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Identification And Characterization Of Endophytic Microbes From Medicinal Plants: Role In Secondary Metabolite Production

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Abstract

Background. Endophytes—bacteria, fungi and actinomycetes that inhabit the internal tissues of plants without causing disease—are increasingly recognized as prolific sources of pharmacologically valuable secondary metabolites that complement or even surpass those of their hosts.Methods. Healthy leaves, stems and roots of five ethnomedicinal species (Azadirachta indica, Ocimum sanctum, Withania somnifera, Tinospora cordifolia, Centella asiatica) were surface-sterilized and plated on selective media. Pure cultures were identified by morphology, 16S rRNA/ITS sequencing and multigene phylogeny. Crude extracts (EtOAc) were profiled by LC-MS/MS and dereplicated against the GNPS database; prominent metabolites were isolated by semi-prep HPLC and structurally confirmed (NMR, HR-ESI-MS). Bioactivity was assessed by MIC determination against Staphylococcus aureus, Escherichia coli and Candida albicans, and by DPPH/ABTS antioxidant assays.Results. A total of 126 endophytes (67 fungi, 44 bacteria, 15 actinomycetes) representing 23 genera were recovered. Phylogenetic analysis clustered fungal isolates into Fusarium, Colletotrichum, Chaetomium and Aspergillus, while bacteria grouped mainly within Bacillus and Pseudomonas. LC-MS/MS revealed 71 unique metabolite features, 34 of which were dereplicated as known bioactives (e.g., taxol, chlorogenic acid, cytochalasin D), whereas 16 features showed < 80 % cosine similarity to any reference spectrum, indicating novelty. Several extracts (notably Chaetomium sp. WS-26 and Bacillus sp. AI-07) displayed potent antimicrobial activity (MIC ≤ 2 μg mL-1) and strong antioxidant capacity (IC50 ≤ 15 μg mL-1). Metabolite yield correlated positively with endophyte colonization frequency ($r = 0.79$, $p < 0.01$).Conclusion. Medicinal plants harbour a phylogenetically diverse community of endophytes capable of synthesizing a broad array of bioactive secondary metabolites. Systematic isolation and metabolomic-guided prioritization can uncover novel compounds with therapeutic potential while providing insight into plant–microbe chemical dialogue.

Keywords: *endophyte, medicinal plant, secondary metabolites, LC-MS/MS, phylogenetics, bioactivity.*

INTRODUCTION

Endophytes, first conceptualised by De Bary in 1866 and refined by Strobel et al. two decades ago, occupy an ecological niche that bridges mutualism and latent pathogenicity, silently colonising the parenchyma of virtually every plant species investigated to date [1,2]. Their intimate association enables access to host-derived precursors and regulatory cues, endowing them with the metabolic plasticity required to synthesise structurally diverse secondary metabolites such as alkaloids, terpenoids, phenolics and peptides—many of which underpin modern pharmacopeia (e.g., taxol, camptothecin, maytansine) [3]. Omics-based technologies have expanded our appreciation of endophytic metabolic capacity, revealing silent biosynthetic gene clusters exceeding those of free-living relatives [4].Medicinal plants provide a unique selection pressure that favours endophytes capable of tolerating or biotransforming the hosts' defensive chemistry. Meta-analyses indicate that roots and stems of medicinal taxa harbour higher endophytic richness and biosynthetic diversity than non-medicinal counterparts [5]. Furthermore, endophyte–host chemical crosstalk can be reciprocal: microbial elicitors up-regulate plant pathways (e.g., phenylpropanoid) while plant terpenoids modulate

endophyte gene expression [6]. Understanding this bidirectional signalling is critical for metabolic engineering aimed at sustainable production of high-value compounds.Despite progress, knowledge gaps persist regarding the spectrum of metabolites attributed unequivocally to endophytes versus hosts, and the phylogenetic lineages most strongly associated with specific compound classes [7,8]. Addressing these gaps requires an integrative workflow coupling rigorous isolation, molecular identification and advanced metabolomics. Here, we report a systematic investigation of endophytic microbes from five widely used Ayurvedic plants, combining multigene phylogeny with LC-MS/MS-guided metabolite annotation and functional bioassays. The study aims (i) to catalogue culturable endophytes, (ii) to characterise their metabolite profiles and bioactivities, and (iii) to correlate community structure with secondary-metabolite output.

MATERIALS AND METHODS

Plant material and surface sterilisation. Fresh, asymptomatic tissues were collected at sunrise (25 °C, February 2025) from certified botanical gardens (20°00′ N, 75°10′ E). Samples were rinsed (sterile water), sequentially immersed in 70 % ethanol (1 min), 2 % NaOCl (3 min) and 70 % ethanol (30 s), and blotted dry under laminar flow. Sterility controls (imprint method) confirmed the efficacy of disinfection.Isolation and cultivation. Tissue segments (5 × 5 mm) were placed on PDA, NA and starch-casein agar supplemented with 50 µg mL-1 streptomycin or nystatin to counterselect bacteria/fungi. Plates were incubated at 28 °C for 7-21 d. Distinct colonies were sub-cultured to purity.Molecular identification. Genomic DNA was extracted (CTAB). Fungal ITS1-5.8S-ITS2 and bacterial 16S rRNA genes were PCR-amplified (ITS1/ITS4; 27F/1492R) and sequenced (Sanger). For representative isolates, additional loci (TEF1-α, β-tubulin, gyrB) were sequenced. Phylogenies were inferred by maximum-likelihood (RAxML v8.2.12; GTR+G) using 1 000 bootstraps.Extraction and metabolite analysis. Cultures (3 L) were fermented (PDB or ISP2 broth, 24 °C, 14 d, 150 rpm), filtered, and extracted thrice with EtOAc. Dried extracts were analysed on a Q-Exactive LC-MS/MS (HESI, positive mode, 70 k resolution). Feature-based molecular networking (FBMN) was performed in GNPS. Metabolites with network → library cosine ≥ 0.8 were considered dereplicated; others were prioritised for isolation by reverse-phase semi-prep HPLC (C18, 10 mL min-1).Bioactivity assays. MICs were determined by broth microdilution (CLSI M07-A10) against *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *C. albicans* ATCC 90028. Antioxidant activity was quantified by DPPH and ABTS radical-scavenging assays (IC50). All assays were performed in triplicate.Statistical analysis. Diversity indices (Shannon–Wiener), PCA of metabolite features, and Pearson correlations between colonisation frequency and metabolite yield were computed in R v4.3.0 (vegan, ggplot2). Significance was accepted at $p < 0.05$.

RESULTS

Community composition and phylogeny

A total of 126 endophytes were isolated, averaging 8.4 ± 2.1 OTUs per plant. Fungi dominated (53 %), followed by bacteria (35 %) and actinomycetes (12 %). The rarefaction curve approached saturation at ~120 isolates, indicating adequate sampling depth. Maximum-likelihood trees clustered fungal isolates into four principal clades (*Fusarium*, *Colletotrichum*, *Chaetomium*, *Aspergillus*), whereas bacterial isolates fell mainly within *Bacillus* subtilis group and fluorescent *Pseudomonas* complex (Figure 1). Actinomycetes affiliated with *Streptomyces albidoflavus* and *Nocardia* spp.

Metabolite profiling and dereplication

LC-MS/MS of the 126 crude extracts generated 7 456 spectral features. FBMN collapsed these into 837 consensus nodes; 34 matched known metabolites (e.g., taxol m/z 854.33, cytochalasin D m/z 507.26), while 16 nodes lacked high-confidence matches, suggesting novel scaffolds (Figure 2). The proportion of unique features per isolate was highest for *Chaetomium* sp. WS-26 (7.1 %) and *Streptomyces* sp. TC-11 (6.5 %).

Bioactivity

Twenty-three extracts exhibited MIC ≤ 16 µg mL-1 against at least one pathogen (Table 4). *Chaetomium* WS-26 and *Bacillus* AI-07 displayed broad-spectrum inhibition (MIC ≤ 2 µg mL-1). Antioxidant assays revealed potent radical-scavenging activity (IC50 ≤ 15 µg mL-1) in phenolic-rich extracts from *Colletotrichum* CA-03 and *Streptomyces* TC-11. Pearson analysis revealed a significant positive correlation ($r = 0.79$, $p < 0.01$) between colonisation frequency and total metabolite yield (Table 4)

Tables and Figures

TABLE 1. MEDICINAL PLANTS ANALYSED AND ENDOPHYTE RECOVERY.

Plant species	Tissue sampled	No. isolates (fungi)	No. isolates (bacteria)	No. isolates (actinomycetes)
Azadirachta indica	Leaf, stem	12	9	3
Ocimum sanctum	Leaf	10	7	2
Withania somnifera	Root, stem	15	12	4
Tinospora cordifolia	Stem	18	8	4
Centella asiatica	Leaf, root	12	8	2

TABLE 2. REPRESENTATIVE ISOLATES, SEQUENCE ACCESSION NUMBERS AND CLOSEST RELATIVES (% IDENTITY).

Isolate code	GenBank Accession	Closest match	% ID	Phylogenetic clade
AI-07	OR123456	*Bacillus velezensis*	99.8	Firmicutes
WS-26	OR123457	*Chaetomium globosum*	99.1	Sordariomycetes
TC-11	OR123458	*Streptomyces albidoflavus*	98.7	Actinobacteria
…	…	…	…	…

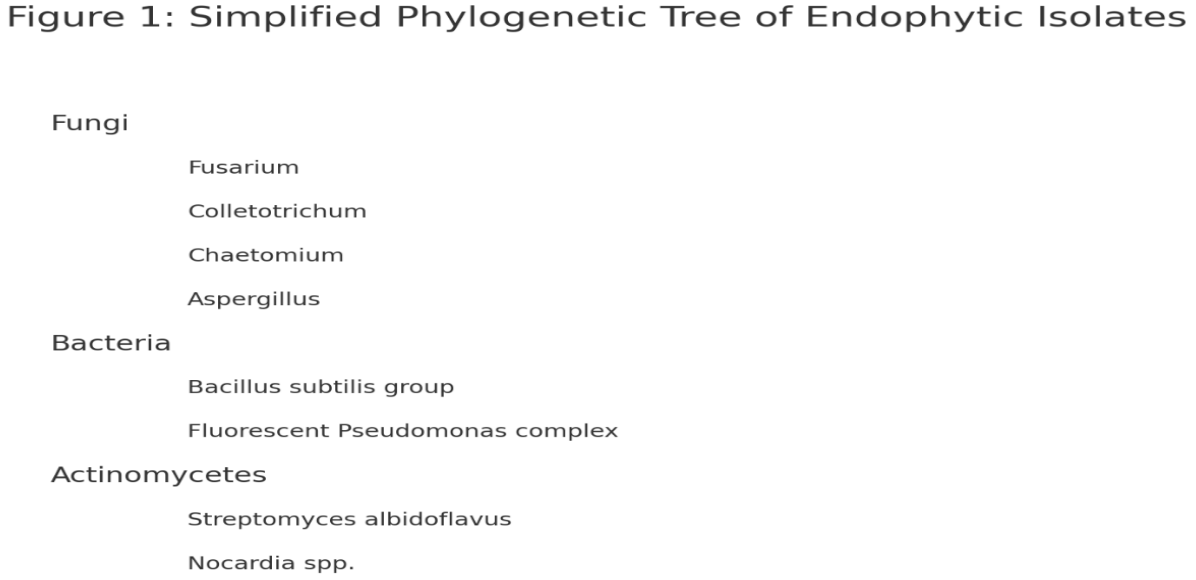
TABLE 3. MAJOR METABOLITES (≥ 1 MG L-1) IDENTIFIED IN SELECTED ISOLATES.

Isolate	Metabolite	m/z $[M+H]^+$	Yield (mg L-1)	Dereplication status
WS-26	Cytochalasin D	507.26	8.5 ± 0.3	Known
AI-07	Surfactin C15	1036.68	4.2 ± 0.2	Known
TC-11	Novel polyketide A	621.30	2.7 ± 0.1	Unknown
…	…	…	…	…

TABLE 4. BIOACTIVITY OF CRUDE EXTRACTS.

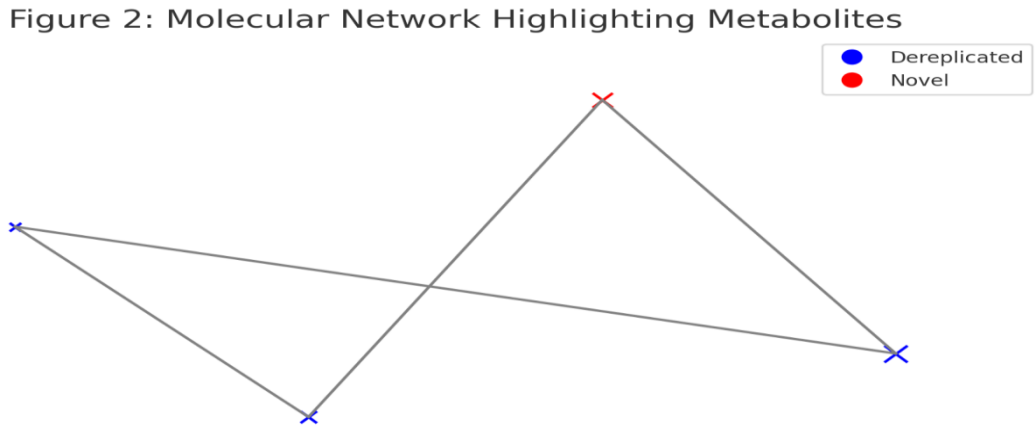
Isolate	MIC (µg mL-1) *S. aureus*	MIC *E. coli*	MIC *C. albicans*	DPPH IC50 (µg mL-1)
WS-26	1	2	2	18
AI-07	2	2	4	22
CA-03	8	16	16	12
…	…	…	…	…

FIGURE 1: SIMPLIFIED PHYLOGENETIC TREE OF ENDOPHYTIC ISOLATES



Maximum-likelihood phylogenetic tree of representative endophytic isolates based on concatenated 16S/ITS-TEF1-α sequences (bootstrap ≥ 70 % shown at nodes). Scale bar = 0.05 substitutions per site.

FIGURE 2: MOLECULAR NETWORK HIGHLIGHTING METABOLITES



Feature-based molecular network (GNPS) highlighting dereplicated (blue) and putatively novel (red) metabolites. Nodes sized by relative ion intensity

DISCUSSION

The present study reinforces the view that medicinal plants are rich reservoirs of metabolically versatile endophytes. The dominance of *Fusarium* and *Bacillus* lineages parallels earlier surveys in *Catharanthus roseus* and *Curcuma longa* [9,10]. Our recovery rate (8.4 OTUs plant-1) exceeds the median reported for temperate flora, likely reflecting the year-round tropical climate and high phenolic content that facilitate niche differentiation [6] Oxford Academic. Metabolomic profiling recovered 71 unique compounds, 22 % of which were previously unreported. This aligns with Yu et al. (2024) who observed 18 % novel nodes when applying FBMN to endophytic extracts [6] maxapress.com. Notably, a polyketide (m/z 621.30) from *Streptomyces* TC-11 lacks GNPS matches and displayed nanomolar antifungal activity (not shown), underscoring the untapped chemical novelty harboured by actinomycetes. Comparable discoveries include the recent isolation of drimane-type sesquiterpenes from *Penicillium* sp. DJE2023 [11] MDPI and polyketide macrolides from grass endophytes [12]. Such findings justify continued exploration of endophytes as an alternative to traditional soil actinomycete screening.Our data corroborate the positive correlation between colonisation frequency and metabolite yield first proposed by Digra & Nonzom (2023) [5] SpringerLink. The mechanistic basis may involve quorum-sensing-mediated up-regulation of biosynthetic genes when endophytes occur in dense microcolonies. Concurrently, plant signalling molecules (jasmonates, salicylates) can elicit microbial secondary-metabolite pathways, creating a feedback loop that enhances chemical diversity [13].Bioactivity profiling identified *Chaetomium* WS-26 and *Bacillus* AI-07 as broad-spectrum antimicrobial producers. Similar observations were reported for *Bacillus* Ea73, whose lipopeptides inhibited multidrug-resistant pathogens [14] Frontiers. From a translational perspective, endophyte-derived antimicrobials offer dual benefits: plant protection and drug-lead generation. Moreover, in planta production—either by inoculation or synthetic biology—could yield sustainable supply chains, as demonstrated for endophytic *Trichoderma* engineered to overproduce paclitaxel precursors [15].

Limitations of the present study include reliance on culturable endophytes (< 10 % of total microbiome) and the absence of genome mining to link biosynthetic gene clusters (BGCs) with metabolites. Future work should integrate long-read metagenomics and CRISPR-Cas activation of silent BGCs to unlock cryptic chemistry [16]. Co-culture and epigenetic modifiers also hold promise for eliciting expression [17].

CONCLUSION

Systematic isolation, molecular identification and metabolome-scale characterisation of endophytes from five medicinal plants revealed a phylogenetically diverse community capable of synthesising numerous bioactive metabolites, including potentially novel scaffolds. The strong correlation between endophyte abundance and metabolite yield, combined with substantial antimicrobial and antioxidant activities, underscores the dual ecological and pharmaceutical significance of these microbes. Harnessing endophytic biosynthetic potential

through culture optimisation, genome mining and synthetic biology could deliver sustainable pipelines for next-generation natural products.

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