Molecular and functional characterization of plant growth-promoting bacterial endophytes from Curcuma longa rhizomes for microbiome-based agricultural solutions

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Abstract

Curcuma longa (turmeric) is a globally significant medicinal and culinary crop, prized for its bioactive rhizomes rich in pharmacologically active compounds. Despite its importance, the rhizome-associated microbiome, particularly the endophytic bacterial species, remains poorly characterized in tropical soils. These endophytes may play critical roles in enhancing rhizome bioactivity and plant resilience to stresses that commonly limit productivity. Although endophyte-derived microbial inoculants have garnered increasing attention, their success has often been constrained by the use of non-native strains poorly adapted to local agroecological conditions. Here, we characterize and identify isolated native endophytic bacteria from turmeric rhizomes with the potential to enhance rhizome bioactivity under stressed conditions. Six potential bacterial strains were characterized and identified via full-length 16S rDNA sequencing as *Pseudomonas putida* (CaTb1), *Priestia megaterium* (CeDs1), *Brevundimonas bullata* (CeNk1), *Pseudomonas syringae* (KbNk1 and GlNk1), and *Leucobacter aridicollis* (CeBe1). These bacterial strains exhibited tolerance to high salinity (15% NaCl), acidity (pH 4), and aluminum toxicity (100 mM Al^{3+}). All strains produced 1-aminocyclopropane-1-carboxylate (ACC) deaminase and ammonia, while phosphorus solubilization was observed only in *Pseudomonas* species. Indole-3-acetic acid (IAA) was produced by *P. syringae* and *L. aridicollis.* All strains expressed catalase, urease, gelatinase, and most produced protease, with cellulase and amylase production seen in select strains. Additionally, all isolates demonstrated arginine and ornithine decarboxylase activities, indicating biostimulant potential. Given these characteristics, the selected endophytes—excluding the pathogenic *P. syringae*—represent promising candidates for development of microbiome-based products as biofertilizers/biocontrol agents to sustainably boost plant bioactivity, production, and stress resilience in tropical agricultural systems.

1. Introduction

Curcuma longa (turmeric) is a globally significant medicinal and culinary crop, “the golden spice of life,” valued for its bioactive rhizomes. It is a perennial herbaceous plant of the Zingiberaceae family, native to South Asia, particularly India, and cultivated for its underground rhizome (Dhakre and Sharma 2009). The medicinal properties of *C. longa* are primarily attributed to its bioactive compounds, particularly curcuminoids and sesquiterpenoids in the rhizomes. Among these, curcumin—the principal curcuminoid—is well recognized for its potent antioxidant, antimicrobial, and anti-inflammatory activities and has also demonstrated therapeutic potential against cancer and HIV (Iweala et al. 2023).

C. longa is mainly cultivated in tropical and subtropical regions for its rhizomes that grow below the soil, producing rhizomes 2.5–7.0 cm long and 2.5 cm thick (Iweala et al. 2023). The production of *C. longa* rhizomes is often limited by a range of abiotic and biotic constraints, including poor soil fertility, salinity, soil acidity, aluminum toxicity, drought stress, and infections caused by pathogens such as *Pythium*, *Fusarium*, and *Rhizoctonia* spp., all of which can significantly reduce yield and rhizome quality (Cardoso and Kuyper 2006; Bationo and Waswa 2011; Adesemoye and Egamberdieva 2013; Kumar et al. 2016; Deshmukh et al. 2018; Iweala et al. 2023). To adress this challenge, chemical inputs have been widely

used to improve tumeric growth but with negative consequences for human health and environment (Bationo and Waswa 2011; Adesemoye and Egamberdieva 2013; Jabborova et al. 2021).

Harnessing the beneficial properties of plant-associated microorganisms for microbiome-based solutions is important either to increase environment-friendly crop quality and yields, stimulate assimilation of soil nutrients, act as abiotic stressors and promoters of drought tolerance, control plant diseases or pests (Nwaga et al. 2010), or adapt plants to appropriate growth conditions (Deshmukh et al. 2018). Bacteria that promote plant growth, collectively known as plant growth-promoting rhizobacteria (PGPR), have been documented across diverse agricultural systems (Khan et al. 2023). A critical factor underlying the success of PGPR is their ability to effectively colonize the plant's rhizosphere, rhizoplane, or root interior (Vacheron et al. 2013). Among these, certain bacterial strains are capable of penetrating root tissues and establishing stable endophytic populations. These endophytes not only demonstrate adaptability to the internal root environment but also confer functional benefits to their host plants, such as improved growth and stress tolerance (Nwaga et al. 2010; Adesemoye and Egamberdieva 2013).

The characterization and molecular identification of beneficial bacterial endophytes associated with *C. longa* (turmeric) are of growing interest due to the plant's dual role as a high-value medicinal crop and a culturally significant food ingredient. Although *C. longa* has been studied to some extent, most endophyte research within the Zingiberaceae family has focused on other species, limiting our understanding of its rhizome microbiome (Khan et al. 2023; Misrahanum et al. 2025). Endophytic bacteria inhabiting turmeric rhizomes may contribute to plant health by promoting growth, enhancing nutrient uptake, and improving tolerance to abiotic and biotic stresses (Cardoso and Kuyper 2006; Deshmukh et al. 2018; Sontsa-Donhoung et al. 2022). Moreover, these endophytes can influence the biosynthesis of key secondary metabolites such as curcumin, which holds therapeutic and commercial value for humans and one health (Adesemoye and Egamberdieva 2013; Deshmukh et al. 2018; Sontsa-Donhoung et al. 2022).

Despite growing interest in microbiome-based solutions for sustainable agriculture, the development of microbial inoculants has largely relied on non-native, lab-cultured strains with limited ecological compatibility (Adesemoye and Egamberdieva 2013). Previous studies have isolated effective native bacterial endophytes for crops such as oil palm, maize, and sorghum (Fankem et al. 2006; Maïmouna et al. 2016). However, these studies did not identify the specific species responsible for the observed crop improvements, nor did they focus on rhizomatous plants like *C. longa*. Yet, we remain far from fully understanding which microbial inoculant species can reliably enhance plant growth. Zhou et al. (2024) demonstrated that microbial consortia constructed from native core soil microbiomes consistently outperform non-native inoculants in colonization efficiency, ecological persistence, and functional capabilities such as nitrogen fixation, phosphorus solubilization, and indole-3-acetic acid (IAA) production. These insights are directly relevant to *C. longa*, whose rhizome-associated endophytic bacteria remain undercharacterized, particularly in tropical agroecosystems. While research on *C. longa* has predominantly focused on rhizosphere-associated bacteria, its internal endophytic microbial

communities–which may offer greater specificity and functional synergy with the host–have received comparatively less attention.

A few studies have identified bacterial endophytes from *C. longa* rhizomes, including *Paenibacillus* sp., which is known for IAA production and stress mitigation, and *Pseudomonas aeruginosa*, which has shown strong biocontrol potential against rhizome rot and leaf blight pathogens, as reviewed in Thankam and Manuel (2023); Khan et al. (2023); Misrahanum et al. (2025). However, the identity and PGP traits of these endophytes often vary across agro-ecosystems, reflecting the influence of local environmental conditions on their potential. This variation underscores the need for location-specific characterization of *C. longa* endophytes to fully harness their agricultural and biotechnological potential. However, traditional top-down approaches, which apply commercially available or model strain-based inoculants, often rely on non-native microorganisms that fall short of adapting to specific soil environments or establishing effective plant-microbe interactions (Zhou et al. 2024). In contrast, bottom-up strategies focused on isolating, characterizing, and identifying native microbiomes offer a more ecologically grounded alternative (Zhou et al. 2024). Native endophytes of *C. longa* are likely to be better adapted to local conditions and plant physiology, thereby enhancing growth, nutrient uptake, and stress tolerance (Adesemoye and Egamberdieva 2013; Deshmukh et al. 2018; Sontsa-Donhoung et al. 2022). The integration of such bottom-up approaches holds promise for the development of more effective, locally tailored bioinoculants that can address the limitations of generalized top-down solutions. The aim of this study was to characterize and identify beneficial native bacterial endophytes from *C. longa* rhizomes to support the development of effective, locally adapted bioinoculants for sustainable turmeric production in tropical agroecosystems.

2. Material and methods

2.1 Selected biological material

Six endophytic strains from the bank of the Biotechnology Centre of the University of Yaoundé I were used in this study. According to Sontsa-Donhoung et al. (2021), they were previously isolated from *Curcuma longa* rhizomes that were obtained in the greenhouse of the University of Yaounde I (Cameroon). The soils used for this greenhouse cultivation originated from three distinct agro-ecological zones representative of tropical environments zone 2 (for ClCaTb1), zone 3 (for ClCeDs1), and zone 4 (for ClCeNK1, ClGINK1, ClKbNK1, and ClCeBe1) (Sontsa-Donhoung et al. 2021). These zones reflect the environmental diversity typical of tropical soil systems, including variations in acidity, nutrient content, and climatic stress (Sontsa-Donhoung et al. 2021). These endophytes were selected based on their demonstrated potential to enhance plant growth, rhizome yield, curcumin content, reduced glutathione levels, total thiols, and carotenoids in turmeric (Sontsa-Donhoung et al. 2022).

2.2 Molecular analysis of bacterial endophytes

Bacterial genomic DNA extraction:

Genomic DNA was extracted from fresh bacterial cultures that had been in LB broth for 18 hours. Mechanical cell lysis was performed using bead beating for 1 min with the Mini Bead Beater-8 homogenizer (BioSpec, Bartlesville, OK, USA) in order to improve DNA extraction. The bacterial DNA was then extracted using the High Pure PCR Template Preparation kit (Roche Life Science, Mannheim, Germany). The SpectraMax® QuickDrop™ Micro-Volume Spectrophotometer (Molecular Devices, San Jose, CA, USA) was used to quantify DNA(Boiu-Sicuia et al. 2023).

Bacterial strain identification using full-length 16 S rDNA gene sequencing

The endophytic bacterial strains were identified at the species level based on the highly conserved 16S rDNA region. For this scope, the bacterial universal primers 27F (5'-AGAGTT TGA TCC TGG CTC AG-3') and 1492R (5'-ACG GCT ACC TTG TTA CGA CTT-3') were used to amplify the 16S rDNA sequence (Zhou et al. 2022). The PCR was performed in a 25 µL reaction volume with 20 ng of template DNA. The PCR mix contained 1X buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs (Thermo-Scientific, Baltics, UAB, Vilnius, Lithuania), 0.5 µM of each primer, and 0.25 U of MangoTaq DNA Polymerase (BioLine, London, UK), all mixed in MilliQ water. The thermocycling parameters begin with an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 0.5 minutes, annealing of the primers at 55°C for 0.5 minutes, and elongation at 72°C for 1 minute, followed by a final elongation step conducted at 72°C for 10 minutes. The PCR products were revealed through agarose gel electrophoresis, then purified and sequenced through the Sanger dideoxy sequencing method by CeMIA (Cellular and Molecular Immunological Applications, Greece) (Sanger et al. 1977).The partial sequences obtained with forward and reverse primers were aligned using the BioEdit Sequence Alignment Editor program. The assembled sequences were further subjected to online NBLAST (Nucleotide Basic Local Alignment Search Tool) software available from the National Center for Biotechnology Information (NCBI) for taxonomic identification based on the sequence's similarity with other microorganisms found in the NCBI database. The phylogenetic analysis was performed using MEGA XI software (Kumar et al. 2018), and the 16S rDNA sequence's alignment was made with ClustalW, while the phylogenetic tree was built using the Neighbor-Joining method (Saitou and Nei 1987).

2.3 Physiological characterization of bacterial endophytes

Abiotic stress tolerance tests against varying pH, salt, and aluminum levels were performed on each isolate (Kumar et al. 2016). The test cultures were subjected to a range of salt stress growth conditions, from 7.5 to 15% NaCl incorporated nutrient agar (NA). After spot-inoculating the test endophytes onto the plates and incubating them for 48 hours, the growth was noted. A pH meter was used to modify the medium for the stress tolerance study on pH (6 to 4). Growth was recorded after test cultures were inoculated and incubated for 48 hours. At 50 mM, 75 mM, and 100 mM of Al^{3+} NA plates inoculated with test organisms, the tolerance to aluminum stress was investigated. Growth was observed while the plates were incubated for 48 hours.

2.4 Plant growth-promoting traits of bacterial endophytes

Phosphate solubilization: In order to test microorganisms for phosphate solubilization, the method outlined by Jasim et al. (2013); Kumar et al. (2016) was used. Bacteria were cultivated on solid NBRIP medium (1% glucose; 0.5% $Ca_3(PO_4)_2$; 0.5% $MgCl_2$; 0.01% $(NH_4)_2SO_4$; 0.025% $MgSO_4.7H_2O$; 0.02% KCl; 1.5% agar; pH 7±0.2), where growth is associated with the ability to use inorganic phosphate in the form of $Ca_3(PO4)_2$ as the sole phosphate source. Plates were grown at 28°C for 5 days. A clear zone around the bacterial colony indicates phosphorus solubilization. Solubilization index (SI) estimation was determined according to the formula SI = Z+n/n, where *Z* is the diameter of the clear zone and *n* is the diameter of the colony (Ullah et al. 2018).

Indole: For indole production, peptone water (peptone 10g/L; NaCl 5g/L, Na_2HPO_4 3.5g/L, KH_2PO_4 1.5g/L, pH 7±0.2) was prepared and distributed in sterile test tubes, inoculated with bacteria, and incubated at 30°C for 2 days. A few drops of Kovac's reagent were added for revelation. The development of a red ring on the surface of the tubes indicates the presence of indole (Marchal et al. 1982). The optical density at 530 nm was measured using a spectrophotometer (Marag and Suman 2018).

ACC deaminase: The ACC deaminase production of bacteria was screened using the methods described by Jasim et al. (2013). The isolates were inoculated onto DF salts minimal medium (KH_2PO_4 4 g/L, Na_2HPO_4 6 g/L, $MgSO_4$,$7H_2O$ 0.2 g/L, $FeSO_4$,$7H_2O$ 0.1 g/L, boric acid 10 µg/L, $MnSO_4$ (II) 10 µg /L, $ZnSO_4$ 70 µg /L, $CuSO_4$ 50 µg/L, molybdenum (VI) oxide 10 µg /L, glucose 2 g/L, gluconic acid 2 g/L, citric acid 2 g/L, agar 12 g/L, pH 6.8) supplemented with 0.2 % $(NH_4)_2SO_4$ (w/v). The test endophytes were incubated for 2-5 days, and the growth was recorded as a positive result.

Ammonia Production: For ammonia production, bacteria cultures were grown in peptone water and incubated for 2-5 days at 30°C to determine the ammonia production potential. After the addition of Nessler's reagent, the development of a yellow to brown color indicated positive activity (Marques et al. 2010).

2.5 Plant protection traits

Catalase: The 24h old bacterial colony was mixed with a drop of 3% hydrogen peroxide on a glass slide by using a sterile toothpick. The formation of bubbles was considered positive for catalase activity (Ullah et al. 2018).

Cellulase: Bacteria were cultured in the center of a cellulose-based medium (10g carboxymethyl cellulose (CMC); 0.5g NaCl; 1g KH_2PO4; 0.5g $MgSO_4.7H_2O$; 0.01g $MnSO_4.H_2O$; 0.3g NH_4NO_3; 0.01g $FeSO_4.7H_2O$; 12 g agar per liter of distilled water; final pH 7.0) (Ariffin et al. 2006). At the end of the incubation, the medium was flooded with an aqueous solution of Congo red (0.1% w/v) for 30 minutes. The Congo Red solution was then drained, and the plates were treated by immersion in 1 M NaCl for 30 minutes. The formation of a clear zone of hydrolysis indicates cellulose degradation.

Protease: Fresh cultures of bacterial isolates were plated on the center of skim milk agar (15g skim milk; 0.5g yeast extract; 9.3g agar; final pH 6.8 ± 0.2) and incubated for 5 days. Colorless halo zones around the colonies were indicative of protease production (Ullah et al. 2018).

Urease: An overnight culture of bacterial isolates were plated on the surface of Christensen's urea agar (1 g/L peptone, 5 g/L NaCl, 1.2 g/L Na_2HPO_4, 0.8 g/L KH_2PO_4, 1 g/L glucose, 20 g/L urea, 0.012 g/L phenol red, 15 g/L agar, pH 6.8–7.0). The plates were incubated at room temperature (28 ± 2 °C) for 24–48 h. Distinct colonies with a bright fuchsia color appearance around the colonies indicating urease activity (Leeprasert et al. 2022).

Gelatinase: Bacteria were grown for three days on gelatin agar medium containing 5 g/L peptone, 3 g/L beef extract, 4 g/L gelatin, and 18 g/L agar. After incubation, plates were flooded with 15% $HgCl_2$ in 20% HCl solution as a protein precipitating agent. Mercuric chloride solution reacted with gelatin and produced a white precipitate, revealing clearing zones of protein degradation. Enzyme activity was quantified by measuring the clearing zones that indicated the extracellular protease activity. Semi-quantitative estimation was possible using the following formula: EA = D-d, where *D* is the diameter of the clearing zone and *d* is the microbial colony diameter (Hasan et al. 2013).

Amylase: Bacteria were grown on NA + 2% starch and incubated at room temperature until microbial growth was observed. After microbial growth, a solution of Lugol's was added to the plate. The presence of a yellow halo indicates a positive test.

2.6 Arginine and ornithine-decarboxylase activities

They were evaluated for plant stimulation. Arginine-decarboxylase as well as ornithine-decarboxylase activities were performed on solid media (5g/L bacto-peptone, 5g/L beef extract, 0.5g/L glucose, 5mg/L pyridoxine, 20mg/L phenol red and 18g/L agar to one liter of distilled water, final pH 6.0) containing 0.2% L-arginine hydrochloride or 0.2% L-ornithine hydrochloride, respectively (Sicuia et al. 2015). The enzymatic activity can be evaluated after one day of incubation due to the color change of the medium from yellow to pink.

2.7 Statistical Analyses

All statistical analyses were performed using R version 4.4.1 within the RStudio environment (R Core Team 2016). Prior to analysis, data were tested for normality and homogeneity of variances using the Shapiro–Wilk and Levene's tests, respectively. A one-way analysis of variance (ANOVA) was conducted to assess differences among treatments, followed by Tukey's post hoc test for multiple comparisons at a significance level of $p < 0.05$. Distinct letters were assigned to indicate statistically significant differences between isolates.

3. Results

3.1. Bacterial Identification by Sequencing

On the basis of 16S rRNA gene sequence, isolates were identified (Table 1). *Pseudomonas putida* was identified as the CaTb1 strain. Its partial 16S DNA sequence revealed 100% identity and query match with the AB680123.1 strain and 44 other strains of *P. putida* from the NCBI database. The NCBI database provides the *P. putida* CaTb1 strain's sequences under accession number PV449777. *Priestia megaterium* was identified as the CeDs1 strain. Its partial 16S DNA sequence showed 100% identity and query match with the CP045272.1 strain and 61 other *P. megaterium* strains from the NCBI database. The NCBI database contains the *P. megaterium* CeDs1 strain's sequences under accession number PV449782. *Brevundimonas bullata* was identified as the CeNk1 strain. Its partial 16S DNA sequence indicated 100% identity and query match with the KX083526.1 strain and 75 other *B. bullata* strains from the NCBI database. The *B. bullata* CeNk1 strain's sequences are available in the NCBI database with accession number PV449786. The sequence analysis of 16S rDNA sequences of KbNK1 and GlNk1 strains showed 99.5% identity and 100% query cover to *Pseudomonas syringae* with 94 and 92 other strains of *P. syringae* from the NCBI database, including CP028490.1 and CP047267.1 strains, respectively. The sequences of *P. syringae* KbNK1 and GlNk1 strains can be found in the NCBI database under the accession GenBank no. PV449792 and PV449789, respectively. *Leucobacter aridicollis* was identified as the CeBe1 strain. Its partial 16S DNA sequence showed 100% identity and query cover with the PQ187040.1 strain and 62 other strains of *L. aridicollis* from the NCBI database. The sequences of *L. aridicollis* CeNk1 strain can be found in the NCBI database under the accession GenBank no. PV449781.

Table 1
16S rDNA sequence analysis of endophytic bacterial isolates from turmeric root

Endophytic isolate	NCBI accession number	Sequence Analysis		
		Closest NCBI database match with accession number	Query cover	Percentage of identity
CaTb1	PV449777	*Pseudomonas putida* AB680123.1	100%	100%
CeDs1	PV449782	*Priestia megaterium* CP045272.1	100%	100%
CeNk1	PV449786	*Brevundimonas bullata* KX083526.1	100%	100%
KbNk1	PV449792	*Pseudomonas syringae* CP028490.1	100%	99.5%
CeBe1	PV449781	*Leucobacter aridicollis* PQ187040.1	100%	100%
GlNK1	PV449789	*Pseudomonas syringae* CP047267.1	100%	99.5%

(Table 1 here)

Using MEGA 11, the 16S rDNA sequences of the isolates and the sequences obtained from the NCBI were phylogenetically analyzed using the Neighbor-Joining method with 1,000 bootstrap repetitions and ClustalW alignment. Phylogenetic analysis revealed that the isolates were well clustered (Fig. 1).

(Fig. 1 here)

3.2. Physiological characterization

In this study, the tolerance assay showed that all the endophytic bacterial strains revealed a high level of adaptation to local environment factors (Table 2). *P. putida* (CaTb1), *P. megaterium* (CeDs1), *B. bullata* (CeNk1), *P. syringae* (KbNk1 and GlNk1) and *L. aridicollis* (CeBe1) exhibited high tolerance (+++) to aluminum (Al^{3+}, 100 mM), acidity (pH 4), and salinity (NaCl, 15%) stress.

Table 2
Sensitivity of selected bacteria endophytes to local environmental factors

Isolates names	Isolate Identified as	Al^{3+}tolerance (100mM)	NaCl tolerance (15%)	Acidity tolerance (pH 4)
CaTb1	*Pseudomonas putida*	+++	+++	+++
CeDs1	*Priestia megaterium*	+++	+++	+++
CeNk1	*Brevundimonas bullata*	+++	+++	+++
KbNk1	*Pseudomonas syringae*	+++	+++	+++
CeBe1	*Leucobacter aridicollis*	+++	+++	+++
GlNK1	*Pseudomonas syringae*	+++	+++	+++
(-) negative (+) low (++) medium (+++) high				

(Table 2 here)

3.3. Plant growth-promoting properties

ACC deaminase activity and ammonia (NH_3) production were observed in all bacterial strains (Table 3). All the endophytic strains produced indole-3-acetic acid (IAA), with the highest concentration (3.3 $\mu g.mL^{-1}$) recorded in *P. syringae* (GlNk1) and the lowest (0.82 $\mu g.mL^{-1}$) in *P. syringae* (KbNk1); IAA levels in the remaining four strains fell within this range. All the strains were able to solubilize tricalcium phosphate except *B. bullata* (CeNk1). Among the solubilizing strains, *P. syringae* (KbNk1) exhibited the highest solubilization index (4.83), followed by *P. putida* (CaTb1) (4.25) and *P. syringae* (KbNk1) (4.25).

Table 3
Plant growth-promoting properties of selected bacterial endophytes

Isolates names	Isolate Identified as	Phosphate solubilization (SI)	IAA production ($\mu g \cdot mL^{-1}$)	ACC production	NH_3 production
CaTb1	*Pseudomonas putida*	4.25 ± 0.61^{a}	0.90 ± 0.01^{c}	+	+
CeDs1	*Priestia megaterium*	2.50 ± 0.33^{c}	2.73 ± 0.15^{b}	+	+
CeNk1	*Brevundimonas bullata*	-	2.51 ± 0.09^{b}	+	+
KbNk1	*Pseudomonas syringae*	4.25 ± 0.12^{a}	0.82 ± 0.00^{c}	+	+
CeBe1	*Leucobacter aridicollis*	3.50 ± 0.20^{b}	2.80 ± 0.08^{b}	+	+
GlNK1	*Pseudomonas syringae*	4.83 ± 0.07^{a}	3.30 ± 0.05^{a}	+	+
(-) negative (+) positive					

(Table 3 here)

3.4. Biocontrol and biostimulating properties of bacterial endophytes

Bacterial endophytes were screened for their biocontrol potential, focusing on the production of extracellular enzymes (Table 4 ; Fig. 2). Catalase, urease, and gelatinase activities were detected in all bacterial isolates. The largest gelatinase hydrolysis halo (24 mm) was observed in *P. syringae* (KbNK1) followed by *P. megaterium* (CeDs1) (22 mm), whereas the smallest halo (5 mm) was recorded in *B. bullata* (CeNk1). All strains produced protease except *P. megaterium* (CeDs1). Among protease-positive strains, *P. putida* (CaTb1) exhibited the largest hydrolysis halo (11 mm), while the smallest (3 mm) was recorded for *P. syringae* (GlNk1). Amylase activity was detected only in *B. bullata* (CeNk1) (8.5 mm) and *P. syringae* (GlNk1) (4 mm). Similarly, cellulase activity was limited to *B. bullata* (CeNk1) (13 mm) and *L. aridicollis* (CeBe1) (12.5 mm), with no production observed in the remaining isolates.

Table 4
Enzymatic activities of selected bacterial endophytes

Isolates names	Isolate Identified as	Cellulase (mm)	Protease (mm)	Urease	Catalase	Amylase (mm)	Gelatinase (mm)
CaTb1	*Pseudomonas putida*	-	11 ± 0.0[a]	+	+	-	6 ± 0.1[d]
CeDs1	*Priestia megaterium*	-	-	+	+	-	22 ± 0.1[b]
CeNk1	*Brevundimonas bullata*	13 ± 0.4[a]	10 ± 0.1[a]	+	+	8.5 ± 0.1[a]	5 ± 1.1[e]
KbNk1	*Pseudomonas syringae*	-	4.5 ± 0.1[c]	+	+	-	24 ± 0.2[a]
CeBe1	*Leucobacter aridicollis*	12.5 ± 0.3[a]	9 ± 0.4[b]	+	+	-	7 ± 0.9[d]
GlNK1	*Pseudomonas syringae*	-	3 ± 0.2[d]	+	+	4 ± 0.6[b]	13 ± 1.2[c]
(-) negative (+) positive							

(Table 4 here)

(Fig. 2 here)

The isolates were also screened for biostimulatory activities. All strains exhibited arginine-decarboxylase (Fig. 3), with the highest production observed in *P. syringae* (KbNk1 and GlNk1). Based on the pink coloration developed on specific media, all strains tested positive for ornithine decarboxylase activity (Fig. 4), with the strongest activity with *P. syringae* (KbNk1).

(Fig. 3 here)

(Fig. 4 here)

4. Discussion

The endophytic bacterial strains used in this study were molecularly identified at the species level based on sequence similarity of their 16S rDNA genes to known taxa in the NCBI database. BLAST analysis was conducted to compare the obtained sequences with reference sequences. Given its high level of conservation and taxonomic resolution, the 16S rDNA gene is widely recognized as a reliable and efficient marker for the rapid identification and phylogenetic classification of bacterial species, including differentiation at the subspecies level (Clarridge 2004). Numerous studies reported in the literature have previously shown that *Pseudomonas sp*. is a plant growth-promoting bacterium (PGPB) (Taghavi et al.

2009; Molina et al. 2020). Some strains in our study were recognized as *P. putida* (CaTb1 strain) and *P. syringae* (KbNk1 and GlNk1 strains) based on the 16S rDNA sequence analysis. However, *P. syringae* is one of the best-studied plant pathogens and serves as a model for understanding host–microorganism interactions, bacterial virulence mechanisms, and host adaptation of pathogens, as well as microbial evolution, ecology, and epidemiology (Xin et al. 2018). Therefore, it can't be used as an inoculant in agriculture. *P. putida* is a plant growth-promoting rhizobacteria (PGPR) that establishes commensal relationships with plants. The relationship includes a number of basic gene-encoded processes that promote effective niche colonization, nutrition mobilization, and pathogen protection. Some *Pseudomonas putida* strains harbor accessory genes that confer specific biodegradative properties, and because these microorganisms can thrive on the roots of plants, they can be exploited to remove pollutants via rhizoremediation, making the consortium plant/Pseudomonas a useful tool to combat pollution (Molina et al. 2020). *Priestia megaterium* was identified as strain CeDs1. With several patents and industrial uses in biotechnology, it is a common gram-positive strain (Biedendieck et al. 2021). *P. megaterium's* enormous potential for biotic/abiotic control and plant growth promotion (PGP) in agriculture has been repeatedly shown in a variety of crops (Wang et al. 2020). By altering the root architecture, encouraging photosynthesis, reducing oxidative and osmotic stress, and fostering systemic resistance, *P. megaterium* enhances plant development under salt stress, according to earlier research (Shi et al. 2022). One of the most varied and common bacterial genera is Brevundimonas, whose species have been isolated worldwide in a variety of environments, including soils, water, and plant roots (Kumar and Gera 2014; Menéndez et al. 2017). Brevundimonas has the potential to be used for a wide range of activities, including soil bioremediation (Rathi and Yogalakshmi 2021), water pollutant treatment (Wang et al. 2015), and plant growth promotion for sustainable agriculture (Kumar and Gera 2014; Naqqash et al. 2020). CeNk1 strain was identified as *Brevundimonas bullata.* Their presence as endophytes has not been reported in turmeric. However, other species of the Brevundimonas genus have been isolated as endophytes from various plants, specifically medicinal plants just like the study plant. These contribute to the improvement of their capacity to produce hydrolytic enzymes, IAA and to solubilize phosphorus (Liu et al. 2016; Zaim and Bekkar 2023). Therefore, *B. bullata* possesses endophytic interactions that are beneficial to plants. The potential advantages of the endophyte *Leucobacter aridicollis* in agriculture have been investigated. It has been found to reduce mycelial growth of pathogens, improve germination rate and root development, and increase ramification number in plants (Abdul et al. 2022; Chabbi et al. 2024). The CeBe1 isolate in this study was determined to be *Leucobacter aridicollis*. Both species with a broad range of host specificity and those with a restricted distribution can be considered to be endophytic bacteria found in turmeric. These critters are even more fascinating because they are found in a particular environment for turmeric. This is due to the fact that these strains are expected to have particular plant growth-promoting properties.

However, microbial activity in soils is commonly limited by several abiotic stress factors, including acidic pH, elevated temperatures, nutrient deficiencies, salinity, and the presence of toxic elements such as aluminum and iron. Therefore, selecting microbial strains for agricultural application requires careful evaluation of their physiological tolerance to these environmental constraints. All isolates demonstrated

the ability to grow in the presence of 100 mM Al^{3+}, although tolerance tended to decline as aluminum concentrations increased. This observation is consistent with previous studies indicating that some bacterial isolates can survive under low to moderate aluminum toxicity (Kumar et al. 2016). Salinity represents another major challenge to global agricultural productivity, particularly in regions affected by climate extremes and unsustainable land use practices (Wicke et al. 2011). The endophytes tested in this study exhibited varying degrees of salt tolerance, with all strains capable of growing in media containing up to 15% NaCl. However, growth performance varied among isolates, and tolerance generally decreased at higher salinity levels. These results align with prior findings demonstrating high salt tolerance among certain endophytic bacteria (Marques et al. 2010; Kumar et al. 2018). Soil acidity is also a critical concern in tropical agriculture, where over 60% of arable land is classified as acidic (Nwaga et al. 2010; Adesemoye and Egamberdieva 2013). The present study showed that all tested endophytic strains could tolerate a pH as low as 4.0. However, microbial growth declined significantly at lower pH levels, supporting earlier observations that most microorganisms are unable to thrive under extreme acidity, such as pH 3.5 (Appunu et al. 2010). These findings highlight the potential of the tested endophytes for application in acidic, aluminum-rich, and saline tropical soils, where conventional microbial inoculants may fail to persist or function effectively.

Because of their ability to promote plant development, microorganisms are increasingly gaining attention. By controlling phytohormone levels, they enhance plant development by either lowering ethylene levels or raising the synthesis of auxins, cytokines, and gibberellins (Santoyo et al. 2016). Additionally, they can aid in the absorption of organic nutrients. One crucial approach for using microorganisms to promote plant development is phosphorus solubilization (Li et al. 2016). One of the macronutrients that is needed in significant quantities to promote plant development is phosphorus. Phosphorus is often found in the soil in an insoluble inorganic form, but it's interesting to note that different microorganisms may change this inaccessible supply into one that plants can absorb (Fan et al. 2018). *P. syringae* (GlNk1) (SI = 4.81) was found to be the plant growth-promoting bacterium with the greatest phosphorus solubilization efficiency among all isolates in this investigation. Based on reports, microorganisms solubilize inorganic phosphorus by lowering pH by eliminating organic acid activity, and they solubilize organic phosphorus by producing different phosphatases, which improves plant growth and yield.

IAA is the main phytohormone responsible for cell growth, building xylem and phloem tissues, promoting and increasing root growth and elongation, which in turn promotes plant growth through nutrient uptake (Vacheron et al. 2013). All the isolates produced indole in varying proportions. These results agree with those of Ahemad and Khan (2010) and Jasim et al. (2013). In fact, Mercado-Blanco and Lugtenberg (2014) estimated that up to 80% of rhizosphere bacteria can synthesize auxins, and Ripa et al. (2019) reported that 56% of endophytic fungi isolated from *Triticum aestivum* L. can produce IAA. In addition to IAA, ammonia production is one of the essential mechanisms for promoting plant growth (Li et al. 2016). The ammonia produced can be taken up by plants as a nitrogen source for root and shoot elongation, thereby promoting plant growth, or it can promote plant growth indirectly by suppressing plant pathogens (Deepa et al. 2010; Nain et al. 2012). The study indicates that isolates produce ammonia.

1-aminocyclopropane-1-carboxylic acid (ACC) plays an important role in the biosynthesis of ethylene, a plant growth-inhibiting hormone. In this study, all the selected microorganisms exhibited ACC deaminase activity as determined by a qualitative test. Previous findings of ACC deaminase synthesis by many isolated strains (Gupta and Pandey 2019) corroborate the results observed. ACC deaminase cleaves ACC, the immediate precursor of the plant hormone ethylene, to produce α-ketobutyrate and ammonia (Todorovic and Glick 2008). Previous work has reported that inoculation of plants with ACC deaminase-producing microorganisms reduces ethylene levels, leading to reduced inhibition of plant growth under biotic and abiotic stresses (Farwell et al. 2007).

In addition to their plant growth-promoting attributes, bacterial endophytes are increasingly recognized for their biocontrol potential against plant pathogens. Several endophytic isolates produce hydrolytic enzymes capable of degrading structural components of pathogenic fungi, thereby inhibiting their growth and infection capacity (Glick 2012). In this study, all selected isolates tested positive for catalase activity. Catalase, produced by aerobic and facultative anaerobic bacteria, plays a crucial role in detoxifying reactive oxygen species (ROS) generated during aerobic metabolism. This enzymatic activity enhances bacterial resilience to chemical, mechanical, and environmental stressors (Kumar et al. 2011), making it a critical defense mechanism. It is widely regarded as the first line of defense against oxidative stress caused by both biotic and abiotic factors, indirectly supporting plant development by maintaining microbial viability under hostile conditions (Ramesh et al. 2014).

Protease production, another important biocontrol trait, was detected in most isolates except *P. megaterium.* Notably, *P. putida* and *B. bullata* exhibited the highest proteolytic activity. These findings align with previous studies reporting that a subset of alkaliphilic bacterial isolates are capable of protease production on skim milk agar (SMA) medium (Gomaa 2013). Similarly, only 23 out of 91 bacterial isolates from dune plant roots were found to express protease activity (Dudeja et al. 2012), highlighting the selective distribution of this trait.

Urease, an enzyme that catalyzes the degradation of urea into ammonia and carbon dioxide via the unstable intermediate carbamic acid, was also generated by all examined bacterial strains. The resultant ammonium (NH_4^+) improves soil fertility and plant nutrition by becoming a readily accessible nitrogen source for plant uptake.

Amylase activity, which facilitates extracellular digestion of starch, was observed only in *B. bullata* (strain CeNk1) and *P. syringae* (strain GlNK1). This enzyme plays an important role in starch degradation, contributing to increased sugar availability and promoting early seed germination (Poli et al. 2006; Gholami et al. 2009). Cellulase production, essential for breaking down cellulose, the most abundant organic carbon source in plant biomass, was detected in *L. aridicollis* and *B. bullata*. Cellulose, a major component of lignocellulosic biomass, is hydrolyzed by cellulases into fermentable sugars, supporting microbial growth and nutrient cycling (Sadhu and Maiti 2013; Susilowati et al. 2015). The presence of cellulolytic activity in these isolates is consistent with previous findings in which 96% of endophytic diazotrophs from rice roots exhibited cellulase production (Prakamhang et al. 2009).

Lastly, gelatinase activity was observed in all bacterial isolates tested. Gelatinase breaks down gelatin, a derivative of collagen found in the peritrophic membrane and cuticle of insect larvae and pupae, thus contributing to biological pest suppression (Brammacharry and Paily 2014). This enzymatic versatility highlights the multifaceted roles of endophytic bacteria in plant protection and reinforces their potential as eco-friendly biocontrol agents.

The selected bacterial strains were also characterized for their biostimulating capacity. Arginine-decarboxylase and ornithine-decarboxylase enzymes are able to initiate polyamine biosynthetic pathways. These polyamines are involved in many important cellular processes (Forouhar et al. 2010). Moreover, they can represent the plant's biostimulating activity against abiotic stress (Boiu-Sicuia et al. 2023).

The functional diversity and environmental adaptability of these native endophytes provide a strong foundation for developing microbiome-based solutions tailored to specific agroecosystems (Zhou et al. 2024). By integrating these strains into customized bioformulations, it is possible to design locally adapted biofertilizers, biopesticides, and biostimulants that work synergistically with the plant and native soil microbiota. Such targeted microbial consortia can not only improve plant health and productivity under stress conditions but also align with sustainable agriculture and one health goals.

5. Conclusion

The characterization of bacterial endophytes from *C. longa* rhizomes and their molecular identification offer valuable insights into their roles in promoting plant growth and enhancing resilience to environmental stressors. This research exemplifies a bottom-up approach, in which native microbial strains are isolated, functionally evaluated, and prioritized for application based on their ecological compatibility and performance under local conditions. Among the six endophytic strains, *P. putida*, *P. megaterium*, and *L. aridicollis* are strong biofertilizer candidates; *P. putida*, *B. bullata*, and *L. aridicollis* show potential as biopesticides; all strains exhibit biostimulant traits, while *P. syringae*, a known phytopathogen, should be excluded from beneficial applications. Their adaptability to local soil constraints, including high salinity, acidity, and aluminum toxicity, further underscores their practical potential. Such strategies contrast with traditional top-down methods that rely on generalized, non-native inoculants, which often fail under field conditions. Moreover, these findings contribute to the broader goal of developing native core microbiome-based solutions. Advancing these native, microbially driven approaches can reduce dependency on synthetic agrochemicals, improve soil health, and enhance food security, particularly in regions facing climate and resource-related challenges.

Declarations

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Author contributions

Alain-Martial Sontsa-Donhoung, Oana-Alina Sicuia, and Dieudonné Nwaga performed the research. The first draft of the manuscript was written by Alain-Martial Sontsa-Donhoung. Simon Thierry Okiobe and Oana-Alina Sicuia scrutinized and refined the manuscript. Alain-Martial Sontsa-Donhoung, Oana-Alina Sicuia and Simon Thierry Okiobe analyzed the data and prepared supporting illustrations including graphical abstract. All authors read and approved the final manuscript.

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Competing interests

The authors declare no conflicts of interest.

Data Availability

The datasets generated during the current study are available from the corresponding authors on reasonable request.

Ethics approval and consent to participate Ethical approval of the study was not necessary.

References

1. Abdul S, Parthiban VK, Parthiban V, Johnson I, Karthikeyan M, Kavitha C (2022) *Leucobacter aridicollis* strain SASBG215: A Novel biocontrol agent against *Colletotrichum orbiculare*. Biol Forum– Int J 14(2):905–911
2. Adesemoye AO, Egamberdieva D (2013) Beneficial Effects of Plant Growth-Promoting Rhizobacteria on Improved Crop Production: Prospects for Developing Economies. In: Maheshwari DK, Saraf M, Aeron A (eds) Bacteria in Agrobiology: Crop Productivity. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 45–63 https://doi.org/10.1007/978-3-642-37241-4_2
3. Ahemad M, Khan MS (2010) Plant growth promoting activities of phosphate-solubilizing *Enterobacter asburiae* as influenced by fungicides. EurAsian J BioSci 4:88–95 https://doi.org/10.5053/ejobios.2010.4.0.11
4. Appunu C, Reddy L, Reddy C, Sen D, Dhar B (2010) Symbiotic diversity among acid-tolerant bradyrhizobial isolates with cowpea. J Agric Sci – Sri Lanka 4(3):126–131. https://doi.org/10.4038/jas.v4i3.1651

5. Ariffin H, Abdullah N, Umi Kalsom MS, Shirai Y, Hassan MA (2006) Production and characterization of cellulase by *Bacillus pumilus* EB3. Int J Eng Technol 3(1):47–53
6. Bationo A, Waswa BS (2011) New Challenges and Opportunities for Integrated Soil Fertility Management in Africa. In: Bationo A, Waswa B, Okeyo JM, Maina F, Kihara JM (eds) Innovations as Key to the Green Revolution in Africa. Springer Netherlands, Dordrecht, pp 3–17 https://doi.org/10.1007/978-90-481-2543-2_1
7. Biedendieck R, Knuuti T, Moore SJ, Jahn D (2021) The "beauty in the beast"—the multiple uses of Priestia megaterium in biotechnology. Appl Microbiol Biotechnol 105(14–15):5719–5737. https://doi.org/10.1007/s00253-021-11424-6
8. Boiu-Sicuia O-A, Toma RC, Diguță CF, Matei F, Cornea CP (2023) In Vitro Evaluation of Some Endophytic Bacillus to Potentially Inhibit Grape and Grapevine Fungal Pathogens. Plants 12(13):2553. https://doi.org/10.3390/plants12132553
9. Brammacharry UB, Paily KP (2014) Gelatinase activity of metabolites of Pseudomonas fluorescens migula on larvae and pupae of culex Quinquefasciatus (Diptera: Culicidae).
10. Cardoso IM, Kuyper TW (2006) Mycorrhizas and tropical soil fertility. Agric Ecosyst Environ 116(1–2):72–84 https://doi.org/10.1016/j.agee.2006.03.011
11. Chabbi N, Chafiki S, Labbassi S, Bouharroud R, Tahiri A, Mentag R, El Amri M, Mimouni A, Aabd NA, Quessaoui R (2024) Plant-Growth-Promoting Rhizobacteria Improve Seeds Germination and Growth of Argania spinosa. Plants, 13(15): 1–19. https://doi.org/10.3390/plants13152025
12. Clarridge JE (2004) Impact of 16S rRNA Gene Sequence Analysis for Identification of Bacteria on Clinical Microbiology and Infectious Diseases. Clin Microbiol Rev. 17(4):840-862. https://doi.org/10.1128/CMR.17.4.840-862.2004
13. Deepa CK, Dastager SG, Pandey A (2010) Isolation and characterization of plant growth promoting bacteria from non-rhizospheric soil and their effect on cowpea (*Vigna unguiculata* (L.) Walp.) seedling growth. World J Microbiol Biotechnol 26(7):1233–1240. https://doi.org/10.1007/s11274-009-0293-y
14. Deshmukh AG, Patil VB, Kale SK, Dudhare MS (2018) Isolation, characterization and identification of endophytes from Curcuma longa. Int J Curr Microbiol Appl Sci 6:1040–1050
15. Dhakre S, Sharma A (2009) Growth and instability analysis of ginger production in north-east: region. Agric Situat India 66(8):463–466
16. Dudeja SS, Giri R, Saini R, Suneja-Madan P, Kothe E (2012) Interaction of endophytic microbes with legumes. J Basic Microbiol 52(3):248–260. https://doi.org/10.1002/jobm.201100063
17. Fankem H, Nwaga D, Deubel A, Dieng L, Merbach W, Etoa F-X (2006). Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree (*Elaeis guineensis*) rhizosphere in Cameroon. Afr J Biotechnol, 5 (24): 2450-2460.
18. Fan M, Liu Z, Nan L, Wang E, Chen W, Lin Y, Wei G (2018) Isolation, characterization, and selection of heavy metal-resistant and plant growth-promoting endophytic bacteria from root nodules of *Robinia*

pseudoacacia in a Pb/Zn mining area. Microbiol Res 217:51–59. https://doi.org/10.1016/j.micres.2018.09.002

19. Farwell AJ, Vesely S, Nero V, Rodriguez H, McCormack K, Shah S, Dixon DG, Glick BR (2007) Tolerance of transgenic canola plants (*Brassica napus*) amended with plant growth-promoting bacteria to flooding stress at a metal-contaminated field site. Environ Pollut 147(3):540–545. https://doi.org/10.1016/j.envpol.2006.10.014
20. Forouhar F, Lew S, Seetharaman J, Xiao R, Acton TB, Montelione GT, Tong L (2010) Structures of bacterial biosynthetic arginine decarboxylases. Struct Biol Cryst Commun 66(12):1562–1566 https://doi.org/10.1107/S1744309110040649
21. Gholami A, Shahsavani S, Nezarat S (2009) The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. Int J Biol Life Sci 1(1):35–40
22. Glick BR (2012) Plant Growth-Promoting Bacteria: Mechanisms and Applications. Scientifica 2012:1–15. https://doi.org/10.6064/2012/963401
23. Gomaa EZ (2013) Optimization and characterization of alkaline protease and carboxymethyl-cellulase produced by *Bacillus pumillus* grown on *Ficus nitida* wastes. Braz J Microbiol 44:529–537. https://doi.org/10.1590/S1517-83822013005000048
24. Gupta S, Pandey S (2019) ACC deaminase producing bacteria with multifarious plant growth promoting traits alleviates salinity stress in French bean (*Phaseolus vulgaris*) plants. Front Microbiol 10:1506. https://doi.org/10.3389/fmicb.2019.01506
25. Hasan S, Ahmad A, Purwar A, Khan N, Kundan R, Gupta G (2013) Production of extracellular enzymes in the entomopathogenic fungus *Verticillium lecanii*. Bioinformation 9(5):238. https://doi.org/10.6026/97320630009238
26. Iweala EJ, Uche ME, Dike ED, Etumnu LR, Dokumnu TM, Oluwapelumi AE, Okoro BC, Dania OE, Adebayo AH, Ugbogu EA (2023) *Curcuma longa* (Turmeric): Ethnomedicinal uses, phytochemistry, pharmacological activities and toxicity profiles—A review. Pharmacol Res - Mod Chin Med 6:100222. https://doi.org/10.1016/j.prmcm.2023.100222
27. Jabborova D, Sulaymanov K, Sayyed RZ, Alotaibi SH, Enakiev Y, Azimov A, Jabbarov Z, Ansari M, Fahad S, Danish S (2021) Mineral Fertilizers Improves the Quality of Turmeric and Soil. Sustainability 13(16):9437. https://doi.org/10.3390/su13169437
28. Jasim B, John Jimtha C, Jyothis M, Radhakrishnan EK (2013) Plant growth promoting potential of endophytic bacteria isolated from *Piper nigrum*. Plant Growth Regul 71(1):1–11. https://doi.org/10.1007/s10725-013-9802-y
29. Khan S, Ambika, Rani K, Sharma S, Kumar A, Singh S, Thapliyal M, Rawat P, Thakur A, Pandey S, Thapliyal A, Pal M, Singh Y (2023) Rhizobacterial mediated interactions in *Curcuma longa* for plant growth and enhanced crop productivity: a systematic review. Front Plant Sci 14:1231676. https://doi.org/10.3389/fpls.2023.1231676
30. Kumar A, Dora J, Singh A (2011) A review on spice of life Curcuma longa (turmeric). Int J Appl Biol Pharm 2(4):371–379

31. Kumar A, Singh R, Yadav A, Giri DD, Singh PK, Pandey KD (2016) Isolation and characterization of bacterial endophytes of *Curcuma longa* L. 3 Biotech 6(1):60. https://doi.org/10.1007/s13205-016-0393-y
32. Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 35(6):1547–1549. https://doi.org/10.1093/molbev/msy096
33. Kumar V, Gera R (2014) Isolation of a multi-trait plant growth promoting *Brevundimonas sp*. and its effect on the growth of Bt-cotton. 3 Biotech 4(1):97–101. https://doi.org/10.1007/s13205-013-0126-4
34. Leeprasert L, Chonudomkul D, Boonmak C (2022) Biocalcifying Potential of Ureolytic Bacteria Isolated from Soil for Biocementation and Material Crack Repair. Microorganisms, 10(5), 1-15. https://doi.org/10.3390/microorganisms10050963
35. Li X, Geng X, Xie R, Fu L, Jiang J, Gao L, Sun J (2016) The endophytic bacteria isolated from elephant grass (P*ennisetum purpureum* Schumach) promote plant growth and enhance salt tolerance of Hybrid Pennisetum. Biotechnol Biofuels 9(1):190. https://doi.org/10.1186/s13068-016-0592-0
36. Liu Y-H, Guo J-W, Salam N, Li L, Zhang Y-G, Han J, Mohamad OA, Li W-J (2016) Culturable endophytic bacteria associated with medicinal plant *Ferula songorica*: molecular phylogeny, distribution and screening for industrially important traits. 3 Biotech 6(2):209. https://doi.org/10.1007/s13205-016-0522-7
37. Maimouna A, Megueni C, Nwaga D, Wassouni F, kongmeneck BA, Manchang TK (2016) Impact of selecting phosphate Solubilizing microorganisms on the growth of maize and sorghum using Vivianite as inorganic phosphate supply. Int. J. Curr. Res, 8(10): 39579-39591.
38. Marag PS, Suman A (2018) Growth stage and tissue specific colonization of endophytic bacteria having plant growth promoting traits in hybrid and composite maize (*Zea mays* L.). Microbiol Res 214:101–113. https://doi.org/10.1016/j.micres.2018.05.016
39. Marchal N, Bourdon J-L, Richard C (1982) Les milieux de culture pour l'isolement et l'identification biochimique des bactéries. Doin, Paris
40. Marques AP, Pires C, Moreira H, Rangel AO, Castro PM (2010) Assessment of the plant growth promotion abilities of six bacterial isolates using Zea mays as indicator plant. Soil Biol Biochem 42(8):1229–1235. https://doi.org/10.1016/j.soilbio.2010.04.014
41. Menéndez E, Pérez-Yepes J, Carro L, Fernández-Pascual M, Ramírez-Bahena M-H, Klenk H-P, León Barrios M, Peix A, Velázquez E (2017) *Brevundimonas canariensis* sp. nov., isolated from roots of *Triticum aestivum*. Int J Syst Evol Microbiol 67(4):969–973. https://doi.org/10.1099/ijsem.0.001725
42. Mercado-Blanco J, Lugtenberg B (2014) Biotechnological Applications of Bacterial Endophytes. Curr Biotechnol 3(1):60–75. https://doi.org/10.2174/22115501113026660038
43. Misrahanum M, Fitri L, Ginting B (2025) A Systematic Review of Endophytic Bacteria in Seven Zingiberaceae Species: Current Insights and Future Directions. IOP Conf Ser Earth Environ Sci 1476(1):012015. https://doi.org/10.1088/1755-1315/1476/1/012015

44. Molina L, Segura A, Duque E, Ramos JL (2020) Chapter Four -The versatility of Pseudomonas putida in the rhizosphere environment. Adv Appl Microbiol 110:149-180. https://doi.org/10.1016/bs.aambs.2019.12.002
45. Nain L, Yadav RC, Saxena J (2012) Characterization of multifaceted *Bacillus sp*. RM-2 for its use as plant growth promoting bioinoculant for crops grown in semi arid deserts. Appl Soil Ecol 59:124–135. https://doi.org/10.1016/j.apsoil.2011.08.001
46. Naqqash T, Imran A, Hameed S, Shahid M, Majeed A, Iqbal J, Hanif MK, Ejaz S, Malik KA (2020) First report of diazotrophic *Brevundimonas spp*. as growth enhancer and root colonizer of potato. Sci Rep 10(1):12893. https://doi.org/10.1038/s41598-020-69782-6
47. Nwaga D, Jansa J, Angue MA, Frossard E (2010) The Potential of Soil Beneficial Micro-Organisms for Slash-and-Burn Agriculture in the Humid Forest Zone of Sub-Saharan Africa. In: Dion P (ed) Soil Biology and Agriculture in the Tropics. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 81–107. https://doi.org/10.1007/978-3-642-05076-3_5
48. Poli A, Esposito E, Lama L, Orlando P, Nicolaus G, De Appolonia F, Gambacorta A, Nicolaus B (2006) *Anoxybacillus amylolyticus* sp. nov., a thermophilic amylase producing bacterium isolated from Mount Rittmann (Antarctica). Syst Appl Microbiol 29(4):300–307. https://doi.org/10.1016/j.syapm.2005.10.003
49. Prakamhang J, Minamisawa K, Teamtaisong K, Boonkerd N, Teaumroong N (2009) The communities of endophytic diazotrophic bacteria in cultivated rice (*Oryza sativa* L.). Appl Soil Ecol 42(2):141–149. https://doi.org/10.1016/j.apsoil.2009.02.008
50. R Core Team (2024) R: A language and environment for statistical computing (Version 4.4.1) [Computer software]. R Foundation for Statistical Computing. Accessed 4 Aug 2025
51. Ramesh A, Sharma SK, Sharma MP, Yadav N, Joshi OP (2014) Plant Growth-Promoting Traits in E*nterobacter cloacae* subsp. dissolvens MDSR9 Isolated from Soybean Rhizosphere and its Impact on Growth and Nutrition of Soybean and Wheat Upon Inoculation. Agric Res 3(1):53–66. https://doi.org/10.1007/s40003-014-0100-3
52. Rathi M, Yogalakshmi KN (2021) Brevundimonas diminuta MYS6 associated Helianthus annuus L. for enhanced copper phytoremediation. Chemosphere 263:128195 https://doi.org/10.1016/j.chemosphere.2020.128195
53. Ripa FA, Cao W, Tong S, Sun J (2019) Assessment of Plant Growth Promoting and Abiotic Stress Tolerance Properties of Wheat Endophytic Fungi. BioMed Res Int 2019:1–12. https://doi.org/10.1155/2019/6105865
54. Sadhu S, Maiti T (2013) Cellulase production by bacteria: a review. Microbiol Res J Int 3(3): 235-258. https://doi.org/10.9734/BMRJ/2013/2367
55. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4(4):406–425
56. Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A 74(12):5463–5467. https://doi.org/10.1073/pnas.74.12.5463

57. Santoyo G, Moreno-Hagelsieb G, del Carmen Orozco-Mosqueda M, Glick BR (2016) Plant growth-promoting bacterial endophytes. Microbiol Res 183:92–99. https://doi.org/10.1016/j.micres.2015.11.008
58. Shi L-N, Lu L-X, Ye J-R, Shi H-M (2022) The endophytic strain ZS-3 enhances salt tolerance in Arabidopsis thaliana by regulating photosynthesis, osmotic stress, and ion homeostasis and inducing systemic tolerance. Front Plant Sci 13:820837. https://doi.org/10.3389/fpls.2022.820837
59. Sicuia O-A, Grosu I, Constantinescu F, Voaideş C, Cornea CP (2015) Enzymatic and genetic variability in Bacillus spp. strains with plant beneficial qualities. AgroLife Sci J 4(2):124–131
60. Sontsa-Donhoung A-M, Bahdjolbe M, Hawaou, Nwaga D (2022) Selecting Endophytes for Rhizome Production, Curcumin Content, Biocontrol Potential, and Antioxidant Activities of Turmeric (*Curcuma longa*). BioMed Res Int 2022:8321734. https://doi.org/10.1155/2022/8321734
61. Sontsa-Donhoung A-M, Hawaou, Bahdjolbe M, Nekou GN, Tadjouo IK, Nwaga D (2021) Growing *Curcuma longa* for Rhizome Production on Diverse Arable Soil Types in Cameroon: Agronomic and Microbial Parameters. Agric Sci 12(05):464–480. https://doi.org/10.4236/as.2021.125030
62. Susilowati DN, Sudiana IM, Mubarik NR, Suwanto A (2015) Species and functional diversity of rhizobacteria of rice plant in the coastal soils of Indonesia. Indones J Agric Sci 16(1):39–50. doi 10.21082/ijas.v16n1.2015.39-50
63. Taghavi S, Garafola C, Monchy S, Newman L, Hoffman A, Weyens N, Barac T, Vangronsveld J, Van Der Lelie D (2009) Genome Survey and Characterization of Endophytic Bacteria Exhibiting a Beneficial Effect on Growth and Development of Poplar Trees. Appl Environ Microbiol 75(3):748–757. https://doi.org/10.1128/AEM.02239-08
64. Thankam SR, Manuel SG (2023) Identification and Characterization of Endophytic Bacteria Isolated from *Curcuma longa*. Proc. Natl. Acad. Sci. India B - Biol. Sci.. 93(3):763–774. https://doi.org/10.1007/s40011-023-01466-3
65. Todorovic B, Glick BR (2008) The interconversion of ACC deaminase and d-cysteine desulfhydrase by directed mutagenesis. Planta 229(1):193–205. https://doi.org/10.1007/s00425-008-0820-3
66. Ullah A, Mushtaq H, Ali U, Hakim AE, Mubeen S, Chaudhary HJ (2018) Screening, isolation, biochemical and plant growth promoting characterization of endophytic bacteria. Microbiol Curr Res 2(3):62–68. https://doi.org/10.4066/2591-8036.18-368
67. Vacheron J, Desbrosses G, Bouffaud M-L, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dyé F, Prigent-Combaret C (2013) Plant growth-promoting rhizobacteria and root system functioning. Front Plant Sci 4:356. https://doi.org/10.3389/fpls.2013.00356
68. Wang B, Zhang D, Chu S, Zhi Y, Liu X, Zhou P (2020) Genomic Analysis of *Bacillus megaterium* NCT-2 Reveals Its Genetic Basis for the Bioremediation of Secondary Salinization Soil. Int J Genomics 2020:1–11. https://doi.org/10.1155/2020/4109186
69. Wang C, Zhang M, Cheng F, Geng Q (2015) Biodegradation characterization and immobilized strains' potential for quinoline degradation by *Brevundimonas sp.* K4 isolated from activated sludge of

coking wastewater. Biosci Biotechnol Biochem 79(1):164–170. https://doi.org/10.1080/09168451.2014.952615

70. Wicke B, Smeets E, Dornburk V, Vashev B, Gaiser T, Turkenburg W, Faij A (2011) The global technical and economic potential of bioenergy from salt-affected soils. Energy Environ Sci 4(8):2669-2681. https://doi.org/10.1039/C1EE01029H
71. Xin X, Kvitko B, He S (2018) *Pseudomonas syringae*: what it takes to be a pathogen. Nat Rev Microbiol 16:316–328. https://doi.org/10.1038/nrmicro.2018.17
72. Zaim S, Bekkar AA (2023) Advances in research on the use of *Brevundimonas spp*. to improve crop and soil fertility and for soil bioremediation. Algerian J Biosci 4(1):045–051. https://doi.org/10.57056/ajb.v4i1.109
73. Zhou Y, Liu D, Li F, Dong Y, Jin Z, Liao Y, Li X, Peng S, Delgado Baquerizo M, Li X (2024) Superiority of native soil core microbiomes in supporting plant growth. Nat Commun 15(1):6599. https://doi.org/10.1038/s41467-024-50685-3
74. Zhou Z, Xiaozhen X, Jiayi L, Yiyin Z, Yaohua H, Wenping Z, Yu S, Junxia W, Shaohua C (2022) A novel quorum quencher, *Rhodococcus pyridinivorans* XN-36, is a powerful agent for the biocontrol of soft rot disease in various host plants. Biol Control 169: 104889. https://doi.org/10.1016/j.biocontrol.2022.104889

Figures

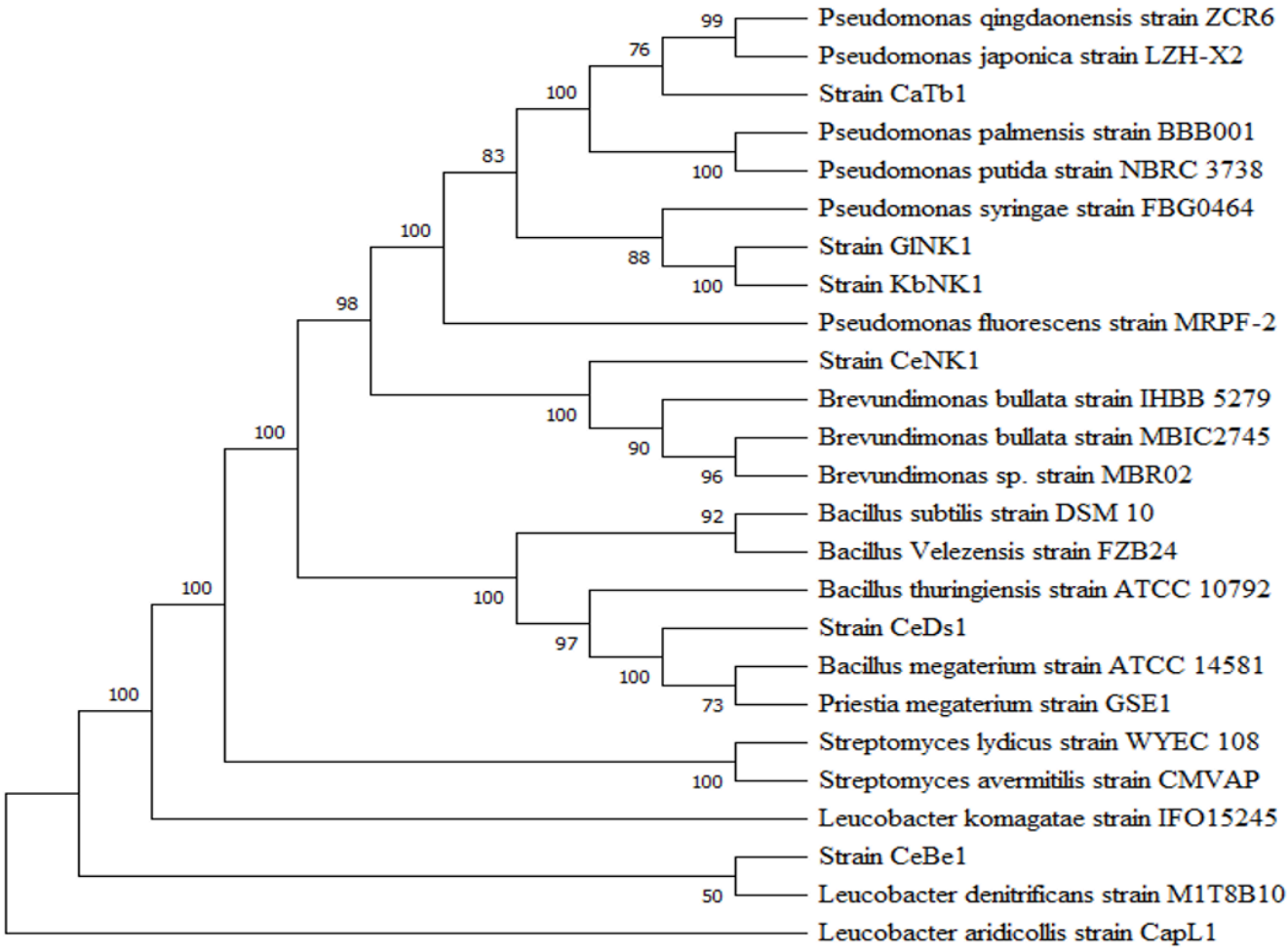

Figure 1

Phylogenic tree constructed with Mega 11 based on the 16 rDNA sequences using the ClustalW alignment tool and Neighbor-Joining method

The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site

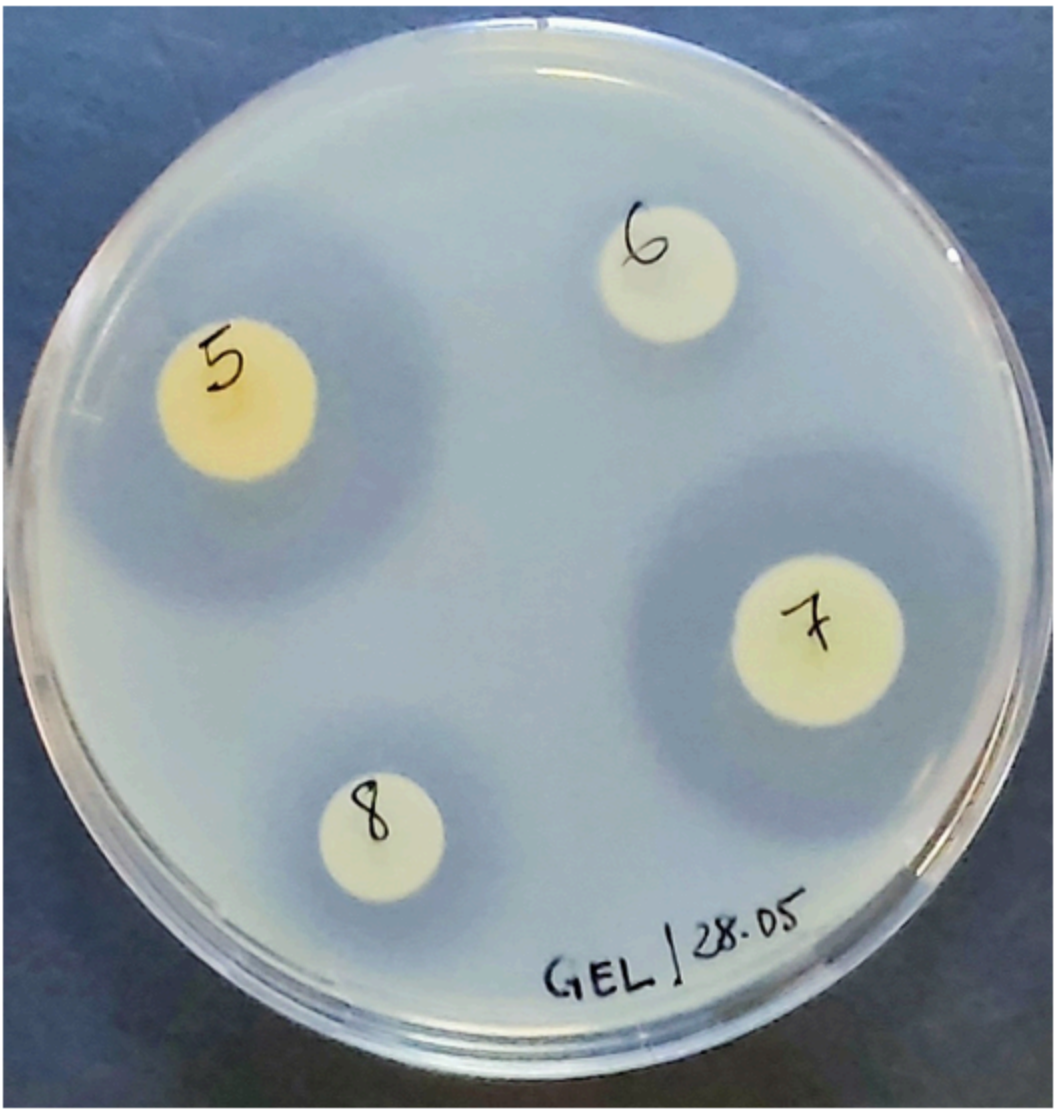

Figure 2

Gelatinase production of bacteria (5) *Pseudomonas syringae* (KbNk1); (6) *B. bullata* (CeNk1) (7); *P. megaterium* (CeDs1); (8) *L. aridicollis* (CeBe1)

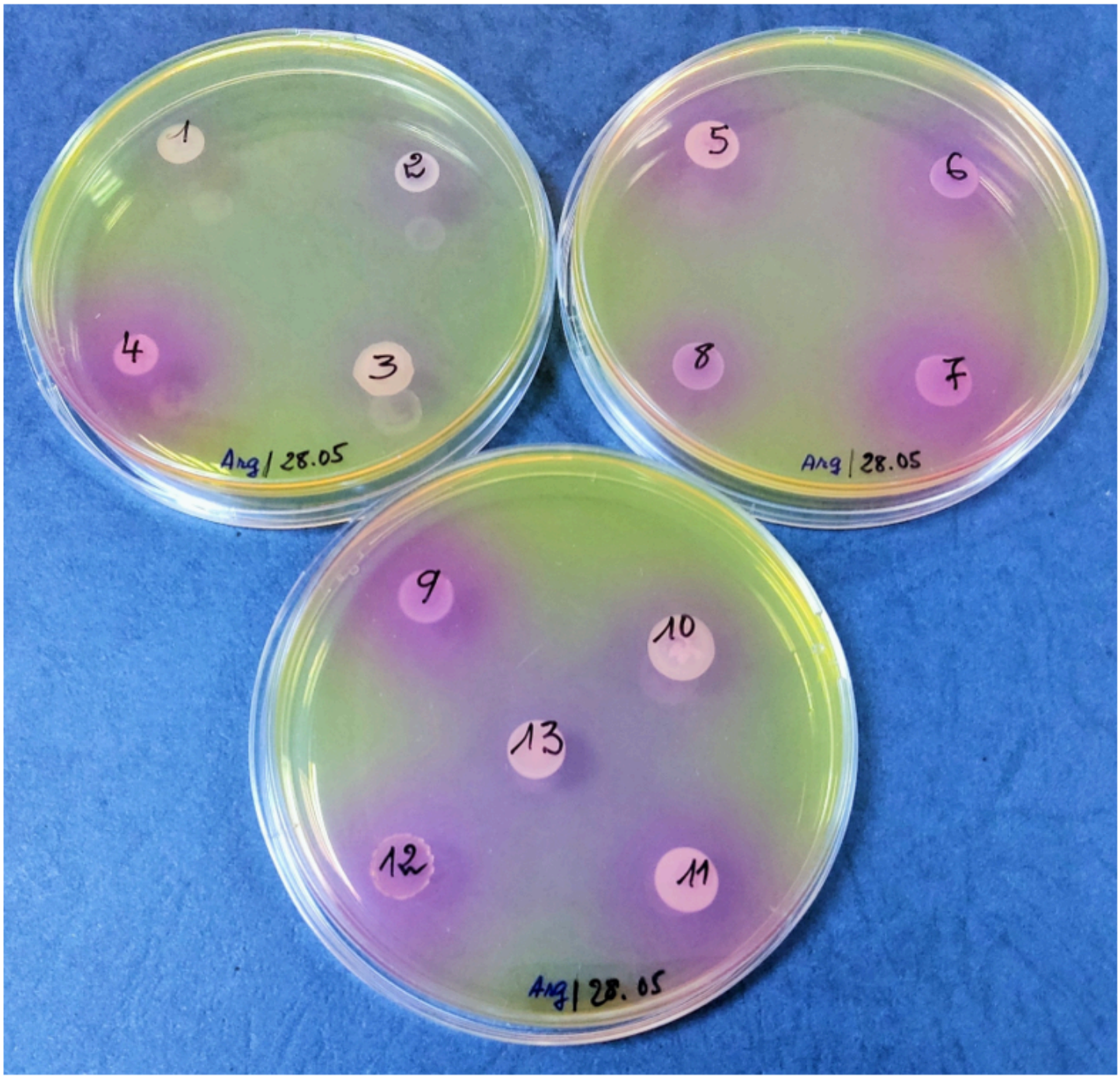

Figure 3

Arginine decarboxylase production of selected bacteria (1 to 4, 9, 10, 13) others microorganisms; (5) *P. syringae* (KbNk1); (6) *B. bullata* (CeNk1) (7); *P. megaterium*(CeDs1); (8) *L. aridicollis* (CeBe1); (11) *P. putida* (CaTb1); (12)*P. syringae* (GlNk1)

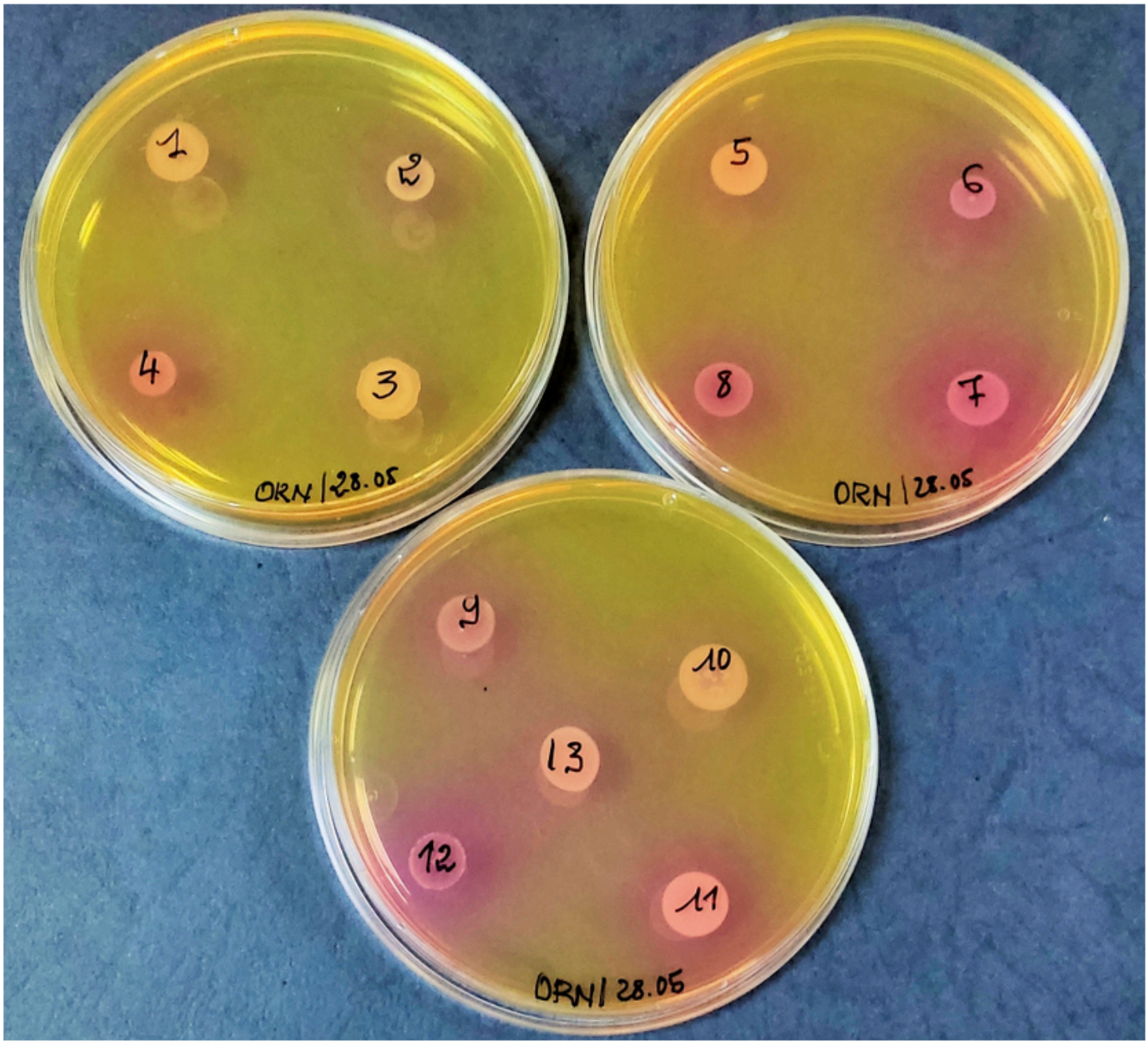

Figure 4

Ornithine decarboxylase production of bacteria (1 to 4, 9, 10, 13) others microorganisms; (5) *P. syringae* (KbNk1); (6) *B. bullata* (CeNk1) (7); *P. megaterium* (CeDs1); (8) *L. aridicollis* (CeBe1); (11) *P. putida* (CaTb1); (12) *P. syringae* (GlNk1)

Supplementary Files

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