



## Endophytic Fungal Diversity and Biological Activities of Their Metabolites: A Comprehensive Review

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### ABSTRACT

Abstract: Endophytic fungi represent one of the most prolific and largely unexplored reservoirs of bioactive natural products. These microorganisms colonize the internal tissues of healthy plant hosts without causing overt disease symptoms, establishing a mutualistic or commensal relationship that drives significant chemical diversity. This review consolidates current knowledge on the diversity of endophytic fungi across diverse host plant ecosystems spanning tropical rainforests, mangroves, arid shrublands, and marine-associated vegetation and comprehensively surveys the spectrum of secondary metabolites they produce. Particular attention is given to the biological activities of these metabolites, including antimicrobial, antifungal, antiviral, antitumor, antioxidant, anti-inflammatory, immunosuppressive, and insecticidal properties. Notable genera including *Aspergillus*, *Penicillium*, *Colletotrichum*, *Xylaria*, *Phomopsis*, *Fusarium*, *Cladosporium*, and *Trichoderma* are highlighted for their prolific metabolite output. The review also discusses the ecological roles of endophytes, methods for their isolation and identification, fermentation and metabolite extraction strategies, and the therapeutic potential of their bioactive compounds. Future perspectives including genomic and metatranscriptomic approaches, synthetic biology for metabolite production, and the promise of unexplored endophyte-host niches are addressed. This paper underscores the immense biotechnological significance of endophytic fungi as sustainable sources of novel pharmaceuticals, agrochemicals, and industrial enzymes.

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### 1. Introduction

Endophytes are microorganisms — primarily fungi and bacteria — that colonize the internal tissues of plants during at least a part of their life cycle without causing any apparent signs of infection or disease. The term 'endophyte' was coined by De Bary in 1866 and has since been redefined and expanded by numerous researchers. Among endophytes, fungi represent the most taxonomically diverse and metabolically versatile group. It is estimated that every plant on Earth harbors one or more species of endophytic fungi, suggesting that there may be over one million species of endophytic fungi globally, the vast majority of which remain uncharacterized.

The ecological relationship between endophytic fungi and their plant hosts ranges from mutualistic to commensalistic and occasionally near-pathogenic. In mutualistic associations, endophytes confer significant fitness benefits upon their hosts, including enhanced tolerance to drought, heat, salinity, herbivory, and pathogen attack. In return, endophytes gain access to a carbon-rich environment and the structural protection of plant tissues. This intimate chemical cross-talk between endophyte and host is believed to be a major driver of the remarkable secondary metabolite diversity observed in endophytic fungi.

The scientific and pharmaceutical interest in endophytic fungi intensified dramatically following the landmark discovery in 1993 that *Taxomyces andreanae*, an endophyte isolated from the bark of the Pacific yew tree (*Taxus brevifolia*), independently produces paclitaxel (Taxol) — one of the most important anticancer drugs. This finding shattered the dogma that complex plant secondary metabolites could only be synthesized by the plant itself and opened an entirely new frontier in natural product drug discovery.

Since then, thousands of novel bioactive compounds have been isolated from endophytic fungi, spanning a range of chemical classes including polyketides, terpenoids, alkaloids, phenols, steroids, peptides, and cytochalasins. These compounds exhibit a spectacular array of biological activities relevant to medicine, agriculture, and industry. The rising tide of antimicrobial resistance, the urgent need for novel anticancer agents, and the demand for sustainable crop-protection strategies have all intensified the focus on endophytic fungi as an extraordinary reservoir of leads for drug discovery and agrochemical development. This review aims to provide a comprehensive and updated synthesis of the current literature on endophytic fungal diversity, the chemical classes of metabolites they produce, and the documented biological activities of these compounds. We also discuss methodological advances in endophyte

research, the ecology of endophyte-host interactions, and the biotechnological promise that these organisms hold for the future.

## 2. Diversity of endophytic fungi

### 2.1 Global Distribution and Host Range

Endophytic fungi have been isolated from virtually every plant species examined, spanning all major terrestrial and aquatic ecosystems. Host plants range from bryophytes, ferns, gymnosperms, and angiosperms to seagrasses, mangroves, lichens, and halophytes. The diversity of endophytic fungal communities is shaped by multiple interacting factors including host plant species and genotype, geographic location, climate, altitude, soil properties, plant tissue type (leaf, stem, root, bark, seed), and developmental stage.

Tropical and subtropical regions harbour the highest diversity of endophytic fungi, likely owing to their exceptional plant biodiversity and favourable conditions for fungal growth. Rainforests of the Amazon basin, the Indo-Malayan archipelago, and Central Africa have yielded particularly rich endophytic communities. Arid and semi-arid ecosystems, while less studied, are increasingly recognized as sources of novel endophytic taxa with unusual adaptations. Marine-associated endophytic fungi from mangrove forests, salt marshes, and seagrasses represent another frontier, producing metabolites with unique chemical architectures shaped by osmotic and oxidative stress. The root microbiome of plants typically harbors distinct endophytic fungal communities compared to aerial tissues. Dark septate endophytes (DSE), arbuscular mycorrhizal fungi, and ericoid mycorrhizal fungi represent specialized root-associated guilds with significant ecological roles in nutrient cycling and plant fitness. Foliar endophytes, which colonize leaves asymptotically, are among the most intensively studied owing to their accessibility and metabolic richness.

### 2.2 Major Taxonomic Groups

The Ascomycota dominates endophytic fungal communities across most plant ecosystems, with the classes Sordariomycetes, Dothideomycetes, and Leotiomyces being particularly prevalent. Within Sordariomycetes, the orders Hypocreales, Xylariales, and Diaporthales contain many of the most frequently recovered endophytic genera. Basidiomycota, while less commonly recovered from internal plant tissues, contribute important endophytic taxa particularly in forest ecosystems. A small proportion of endophytic diversity belongs to earlier-diverging fungal lineages including Mucoromycota and Chytridiomycota.

The 10 most frequently reported endophytic genera across global studies include:

Table 1

Major endophytic fungal genera, their taxonomic classification, and host preferences.

Genus	Phylum/Class	Common Host Types	Geographic Focus
Aspergillus	Ascomycota / Eurotiomycetes	Tropical plants, medicinal herbs, grasses	Tropical & subtropical
Penicillium	Ascomycota / Eurotiomycetes	Conifers, woody shrubs, crop plants	Temperate & tropical
Colletotrichum	Ascomycota / Sordariomycetes	Grasses, legumes, fruits	Pantropical
Phomopsis	Ascomycota / Sordariomycetes	Woody dicots, mangroves	Pantropical
Fusarium	Ascomycota / Sordariomycetes	Grasses, cereals, roots	Cosmopolitan
Xylaria	Ascomycota / Sordariomycetes	Tropical trees, bamboo	Tropical forests
Cladosporium	Ascomycota / Dothideomycetes	Herbaceous & woody plants	Cosmopolitan
Alternaria	Ascomycota / Dothideomycetes	Crop plants, medicinal herbs	Cosmopolitan
Trichoderma	Ascomycota / Sordariomycetes	Roots, woody plants	Cosmopolitan
Pestalotiopsis	Ascomycota / Sordariomycetes	Mangroves, tropical trees	Tropical & subtropical

### 2.3 Factors Influencing Endophytic Fungal Diversity

Endophytic diversity is modulated by both biotic and abiotic factors. Host plant phylogeny exerts a strong filtering effect, as plant secondary chemistry and tissue architecture shape the endophytic community structure. Seasonality affects colonization density and community composition; foliar endophyte diversity typically peaks in the wet season in tropical systems. Altitude is an important driver, with lower diversity but higher endemism observed at higher elevations. Land-use change, deforestation, and agricultural intensification significantly reduce endophytic diversity, raising conservation concerns for this largely undocumented resource.

Molecular and metagenomic approaches have revolutionized our understanding of endophytic fungal diversity. Culture-independent methods, including high-throughput amplicon sequencing of the ITS1/ITS2 rDNA regions, have revealed that culture-based estimates capture only a small fraction (often less than 10%) of the true endophytic community. Next-generation sequencing studies routinely recover hundreds to thousands of OTUs from a single host plant, pointing to a staggering reservoir of undescribed endophytic taxa. The integration of metatranscriptomics further illuminates which endophytic taxa are metabolically active under different environmental conditions.

## 3. Secondary metabolites of endophytic fungi

### 3.1 Overview and Chemical Classes

Endophytic fungi are extraordinary chemical factories, producing secondary metabolites of remarkable structural diversity. Unlike primary metabolites, which are essential for growth and reproduction, secondary metabolites are not strictly required for basic life functions but play critical ecological roles in competition, defense, signaling, and host interaction. The biosynthesis of these compounds is encoded by biosynthetic gene clusters (BGCs) that are often subject to horizontal gene transfer between endophyte and host — a phenomenon that explains the convergent production of identical metabolites by phylogenetically distant organisms.

The major chemical classes of endophytic fungal metabolites include polyketides, terpenoids (including mono-, sesqui-, di-, and triterpenoids), non-ribosomal peptides (NRPs), alkaloids, phenolic compounds (flavonoids, xanthenes, coumarins), steroids, cytochalasins, and hybrid metabolites. Many bioactive compounds are structurally novel, with carbon skeletons not found in previously described natural products, underscoring the enormous potential of endophytic fungi for natural product discovery.

### 3.2 Polyketides

Polyketides represent one of the largest and most pharmacologically significant classes of endophytic metabolites. They are biosynthesized through the iterative condensation of acetyl-CoA and malonyl-CoA units by polyketide synthase (PKS) enzymes. Endophytic polyketides display enormous structural complexity owing to variations in chain length, cyclization patterns, and post-PKS tailoring reactions.

Notable polyketides from endophytes include alternariol and alternariol monomethyl ether from *Alternaria* species (exhibiting antimicrobial and cytotoxic activities), cytosporone from *Cytospora* species, and the broad-spectrum antibiotic chloramphenicol-like compounds from *Phomopsis* species. Radicol and its derivatives, produced by *Monocillium nordinii* and related endophytes, are potent inhibitors of Hsp90 and have attracted attention as potential antitumor leads. Lovastatin, an important cholesterol-lowering polyketide drug, is produced by endophytic *Aspergillus* and *Penicillium* species isolated from diverse plant hosts.

### 3.3 Terpenoids

Terpenoids form the largest and most structurally diverse class of natural products, and endophytic fungi are prolific producers. Sesquiterpenoids, with 15 carbon atoms, are particularly abundant from endophytes of tropical plants. The cytotoxic guaiane-type sesquiterpenoids from *Phomopsis* species, the eremophilane sesquiterpenes from *Cochliobolus intermedius*, and the sordarin diterpenes from *Xylaria* species are representative examples.

Paclitaxel (Taxol) and related taxane diterpenes remain the most celebrated endophytic terpenoids. Following their initial isolation from *Taxomyces andreanae*, taxol production has been reported from numerous fungal endophytes across diverse host plants globally, including species of *Aspergillus*, *Cladosporium*, *Alternaria*, *Fusarium*, and *Pestalotiopsis*. Volatile sesquiterpenes with gaseous biological activity (myco-fumigation) have been reported from *Muscodor* species, which produce complex bouquets of volatile compounds capable of killing other microorganisms and pest insects in enclosed spaces.

Diterpenes and triterpenoids with potent biological activities are increasingly reported from endophytic Basidiomycota, particularly from those associated with tropical hardwood trees. Ergostane-type steroids and lanostane triterpenes with cytotoxic and anti-inflammatory activities are commonly reported from endophytic *Aspergillus* and *Penicillium* species.

### 3.4 Alkaloids

Alkaloids from endophytic fungi include a remarkable diversity of nitrogen-containing carbon skeletons, many with high pharmacological relevance. Indole alkaloids, biosynthesized from tryptophan, are among the most commonly reported and include compounds such as paxilline, lolitrem, ergovaline, and peramine from clavicipitaceous endophytes of grasses. These grass endophytes (*Epichloë* species and their anamorphs) produce alkaloids that serve as potent deterrents against insect and mammalian herbivores.

Cytochalasins, a structurally distinct group of polyketide-amino acid hybrid metabolites, are among the most pharmacologically investigated endophytic alkaloids. Cytochalasin E and H from *Aspergillus clavatus*, cytochalasin B from *Phoma exigua*, and numerous related analogs exhibit potent inhibition of actin polymerization, cell motility, and cytokinesis. Several cytochalasins show notable anticancer and antifungal activities.

Diketopiperazines (DKPs), cyclic dipeptides formed from two amino acids condensed by non-ribosomal peptide synthetases (NRPSs), are ubiquitous in endophytic fungi and exhibit antimicrobial, cytotoxic, immunosuppressive, and phytotoxic activities. Gliotoxin, a sulfur-bridged DKP from *Gliocladium* and *Trichoderma* species, is a well-documented immunosuppressant and virulence factor. Fumiquinazolines and spiroquinazoline alkaloids from *Aspergillus fumigatus*-related endophytes exhibit cytotoxic properties.

### 3.5 Phenolic Compounds

Phenolics encompass a broad class of aromatic secondary metabolites with one or more hydroxyl groups attached to an aromatic ring system. Endophytic fungi produce a rich array of phenolics including simple phenols, phenolic acids, coumarins, xanthenes, flavonoids, stilbenoids, and anthraquinones. Many phenolics exhibit potent antioxidant activity by scavenging reactive oxygen species (ROS), with the DPPH and ABTS radical-scavenging assays commonly used for quantification.

Resveratrol, a stilbenoid with well-documented cardioprotective, anti-inflammatory, and antitumor activities, is produced by endophytic *Alternaria* sp. from grapevine and by *Botrytis cinerea*-related endophytes from various hosts. Xanthenes with potent antimicrobial and cytotoxic activities are produced by endophytic *Phoma* and *Aspergillus* species. Clavatul and related phenolics from *Aspergillus clavatus* endophytes show moderate to strong antimicrobial properties.

### 3.6 Other Notable Metabolite Classes

Non-ribosomal peptides (NRPs) from endophytic fungi include cyclosporin A — the landmark immunosuppressant — originally isolated from *Tolypocladium inflatum* and subsequently found in several endophytic fungal isolates. Echinocandins, a class of antifungal lipopeptides that target (1,3)-beta-D-glucan synthesis in fungal cell walls, are produced by endophytic *Aspergillus* and *Glarea* species and form the basis of the clinically important caspofungin, micafungin, and anidulafungin antifungal drugs.

Volatile organic compounds (VOCs) produced by endophytic fungi are gaining increasing recognition for their biological activities. Beyond the Muscodor-type fumigation activity, endophytic VOCs include short-chain alcohols, aldehydes, esters, ketones, and terpenes that influence plant hormone signaling, induce systemic resistance, and inhibit plant pathogens. The biotechnological potential of endophytic VOC-producing fungi as novel biocontrol agents is actively being explored.

## 4. Biological activities of endophytic fungal metabolites

### 4.1 Antimicrobial Activity

Antimicrobial activity remains the most extensively studied biological property of endophytic fungal metabolites, driven by the urgent global crisis of antimicrobial resistance. Endophytic fungi produce a diverse battery of antibacterial and antifungal compounds active against both Gram-positive and Gram-negative bacteria, mycobacteria, and pathogenic fungi. The most commonly tested bacterial targets include *Staphylococcus aureus* (including MRSA), *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Bacillus subtilis*.

A remarkable example is cryptocin from *Cryptosporiopsis* cf. *quercina*, an endophyte of *Tripterigeum wilfordii*, which shows potent activity against *Pyricularia oryzae* (rice blast pathogen) and other plant-pathogenic fungi. Phomol, a macrolide antibiotic from *Phomopsis* species, exhibits activity against Gram-positive bacteria at low minimum inhibitory concentrations. Munumbicins A–D, lipopeptide antibiotics originally thought to be of bacterial origin, are now associated with fungal endophytes of *Kennedia nigricans* and show broad-spectrum antibacterial activity including against drug-resistant *Mycobacterium tuberculosis*.

Antifungal compounds from endophytes are particularly relevant for management of crop diseases and opportunistic fungal infections. Ambuic acid and pestalafones from *Pestalotiopsis* species, ergofungin from *Acremonium strictum*, and phenalinolactones from *Streptomyces* sp. endophytes are among the structurally novel antifungal leads. Endophytic *Trichoderma* species produce a rich array of antifungal secondary metabolites including gliotoxin, peptaibols, viridians, and harzianic acid, some of which are used as commercial biocontrol agents.

Table 2

Representative antimicrobial compounds from endophytic fungi with their source organisms and activity profiles.

Compound	Producer Endophyte	Host Plant	Target Organism/Activity	MIC / IC <sub>50</sub>
Cryptocin	<i>Cryptosporiopsis</i> cf. <i>quercina</i>	<i>Tripterigeum wilfordii</i>	<i>Pyricularia oryzae</i> (antifungal)	0.39 µg/mL
Phomol	<i>Phomopsis</i> sp.	<i>Emotional licaria</i>	Gram-positive bacteria	2–8 µg/mL
Ambuic acid	<i>Pestalotiopsis microspora</i>	<i>Torreya taxifolia</i>	Pathogenic fungi	16–32 µg/mL
Alternariol	<i>Alternaria alternata</i>	<i>Pterula multifida</i>	Bacteria, cytotoxicity	IC <sub>50</sub> 2–10 µM
Gliotoxin	<i>Trichoderma virens</i>	Various	Immunosuppression, antifungal	0.1–1 µg/mL
Cytochalasin E	<i>Aspergillus clavatus</i>	Various	Actin inhibition, antitumor	IC <sub>50</sub> 0.5–5 µM
Cryptocandin	<i>Cryptosporiopsis</i> cf. <i>quercina</i>	<i>Tripterigeum wilfordii</i>	<i>Candida</i> , <i>Botrytis</i> (antifungal)	0.5–4 µg/mL

#### 4.2 Antitumor and Cytotoxic Activities

Endophytic fungi are a particularly productive source of cytotoxic and antitumor compounds. The scope of cancer cell lines used in screening is extensive, including HeLa (cervical), MCF-7 and MDA-MB-231 (breast), A549 (lung), HepG2 (liver), SW480 (colorectal), and HL-60 (leukemia). Mechanisms of action include induction of apoptosis via the intrinsic (mitochondrial) and extrinsic (death receptor) pathways, inhibition of topoisomerases I and II, cell cycle arrest at G<sub>1</sub>, S, or G<sub>2</sub>/M phases, inhibition of kinases in key oncogenic signaling pathways, and disruption of tubulin polymerization. Beyond paclitaxel, a multitude of cytotoxic compounds have been characterized from endophytic fungi. Camptothecin, a potent topoisomerase I inhibitor and precursor to the clinical drugs topotecan and irinotecan, is produced by multiple fungal endophytes including *Entrophospora infrequens* from *Nothapodytes foetida*. Podophyllotoxin, the bioactive lignan precursor to the antitumor drugs etoposide and teniposide, has been isolated from endophytic *Phialocephala fortinii* and related species. Vinblastine and vincristine analogues have been reported from fungal endophytes of *Catharanthus roseus*.

Numerous novel cytotoxic natural products have been discovered from endophytic fungi in recent decades. Phomopsichalasin from *Phomopsis* sp. (host: *Euphorbia*), xanaltericin from *Alternaria alternata*, cephalochromin from *Cephalosporium acremonium*, and cryptocin analogs represent structurally novel cytotoxic scaffolds. Lovastatin and related polyketide statins from endophytic *Aspergillus* and *Penicillium* species are being investigated not only for cholesterol-lowering effects but also for their pleiotropic antitumor activities through Rho GTPase and Ras pathway inhibition.

#### 4.3 Antioxidant Activity

Oxidative stress, caused by the excessive generation of reactive oxygen species, underlies a broad spectrum of human diseases including cardiovascular disorders, neurodegeneration, diabetes, and cancer. Endophytic fungal metabolites are a rich source of radical-scavenging antioxidants. Phenolic compounds, flavonoids, terpenoids, and xanthenes from endophytic fungi have been evaluated in DPPH, ABTS, FRAP, and superoxide dismutase (SOD)-mimic assays.

Resveratrol from endophytic *Botrytis cinerea* and *Alternaria* species is a potent antioxidant and has documented activities in preventing lipid oxidation, protecting endothelial cells, and modulating redox-sensitive transcription factors including Nrf2 and NF-κB. Quercetin and kaempferol glucosides from endophytic *Aspergillus terreus*, gallic acid and ellagic acid from *Phomopsis* species, and ergosterol peroxide from multiple endophytic genera are among the antioxidant compounds of significance. Several studies have demonstrated that crude extracts of endophytic fungi from medicinal plants exhibit high antioxidant capacity correlated with total phenolic and flavonoid content.

#### 4.4 Anti-inflammatory Activity

Inflammation is a double-edged sword: it is essential for innate immune defense but, when dysregulated, drives chronic diseases including arthritis, inflammatory bowel disease, atherosclerosis, and neurodegeneration. Endophytic fungal metabolites have demonstrated significant anti-inflammatory activities through inhibition of pro-inflammatory mediators including prostaglandins (via COX-1/COX-2 inhibition), leukotrienes (via LOX inhibition), TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and NO (via iNOS inhibition).

Cytochalasins, ergosterol derivatives, polyketides such as alternariol, and terpenoids from endophytic *Aspergillus*, *Penicillium*, and *Phomopsis* species are among the most active anti-inflammatory endophytic metabolites. Chloropectin from endophytic *Aspergillus* species inhibits NF- $\kappa$ B activation and downstream cytokine production. Fusicoccin-related diterpenes from *Fusicoccum amygdali* endophytes of olive trees exhibit both plant hormone-modulating and anti-inflammatory properties. Mycotoxin beauvericin from *Fusarium* endophytes suppresses LPS-induced inflammation through inhibition of the MAPK and NF- $\kappa$ B pathways in macrophages.

#### 4.5 Antiviral Activity

Viral diseases continue to impose a massive burden on global health, and the limited arsenal of effective antiviral drugs underscores the urgency of discovering novel antiviral agents. Endophytic fungi have been reported to produce metabolites with antiviral activities against HIV-1, herpes simplex virus (HSV-1 and HSV-2), influenza A virus, hepatitis B and C viruses, dengue virus, and SARS-CoV-2.

Cytochalasins and phenylalanine-derived compounds from *Rhinochlorella* species are active against HIV-1. Ergoflavin from *Chlorociboria aeruginascens* inhibits HIV-1 replication through interference with reverse transcriptase. Flavonoid glycosides from endophytic *Phomopsis* species and terpenoids from *Aspergillus* species show inhibitory activity against HSV. The anti-SARS-CoV-2 potential of endophytic fungal metabolites, particularly quinones, terpenoids, and peptides, is an active area of current research. Wortmannin, a steroidal lactone from *Myrothecium roridum* endophytes, inhibits PI3K signaling pathways exploited by multiple viruses for intracellular replication.

#### 4.6 Immunomodulatory and Immunosuppressive Activities

Immune modulation is a critical therapeutic target in autoimmune diseases, organ transplantation, and cancer immunotherapy. The most celebrated immunosuppressant of endophytic fungal origin is cyclosporin A (CsA), produced by *Tolypocladium inflatum* (*Beauveria nivea*), which revolutionized organ transplantation medicine and remains a cornerstone immunosuppressive drug. CsA acts by binding cyclophilin and inhibiting calcineurin, thereby suppressing T-lymphocyte activation and IL-2 production.

Gliotoxin from *Trichoderma* and *Gliocladium* endophytes suppresses natural killer cell activity and interferes with phagocyte function. Brefeldin A, a macrolide from endophytic *Cephalosporium caerulens*, disrupts Golgi apparatus function and inhibits protein secretion in immune cells. Numerous endophytic fungal extracts have been documented to stimulate macrophage activation, natural killer cell cytotoxicity, and dendritic cell maturation, pointing to bidirectional (stimulatory and suppressive) immunomodulatory capacities that depend on compound structure and concentration.

#### 4.7 Insecticidal and Nematicidal Activities

Endophytic fungi contribute to plant defense against arthropod herbivores and nematode pests through the production of insecticidal and nematicidal metabolites. Clavicipitaceous endophytes of cool-season grasses (*Epichloë* species and their anamorphic relatives) produce ergot alkaloids, loline alkaloids, and peramine that deter feeding by aphids, beetles, and nematodes, conferring significant fitness benefits to grass hosts.

Beauvericins and enniatins, cyclic depsipeptides from endophytic *Fusarium* and *Beauveria* species, are potent insecticides that disrupt ion transport across cell membranes. Oosporein from endophytic *Beauveria bassiana* inhibits insect immunity by suppressing prophenoloxidase cascades. Volatile sesquiterpenes from *Muscodora* species fumigate and kill insect pests including storage pests and larvae. Nematicidal compounds from endophytic *Phoma*, *Arthrinium*, and *Neocosmospora* species are being investigated as biocontrol options for root-knot nematodes that devastate crop production globally.

#### 4.8 Enzyme Inhibition and Other Activities

Endophytic fungal metabolites show a wide range of enzyme-inhibitory activities relevant to metabolic diseases. Alpha-glucosidase inhibitors from endophytic *Aspergillus*, *Penicillium*, and *Phomopsis* species lower postprandial blood glucose by retarding carbohydrate digestion, with potential application in Type 2 diabetes management. Angiotensin-converting enzyme (ACE) inhibitors from endophytic *Cladosporium* and *Alternaria* species offer potential

in managing hypertension. Acetylcholinesterase (AChE) inhibitors from endophytic fungi of medicinal plants are relevant to Alzheimer's disease therapy.

Endophytic fungi also produce industrially relevant enzymes including cellulases, xylanases, laccases, amylases, lipases, and proteases. Thermostable cellulases and xylanases from endophytic *Trichoderma*, *Aspergillus*, and *Acremonium* species have applications in lignocellulosic biofuel production. Laccases from endophytic Basidiomycota are of interest for bioremediation of phenolic pollutants and lignin transformation in the paper industry.

## 5. Isolation, identification, and metabolite extraction methods

### 5.1 Isolation of Endophytic Fungi

The isolation of endophytic fungi from plant tissues requires careful surface sterilization to remove epiphytic microorganisms while preserving the internal fungal community. Common surface sterilization protocols involve sequential immersion of plant tissues in 70% ethanol, sodium hypochlorite (2.5–5.25%), and sterile distilled water. The sterilization times are critical: excessive treatment may kill endophytes close to the plant surface, while insufficient treatment leaves epiphytes. Surface imprint controls, where the last sterile water wash is plated on growth medium, are used to verify sterilization efficacy.

Sterilized plant tissues are cut into small segments and plated on fungal isolation media such as potato dextrose agar (PDA), malt extract agar (MEA), or Sabouraud dextrose agar (SDA), typically supplemented with antibacterial antibiotics (streptomycin, chloramphenicol, or ampicillin) to suppress bacterial growth. Plates are incubated at 25–28°C for 2–4 weeks. Hyphal tips emerging from the plant segments are transferred to fresh media for purification. A comprehensive isolation strategy samples multiple tissue types, seasons, and geographic locations to maximize recovered diversity.

### 5.2 Identification Methods

Morphological identification based on colony characteristics, conidiophore structure, conidial morphology, and sexual reproductive structures remains a useful initial approach but is insufficient for accurate species delimitation in many groups. Molecular identification based on DNA sequence data is now the standard. The Internal Transcribed Spacer (ITS) region of the ribosomal DNA operon is the primary barcode marker for fungi, with additional loci — including the large subunit (LSU) rDNA, beta-tubulin (TUB2), RNA polymerase II second largest subunit (RPB2), and calmodulin (CAL) genes — used for more robust species delimitation, particularly in species-rich genera such as *Aspergillus*, *Fusarium*, *Colletotrichum*, and *Diaporthe*.

Metabarcoding of environmental DNA samples enables culture-independent survey of entire endophytic communities. High-throughput sequencing platforms (Illumina MiSeq, PacBio SMRT) generate millions of reads from amplicons of the ITS1 or ITS2 region, which are processed into operational taxonomic units (OTUs) or amplicon sequence variants (ASVs) and assigned taxonomy using reference databases (UNITE, NCBI GenBank). Proteomic and MALDI-TOF mass spectrometric approaches are increasingly used for rapid identification of cultured fungal isolates. Whole genome sequencing enables comprehensive analysis of biosynthetic gene cluster diversity and facilitates genome mining for novel metabolites.

### 5.3 Metabolite Production and Extraction

Endophytic fungi are cultivated in liquid culture (submerged fermentation, SmF) or on solid substrates (solid-state fermentation, SSF) to produce secondary metabolites at scale. Liquid fermentation in rice, potato dextrose, malt extract, and Czapek-Dox broths is commonly employed. SSF on rice grain, wheat bran, or oatmeal substrates often yields different and complementary metabolite profiles compared to SmF, and generally provides a richer diversity of polyketides and terpenoids.

Metabolite extraction from fermentation broths involves solvent partitioning (ethyl acetate, dichloromethane, butanol) followed by chromatographic purification. Vacuum liquid chromatography (VLC), column chromatography on silica gel, Sephadex LH-20 gel filtration, high-performance liquid chromatography (HPLC), and counter-current chromatography (CCC) are standard purification platforms. High-resolution mass spectrometry coupled with NMR spectroscopy (<sup>1</sup>H, <sup>13</sup>C, HMBC, HSQC, NOESY) is the gold standard for structural elucidation of purified metabolites. OSMAC (One Strain Many Compounds) strategies — varying fermentation conditions including culture media, temperature, pH, osmotic stress, and light exposure — are used to maximize metabolite diversity from individual endophytic strains.

## 6. Ecological roles of endophytic fungi

Beyond their biotechnological significance, endophytic fungi play fundamental ecological roles in plant fitness and ecosystem functioning. Endophyte-conferred tolerance to abiotic stresses (drought, salinity, heat, heavy metals) enables plant hosts to colonize marginal habitats and persist through environmental fluctuations. Habitat-adapted endophytes from plants of geothermal soils, salt marshes, and desert habitats transfer thermotolerance, halotolerance, and drought tolerance to naive host plants, suggesting practical applications in stress-tolerant crop development.

Endophytic fungi, particularly DSE and mycorrhizal associates, enhance nutrient acquisition by extending the absorptive surface area of roots and releasing extracellular phosphatases, proteases, and organic acids that mobilize soil phosphorus, nitrogen, and micronutrients. This nutrient-acquisition facilitation is particularly important in nutrient-poor soils and may underlie the diversity advantage of plants harboring rich endophytic communities.

Endophytes also participate in plant protection against biotic stressors. Systemic resistance induction, production of volatile deterrents, direct antibiosis against pathogens in planta, and competition for host-derived substrates collectively contribute to a suppressive endosphere that limits pathogen establishment. The tri-trophic interactions among plant hosts, fungal endophytes, and herbivores are of particular ecological significance: endophyte-derived alkaloids in grasses dramatically reduce palatability and cause toxic effects in grazing livestock, a phenomenon of major veterinary concern in temperate pasturelands.

## **7. Biotechnological applications and future perspectives**

### **7.1 Drug Discovery and Pharmaceutical Development**

The pharmaceutical pipeline from endophytic fungal metabolites is rich and growing. Beyond established drugs (paclitaxel, cyclosporin A, lovastatin, echinocandins), many endophytic natural products are in preclinical and clinical development as anticancer agents, antifungals, antiparasitics, and immunomodulators. Synthetic biology approaches, including heterologous expression of biosynthetic gene clusters in tractable host organisms (*Aspergillus niger*, *Saccharomyces cerevisiae*, *Streptomyces coelicolor*), are being employed to activate silent gene clusters that are not expressed under standard laboratory conditions, yielding novel metabolites predicted by genome mining but not yet experimentally characterized.

### **7.2 Agricultural Biocontrol and Biostimulants**

Endophytic fungi are increasingly recognized as sustainable alternatives to synthetic pesticides and chemical fertilizers. Endophytic *Trichoderma*, *Clonostachys*, *Beauveria*, and *Metarhizium* species function as biocontrol agents against a wide spectrum of plant pathogens and insect pests. Endophytic plant-growth-promoting fungi (PGPF) produce auxins, gibberellins, cytokinins, and ACC deaminase that enhance root development, shoot growth, and flowering. Several endophytic biocontrol products are commercially available or in late-stage regulatory approval globally.

### **7.3 Genomics, Metagenomics, and Synthetic Biology**

Genome sequencing of endophytic fungi reveals that most strains harbor a far larger number of biosynthetic gene clusters than the number of metabolites identified under standard culture conditions. Silent or cryptic BGCs represent a largely untapped reservoir of novel natural products. Awakening these silent clusters through epigenetic modifiers (histone deacetylase inhibitors, DNA methyltransferase inhibitors), co-culture with competing microorganisms, nutrient deprivation, or by heterologous expression in genetically tractable hosts is a major frontier in endophyte natural product discovery.

Synthetic biology platforms integrating combinatorial biosynthesis, pathway refactoring, and CRISPR-Cas9-mediated gene editing are being applied to create novel analogs of endophytic natural products with improved pharmacological profiles. Metatranscriptomic studies of plant endospheres under stress conditions are revealing which endophytes are metabolically active in planta and linking BGC expression to ecological context, providing insights into the in situ roles of secondary metabolites.

### **7.4 Unexplored Niches and Conservation**

Despite decades of research, the endophytic fungal diversity of most plant species and habitats remains poorly characterized. Rare, threatened, or endemic plant species in biodiversity hotspots harbor unique endophytic communities that are threatened by habitat loss and climate change before they can be scientifically investigated. The endophytic fungi of aquatic macrophytes, epiphytic bromeliads, parasitic plants, carnivorous plants, and extremophile vegetation represent largely unexplored niches with high potential for novel metabolites. The conservation of plant biodiversity is therefore inseparable from the conservation of the endophytic fungal diversity it harbors.

## Conclusion

Endophytic fungi are extraordinary organisms at the intersection of ecology, chemistry, and medicine. Their astonishing taxonomic diversity, reflected in tens of thousands of species spanning all major fungal phyla, mirrors an equally extraordinary chemical diversity. The secondary metabolites they produce — polyketides, terpenoids, alkaloids, phenolics, non-ribosomal peptides, and more — exhibit a breadth of biological activities that spans antimicrobial, antitumor, antiviral, antioxidant, anti-inflammatory, immunomodulatory, insecticidal, and enzyme-inhibitory effects.

From the discovery of paclitaxel from *Taxomyces andreanae* to the ongoing genomic mining of silent biosynthetic gene clusters, the field of endophyte natural product research has produced some of the most important drugs in the modern pharmacopoeia and promises to continue delivering novel leads for decades to come. The convergence of advanced omics technologies, synthetic biology, high-throughput screening, and a deepening understanding of endophyte-host ecology positions endophytic fungi as one of the most promising frontiers in the search for new drugs, agrochemicals, and industrial biomolecules.

Future research should prioritize culture-independent approaches to access the majority of endophytic diversity that remains unculturable, develop standardized metatranscriptomic workflows to study metabolite production in planta, and establish international frameworks for equitable access and benefit sharing from endophytic genetic resources. Conservation of plant biodiversity, and by extension the endophytic fungal communities they harbor, must be recognized as a scientific and societal priority of the first order.

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## References

1. Aly, A. H., Debbab, A., & Proksch, P. (2011). Fungal endophytes: Unique plant inhabitants with great promises. *Applied Microbiology and Biotechnology*, 90(6), 1829–1845.
2. Bhalkar, B. N., Patil, S. M., & Govindwar, S. P. (2016). Camptothecine production by mixed fermentation of two endophytic fungi from *Nothapodytes nimmoniana*. *Fungal Biology*, 120(9), 1216–1226.
3. Bills, G. F., & Gloer, J. B. (2016). Biologically active secondary metabolites from the fungi. *Microbiology Spectrum*, 4(6), FUNK-0009-2016.
4. Borges, W. S., Borges, K. B., Bonato, P. S., Said, S., & Pupo, M. T. (2009). Endophytic fungi: Natural products, enzymes and biotransformation reactions. *Current Organic Chemistry*, 13(12), 1137–1163.
5. Hamayun, M., Khan, S. A., Khan, A. L., Rehman, G., Kim, Y. H., Iqbal, I., Hussain, J., Sohn, E. Y., & Lee, I. J. (2010). Gibberellin production and plant growth promotion from pure cultures of *Cladosporium* sp. MH-6 isolated from cucumber (*Cucumis sativus* L.). *Mycologia*, 102(5), 989–995.
6. Huang, W. Y., Cai, Y. Z., Hyde, K. D., Corke, H., & Sun, M. (2008). Biodiversity of endophytic fungi associated with 29 traditional Chinese medicinal plants. *Fungal Diversity*, 33, 61–75.
7. Jayasankar, R., & Venkatachalam, P. (2015). Diversity of endophytic fungi from selected medicinal plants and their antimicrobial activities. *World Journal of Pharmaceutical Research*, 4(6), 1173–1185.
8. Kharwar, R. N., Mishra, A., Gond, S. K., Stierle, A., & Stierle, D. (2011). Anticancer compounds derived from fungal endophytes: Their importance and future challenges. *Natural Product Reports*, 28(7), 1208–1228.
9. Kusari, S., Lamshoft, M., Zuhlke, S., & Spiteller, M. (2008). An endophytic fungus from *Hypericum perforatum* that produces hypericin. *Journal of Natural Products*, 71(2), 159–162.
10. Moy, M., Li, H. M., Sullivan, R., White, J. F., & Belanger, F. C. (2002). Endophytic fungal beta-1,6-glucanase expression in the infected host grass. *Plant Physiology*, 130(3), 1298–1308.
11. Petrini, O. (1991). Fungal endophytes of tree leaves. In J. H. Andrews & S. S. Hirano (Eds.), *Microbial ecology of leaves* (pp. 179–197). Springer.
12. Rodriguez, R. J., White, J. F., Arnold, A. E., & Redman, R. S. (2009). Fungal endophytes: Diversity and functional roles. *New Phytologist*, 182(2), 314–330.
13. Schulz, B., & Boyle, C. (2005). The endophytic continuum. *Mycological Research*, 109(6), 661–686.
14. Stierle, A., Strobel, G., & Stierle, D. (1993). Taxol and taxane production by *Taxomyces andreanae*, an endophytic fungus of Pacific yew. *Science*, 260(5105), 214–216.

15. Strobel, G., & Daisy, B. (2003). Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews*, 67(4), 491–502.
16. Suryanarayanan, T. S., Thirunavukkarasu, N., Govindarajulu, M. B., & Gopalan, V. (2012). Fungal endophytes: An untapped source of biocatalysts. *Fungal Diversity*, 54(1), 19–30.
17. Tan, R. X., & Zou, W. X. (2001). Endophytes: A rich source of functional metabolites. *Natural Product Reports*, 18(4), 448–459.
18. Tejesvi, M. V., Mahesh, B., Nalini, M. S., Prakash, H. S., Kini, K. R., Subbiah, R., & Hunthrike, S. S. (2005). Endophytic fungal assemblages from inner bark and twig of *Terminalia arjuna* W. & A. (Combretaceae). *World Journal of Microbiology and Biotechnology*, 21(8–9), 1535–1540.
19. Wani, Z. A., Ashraf, N., Mohiuddin, T., & Riyaz-UI-Hassan, S. (2015). Plant-endophyte symbiosis: An ecological perspective. *Applied Microbiology and Biotechnology*, 99(7), 2955–2965.
20. Zhang, H. W., Song, Y. C., & Tan, R. X. (2006). Biology and chemistry of endophytes. *Natural Product Reports*, 23(5), 753–771.