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ISOLATION OF ENDOPHYTIC BACTERIA FROM *CURCUMA LONGA* COLLECTED FROM AGRICULTURAL LAND OF ARDHAPUR, NANDED DISTRICT, MAHARASHTRA, INDIA

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Abstract:

Endophytic bacteria live inside tissues such as leaves, roots, rhizomes, and cells without harming the host plant. Medicinal plants harbor specialized endophytes of notable agricultural and biotechnological importance. Among these, Curcuma longa (turmeric) serves as a valuable source of specialized microorganisms. This study isolated endophytic bacteria from Curcuma longa leaves and rhizomes collected from agricultural land in Ardhapur, Nanded District, Maharashtra, India. Samples were surface-sterilized using 80% ethanol and 0.1% $HgCl_2$, then rinsed three times with sterile distilled water. Sterilization effectiveness was confirmed when unsliced leaf and rhizome segments placed on nutrient agar showed no growth. Sterilized tissues were enriched in Nutrient Broth and Tryptic Soy Broth at 37 °C for 8–10 days; resulting turbidity confirmed microbial growth. Two experimental sets were evaluated. In Set TP-I, enriched segments were plated directly on Nutrient Agar and Tryptic Soy Agar, yielding seven distinct bacterial colonies from leaves and six from rhizomes. In Set TP-II, enriched suspensions were streaked across both agars, producing four colonies from leaves and five from rhizomes. A total of 22 distinct bacterial isolates were obtained, maintained as pure cultures on agar slants, and subjected to morphological characterization.

Keywords: *Curcuma longa Leaves, Curcuma Longa Rhizome, Endophytes.*

1. Introduction

Endophytic bacteria are those that live within the cells or tissues of the host plant. Strictly speaking, endophytes may be defined as organisms capable of living in the interior of tissues. In reality, these organisms live therein for at least a portion of their life history without producing any damage to the host plant. Such organisms can be isolated from roots, stems, leaves, seeds, rhizomes, etc., of both vascular plants and lower cryptogams. Endophytic

bacteria assume great importance as they are frequently of advantage to the host by stimulating growth and promoting the absorption of plant food materials (1). Endophytic bacteria are microorganisms that live in the internal tissues of plants without any visible adverse effect on the host. Such bacteria reside in various tissues of the medicinal plant *Curcuma longa* (turmeric), including the rhizome, root, stamen, and leaves. Turmeric is reputed for its medicinal properties and bioactive compounds, especially curcumin (2).

The bacterium *Cronobacter* species is an endophyte. Endophytic bacteria from *Curcuma longa* have not been extensively studied, though such bacteria may offer substantial benefits to the plant by promoting growth, cycling nutrients, or protecting the host from phytopathogens. Some of the endophytes of *Curcuma longa* can produce secondary metabolites and chemical compounds similar to those of their host, potentially contributing to turmeric's value in medicine and health (3).

In the present study, endophytic bacteria were isolated from the leaves and rhizomes of *Curcuma longa* collected from agricultural farmland in Ardhapur, Nanded District, Maharashtra, India. The plant parts were subjected to surface sterilization using ethanol, mercuric chloride ($HgCl_2$), and sterile distilled water to remove extraneous microorganisms. The efficacy of surface sterilization was determined to ensure the isolation of only internal microbes. Collected leaves and rhizomes were enriched in Nutrient Broth and Tryptic Soy Broth and cultured on Nutrient Agar and Tryptic Soy Agar. The characteristic bacterial colonies obtained after incubation were recorded and subsequently characterized based on their morphological profiles.

2. Material and Methods

2.1 Sample collection

The leaves and rhizomes of *Curcuma longa* were collected from agricultural farmlands in Ardhapur, Nanded District (MS), India. The plants were harvested in the month of March 2025 at an approximate age of six months. They were carefully dug up in such a manner as to retain intact rhizomes and surrounding root systems, placed in sterile glass containers, and processed in the laboratory within 12–24 hours (4).

2.2 Enrichment

Plant leaves and rhizomes of *Curcuma longa* were collected and utilized for the isolation of endophytic bacteria. Samples were aseptically surface-sterilized by immersion for 1 min in 80% ethanol, followed by rinsing in tap water. The samples were then treated with 0.1% (w/v) mercuric chloride ($HgCl_2$) for 5 min to disinfect them, followed by a 10 min rinse with sterile distilled water to thoroughly remove residual disinfectant (2).

The efficiency of surface sterilization was verified by plating surface-sterilized, unsliced plant tissues and rhizome segments onto nutrient agar plates prior to endophyte isolation. The absence of microbial growth confirmed the effectiveness and validity of the sterilization process (5). The surface-sterilized leaves and rhizome pieces of *Curcuma longa* were aseptically cut into small segments of approximately 5 mm in size. The segments were then inoculated into Tryptic Soy Broth (TSB) and Nutrient Broth (NB) and incubated at 37 °C for 8–10 days to facilitate the enrichment of the microbial population. After the incubation period, the enriched cultures were serially diluted up to 10^{-3} using sterile distilled water. The diluted cultures were subsequently inoculated onto suitable culture media for the isolation of endophytic bacteria, followed by purification and further characterization.

2.3 Isolation and purification

In the first set of experiments, enriched portions of leaves and rhizomes of *Curcuma longa* were placed aseptically onto sterile nutrient agar. In the second set of experiments, a 10^{-3} dilution of the enriched culture suspension was gently streaked onto sterile nutrient agar and incubated at 37 °C for 48 hours. After incubation, bacterial colonies

that developed on and around the tissue were carefully picked and streaked onto fresh nutrient agar plates. From these plates, purified colonies were subsequently obtained, characterized, and preserved on agar slants (2, 6).

2.4 Morphological evaluation

Potential bacterial isolates obtained from the leaves and rhizomes of *Curcuma longa* were characterized based on colony features. Pure cultures of selected isolates were established by streaking isolated colonies onto nutrient agar medium.

After incubation, individual colonies were examined for their morphological characteristics, including colony color, shape, margin, elevation, and texture, following standard microbiological procedures (7, 8).

3. Result and Discussion

Healthy leaves and rhizomes of *Curcuma longa* (approximately six-month-old plants) were collected from agricultural farmlands in Ardhapur, Nanded District, Maharashtra, India. At this growth stage, the plants possess fully developed rhizomes and active microbial associations. Healthy and disease-free plant parts were selected to ensure the recovery of natural endophytic microflora and to avoid contamination of the plant samples with phytopathogens. The collected samples were promptly transported to the laboratory for experimental analysis. Previously, Jain *et al.* (4) isolated endophytic microbes from seven different medicinal plants, namely *Codiaeum variegatum* pictum (Croton), *Adhatoda vasica* Nees (Adulsa), *Neolamarckia cadamba* (Kadamba), *Azadirachta indica* (Neem), *Curcuma longa* (Turmeric), *Hibiscus rosa-sinensis* (Hibiscus), and *Saraca asoca* (Ashoka), collected from the botanical garden of Birla College, Kalyan.

Figure 1: *Curcuma longa* leaves

Figure 2: *Curcuma longa* rhizome

Leaves and rhizomes of *Curcuma longa* were surface-sterilized with 80% ethanol and 0.1% $HgCl_2$, followed by three successive rinses with sterile distilled water. This treatment effectively eliminated surface contaminants from the collected plant samples. Rigorous surface sterilization ensures the destruction of epiphytic organisms while preserving internal endophytic flora. A comparable sterilization protocol utilizing ethanol, $HgCl_2$, and distilled water with varying concentrations and exposure durations was reported by Deshmukh *et al.* (2), demonstrating the efficiency and reliability of this procedure for plant tissue surface sterilization.

To confirm the effectiveness of the surface sterilization protocol, surface-sterilized, unsliced plant leaves and rhizome segments were inoculated onto nutrient agar plates prior to endophyte isolation. No microbial colonies appeared on the plates, confirming that surface sterilization was completely successful and that all subsequently recovered microorganisms represented true endophytes. This validation procedure for confirming the efficacy of surface sterilization in endophyte research was likewise detailed by Schulz *et al.* (5).

Two sets of isolation procedures were conducted for the recovery of endophytic microflora from the leaves and rhizomes of *Curcuma longa*. In the first set (designated TP-I), enriched segments of leaves and rhizomes were

directly plated onto the culture media. In the second set (designated TP-II), enriched broth suspensions prepared from the leaves and rhizomes of the plant were streaked onto the culture media. Both sets of procedures together yielded a total of 22 isolates.

Figure 3: Enrichment of *Curcuma longa* leaves and rhizome in NB and TSB

Table 1: Isolation of endophytic microbes from leaves and rhizome of *Curcuma longa*.

TP-I		**TP-II**	
Leaves	**Rhizome**	**Leaves Suspension**	**Rhizome Suspension**
07	06	04	05
Total = 22			

Similar results were reported by Deshmukh *et al.* (2) and Anggraeni *et al.* (3), who isolated endophytic bacteria from *Curcuma longa*. Sun *et al.* (6) documented the recovery of 973 isolates of endophytic fungi from tissue fragments of six medicinal plant species, further corroborating that medicinal plants serve as exceptionally rich reservoirs of endophytic microorganisms.

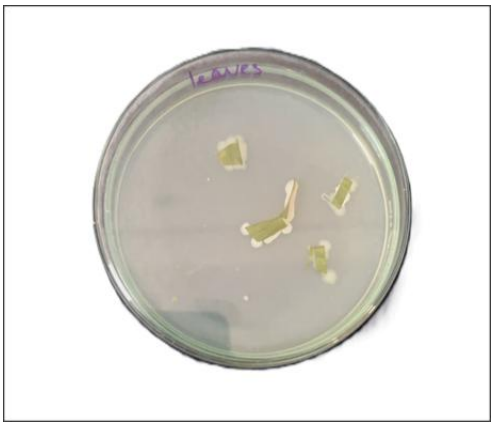

Figure 4: Isolation of endophytic microbes from *Curcuma longa* leaves and rhizome

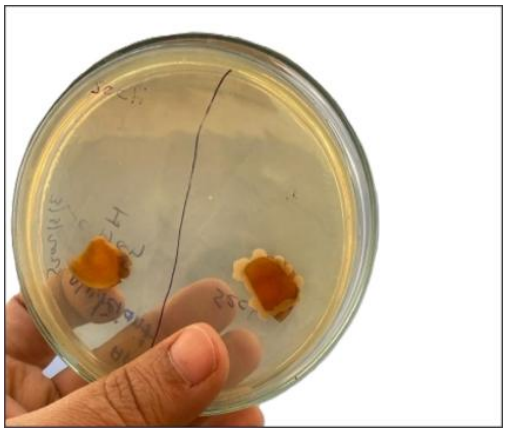

Figure 5: Microbial isolates

A total of 22 distinct microbial isolates from the leaves and rhizomes of *Curcuma longa* were characterized morphologically. Colony characteristics, including color, shape, margin, elevation, and texture, were evaluated on nutrient agar medium. The observable diversity in colony morphology among the microbial endophytes enabled

effective differentiation between isolates and confirmed that a substantial variety of endophytic microbes reside within the host plant tissues. A comparable pattern of colony characterization among endophytic isolates from *Curcuma longa* was reported by Deshmukh *et al.* (2).

Table 2: Colony characterization of bacterial endophytes.

Colonies	Color	Shape	Margin	Elevation	Texture
ET1	Creamy white	Circular	Entire	Flat	Butyrous
ET2	White	Irregular	Undulate	Flat	Dry
ET3	Creamy	Irregular	Undulate	Flat	Slightly rough
ET4	White to creamy	Circular	Irregular	Flat	Granular
ET5	Pale white	Circular	Entire	Flat	Moist
ET6	Orange	Circular	Entire	Slightly raised	Moist
ET7	White to creamy	Circular	Entire	Convex	Mucoid
ET8	Pale yellow	Circular	Entire	Flat	Sticky
ET9	Creamy	Circular	Irregular	Flat	Dry
ET10	Pale white	Irregular	Entire	Convex	Moist
ET11	Creamy	Circular	Entire	Flat	Dry
ET12	White to creamy	Irregular	Entire	Convex	Moist
ET13	Creamy	Circular	Entire	Flat	Mucoid
ET14	Creamy	Irregular	Undulate	Flat	Moist
ET15	Pale white	Circular	Undulate	Flat	Dry
ET16	Pale yellow	Irregular	Entire	Convex	Moist
ET17	White to creamy	Circular	Entire	Flat	Mucoid
ET18	Creamy	Irregular	Undulate	Flat	Sticky
ET19	Creamy to white	Circular	Undulate	Convex	Dry
ET20	Pale white	Circular	Entire	Convex	Sticky
ET21	Pale yellow	Irregular	Entire	Flat	Dry
ET22	White to creamy	Circular	Undulate	Convex	Mucoid

4. Conclusion

The present study successfully isolated endophytic bacteria from the leaves and rhizomes of *Curcuma longa* collected from agricultural land in Ardhapur, Nanded District, Maharashtra, India. Elimination of epiphytic microorganisms from the plant tissue surface was accomplished using the optimized surface-sterilization protocol. Distinct colonies were obtained on Nutrient Agar and Tryptic Soy Agar and characterized based on their unique cultural and morphological attributes. The findings demonstrate that *Curcuma longa* harbors a diverse community of endophytic bacteria. These bacterial isolates exhibit distinct morphological traits and represent candidates of notable agricultural and biotechnological value.

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