roque, J., Cano, A., La Torre, M.I., Weigend, M. 2009. Factsheet: Datos botánicos de Maca - *Lepidium meyenii* Walp. Proyecto Perúbiodiverso – PBD. Available at https://museohn.unmsm.edu.pe/docs/pub_dico/Maca_factsheet_final.pdf

Ghazy, N., El-Nahrawy, S. 2020. Siderophore production by *Bacillus subtilis* MF497446 and *Pseudomonas koreensis* MG209738 and their efficacy in controlling *Cephalosporium maydis* in maize plants. Archives of Microbiology 203:1195-1209. doi:10.1007/s00203-020-02113-5.

Gonzales, G.F. 2011. Maca, producto de bandera del Perú: de la tradición a la ciencia. Revista Acta Andina 11(1):56-67. Available at https://revistas.upch.edu.pe/index.php/AA/article/view/5888

Huamán-Carrión, M.L., Espinoza-Montes, F., Barrial-Lujan, A., Ponce-Atencio, Y. 2021. Influence of altitude and soil characteristics on organic carbon storage capacity of High Andean natural pastures. Scientia Agropecuaria 12(1):83-90. doi:10.17268/sci.agropecu.2021.010.

Kang, S.-M., Adhikari, A., Lee, K.-E., Park, Y.-G., Shahzad, R., Lee, I.-J. 2019. Gibberellin producing rhizobacteria *Pseudomonas koreensis* MU2 enhance growth of lettuce (*Lactuca sativa*) and Chinese cabbage (*Brassica rapa*, *chinensis*). Journal of Microbiology, Biotechnology and Food Sciences 9(2):166-170. doi:10.15414/jmbfs.2019.9.2.166-170.

Kumar, P., Rani, S., Dahiya, P., Kumar, A., Dang, A.S., Suneja, P. 2022. Whole genome analysis for plant growth promotion profiling of *Pantoea agglomerans* CPHN2, a non-rhizobial nodule endophyte. Frontiers in Microbiology 13:998821. doi:10.3389/fmicb.2022.998821.

Louden, B.C., Haarmann, D., Lynne, A.M. 2011. Use of blue agar CAS assay for siderophore detection. Journal of Microbiology & Biology Education 12:51-53. doi:10.1128/jmbe.v12i1.249.

Luziatelli, F., Ficca, A.G., Cardarelli, M., Melini, F., Cavalieri, A., Ruzzio, M. 2020. Genome sequencing of *Pantoea agglomerans* C1 provides insights into molecular and genetic mechanisms of plant growth-promotion and tolerance to heavy metals. Microorganisms 8:153. doi:10.3390/microorganisms8020153.

Mattos, G., de Sousa, C., da Silva, A.T., Palheta, M., Eduardo, M., Lemos, T.M., et al. 2025. Use of bioinputs in sustainable agriculture: Trends, challenges and perspectives for reducing the use of agrochemicals. Revista de Gestão Social e Ambiental 19(5):e011955. doi:10.24857/rgsa.v19n5-003.

Nautiyal, C.S. 1999. An efficient microbiological growth medium for screening phosphate solubilization microorganisms. FEMS Microbiology Letters 170(1):265-270. doi:10.1111/j.1574-6968.1999.tb13383.x.

Pizarro, S., Requena-Rojas, E., Barboza, E., Peña-Elme, E., Arias-Arredondo, A., Ccopi, D. 2025. Ecological and carcinogenic risk assessment of potentially toxic elements in rangelands and croplands around Lake Junin (Peru): Integrating remote sensing, machine learning, and land cover segmentation. Science of the Total Environment 999:180327. doi:10.1016/j.scitotenv.2025.180327.

Quispe-Díaz, I.M., Ybañez-Julca, R.O., Asunción-Alvarez, D., Enriquez-Lara, C, Polo-Bardales, J.L., Jara-Aguilar, R., et al. 2025. Antioxidant, neuroprotective, and antinociceptive effects of Peruvian black maca (*Lepidium meyenii* Walp.) Antioxidants 14:1214. doi:10.3390/antiox14101214.

Rafikova, G.F., Korshunova, T.S., Minnebaev, L.F., Chetverikov, S.P., Loginov, O.N. 2016. A new bacterial strain, *Pseudomonas koreensis* IB-4, as a promising agent for plant pathogen biological control. Microbiology 85(3):333-341. doi:10.1134/S0026261716030115.

Rawat, P., Das, S., Shankhdhar, D., Shankhdhar, S.C. 2020. Phosphate-solubilizing microorganisms: Mechanism and their role in phosphate solubilization and uptake. Journal of Soil Science and Plant Nutrition 21:49-68. doi:10.1007/s42729-020-00342-7.

Reysenbach, A.-L., Giver, L.J., Wickham, G.S., Pace, N.R. 1992. Differential amplification of rRNA genes by polymerase chain reaction. Applied and Environmental Microbiology 58(10):3417-3418. doi:10.1128/aem.58.10.3417-3418.1992.

Rodríguez, C.A., Buitrago, J.F., Betancourt, A.D., Cardenas, R.L. 2017. Actividad antagonista de *Bacillus* sp. frente a Fusarium oxysporum: un aporte a la agricultura sostenible. Revista Nova 3:9-19. doi:10.23850/25004476.1515.

Rousk, J., Brookes, P.C., Bååth, E. 2009. Contrasting soil pH effects on fungal and bacterial growth suggests functional redundancy in carbon mineralization. Applied and Environmental Microbiology 75(6):1589-1596. doi:10.1128/AEM.02775-08.

Salaheen, S., Biswas, D. 2019. Chapter 2 - Organic farming practices: Integrated culture versus monoculture. In Biswas, D., Micallef, S.A. (eds.) Safety and practice for organic food. p. 23-32. Academic Press, Cambridge, Massachusetts, USA. doi:10.1016/B978-0-12-812060-6.00002-7.

Sartorio, M.G., Repizo, G.D., Cortez, N. 2020. Catalases of the polyextremophilic Andean isolate *Acinetobacter* sp Ver3 confer adaptive response to H_2O_2 and UV radiation. The FEBS Journal 287(20):4525-4539. doi:10.1111/febs.15244.

Valente, I., Wancura, J.H.C., Zabot, G.L., Mazutti, M.A. 2025. Endophytic and rhizospheric microorganisms: An alternative for sustainable, organic, and regenerative bioinput formulations for modem agriculture. Microorganisms 13(4):813. doi:10.3390/microorganisms13040813.

Waheed, Z., Iqbal, S., Irfan, M., Jabeen, K., Umar, A., Aljowaie, R.M., et al. 2024. *Pseudochrobactrum asaccharolyticum* mitigates arsenic induced oxidative stress of maize plant by enhancing water status and antioxidant defense system. BMC Plant Biology 24:832. doi:10.1186/s12870-024-05496-5.

Xu, D., Yin, J., Huang, J., Wang, J., Wu, X., Qiu, Q., et al. 2025. Impact of excessive nitrogen application on growth and rhizosphere microbial community of Tartary buckwheat. Chilean Journal of Agricultural Research 85:783-795. doi:10.4067/S0718-58392025000600783.

Yasin, R., Rasheed, S., Cheema, T.M., Amin, A. 2022. Isolation and characterization of plant growth-promoting bacteria from different rhizospheric soils. The Journal of Microbiology and Molecular Genetics 3(3):179-189. doi:10.3390/plants13152062.

Zúñiga-Dávila, D., Tolentino, J., García, M., Pérez, W., Matsubara, M., Ogata-Gutiérrez, K. 2011. Characterization of rhizospheric bacteria isolated from maca (*Lepidium meyenii* W.) in the highlands of Junin-Peru. p. 21-25. In Mendez-Vilas, A. (ed.) Microorganisms in industry and environment. From scientific and industrial research to consumer products. Formatex Research Center, Badajoz, Spain.