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Endophytic bacteria in *Curcuma caesia* Roxb. and their role in promoting plant growth

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The present study focused on the isolation and characterization of plant growth-promoting traits of endophytic bacteria from *Curcuma caesia* Roxb., followed by the evaluation of biocontrol activity using dual culture and double plate methods and *in vivo* bioassay experiment for plant growth activities. A total of forty-one endophytic bacterial isolates were recovered from different tissues of *C. caesia*. Quantitative assays revealed phosphate solubilization capacities ranging from 1.04 to 7.30 µg/ml, indole-3-acetic acid (IAA) production between 2.01 and 52.57 µg/ml, ammonia (NH_2) synthesis from 1.93 to 38.53 µmol/ml, and siderophore production efficiencies varying from 4.44% to 82.62%. Molecular identification based on 16S rRNA gene sequencing classified the potent isolates as *Bacillus cereus* (CC2), *Bacillus thuringiensis* (CC5), *Bacillus amyloliquefaciens* (CC7), *Klebsiella variicola* (CC18), *Pseudomonas monteilii* (CC20), *Enterobacter ludwigii* (CC21a), and *Bacillus mycoides* (CC35). Isolates with strong enzymatic activity were further assessed for *in vitro* antagonistic effects against three phytopathogenic fungi—*Fusarium solani*, *F. oxysporum*, and *Colletotrichum gloeosporioides* using dual culture and double plate techniques. Among them, *Bacillus amyloliquefaciens* (CC7) exhibited the highest antifungal activity. Subsequent in vivo experiments demonstrated that *B. amyloliquefaciens*, *P. monteilii,* and *B. cereus* significantly enhanced the vegetative growth parameters of *C. caesia*.

Keywords : Antagonistic activity, *Curcuma caesia*, endophytic bacteria, plant growth-promoting traits, *in vivo* plant growth, *Bacillus amyloliquefaciens*, 16S rRNA

INTRODUCTION

With excessive reliance on synthetic fertilizers in modern agriculture, the physical and chemical properties of soil have been degraded and pose health risks due to toxic residue accumulation in the food chain. To overcome these challenges, plant growth-promoting microorganisms (PGPMs) offer a sustainable alternative way by enhancing plant productivity through various mechanisms such as nitrogen fixation, phosphate solubilization, phytohormone production, and induction of systemic resistance against pathogens. Plant-associated microbes are rich sources of bioactivemetabolites that can act as biofertilizers, biostimulants, and biopesticides. Application of these microorganisms play a key role in improving soil health, enhancing crop resilience, and supporting sustainable agricultural principles (Brauer *et al.* 2019; Cullen *et al.* 2019; Omomowo and Babalola, 2019). Endophytic bacteria, in particular, are microorganisms that inhabit plant tissues without causing harm (Le Cocq *et al.* 2017). Plants recruit these bacteria via root exudates, which act as chemical signals (Rosenblueth and Martinez-Romero, 2006). They can colonize roots, seeds, leaves, xylem, stomata, wounds, or other tissues (Mano and Morisaki, 2008).

Like rhizospheric microbes, endophytes promote plant growth through nitrogen fixation, phytohormone production, nutrient acquisition, and enhanced tolerance to biotic and abiotic stresses (Kandel *et al.* 2017). They also protect plants by producing hydrolytic enzymes (chitinase, cellulase) and antimicrobial compounds (Compant *et al.* 2011; Santoyo *et al.*

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2016). In return, plants supply nutrients that support endophyte survival under diverse environmental conditions. Their internal colonization allows rapid and effective benefits, improving stress tolerance, growth, and productivity (Pillay and Nowak, 1997; Deshmukh *et al.* 2018; Yadav and Yadav, 2019).

Curcuma caesia Roxb., commonly known as 'Black Turmeric', is a rare member of the Zingiberaceae species valued for its ethnomedicinal uses in South and Southeast Asia. The plant is native to India, Nepal, Bangladesh, China, Malaysia, and Thailand (Karmakar *et al.* 2011), it is now critically endangered due to overharvesting and habitat loss, which primarily occurred in Northeastern and Central India (Ranemma and Reddy, 2017).The plant possessed a unique rhizome that is bluish black in colour with a strong odor.The rhizome contains essential oils, flavonoids, alkaloids, terpenoids, and phenolic compounds (Sawant *et al.* 2014; Thomas and Jose, 2014; Van Hung and Vo, 2017; Ghosh *et al.* 2018; Gupta *et al.* 2018). These metabolites contribute to various biological activities, including antimicrobial, anticancer, anticonvulsant, analgesic, antipyretic, neuro-depressive, anti-hyperglycemic, and anti-inflammatory activities (Arulmozhi *et al.* 2006; Sarangthem and Haokip, 2010; Karmakar *et al.* 2011; Dewangan, 2014; Venugopal, 2017). Traditionally, it is used to treat inflammation, tonsillitis, glandular swelling, tuberculosis, snake bites, joint pain, dental pain, spleen disorders, allergic eruptions, and gastrointestinal ailments (Sahu and Saxena, 2013; Dennis, 2021).

Despite its pharmacological potential, research on *C. caesia* has mainly focused on its phytochemistry, with limited studies on its endophytic microbes and their potential as biofertilizers. This study aims to isolate endophytic bacteria from *C. caesia* and evaluate their plant growth-promoting potential.

MATERIALS AND METHODS

Collection of Plant Samples

The plant samples were collected from the Ri-Bhoi and East Khasi Hills districts of Meghalaya and deposited in the Herbarium of the Eastern Regional Centre, Botanical Survey of India, Shillong, Meghalaya (Accession No. 101654). Different parts of the fresh sample (leaf, rhizome, and root) were surface sterilized following the protocol of Jalgaonwala and Mahajan (2011). In brief, the plant parts were first washed under running tap water to remove dirt and soil particles, then immersed in 70% ethanol for 2 minutes, followed by treatment with 2% sodium hypochlorite solution for 2 minutes. They were again dipped in 70% ethanol for 5–10 seconds, rinsed three times with sterile distilled water, and finally dried using sterile filter paper.Sterilization efficiency was confirmed by plating 0.1 ml of the last rinse water; absence of microbial growth indicates successful removal of epiphytic microorganisms and endophytic bacterial colonization was confirmed by isolating the strain from the sterilized plant tissues.

Isolation of endophytic bacteria

Endophytic bacteria were isolated following the method described by Vinayarani and Prakash (2018), with slight modifications, using Nutrient Agar (Himedia). One gram of the sample was homogenized using a sterile pestle and mortar, and 10 ml of sterile distilled water was added to the homogenate. Serial dilutions were prepared up to 10^3 for leaf samples, 10 for root samples, and 10^1 for rhizome samplesas roots have higher bacterial colonization frequency. From each dilution, 0.1 ml of the aliquot was spread evenly on Nutrient Agar plates using a sterile spreader. The plates were then incubated at 28°C for 2–3 days until bacterial colonies appeared. Distinct colonies were purified and maintained as pure cultures, which were stored at 4°C for further studies.

Plant growth promoting traits

Phosphate Solubilization

The isolated bacterial strains were screened for their phosphate-solubilizing ability following the method described by Pikovskaya (1948). A loopful of actively grown culture was inoculated onto Pikovskaya agar medium supplemented with tricalcium phosphate and incubated at 28°C for 4–5 days. The appearance of a clear halo zone around the bacterial colony indicated positive phosphate solubilization activity.

Quantitative estimation of phosphate solubilization by the bacterial endophytes was carried out following the method of Subba Rao (1988). Fresh bacterial inoculum was cultured in Pikovskaya broth containing 0.5 mg/ml tricalcium phosphate and incubated at 28°C for 5 days. After incubation, the culture was centrifuged at 10,000 rpm for 20 minutes. From the supernatant, 0.1 ml was mixed with 0.25 ml of Barton's reagent, and the final volume was made up to 5 ml with sterile distilled water. The mixture was allowed to stand for 10 minutes, and absorbance was recorded at 430 nm using a UV–Vis spectrophotometer. The concentration of solubilized phosphate was determined using a standard curve prepared with potassium dihydrogen phosphate.

Indole Acetic Acid (IAA) Production

Production of indole acetic acid by the isolated endophytic bacteria was carried out following the method described by Bric *et al.* (1991). Using a sterile inoculating loop, the bacterial isolates were inoculated into sterile Luria Bertani (LB) broth supplemented with 0.1% tryptophan (precursor of IAA). The cultures were incubated at 28°C for 48 hours. After incubation, the cultures were centrifuged at 10,000 rpm for 10 minutes. Two milliliters of the supernatant were mixed with 4 ml of Salkowski's reagent (prepared by combining 49 ml of 35% perchloric acid with 1 ml of 0.4 M ferric chloride) followed by the addition of a drop of orthophosphoric acid. The reaction mixture was incubated in the dark at room temperature for 30 minutes. Development of a pink to reddish coloration indicated a positive result for IAA production. Quantitative estimation of IAA was determined by measuring the color intensity at 530 nm using a UV spectrophotometer.

Ammonia Production

Ammonia production by the bacterial isolates was determined following the method of Cappuccino and Sherman (1992). The isolates were inoculated into 10 ml of sterile peptone water and incubated at 28°C for 48 hours. After incubation, the cultures were centrifuged at 10,000 rpm for 10 minutes. To 5 ml of the supernatant, 0.5 ml of Nessler's reagent was added. The formation of a yellow to brown coloration indicated positive ammonia production. Quantitative estimation was performed by measuring the color intensity at 450 nm using a UV spectrophotometer.

Siderophore Production

Screening for siderophore production by the endophytic bacterial isolates was conducted using the method described by Schwyn and Neilands (1987). The bacterial isolates were inoculated on nutrient agar plates supplemented with CAS reagent (9:1) and incubated for 3–5 days at 28ºC. The appearance of an orange halo around the colonies indicated positive siderophore production. For quantitative estimation, the isolates were inoculated into nutrient broth and incubated for 2–3 days at 28°C. The cultures were centrifuged at 10,000 rpm for 10 minutes, and 2 ml of the supernatant was mixed with 2 ml of CAS reagent. Absorbance was measured at 630 nm using a UV spectrophotometer.

Molecular identification of endophytic bacteria

Bacteria selected based on their performance in various plant growth-promoting assays were subjected to molecular identification using the lysozyme method. Briefly, the isolates were inoculated into nutrient broth and incubated for 48 hours. Two ml of the culture were centrifuged at 13,000 rpm for 5 minutes, and the resulting pellets were washed twice with 0.5 ml of distilled water. Subsequently, 3.6 µl of lysozyme and 96.4 µl of distilled water were added to the pellets, followed by incubation at 35°C for 1 hour and then at 100°C for 5 minutes. After centrifugation, the supernatant was collected, and DNA purity was assessed using a Nanodrop spectrophotometer (User Manual Nanodrop, 2009; Desjardin *et al.* 2011).

High-quality DNA obtained from this process was used for PCR amplification of the 16S rRNA gene. The PCR reaction was carried out in a thermal cycler (Bio-Rad C1000 Touch, USA) using a mixture containing 1X PCR buffer, 1.5 mM MgCl, 200 µM dNTPs, 0.2 µM of each primer, 0.15 µl of 5U Taq polymerase, and 1 µl of DNA (50 ng). Universal primers 27F (5'AGAGTTTGA-TCMTGGCTCAG3') and 1492R (5'TACGGYTA-CCTTGTTACGACTT3') were used for amplification. The PCR products were resolved on a 1% agarose gel at 70V for 1.5 hours. The amplified products were then sequenced by Eurofins, India, and the resulting sequences were analysed using NCBI BLAST software.

Extracellular enzymatic activities

Protease activity

Activity of endophytic bacteria isolates was initially screened on Skim Milk Agar plates incubated at 28-30°C for 48 hours, with clear halo formation indicating a positive result (Sgroy *et al.,* 2009). Quantitative protease activity was determined by measuring tyrosine release from casein substrate after incubation of culture supernatant with phosphate buffer at 50°C, followed by reaction with trichloroacetic acid, sodium carbonate, and Folin's reagent, with absorbance read at 660 nm (Rupali, 2015). One unit (IU) of protease activity was defined as the amount of enzyme required to release 1 µmole of tyrosine per minute per ml of crude supernatant under the assay conditions.

Cellulase activity

Cellulase activity screening was performed on 1% carboxymethyl cellulose (CMC) agar by Congo red staining, with orange halos indicating positive results (Shaikh *et al.,* 2013). Quantitative cellulase activity was measured by incubating culture supernatant with 0.05/ M citrate phosphate buffer (pH 7) and 1 ml of 1% CMC prepared in the same buffer, followed by incubation at 37°C for 30 minutes. The reaction was stopped using DNS reagent, and absorbance was recorded at 540 nm (Miller, 1959).

Chitinase activity

Chitinase activity was screened on agar containing 1% colloidal chitin stained with iodine, with halos indicating activity (Chernin *et al.* 1995). Quantitative chitinase was determined by incubating culture supernatant with colloidal chitin in 0.1 M citrate buffer (pH 7) at 37°C for 30 minutes. The reaction was terminated by adding 2 ml of DNS reagent and heating in a boiling water bath for 10 minutes, and measuring absorbance at 540 nm (Shivalee *et al.* 2016).

Amylase activity

Amylase activity was qualitatively assessed on Starch Agar with iodine staining showing clear zones around colonies (Hankin and Anagnostakis, 1975). The quantitative assay involved incubating the supernatant with a potato starch substrate in 0.2 M phosphate buffer (pH 7.0) at 40°C for 10 minutes. The reaction was stopped by adding 2 ml of DNS reagent, followed by boiling for 5 minutes and cooling in an ice bath for 20 minutes. Absorbance was measured at 540 nm using a reagent blank as a control (Naiola, 2008).

HCN production

Hydrogen cyanide (HCN) production was qualitatively evaluated by growth in King's B medium with glycine, using picric acid-soaked filter paper in the petri dish lid. Plates were sealed with parafilm and incubated at 28°C for 2–3 days. Color changes from yellow to brown or reddish brown indicate positive HCN production (Baker and Schipper, 1987).

Antagonistic activity

Isolation of pathogens

Infected plant parts were collected and washed thoroughly with sterile distilled water. Surface sterilization was carried out by immersing the samples in 70% ethanol for 2 minutes, followed by 2% sodium hypochlorite solution for 1 minute, a quick dip in 70% ethanol for 30 seconds, and then rinsing three times with sterile distilled water. The samples were allowed to dry, cut into small pieces, and inoculated onto Petri plates containing Potato Dextrose Agar (PDA). Plates were incubated at 30°C for 7 days. Pure cultures of each pathogen were maintained at 4°C.

Fungal DNA extraction

Genomic DNA of the isolated fungal pathogens was extracted using a manual lysis method. Fresh mycelium was ground in sterile conditions, lysed with buffer (pH 8.0) and the mixture was incubated at 65°C for 1 hour. The samples were subjected to sequential centrifugations with phenol: chloroform: isoamyl alcohol (25:24:1) and treatment with chloroform and sodium acetate (1/10th). DNA was then precipitated using cold ethanol, pellet, air-dried, and resuspended in TE buffer following the method given by National Burea of Agricultural Important Microorganisms (NBAIM).The purity of extracted DNA was assessed using a Nanodrop spectrophotometer. For PCR amplification, 50 ng of DNA was subjected to PCR using universal primers ITS1 (5' TCCGTAGGTGAACCTGCGG 3') and ITS4 (5' TCCTCCGCTTATTGATATGC 3'). The PCR mixture contained 1X PCR buffer, 1.5 mM MgCl, , 200 µM dNTPs, 0.2 µM of each primer, 0.15 µL of Taq polymerase, and 1 µL of DNA. Amplified products were sequenced at Eurofins, India, and analysed using NCBI BLAST for identification.

Dual culture method

A 5-mm plug from a fresh culture of the fungal pathogen was placed at the centre of a PDA plate and incubated at 28°C for 48 hours. A loopful of the selected bacterial isolate was streaked in a zig-zag pattern on both sides of the fungal plug. Plates were sealed with parafilm and incubated at 28°C for 7 days (Gupta *et al.* 2001). Control plates were prepared by inoculating the fungal pathogen alone. The experiment was performed in triplicate, and the percentage of growth inhibition was calculated using the following formula:

$$\text{Growth inhibition (\%)} = \frac{Rc - Rt}{Rc} \times 100$$

Where, R_c is the diameter of the control fungal colony R_t is the diameter of the treated fungal colony

Double plate method

The double plate (sandwich) method was performed following the protocol described by Ebadzadsahrai *et al.* (2020). The fungal pathogen was inoculated onto Petri dishes containing PDA, while the selected endophytic bacteria were inoculated onto Petri dishes containing NA. The dishes were then placed facing each other and tightly sealed with parafilm. They were incubated at 28°C for 7 days. The diameter of the fungal colonies was measured, and growth inhibition was determined relative to the control. The experiment was conducted in triplicate, and the percentage of inhibition was calculated using the following formula :

$$\text{Growth inhibition (\%)} = \frac{Rc - Rt}{Rc} \times 100$$

Where, R_c is the diameter of the control fungal colony

R_t is the diameter of the treated fungal colony

Scanning electron microscopy (SEM)

SEM analysis was conducted to investigate the interactions between the selected bacterial isolates and fungal pathogens. Briefly, 5-mm plugs of both treated and untreated fungal pathogens were excised, fixed in glutaraldehyde, washed with 0.1 M sodium cacodylate buffer, and dehydrated through a graded series of acetone. Fixation was further carried out using tetramethylsilane (TMS). The samples were then gold-coated and examined under a Scanning Electron Microscope at SAIF, North-Eastern Hill University, Shillong, Meghalaya, India.

In vivo plant growth promotion

Bacterial isolates exhibiting high potential in PGP assays were selected as endophytic inoculants for promoting plant growth. Each isolate was cultured separately in 250 ml of nutrient broth (Himedia) on a rotary shaker at 130 rpm for 48 hours. The bacterial suspensions were adjusted to a concentration of 10^6 CFU ml using sterile distilled water. Fresh and healthy rhizomes were chosen and surface-sterilized by sequential washing: first in 2% sodium hypochlorite solution for 2 minutes, then in 70% ethanol for 1 minute, followed by three rinses with sterile distilled water. To confirmed the effectivity of surface sterilization, 0.1 ml of the last rinse water was added on nutrient agar plate and incubate for 48 hours at 28ºC which shows absence of microbial growth. The sterilized rhizomes were soaked in the bacterial suspension for 8 hours, dried under laminar air flow, and then planted in polythene bags containing 5 kg of autoclaved soil within a polyhouse, with appropriate replicates. Additionally, 250 ml of bacterial inoculum (10^6 CFU/ml) was applied every 30 days to enhance plant growth. After six months, plant growth parameters including shoot length, rhizome fresh weight, and rhizome dry weight were recorded and compared with the control.The experiment were conducted in three replicates for each including the control.

Statistical analysis

The data were analyzed statistically using MS-Excel v.2019 (Microsoft, Washington, DC, USA), and SPSS 16.0. The data obtained were subjected to variance analysis and Tukey's HSD test at $p \leq 0.05$.

RESULTS

In vitro analysis of Plant Growth Promoting traits

Primary screening for plantgrowth-promoting traits

A total of 41 endophytic bacterial isolates were obtained from different parts of *Curcuma caesia*. Qualitative screening of these isolates for different plant growth-promoting (PGP) traits revealed that 25 isolates were positive for phosphate solubilization, 34 isolates for IAA production, 32 isolates for ammonia production, and 38 isolates showed positive for siderophore production (Fig.1).

Quantitative estimation of plantgrowth-promoting traits

The isolates showing positive in the primary PGP screening were further assessed quantitatively. Indole-3-acetic acid (IAA) production ranged from 2.01 to 52.57 µg/ml, with isolate CC35 (*Bacillus mycoides*) showing the highest production. Ammonia production varied from 1.93 to 38.53 µmol/ml, with CC2 (*Bacillus cereus*) producing the maximum amount. Phosphate solubilization ranged between 1.04 and 7.30 µg/ml, with CC21a (*Enterobacter ludwigii*) exhibiting the highest activity. Siderophore production ranged from 4.44 to 82.62, with CC35 (*Bacillus mycoides*) again showing the highest level. A detailed list of the endophytic bacteria and their PGP traits is provided in Table 1.

Molecular identification

The seven selected endophytic bacterial isolates were molecularly identified through amplification and sequencing of the 16S rRNA gene. The resulting sequences were analysed using the BLAST (Basic Local Alignment Search Tool) algorithm against the NCBI GenBank database to determine their closest phylogenetic relationships. Based on the BLAST analysis, the isolates were identified as *Bacillus cereus* (CC2), *Bacillus thuringiensis* (CC5), *Bacillus amyloliquefaciens* (CC7), *Bacillus mycoides* (CC35),*Klebsiella variicola* (CC18), *Pseudomonas monteilii* (CC20), and *Enterobacter ludwigii* (CC21a), and *Bacillus mycoides* (CC35). The 16S rRNA gene sequences of all seven isolates were subsequently submitted to the NCBI GenBank database, and accession numbers were obtained (Table 2).

Enzymatic activities

The selected isolates were also evaluated for various enzymatic activities, including protease, cellulase, amylase, chitinase, and HCN production (Fig. 2). Quantitative analysis revealed that the isolates exhibited differing levels of hydrolytic enzyme activity. Protease activity ranged from 0.091 to 0.259 U/ml, with *B. cereus* showing the highest activity. Cellulase activity varied between 0.54 and 1.27 U/ml, while chitinase activity ranged from 0.03 to 0.12 U/ml. Amylase activity ranged from 0.25 to 1.89 U/ml, with *B. amyloliquefaciens* identified as the most efficient producer (Fig.1). For HCN production, the list of isolates with the results has been displayed (Table 3).

Antagonistic activity

Three pathogens causing leaf diseases and rhizome rot were isolated from the infected leaves and rhizome of *C. caesia* and were identified as *Fusarium oxysporum* (PV169199), *F. solani* (PV168486), and *Colletotrichum gloeosporioides*

(PV169203). The selected bacterial isolates were treated for antagonistic potential with all these pathogens using Dual culture and Double plate techniques.

Dual Culture Assay

In dual culture assays, all selected isolates exhibited varying degrees of inhibitory activity against the tested pathogens. The mycelial growth inhibition ranged from 10.38% to 62.51% against *F. oxysporum*, 14.91% to 63.42% against *F. solani*, and 23.88% to 69.65% against *C. gloeosporioides* (Table 4). Among the isolates, *Bacillus amyloliquefaciens* (CC7) consistently showed the highest antifungal activity against all three pathogens (Fig. 2). Conversely, *B. mycoides* displayed the lowest inhibition, indicating comparatively weaker antagonistic potential.

Double plate assay

The effect of volatile organic compounds (VOCs) produced by the bacterial isolates was evaluated using the double plate method. The results indicated inhibition percentages ranging from 26.39% to 57.34% against *F. oxysporum*, 15.26% to 52.83% against *F. solani*, and 15.38% to 46.57% against *C. gloeosporioides* (Table 4).

Scanning electron microscopy (SEM)

The structural interactions between the bacterial isolates and fungal pathogens were examined using scanning electron microscopy. Compared to the control, the hyphal structures of the pathogens treated with the bacterial isolates showed noticeable alterations. The results demonstrated shrinkage, wrinkling, and irregular twisting of the hyphae upon treatment with the antagonists (Fig.3).

In vivo plant growth

All tested strains caused a statistically significant improvement in multiple plant growth parameters compared to the control (uninoculated)(Fig.4). Among them, *B. amyloliquefaciens* showed the highest PGP activity, followed by *P. monteilii* and *B. cereus*. The specific effects of these endophytic bacteria on key morphological and physiological traits, including shoot length and biomass accumulation, are summarized in detail in Table 5.

DISCUSSION

The present study evaluated the plant growth-promoting and biocontrol potential of endophytic bacteria isolated from *Curcuma caesia*. Although phosphorus is vital for plant growth, only about 0.1% of soil phosphorus is readily available to plants. Phosphate-solubilizing bacteria can enhance phosphorus availability, particularly under stress conditions, by releasing organic acids or protons that lower the surrounding pH and promote solubilization (Hariprasad and Niranjana, 2009; Kour *et al.* 2019, 2020). In our findings, *Enterobacter ludwigii* (CC21a) exhibited the highest phosphate solubilization (7.30 µg/ml) compared to the others and also consistent with its previously reported biofertilizer potential Lee *et al.* (2019). Variations in other growth-promoting traits was also observed as PGP traits are governed by independent metabolic pathways (Glick, 2012). *Bacillus mycoides* producing the highest (52.67 µg/ml), aligned with Van *et al.* (2021), who reported high IAA production in this species. Endophytic ammonia-producing bacteria can enhance plant nitrogen availability by releasing ammonia into the surrounding tissues (Guha and Biswas, 2025). In our study, *Bacillus cereus* (CC2) produced the highest ammonia levels (38.37 µmol/ml) compared with other isolates. This was aligned with reports by Gohil *et al.* (2022) and Chhetri *et al.* (2025), linking ammonia production to improved plant growth and enhanced resistance to biotic stresses. Siderophores are compounds that strongly chelate Fe (III) ions, facilitating iron acquisition in low-iron environments produced by both microbes and gramineous plants to meet their iron needs and thereby improving plant growth (Hider and Kong, 2010). Our findings showed that *Bacillus mycoides* generated the highest siderophore output at 82.97%, in agreement with Yi *et al.* (2018), who identified the relevant biosynthetic gene cluster in this species.

Additionally, *Bacillus amyloliquefaciens*, *Pseudomonas monteilii*, and *Bacillus*

Table 1: Quantitative analysis of the endophytic bacteria for various plant growth promoting properties

Bacterial Isolates	Phosphates Solubilization (µg/ml)	IAA Production (µg/ml)	Ammonia Production (µmol/ml)	Siderophore Production (%)
CC1	3.02±0.02	7.76±0.86	4.41±0.64	27.92±2.15
CC2	3.21±0.65	6.89±0.86	38.53±0.19	23.36±2.25
CC3	3.14±0.06	12.38±0.04	5.51±0.06	18.27±2.24
CC4	2.39±0.03	-	7.42±0.00	34.85±3.16
CC5	2.86±0.04	4.31±0.86	3.31±0.63	22.43±3.75
CC6	2.59±0.03	11.21±0.86	4.78±0.00	14.85±1.43
CC7	1.19±0.03	19.83±0.86	5.88±0.11	31.85±1.69
CC8	-	6.03±0.86	6.61±0.64	9.46±2.63
CC9	4.28±0.07	5.17±0.86	-	4.59±2.82
CC11a	1.94±0.005	10.34±0.86	-	12.9±1.81
CC11d	-	-	-	11.70±2.41
CC12a	6.19±0.17	-	-	19.58±0.52
CC12c	2.71±0.18	17.81±2.63	-	13.82±1.14
CC12d	6.21±0.00	16.09±0.49	14.95±0.06	24.56±1.87
CC13	-	11.78±0.24	15.68±0.06	-
CC15	4.39±0.03	3.16±0.49	-	6.92±2.17
CC16	7.10±0.38	-	-	25.97±2.01
CC17a	3.05±0.04	4.77±0.12	6.50±0.06	38.42±1.77
CC17b	1.04±0.005	4.65±0.31	6.06±0.27	9.90±2.59
CC18	2.65±0.04	29.31±0.86	5.88±0.00	23.11±3.37
CC19	-	4.31±0.86	3.06±0.00	52.46±0.61
CC20	2.23±0.04	14.65±0.86	2.15±.06	5.13±1.69
CC21a	7.30±0.21	2.01±0.50	-	34.16±4.47
CC21b	3.43±0.19	30.17±3.10	-	41.95±2.28
CC22	3.32±0.04	28.73±0.49	3.28±0.13	34.24±4.26

(*Contd. Part of Table* 1)

CC23	-	46.55±0.00	5.40±0.06	-
CC24	-	2.47±0.36	7.71±0.46	-
CC25	-	9.45±0.05	10.71±0.00	19.05±1.55
CC26	-	8.62±0.00	25.42±0.00	4.44±2.71
CC27	6.25±0.08	10.05±0.49	8.30±0.00	15.54±1.23
CC28	3.06±0.10	4.31±0.86	4.70±0.13	20.97±1.62
CC29	-	-	14.00±0.00	11.69±2.90
CC30	-	11.35±0.52	6.02±0.13	11.43±0.97
CC31	-	-	7.75±0.00	44.88±2.15
CC33	3.70±0.04	12.93±0.86	14.77±0.00	15.51±2.31
CC34	-	-	3.03±0.00	18.93±2.97
CC35	-	52.67±0.14	3.28±0.06	82.62±2.68
CC36	-	12.07±0.00	6.61±0.06	18.58±1.76
CC37	-	18.10±0.00	21.32±0.06	69.68±2.69
CC41	1.52±0.04	3.73±0.46	7.42±0.29	40.66±1.68
CC43	2.85±0.04	19.25±0.49	1.93±0.00	12.00±1.76

Note: Data are mean of three replicates with standard deviation.

thuringiensis showed as promising endophytes with good potential in all plant growth-promoting traits and can be considered effective biofertilizers. Their production of phytohormones and volatile organic compounds enhances root development, regulates plant cell growth, and improves tolerance to biotic and abiotic stresses (Raddadi *et al*., 2008; Lou *et al.* 2022; Ramirez *et al.* 2024).

Within the Zingiberaceae family, *Fusarium solani* is the primary cause of soft rot, or rhizome rot, a major constraint on ginger production in both fields and storage (Reddy *et al.* 2003; Liu *et al.* 2016; Chenniappan *et al.* 2020). *Colletotrichum gloeosporioides* can also induce leaf spot disease, causing symptoms similar to those observed in *Curcuma longa* (Chawda *et al.,* 2012, Mahanta *et al.* 2022). Biological control has gained recognition as a safer, more sustainable alternative to chemical pesticides, reducing harmful residues in soils and plant tissues (Nogorska *et al*. 2007; Khan *et al.* 2020; Hita *et al.* 2020). Endophytic bacteria used in biocontrol suppress phytopathogens by producing bioactive compounds, including extracellular lytic enzymes, siderophores, salicylic acid, antibiotics, and volatile metabolites like hydrogen cyanide (Raaijmakers *et al.* 2002; Kishore *et al.* 2005; Afsharmanesh *et al.* 2006; Imran *et al.* 2019; Khan *et al.* 2020; Hita *et al.* 2020). These bacteria inhibit fungal growth through hydrolytic enzymes that degrade fungal cell walls (Weller, 2007; Elshafie *et al.,* 2012) and hydrogen cyanide production, which disrupts fungal respiration by inhibiting cytochrome c oxidase (Blumer and Haas, 2000).

Table 2: Molecular identification of Bacterial isolates based on 16S rRNA gene sequencing

Isolates	Species name	Accession no.	Closest sequence	Similarity (%)
CC2	*Bacillus cereus*	PV202109	*Bacillus cereus* strain IARI-L19	99.84
CC5	*Bacillus thuringiensis*	PV242111	*Bacillus thuringiensis* strain NBAIR BT-R13	100
CC7	*Bacillus amyloliquefaciens*	PV687616	*Bacillus amyloliquefaciens* strain 24YG58	99.87
CC18	*Klebsiella variicola*	PV242113	*Klebsiella variicola* strain NGB-FR87	100
CC20	*Pseudomonas monteilii*	PV242114	*Pseudomonas monteilii* strain SND305	100
CC21	*Enterobacter ludwigii*	PV242112	*Enterobacter ludwigii* strain IHB B 13554	99.79
CC35	*Bacillus mycoides*	PV242110	*Bacillus mycoides* strain YR319	99.76

Table 3 : Screening of isolates for HCN production

Isolates	HCN production
CC2	(++)
CC5	(+++)
CC7	(+++)
CC18	(+++)
CC20	(++)
CC21a	(++)
CC35	(++)

Note: ++ indicates moderately positive, +++ indicates highly positive, based on the colour changes in filter paper.

Among the isolates, *B. amyloliquefaciens*, showed to be the most effective in antagonistic activity followed by *B. cereus*, highlighting their potential to produce direct interactions such as antibiosis, hydrolytic enzyme secretion, siderophore production, and competition for nutrients and also produce VOCs with strong antifungal activity, all of which collectively contribute to pathogen suppression. Its strong suppression of fungal growth is attributed to the production of a wide range of extracellular hydrolytic enzymes and bioactive metabolites with antifungal properties. Notably, *B. amyloliquefaciens* exhibited the highest enzymatic activity, particularly in cellulase and chitinase, which degrade fungal cell walls, enhancing its biocontrol potential. Chitinase is widely recognized as the most critical enzyme for suppressing both *Fusarium* and *Colletotrichium*, as it directly degrades chitin, a dominant fungal wall component. In addition, proteases degrade structural and membrane-associated fungal proteins, contributing to hyphal damage and improving overall antagonistic activity. Multiple strains of *B. amyloliquefaciens* have been demonstrated significant antagonism against *Fusarium* species (Yuan *et al.* 2012; Kim *et al.* 2015; Wan *et al.* 2017). Beyond direct antifungal action, *B. amyloliquefaciens* also produces diverse antimicrobial metabolites, enhances plant growth, induces systemic resistance, and persists under varied environmental conditions, contributing to its effectiveness in disease management (Meng *et al.* 2012; Chen *et al.* 2014; Sigdel *et al.* 2015).

Table 4: Antagonistic activity of endophytic bacteria against phytopathogens using Dual culture and Double plate techniques.

Growth inhibition (%)						
	Dual culture			Double plate		
Isolates	*F. oxysporum*	*C. gloeosporioides*	*F. solani*	*F. oxysporum*	*C. gloeosporioides*	*F. solani*
CC2	54.75 ± 0.59^{b}	38.29 ± 1.19^{c}	45.31 ± 0.91^{b}	47.95 ± 0.79^{b}	42.58 ± 0.92^{b}	52.83 ± 0.72^{a}
CC5	25.95 ± 2.68^{d}	30.88 ± 1.71^{d}	23.85 ± 0.96^{f}	33.79 ± 2.08^{c}	46.27 ± 0.99^{a}	30.97 ± 2.09^{b}
CC7	62.51 ± 1.84^{a}	69.65 ± 0.46^{a}	63.42 ± 1.01^{a}	57.34 ± 1.63^{a}	46.57 ± 1.48^{a}	20.52 ± 0.53^{c}
CC18	48.92 ± 0.95^{c}	50.78 ± 1.52^{b}	40.33 ± 0.87^{c}	26.39 ± 1.97^{d}	45.62 ± 1.64^{ab}	19.63 ± 2.00^{cd}
CC20	55.63 ± 1.04^{b}	41.30 ± 0.64^{c}	34.25 ± 0.31^{e}	34.07 ± 0.66^{c}	35.73 ± 1.52^{c}	18.74 ± 2.91^{cd}
CC21a	47.21 ± 2.28^{c}	53.20 ± 1.61^{b}	37.56 ± 0.24^{d}	27.55 ± 1.15^{d}	15.38 ± 1.23^{e}	15.26 ± 1.73^{d}
CC35	10.38 ± 1.52^{e}	23.88 ± 1.29^{e}	14.91 ± 1.66^{g}	32.48 ± 0.74^{c}	20.96 ± 0.91^{d}	27.07 ± 0.50^{b}

Note : Data are mean of three replicates with standard deviation. Data showing the same letter within the same column are not significantly different at $p\leq0.05$ as determined by Tukey's HSD test.

Table 5: Effects of the selected endophytes in the growth parameters of *Curcuma caesia*

Isolates	Shoot length (cm)	Rhizome fresh weight (g)	Rhizome dry weight (g)
Control	63 ± 2.00^{e}	78.54 ± 0.41^{g}	18.00 ± 0.06^{g}
Bacillus cereus (CC2)	90.33 ± 3.05^{c}	107.80 ± 0.695^{c}	23.82 ± 0.56^{c}
Bacillus thuringiensis (CC5)	88.66 ± 4.04^{d}	102.78 ± 1.92^{d}	21.52 ± 0.51^{d}
Bacillus amyloliquefaciens(CC7)	108.33 ± 1.52^{a}	134.26 ± 2.01^{a}	29.138 ± 0.29^{a}
Klebsiella variicola(CC18)	81.33 ± 2.08^{d}	93.44 ± 0.37^{e}	19.67 ± 0.09^{e}
Pseudomonas monteilii(CC20)	99.67 ± 3.05^{b}	128.19 ± 3.38^{b}	26.55 ± 0.38^{b}
Enterobacter ludwigii(CC21a)	79.33 ± 1.52^{d}	88.06 ± 0.37^{f}	19.2 ± 0.099^{ef}

Note: Data are mean of three replicates with standard deviation. Data showing the same letter within the same column are not significantly different at $p\leq0.05$ as determined by Tukey's HSD test.

Under *in vivo* condition, *Bacillus amyloliquefaciens* demonstrate superior promoting plant growth followed by *P. monteilii* and *B. cereus*. The reasons were because of the potential activity of these isolates in production of PGP traits as well as enzymatic and biocontrol activity. The results were aligned with other reports being studied. *B. amyloliquefaciens* has been shown to significantly increase crop yield in small millet (Rajkumar *et al.* 2024). Similarly, *P. monteilii* enhances growth and development in *Melia azedarach* (Ramirez *et al.* 2024). Other species, including *B. cereus*, *B. thuringiensis*, *K. variicola*, and *E. ludwigii*, have also been reported in promoting plant growth (Wei *et al.* 2014; Ku *et al.* 2018; Kumar *et al.* 2020; Bandopadhyay, 2020; Bendaha and Belaouni, 2020; Ali *et al.* 2021). *Bacillus amyloliquefaciens* has emerged as an effective biocontrol agent due to its potent

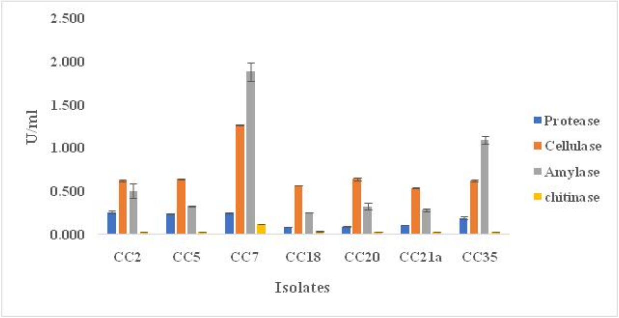

Fig 1: Bar graph showing different enzymatic activities of the identified isolates with standard deviation

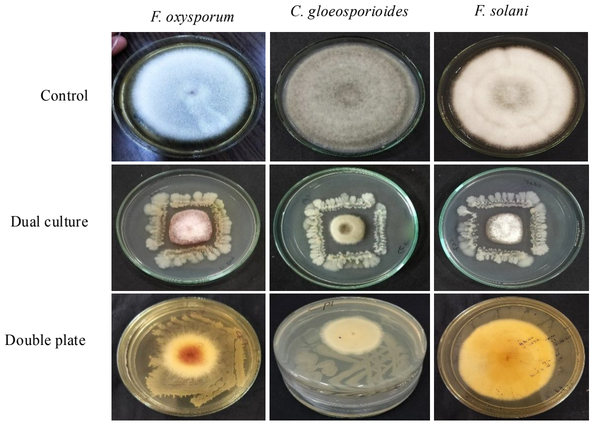

Fig 2: Plates showing antagonistic activity of *B. amyloliquefaciens* against fungal pathogens using Dual culture and Double plate methods.

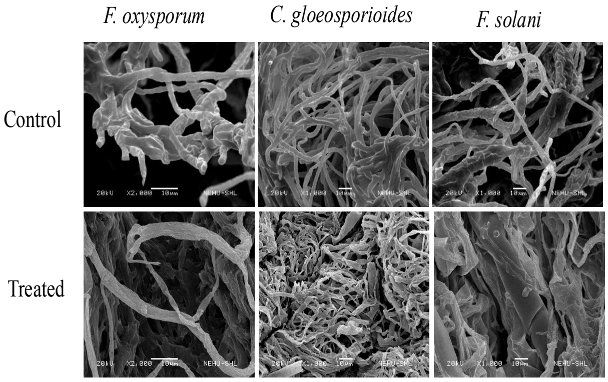

Fig. 3 : SEM picture showing interaction of *B. amyloliquefaciens* with the hyphae of fungal pathogens.

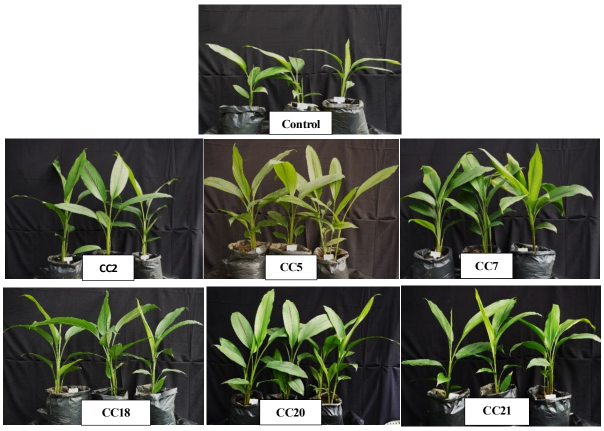

Fig 4: *In vivo* treatment of endophytic bacteria promoting plant growth in *Curcuma caesia*

antifungal activity by producing various lytic enzymes particularly chitinase and cellulase, plant growth-promoting traits, and environmental adaptability. Though some isolates showed highest production in few PGP traits yet those isolates showing uniform traits exhibit better in *in vivo* results. Therefore, overall study revealed that one PGP trait alone cannot predict strong performer but isolates with a balanced combination of multiple traits exhibit superior plant performance which aligned with report given by Vessey (2003) and Compant *et al.* (2005).

CONCLUSION

Bacillus amyloliquefaciens strains emerged as the standout endophyte from *Curcuma caesia*, combining strong plant growth promotion with antifungal activity. Other isolates, including *B. cereus*, *B. thuringiensis*, and *Pseudomonas monteilii* also showed notable PGP and biocontrol traits. These results highlight that endophytes with a balance suite of traits hold promise as natural, eco-friendly tools to boost plant growth and protect against pathogens.

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DECLARATION

Conflict of interest. Authors declare no conflict of interest.

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