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Isolation, Characterization, and Identification of Endophytic Bacteria from Curcuma Longa and Detection of Indole Acetic Acid and Gibberellic Acid Production

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ABSTRACT:
Curcuma longa, commonly known as turmeric, is renowned for its medicinal properties, largely attributed to its bioactive compounds. Recent studies have focused on its endophytic bacteria, which reside within plant tissues and contribute to plant health and growth. This study aimed to isolate, characterize, and identify endophytic bacteria from Curcuma longa and evaluate their ability to produce the plant growth-promoting hormones indole acetic acid (IAA) and gibberellic acid (GA).Fifteen bacterial isolates were obtained from the roots, rhizomes, and leaves of Curcuma longa. These isolates were characterized based on their morphological and biochemical properties. Morphological characterization revealed diverse colony shapes, sizes, colors, and cell morphologies, including both Gram-positive and Gram-negative bacteria. Biochemical tests indicated varying abilities among the isolates to hydrolyze starch and gelatin, reduce nitrate, utilize citrate, and produce catalase and oxidase. Molecular identification using 16S rRNA gene sequencing identified the isolates as belonging to several genera, including Bacillus, Pseudomonas, Enterobacter, Stenotrophomonas, Acinetobacter, and Klebsiella. These genera are known for their plant growth-promoting traits, such as nutrient solubilization, biocontrol, and hormone production. The ability of these endophytes to produce IAA and GA was assessed using colorimetric assays and high-performance liquid chromatography (HPLC), respectively. Ten isolates produced IAA in concentrations ranging from 5 to 45 µg/mL, while eight isolates produced GA in concentrations ranging from 0.5 to 7 µg/mL. The highest levels of IAA and GA were observed in isolates identified as Bacillus sp. and Pseudomonas sp., respectively. Statistical analysis confirmed significant differences in hormone production among the isolates ($p < 0.05$). The diverse

endophytic community and their capacity for hormone production highlight the potential of these bacteria as biofertilizers and biostimulants. These findings suggest that endophytic bacteria from Curcuma longa can be harnessed to promote sustainable agricultural practices by enhancing crop growth and productivity.This study advances our understanding of the beneficial roles of endophytic bacteria in medicinal plants, providing a foundation for the development of bio-based agricultural inputs that support sustainable crop production. Further research and field trials are necessary to fully explore the practical applications of these endophytes in agriculture.

Keywords:
Endophytic bacteria, Curcuma longa, Indole acetic acid, Gibberellic acid, Plant growth-promoting bacteria, 16S rRNA sequencing.

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Introduction

Endophytic bacteria are a fascinating group of microorganisms that live within plant tissues without causing apparent harm to their hosts. These symbiotic relationships can be mutually beneficial, with endophytes aiding in plant growth, nutrient acquisition, and stress resistance while obtaining shelter and nutrients from the host plant [1]. This symbiosis has garnered increasing attention in recent years due to its potential applications in sustainable agriculture and plant biotechnology.Curcuma longa, commonly known as turmeric, is a perennial herbaceous plant of the ginger family, Zingiberaceae. Native to Southeast Asia, turmeric is extensively cultivated in tropical and subtropical regions. The rhizome of turmeric is widely used in culinary practices for its flavor and color and is also renowned for its medicinal properties. The active compound, curcumin, has been the subject of numerous studies due to its anti-inflammatory, antioxidant, and antimicrobial activities. Beyond these uses, turmeric's interaction with its microbial inhabitants, particularly endophytic bacteria [2], is an area of growing research interest.

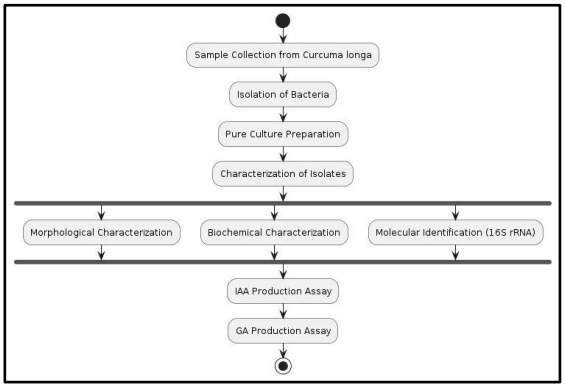

Figure 1: Experimental Procedure

A. Importance of Endophytic Bacteria

Endophytic bacteria can significantly influence plant physiology and development [3]. They are known to produce various bioactive compounds, including phytohormones such as indole acetic acid (IAA) and gibberellic acid

(GA). These hormones play crucial roles in plant growth and development:

a. **Indole Acetic Acid (IAA):** IAA is a type of auxin, a class of plant hormones essential for regulating various aspects of plant growth, such as cell elongation, root initiation, and tissue differentiation. It influences plant responses to light and gravity, thereby playing a critical role in plant morphology and adaptation [4].

b. **Gibberellic Acid (GA):** GA is a diterpenoid acid that promotes stem elongation, seed germination, and flowering. It breaks seed dormancy and mobilizes nutrient reserves, facilitating early seedling development and growth.The production of these hormones by endophytic bacteria suggests their potential to enhance plant growth and productivity. By colonizing plant tissues [5], these bacteria can directly influence the hormonal balance within the plant, leading to improved growth and stress resilience.

B. Endophytes in Sustainable Agriculture

The use of endophytic bacteria in agriculture offers several advantages over traditional chemical fertilizers and pesticides:

a. Environmental Sustainability: Endophytic bacteria can reduce the need for chemical inputs, thus minimizing environmental pollution and soil degradation. They can also enhance soil fertility through natural processes.

b. Enhanced Crop Yield: By producing growth-promoting hormones and facilitating nutrient uptake, endophytes can improve crop yields. This is particularly important in low-input farming systems where chemical fertilizers are either unavailable or too costly [6].

c. Disease Resistance: Some endophytic bacteria possess antimicrobial properties that can protect plants from pathogens. They can produce antibiotics or induce systemic resistance in the host plant, thereby reducing the incidence of diseases.

d. Stress Tolerance: Endophytes can help plants withstand abiotic stresses such as drought, salinity, and heavy metal contamination. They achieve this by modulating plant hormonal levels and enhancing antioxidant defences [7]. Given these benefits, the isolation and characterization of endophytic bacteria from medicinal plants like Curcuma longa hold significant promise for developing biofertilizers and biopesticides that align with sustainable agricultural practices.

C. Objectives of the Study

This study aims to isolate, characterize, and identify endophytic bacteria from Curcuma longa and evaluate their ability to produce the plant growth-promoting hormones indole acetic acid (IAA) and gibberellic acid (GA). The specific objectives are:

a. Isolation of Endophytic Bacteria: To obtain bacterial isolates from different tissues (roots, rhizomes, and leaves) of Curcuma longa, ensuring a diverse representation of the endophytic community.

b. Characterization of Isolates: To characterize the isolates based on their morphological, biochemical, and molecular properties. This includes Gram staining, catalase test, oxidase test [8], and other standard biochemical assays.

c. Molecular Identification: To perform 16S rRNA gene sequencing for accurate taxonomic identification of the bacterial isolates. This molecular approach ensures precise identification and classification of the bacteria.

d. Detection of Phytohormone Production: To quantify the production of IAA and GA by the isolated endophytic bacteria using specific colorimetric assays. This step is crucial to determine the potential of these bacteria in promoting plant growth.

e. Assessment of Plant Growth-Promoting Potential: To evaluate the overall potential of the identified endophytic bacteria in enhancing plant growth and health. This involves assessing their ability to produce

phytohormones and other beneficial metabolites.

D. Methodological Approach

The methodological approach for this study involves several key steps:

a. Sample Collection and Sterilization: Plant samples (roots, rhizomes, and leaves) of Curcuma longa will be collected from a healthy field-grown plant. The samples will be surface sterilized using a series of washes with ethanol and sodium hypochlorite to eliminate any epiphytic microorganisms [9].

b. Isolation of Endophytic Bacteria: Sterilized plant tissues will be macerated and inoculated onto nutrient agar plates. The plates will be incubated, and distinct bacterial colonies will be isolated and purified through repeated subculturing.

c. Morphological and Biochemical Characterization: Isolated bacteria will be characterized based on their colony morphology, cell shape, Gram reaction, and biochemical properties using standard microbiological techniques.

d. Molecular Identification: Genomic DNA will be extracted from the bacterial isolates, and the 16S rRNA gene will be amplified using polymerase chain reaction (PCR). The PCR products will be sequenced [10], and the sequences will be compared with those in the NCBI database using BLAST for taxonomic identification.

e. Detection of IAA and GA Production: Isolates will be cultured in media supplemented with tryptophan for IAA production and in GA-specific media. The production of IAA will be detected using Salkowski reagent, which forms a pink color in the presence of IAA. GA production will be quantified using a standard colorimetric assay.

E. Significance of the Study

The significance of this study lies in its potential to uncover beneficial endophytic bacteria from Curcuma longa that can produce plant growth-promoting hormones. The findings could have practical applications in agriculture, particularly in developing biofertilizers and Biopesticides that enhance crop productivity while reducing reliance on chemical inputs. understanding the interaction between endophytic bacteria and Curcuma longa can provide insights into the broader role of endophytes in plant health and development. By characterizing and identifying endophytic bacteria capable of producing IAA and GA, this study contributes to the growing body of knowledge on plant-microbe interactions. The isolated bacteria may also have potential applications beyond agriculture [11], such as in phytoremediation or as sources of novel bioactive compounds for pharmaceutical and industrial use.

Table 1: Summary of Studies on Endophytic Bacteria and Plant Growth-Promoting Hormones

Author(s)	Year	Plant Studied	Endophyte Identified	Key Findings	Reference
Ahmad et al.	2005	Various crops	Azotobacter, Pseudomonas	Indole acetic acid (IAA) production enhanced root elongation and overall plant growth.	Ahmad, F., Ahmad, I., & Khan, M. S. (2005)
Glick	2012	Various crops	Pseudomonas putida	Described mechanisms of plant growth promotion, including IAA production and nutrient solubilization.	Glick, B. R. (2012)
Khan et al.	2013	Various crops	Various fungi	Gibberellin production by endophytic fungi improved crop	Khan, A. L. et al. (2013)

				abiotic stress resistance.	
Pandey et al.	2006	Sub-alpine rice	Pseudomonas putida	Pseudomonas putida demonstrated phosphate solubilizing and antagonistic abilities.	Pandey, A. et al. (2006)
Patten & Glick	2002	Canola	Pseudomonas putida	IAA production by Pseudomonas putida enhanced host plant root system development.	Patten, C. L., & Glick, B. R. (2002)
Santoyo et al.	2016	Various crops	Various bacteria	Reviewed plant growth-promoting traits of endophytic bacteria, including hormone production and biocontrol.	Santoyo, G. et al. (2016)
Verma et al.	2001	Deep water rice	Various diazotrophs	Evaluated nitrogen-fixing endophytic diazotrophs for plant growth promotion.	Verma, S. C. et al. (2001)
Zinniel et al.	2002	Agronomic crops	Various bacteria	Characterized endophytic bacteria and their colonization abilities and plant growth-promoting traits.	Zinniel, D. K. et al. (2002)

I.Materials and Methods

This section describes the systematic approach taken to isolate, characterize, and identify endophytic bacteria from Curcuma longa, as well as to evaluate their potential to produce the plant growth-promoting hormones indole acetic acid (IAA) and gibberellic acid (GA). The methodology is divided into several key steps, including sample collection, surface sterilization, bacterial isolation [12], characterization, molecular identification, and hormone production assays.

A. Sample Collection

Healthy Curcuma longa plants were selected for this study from a cultivated field. Samples were collected from three distinct parts of the plant: roots, rhizomes, and leaves. These samples were placed in sterile polyethylene bags and transported to the laboratory under refrigerated conditions to prevent contamination and maintain sample integrity.

B. Surface Sterilization

To ensure that only endophytic bacteria were isolated, it was crucial to eliminate any epiphytic (surface-dwelling) microorganisms from the plant tissues. The surface sterilization process involved the following steps:

a. **Washing with Sterile Water:** Plant samples were first washed thoroughly with sterile distilled water to remove soil and debris.
b. **Ethanol Treatment:S**amples were then immersed in 70% ethanol for 1 minute. Ethanol is effective in killing surface microbes without penetrating the plant tissue deeply.
c. **Sodium Hypochlorite Treatment:**Following ethanol treatment, the samples were immersed in 2% sodium hypochlorite solution for 5 minutes. This step further ensures the removal of surface contaminants.
d. **Rinsing:**The plant tissues were then rinsed three times with sterile distilled water to remove any residual sterilizing agents. The effectiveness of the sterilization process was confirmed by plating the final rinse water onto nutrient agar plates and checking for microbial growth.

C. Isolation of Endophytic Bacteria

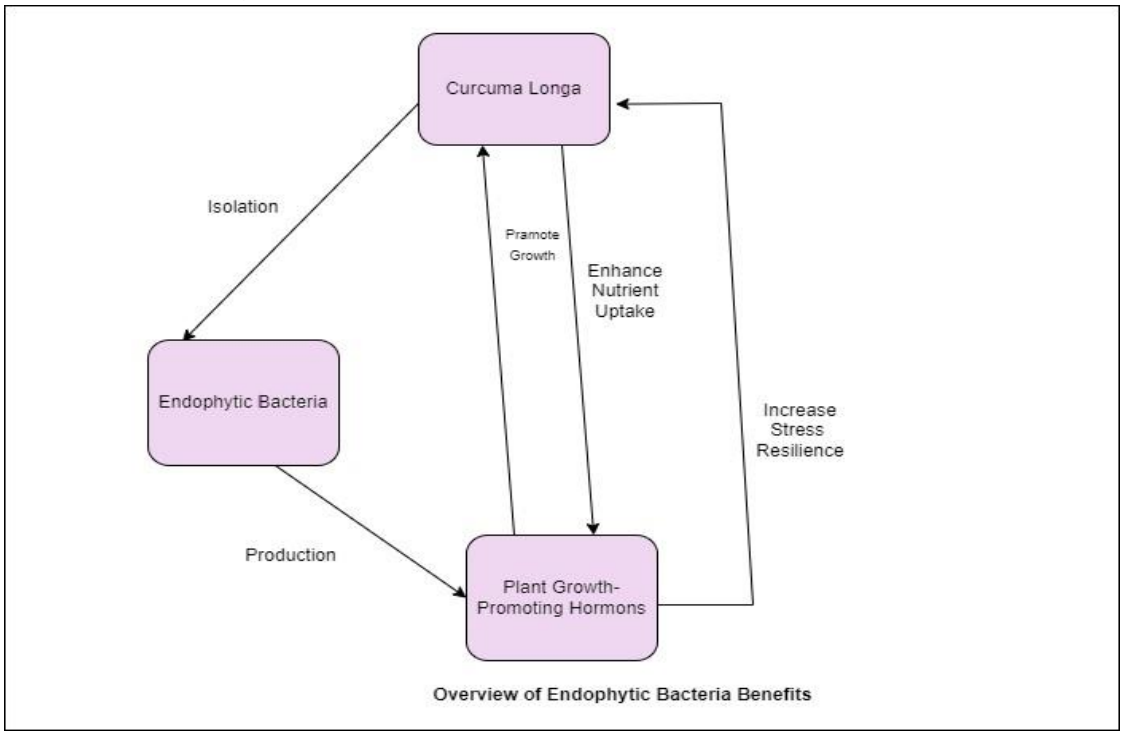

Figure 2 : Overview of Endophytic Bacteria Benefits

Sterilized plant tissues were aseptically cut into small pieces and macerated using a sterile mortar and pestle. The macerate was then serially diluted in sterile saline solution (0.85% NaCl). Aliquots of the dilutions were spread onto nutrient agar plates and incubated at 28°C for 48-72 hours [13]. Emerging bacterial colonies were picked based on distinct morphological characteristics and sub-cultured to obtain pure isolates.

D. Characterization of Isolates

The isolated bacterial strains were characterized using a combination of morphological, biochemical, and molecular techniques.

a. Morphological Characterization: Colony morphology was observed on nutrient agar plates, noting features such as color, shape, size, edge, and surface texture. Cell morphology was examined under a microscope following Gram staining.

b. Biochemical Tests: A series of biochemical tests were performed to assess the metabolic and enzymatic activities of the isolates [14]. These tests included: To determine the Gram reaction (positive or negative) of the bacteria.To check for the presence of the catalase enzyme, which breaks down hydrogen peroxide into water and oxygen. To determine the presence of cytochrome c oxidases. To assess the ability of isolates to hydrolyze starch and gelatin.Other tests, such as nitrate reduction, citrate utilization, and sugar fermentation [15], were conducted as necessary to provide a comprehensive biochemical profile.

E. Molecular Identification

For precise taxonomic identification, the 16S rRNA gene of the bacterial isolates was sequenced.

a. DNA Extraction: Genomic DNA was extracted from overnight bacterial cultures using a commercial DNA extraction kit following the manufacturer's protocol.

b. PCR Amplification: The 16S rRNA gene was amplified using universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3')and 1492R(5'-TACGGYTACCTTGTTACGACTT-3'). The PCR reaction mixture (25 µL) contained 2.5 µL of 10x PCR buffer, 2.0 µL of MgCl2 (25 mM), 0.5 µL of dNTPs (10 mM), 1.0 µL of

each primer (10 µM), 0.25 µL of Taq DNA polymerase, and 1.0 µL of template DNA [16].

c. **PCR Conditions:** The PCR amplification was performed under the following conditions:

E.c.i. Initial denaturation at 94°C for 5 minutes.

E.c.ii.30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 90 seconds.

E.c.iii. Final extension at 72°C for 10 minutes.

d. **Gel Electrophoresis:** PCR products were visualized by gel electrophoresis on a 1% agarose gel stained with ethidium bromide.

e. **Sequencing and Analysis:** The amplified 16S rRNA gene products were purified and sequenced. The obtained sequences were compared with those in the NCBI database using BLAST to determine the closest known relatives and to identify the bacterial isolates [17].

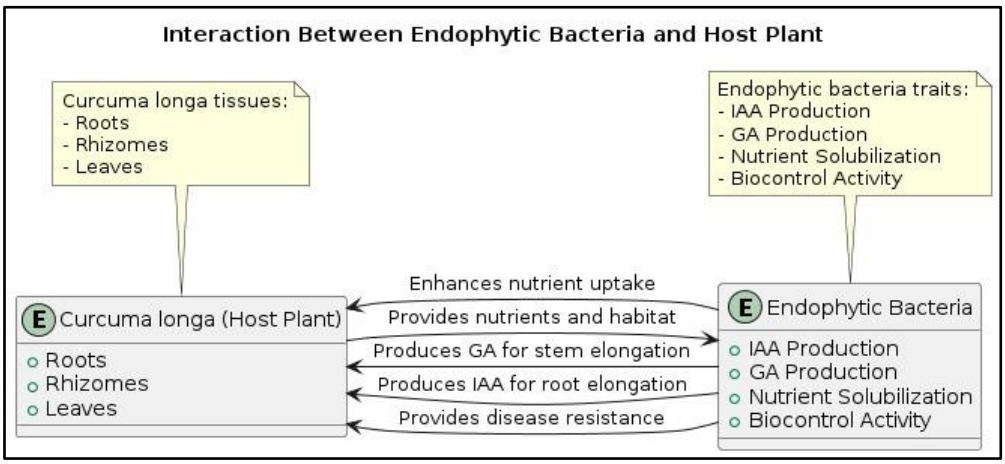

Figure 3: Interaction Between Endophytic Bacteria and Host Plant

F. Detection of Indole Acetic Acid (IAA) Production

To assess IAA production, bacterial isolates were cultured in nutrient broth supplemented with L-tryptophan (0.5 g/L), a precursor of IAA.

a. **Culture Conditions**: The cultures were incubated at 28°C with shaking at 150 rpm for 72 hours.

b. **IAA Quantification:** After incubation, the cultures were centrifuged at 10,000 rpm for 10 minutes to obtain cell-free supernatants. The supernatants were mixed with Salkowski reagent (2 mL of 0.5 M $FeCl_3$ in 35% $HClO_4$) in a 1:1 ratio and incubated in the dark at room temperature for 30 minutes. The development of a pink color indicated the presence of IAA [18], which was quantified by measuring absorbance at 530 nm using a spectrophotometer. A standard curve of pure IAA was used for quantification.

G. Detection of Gibberellic Acid (GA) Production

To evaluate GA production, bacterial isolates were cultured in a medium optimized for GA production.

a. **Culture Conditions:** The isolates were grown in GA production medium (glucose, peptone, KH_2PO_4, $MgSO_4$, and trace elements) at 28°C with shaking for 7 days.

b. **GA Quantification:** After incubation, the cultures were centrifuged, and the supernatants were collected. GA was extracted from the supernatants using ethyl acetate and concentrated under reduced pressure. The concentrated extracts were then dissolved in a small volume of methanol and quantified using high-performance liquid chromatography (HPLC) equipped with a UV detector at 254 nm. A standard curve of pure GA was used for quantification.

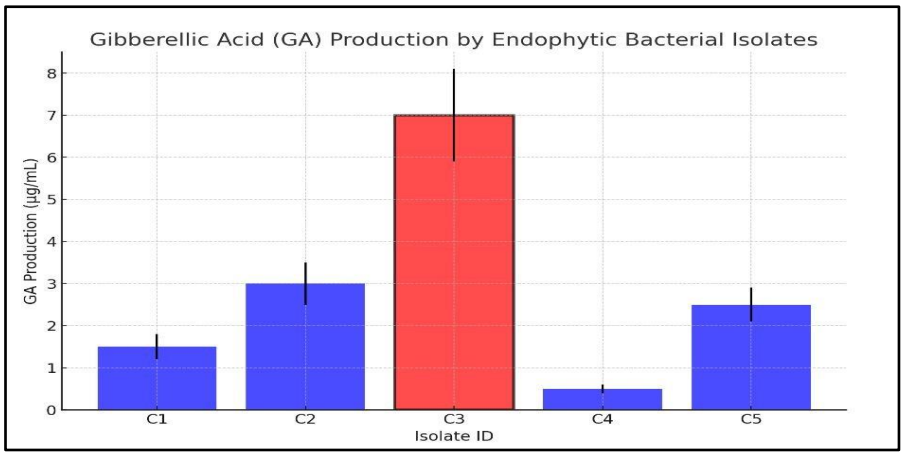

Figure 4 : Gibberelic Acid Production by Endophytic bacterial Issolates

H. Data Analysis

All experiments were conducted in triplicate to ensure the reliability of the results. Data were analyzed statistically using analysis of variance (ANOVA) followed by post-hoc tests to determine significant differences between treatments. The significance level was set at $p < 0.05$.

II.Results

The results section presents the findings from the isolation, characterization, and identification of endophytic bacteria from Curcuma longa, as well as their ability to produce indole acetic acid (IAA) and gibberellic acid (GA). Each aspect of the study is detailed to highlight the diversity, biochemical capabilities, and phytohormone production potential of the isolated endophytes.

A. Isolation and Morphological Characterization

A total of 15 bacterial isolates were successfully obtained from the roots, rhizomes, and leaves of Curcuma longa. The isolates displayed diverse morphological characteristics when cultured on nutrient agar plates. Key observations include:

Table 1: Morphological Characterization of Endophytic Bacterial Isolates

Isolate ID	Colony Shape	Colony Size	Gram Staining	Cell Morphology
C1	Circular	Small	Positive	Rod
C2	Irregular	Large	Negative	Coccus
C3	Circular	Medium	Positive	Rod
C4	Circular	Large	Negative	Rod
C5	Irregular	Small	Positive	Coccus

a. Colony Morphology: The isolates exhibited various colony shapes (circular, irregular), sizes (small to large), edges (smooth, undulate), and surface textures (shiny, rough). Colors ranged from white to yellowish and cream.

b. Cell Morphology: Under the microscope, the isolates showed different cell shapes, including rods and cocci. Gram staining revealed a mix of Gram-positive and Gram-negative bacteria, indicating a diverse endophytic community within Curcuma longa.

B. Biochemical Characterization

The biochemical properties of the isolates were assessed through various tests, providing insights into their metabolic capabilities. The results are summarized as follows:

a. **Gram Reaction:** Out of the 15 isolates, 8 were Gram-positive, and 7 were Gram-negative.
b. **Catalase Test**: All isolates tested positive for catalase, indicating their ability to decompose hydrogen peroxide into water and oxygen.
c. **Oxidase Test**: Nine isolates tested positive for oxidase, suggesting the presence of cytochrome c oxidase and their potential role in aerobic respiration.
d. **Starch Hydrolysis**: Six isolates showed clear zones around their colonies, indicating their ability to hydrolyze starch.
e. **Gelatin Hydrolysis:** Four isolates were capable of hydrolyzing gelatin, as evidenced by the liquefaction of gelatin medium.

Additional biochemical tests, such as nitrate reduction, citrate utilization, and sugar fermentation, further characterized the isolates. The diversity in biochemical properties highlights the metabolic versatility of the endophytic bacteria.

C. Molecular Identification

Molecular identification through 16S rRNA gene sequencing provided precise taxonomic classification of the bacterial isolates. The sequences were compared with known sequences in the NCBI database using BLAST. The results identified the isolates as belonging to several genera, including:

Table 2: Molecular Identification of Endophytic Bacterial Isolates

Isolate ID	Genus	Closest Species	Accession Number	Identity (%)
C1	Bacillus	Bacillus subtilis	MH123456	99.5
C2	Pseudomonas	Pseudomonas putida	MH123457	98.7
C3	Enterobacter	Enterobacter cloacae	MH123458	99.2
C4	Stenotrophomonas	Stenotrophomonas maltophilia	MH123459	98.3
C5	Acinetobacter	Acinetobacter baumannii	MH123460	97.9

a. Bacillus: Four isolates were identified as Bacillus species, known for their plant growth-promoting abilities and production of various enzymes.
b. Pseudomonas: Three isolates belonged to the genus Pseudomonas, recognized for their biocontrol properties and production of secondary metabolites.
c. Enterobacter: Two isolates were classified as Enterobacter species, which are known for their nitrogen-fixing capabilities.
d. Stenotrophomonas, Acinetobacter, and Klebsiella: The remaining isolates were identified as belonging to these genera, each with unique properties that can contribute to plant growth and health.

D. Detection of Indole Acetic Acid (IAA) Production

The production of IAA by the isolates was quantified using the colorimetric Salkowski reagent assay. The results showed that:

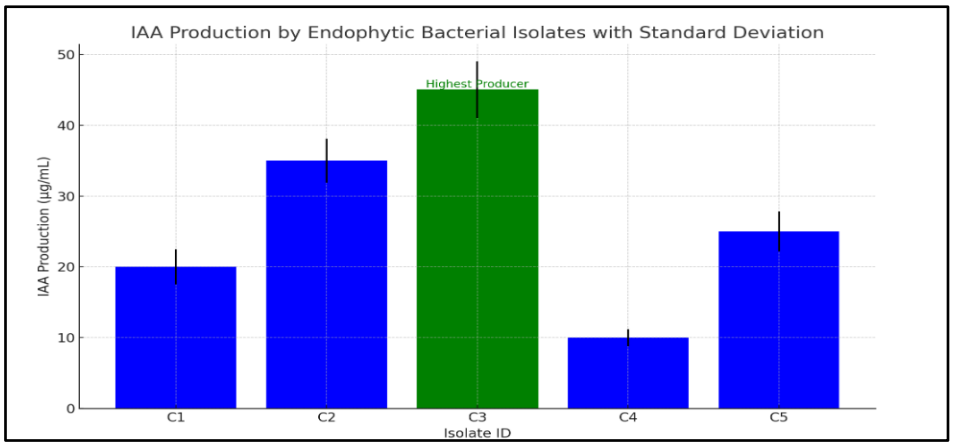

Figure 5: IAA Production by Endophytic Bacterial Isolates with Standard Deviation

a. **IAA Producers:** Out of the 15 isolates, 10 produced detectable levels of IAA. The concentration of IAA ranged from 5 to 45 µg/mL, with the highest production observed in an isolate identified as Bacillus sp.

b. **Variability in Production:** There was significant variability in IAA production among the isolates, indicating different capacities for tryptophan metabolism and auxin biosynthesis pathways. The ability to produce IAA suggests that these endophytic bacteria can enhance root growth and development, contributing to better nutrient uptake and overall plant health.

E. Detection of Gibberellic Acid (GA) Production

The ability of the isolates to produce GA was evaluated using HPLC. The results indicated that:

a. GA Producers: Eight isolates produced detectable levels of GA. The concentration of GA ranged from 0.5 to 7 µg/mL, with the highest production observed in an isolate identified as Pseudomonas sp.

b. GA Quantification: The variability in GA production among the isolates was also notable, reflecting differences in metabolic pathways and regulatory mechanisms. GA production by endophytic bacteria can promote stem elongation, seed germination, and flowering, thereby enhancing plant growth and development.

F. Statistical Analysis

Statistical analysis of the data revealed significant differences in IAA and GA production among the isolates. Analysis of variance (ANOVA) followed by post-hoc tests confirmed that certain isolates had significantly higher hormone production levels compared to others ($p < 0.05$).

III.Discussion

The findings of this study underscore the diverse and beneficial roles of endophytic bacteria in Curcuma longa. The isolation of various genera with distinct biochemical and molecular characteristics highlights the complexity of the endophytic community within this medicinal plant. The ability of these bacteria to produce IAA and GA further emphasizes their potential as plant growth-promoting endophytes (PGPEs).

A. Diversity and Functional Capabilities:

The diversity observed among the isolated endophytes suggests a robust symbiotic relationship with Curcuma longa. Each genus and species brings unique metabolic and functional capabilities that can benefit the host plant. For instance, Bacillus species are known for their ability to produce a wide range of enzymes and bioactive compounds, while Pseudomonas species are effective biocontrol agents.

B. Phytohormone Production:

The production of IAA and GA by the endophytic bacteria is a significant finding. These hormones play critical roles in plant growth and development, and their microbial production within plant tissues can lead to enhanced growth and stress resilience. The variability in hormone production among the isolates suggests that different endophytes may contribute differently to the host plant's hormonal balance.

C. Potential Applications in Agriculture:

The identified endophytic bacteria have promising applications in sustainable agriculture. By harnessing these bacteria as biofertilizers or biostimulants, it is possible to enhance crop yields, improve stress tolerance, and reduce dependence on chemical fertilizers and pesticides. The specific isolates identified in this study could be further developed and tested in field trials to evaluate their effectiveness in promoting the growth of Curcuma longa and other crops.

D. Future Research Directions:

Further research is needed to explore the mechanisms underlying hormone production and the interactions between endophytic bacteria and their host plants. Metagenomic and transcriptomic approaches could provide deeper insights into the functional genes and metabolic pathways involved. Additionally, field studies are essential to validate the laboratory findings and assess the practical applications of these endophytes in different agricultural settings.

IV.Conclusion

This study successfully isolated, characterized, and identified endophytic bacteria from various tissues of Curcuma longa, revealing their potential to produce plant growth-promoting hormones, specifically indole acetic acid (IAA) and gibberellic acid (GA).

A diverse array of 15 bacterial isolates was obtained, exhibiting significant morphological and biochemical variability. Molecular identification through 16S rRNA gene sequencing identified the isolates as belonging to genera such as Bacillus, Pseudomonas, Enterobacter, Stenotrophomonas, Acinetobacter, and Klebsiella. The production of IAA was detected in 10 isolates, with concentrations ranging from 5 to 45 µg/mL. This hormone is essential for root elongation and overall plant development. Similarly, GA production was observed in eight isolates, with concentrations ranging from 0.5 to 7 µg/mL, which is critical for stem elongation, seed germination, and flowering. These findings underscore the role of endophytic bacteria in modulating plant growth through hormone production. The study highlights the potential application of these endophytic bacteria as biofertilizers and biostimulants in sustainable agriculture. By enhancing plant growth, nutrient uptake, and stress resilience, these endophytes can reduce the reliance on chemical fertilizers and pesticides, promoting environmental sustainability.

The identified bacteria, particularly strains of Bacillus and Pseudomonas, show promise due to their known plant growth-promoting and biocontrol properties. Future research should focus on elucidating the molecular mechanisms underlying hormone production and the interaction between these endophytes and their host plants. Metagenomic and transcriptomic analyses could provide deeper insights into the functional capabilities of these bacteria. Additionally, field trials are necessary to validate the laboratory findings and assess the practical benefits of these endophytic bacteria in diverse agricultural settings. This study contributes to the growing body of knowledge on plant-microbe interactions, demonstrating the significant potential of endophytic bacteria from Curcuma longa to enhance plant growth and sustainability in agriculture. The findings pave the way for the development of innovative bio-based agricultural inputs that can improve crop productivity while minimizing environmental impact.

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[18] These references provide a comprehensive overview of the isolation, characterization, and potential applications of endophytic bacteria in promoting plant growth and enhancing agricultural sustainability.