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Research Article

Endophytic Bacterial Diversity of *Cocculus hirsutus* Revealed by 16S rRNA Gene Sequencing: Implications for Pharmacologically Active Metabolites

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ABSTRACT

Background: Endophytic bacteria are recognized as important sources of bioactive secondary metabolites contributing to plant health and therapeutic potential. Cocculus hirsutus, a medicinal climber widely used in traditional medicine, remains poorly explored for its endophytic bacterial diversity. Methods: Healthy leaf and stem tissues of C. hirsutus were collected from Nagpur, India, surface sterilized, and processed for endophytic microbial analysis. Genomic DNA was extracted, and the V3–V4 region of the 16S rRNA gene was sequenced using the Illumina MiSeq platform. Bioinformatic analysis was performed using QIIME2 with DADA2 denoising, and taxonomic classification was carried out using the SILVA 138 database. Results: High-quality sequencing data revealed a diverse endophytic bacterial community dominated by the genera Bacillus (28.5%), Pseudomonas (22.3%), and Streptomyces (15.1%). Alpha diversity metrics, including Shannon index, Faith's phylogenetic diversity, and rarefaction analysis, indicated substantial microbial richness. The diversity observed in C. hirsutus was comparatively higher than that reported for the reference medicinal plant Tinospora cordifolia. Conclusion: The presence of endophytic bacterial genera known for producing antimicrobial, immunomodulatory, and anticancer metabolites highlights C. hirsutus as a promising reservoir of pharmacologically important microorganisms. These findings provide a scientific basis for further bioactivity-guided isolation and drug discovery studies.

INTRODUCTION

Medicinal plants are increasingly recognized as complex biological systems that harbor diverse communities of endophytic microorganisms residing asymptomatically within their internal tissues. These endophytes contribute significantly to plant fitness by enhancing nutrient acquisition, conferring tolerance to biotic and abiotic stress, and participating in the biosynthesis of secondary

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metabolites with therapeutic importance [1–3]. Among them, endophytic bacteria have gained considerable attention due to their metabolic versatility and ability to produce a broad spectrum of bioactive compounds, including antimicrobial, anticancer, immunomodulatory, anti-inflammatory, and antioxidant agents [4–6]. Importantly, several metabolites originally attributed to host plants have later been shown to be synthesized by their associated endophytes, highlighting their relevance in pharmaceutical research and sustainable drug discovery [7,8].

From a pharmaceutical perspective, endophytic bacteria represent an attractive and renewable source of bioactive molecules because they can be cultured under laboratory conditions and manipulated to enhance metabolite production, thereby reducing dependence on plant biomass and ecological exploitation [6,9]. Advances in molecular biology and next-generation sequencing technologies have facilitated culture-independent exploration of endophytic microbial diversity, overcoming limitations associated with traditional cultivation-based approaches [10,11]. In particular, high-throughput 16S rRNA gene sequencing combined with robust bioinformatic pipelines such as QIIME2 and DADA2 enables accurate identification of bacterial taxa and detailed assessment of microbial diversity within plant tissues [12,13].

Cocculus hirsutus (Menispermaceae) is a perennial medicinal climber widely distributed in India and employed in traditional medicine for the management of inflammatory disorders, microbial infections, fever, diabetes, and hepatic ailments. Phytochemical studies on *C. hirsutus* have reported the presence of biologically active alkaloids, flavonoids, and terpenoids with pharmacological significance. However, despite its established therapeutic potential, systematic investigations focusing on the endophytic bacterial diversity associated with *C. hirsutus* remain limited. Considering the growing evidence that medicinal plants serve as reservoirs of pharmaceutically important endophytes, exploration of the endophytic microbiome of *C. hirsutus* is both scientifically and therapeutically relevant [14,15]..

Therefore, the present study aims to characterize the endophytic bacterial community of *Cocculus hirsutus* using high-throughput 16S rRNA gene sequencing and comprehensive bioinformatic analysis. Emphasis is placed on identifying dominant bacterial taxa with known pharmaceutical relevance, thereby providing a scientific foundation for future bioactivity-guided isolation and drug discovery studies involving endophytic bacteria from medicinal plants. [16,17].

2. MATERIALS AND METHODS

2.1 Plant Collection and Surface Sterilization

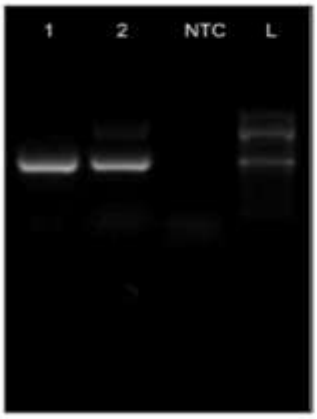
Healthy, disease-free plants of *Cocculus hirsutus* (Menispermaceae) were collected from the Nagpur region, Maharashtra, India. Leaves and stems were selected to ensure representation of internal endophytic microbial communities. The collected samples were transported to the laboratory in sterile containers and processed within 24 h of collection.

Plant materials were initially washed under running tap water to remove adhering soil particles and debris. Surface sterilization was carried out under aseptic conditions by sequential immersion in 70% (v/v) ethanol for 1 min, followed by treatment with 1% (w/v) sodium hypochlorite solution for 2–3 min. Samples were then rinsed five times with sterile distilled water to remove residual sterilizing agents. The effectiveness of

surface sterilization was confirmed by imprinting the sterilized plant tissues onto nutrient agar plates and incubating at 37 °C for 48 h. The absence of microbial growth confirmed successful elimination of epiphytic microorganisms.

2.2 Genomic DNA Extraction

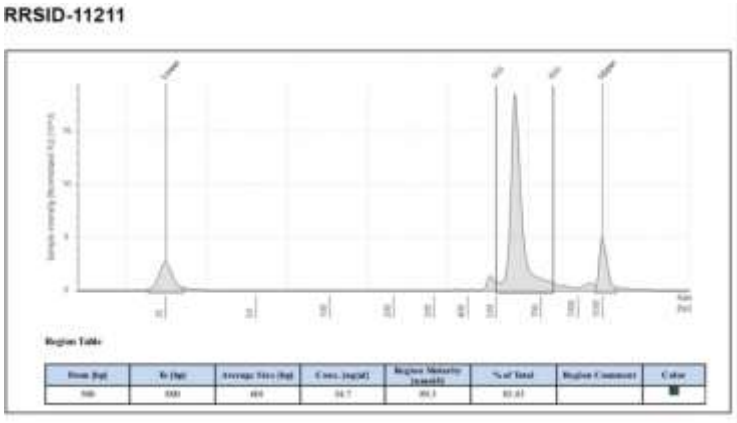
Surface-sterilized leaf and stem tissues were aseptically macerated, and total genomic DNA was extracted using the Qiagen plant DNA extraction kit according to the manufacturer's protocol. The quality and integrity of the extracted DNA were evaluated by electrophoresis on a 1% agarose gel, while DNA concentration and purity were assessed using NanoDrop spectrophotometry based on A260/A280 ratios. Only high-quality DNA samples were used for downstream amplification and sequencing (Fig. 1).



Lane ID	Sample Name	Sample ID
1	Cocculus hirsutus	RRSID-11211
2	Tinospora cordifolia leaf	RRSID-11210
NTC	NTC	NTC
L	100bp Ladder	100bp Ladder

2.3 PCR Amplification and Illumina MiSeq Sequencing

The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using universal primers under optimized PCR conditions. PCR amplification was performed in a thermal cycler, and the amplified products were confirmed by agarose gel electrophoresis, which yielded distinct bands of approximately 600 bp (Fig. 2). Purified PCR amplicons were subjected to paired-end sequencing using the Illumina MiSeq platform following standard library preparation protocols provided by the sequencing facility.



2.4 Bioinformatic Processing of Sequencing Data

Raw paired-end sequencing reads were demultiplexed and imported into the QIIME2 bioinformatics pipeline for downstream analysis.

Quality filtering, denoising, and chimera removal were performed using the DADA2 algorithm to generate high-resolution amplicon sequence variants (ASVs). Low-quality reads and sequencing artifacts were removed to ensure reliable taxonomic assignments.

Taxonomic classification of ASVs was carried out using a naïve Bayes classifier trained on the SILVA 138 reference database. Taxonomic profiles were generated at phylum, class, order, family, and genus levels, and relative abundance plots were constructed to visualize community composition (Fig. 3).



Fig-3 Abundance plot of the provided samples at the genus level

2.5 Diversity and Statistical Analysis

Alpha diversity indices, including observed OTUs, Shannon diversity index, Faith's phylogenetic diversity, and Pielou's evenness, were calculated using QIIME2 to assess microbial richness and diversity within *C. hirsutus* tissues. Rarefaction curves were generated to evaluate sequencing depth sufficiency and sampling completeness (Fig. 4). The diversity data were interpreted in comparison with previously reported endophytic bacterial diversity in medicinal plants, including *Tinospora cordifolia*, to assess the relative bioprospecting potential of *C. hirsutus*.

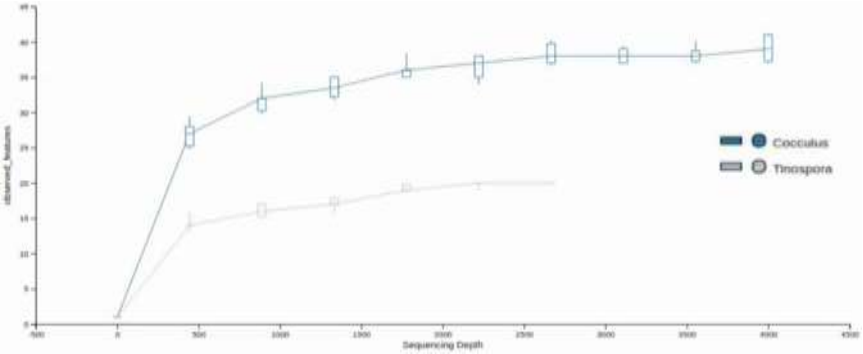


Fig:4 - Rare faction curve for the provided sample

3. RESULTS

3.1 Sequencing Quality Assessment

PCR amplification of the bacterial 16S rRNA gene yielded distinct and reproducible bands of approximately 600 bp, confirming the successful amplification of the targeted hypervariable

regions. Illumina sequencing generated a high number of raw reads, of which the majority passed quality filtering, chimera removal, and denoising steps. The high Phred quality scores across reads indicated minimal sequencing errors and ensured the reliability of downstream taxonomic and diversity analyses. Overall, the sequencing depth and quality were sufficient to accurately characterize the endophytic bacterial community associated with *Cocculus hirsutus*.

3.2 Endophytic Bacterial Community Composition

Taxonomic classification revealed that the endophytic bacterial community of *C. hirsutus* was predominantly represented by the phyla Proteobacteria and Firmicutes, followed by Actinobacteria. These phyla are commonly reported as dominant endophytes in medicinal plants and are known for their metabolic versatility and adaptability to plant internal tissues.

At the genus level, Bacillus (28.5%), Pseudomonas (22.3%), and Streptomyces (15.1%) were identified as the most abundant taxa (Table 1). The predominance of *Bacillus* species suggests their important role in plant growth promotion and stress tolerance, owing to their ability to produce extracellular enzymes, lipopeptides, and antimicrobial compounds. *Pseudomonas* species are well documented for producing phenazine derivatives, siderophores, and bioactive secondary metabolites that contribute to plant defense mechanisms. Similarly, *Streptomyces*, a genus renowned for its prolific secondary metabolite biosynthesis, is a major source of clinically relevant antibiotics and anticancer compounds.

Table 1: Dominant endophytic bacterial genera isolated from *C. hirsutus*

Genus	Relative abundance (%)
Bacillus	28.5
Pseudomonas	22.3
Streptomyces	15.1

The presence of these metabolically active genera underscores the pharmaceutical relevance of the *C. hirsutus* endophytic microbiome and its potential contribution to the medicinal properties of the host plant.

3.3 Alpha Diversity Analysis

Alpha diversity metrics demonstrated a high level of microbial richness and evenness within the endophytic bacterial communities. The number of observed OTUs ranged from 138 to 152, indicating substantial species richness across samples. Shannon diversity index values varied between 3.75 and 3.95, reflecting a well-balanced distribution of taxa. Faith's phylogenetic diversity values (5.0–5.23) further suggested a broad evolutionary diversity among the detected endophytes.

Rarefaction curves approached a plateau for all samples, confirming adequate sequencing depth and comprehensive coverage of the bacterial communities. Notably, the alpha diversity indices observed in *C. hirsutus* were higher than those previously reported for *Tinospora cordifolia*, indicating a comparatively richer and more diverse endophytic bacterial population. This enhanced diversity may contribute to a broader spectrum of bioactive metabolites, thereby increasing the plant's bioprospecting potential.

4. DISCUSSION

The present study reveals that *Cocculus hirsutus* harbors a diverse and taxonomically rich endophytic bacterial community dominated by genera with well-documented pharmaceutical relevance. The prevalence of *Bacillus*, *Pseudomonas*, and *Streptomyces* is consistent with earlier reports on medicinal plants, supporting the

hypothesis that such plants act as reservoirs of bioactive endophytes [12,13,19].

These genera are known to synthesize compounds with antimicrobial, anticancer, antioxidant, and immunomodulatory activities, suggesting that endophytic bacteria may contribute synergistically to the therapeutic properties traditionally attributed to *C. hirsutus* [6,18,20]. The higher microbial diversity compared to *T. cordifolia* further indicates the superior potential of *C. hirsutus* as a source of novel microbial metabolites.

5. CONCLUSION

This study represents the first comprehensive characterization of the endophytic bacterial diversity of *Cocculus hirsutus* using 16S rRNA gene sequencing. The identification of metabolically versatile and pharmaceutically significant bacterial genera highlights the plant's potential as a valuable source of novel bioactive compounds. The observed high microbial diversity and dominance of well-known secondary metabolite producers emphasize the importance of endophytes as contributors to medicinal plant bioactivity.

Future investigations focusing on bioactivity-guided isolation, functional genomics, and pharmacological evaluation of endophytic bacterial metabolites are warranted to fully exploit the therapeutic potential of *C. hirsutus*–associated microbiota.

6. DECLARATIONS

Authors' Contributions: All authors contributed to study design, experimentation, data analysis, manuscript drafting, and approved the final version.

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Conflict of Interest: The authors declare no conflict of interest.

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