Biochemistry and Biophysics Reports 45 (2026) 102385

Contents lists available at ScienceDirect

Biochemistry and Biophysics Reports

journal homepage: www.elsevier.com/locate/bbrep

Unlocking the potential of endophytes in enhancing plant secondary metabolite biosynthesis

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ARTICLE INFO

Keywords:
Endophytes
Plant secondary metabolites
Biosynthetic pathways
Metabolic engineering
Sustainable bio-production

ABSTRACT

Plant secondary metabolites (PSMs) are vital bioactive compounds with wide pharmaceutical, agricultural, and industrial applications. However, their commercial production faces challenges due to low yields, environmental variability, and high extraction costs. Endophytes, microorganisms living within plant tissues, have emerged as key regulators of plant metabolism, enhancing PSM production through activation of biosynthetic gene clusters, secretion of precursor molecules, and modulation of plant stress responses. This narrative review explores recent advances in understanding endophyte-plant interactions, focusing on their mechanisms for stimulating metabolite production. It highlights biotechnological applications, including metabolic engineering, genome editing, and co-cultivation strategies, for optimizing endophyte-mediated biosynthesis. The review also identifies challenges in large-scale application and proposes recommendations for integrating endophytes into sustainable agriculture and pharmaceutical production. Harnessing endophytes offers an eco-friendly, cost-effective approach for scalable natural product biosynthesis, with significant potential for addressing global health, agricultural sustainability, and industrial needs.

1. Introduction

Plant secondary metabolites (PSMs) are a diverse group of bioactive compounds synthesised by plants in reaction to environmental stressors and play critical roles in plant defense, signaling, and adaptation [1]. Unlike primary metabolites, PSMs do not directly help plants grow and reproduce, but they are very important for their survival [2]. Plant secondary metabolites are grouped into three primary groups: terpenoids, phenolics, and nitrogenous chemicals (including alkaloids) [1,3]. Beyond their ecological functions, these metabolites hold immense value in pharmaceuticals, agriculture, and industry. Alkaloids, flavonoids, terpenoids, and phenolics are widely utilized for their antimicrobial, anticancer, and antioxidant properties, forming the basis of many therapeutic and commercial applications [4]. The shikimate, mevalonic, and tricarboxylic acid cycles are some of the metabolic pathways that make PSMs [5]. The synthesis and accumulation of PSMs are affected by abiotic stressors such as heavy metals, temperature extremes, light, salt, and drought [5]. PSMs are vital bioactive compounds with wide-ranging applications in pharmaceuticals, agriculture, and industry. However, the large-scale production of these metabolites remains a challenge due to their low natural abundance, complex biosynthetic pathways, and susceptibility to environmental fluctuations. Conventional production methods, such as chemical synthesis and large-scale plant cultivation, are often costly, inefficient, and unsustainable [6,7].

Endophytes are microbes that live inside plant tissues, forming

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https://doi.org/10.1016/j.bbrep.2025.102385
Received 28 July 2025; Received in revised form 12 November 2025; Accepted 23 November 2025

important symbiotic associations with their host [8]. These can range from mutualistic to parasitic interactions and are essential for enhancing plant growth, activating defense mechanisms, and alleviating abiotic stressors [9]. They generate a wide range of physiologically active metabolites with prospective applications in medicine, agriculture, and industry [10]. New research has focused on figuring out the unique connections between endophytes and the plants that host them, especially how they affect gene expression and metabolism [11]. Recent studies demonstrate that endophytes can modulate plant metabolic pathways by activating biosynthetic gene clusters (BGCs)**,** producing precursor molecules, and eliciting plant stress responses that result in increased metabolite accumulation [12]. Unlike traditional methods, endophyte-mediated biosynthesis of metabolite offers a sustainable and scalable approach to improving the yield and diversity of plant secondary metabolites. Despite these promising findings, the mechanisms underlying endophyte-driven metabolite enhancement remain incompletely understood, especially in terms of how they interact with host plants to activate BGCs, modulate stress responses, and produce precursor molecules. Unraveling these interactions could pave the way for biotechnological applications, including metabolic engineering and synthetic biology approaches to optimize secondary metabolite production. While several studies have highlighted the role of endophytes in increasing PSM yields, there is still a lack of detailed understanding regarding the molecular and epigenetic mechanisms involved. This gap in knowledge limits the effective integration of endophyte-mediated biosynthesis into scalable, sustainable production systems. Therefore, this review aims to address this gap by synthesizing recent advances in endophyte-plant interactions, focusing on the molecular mechanisms driving metabolite enhancement and exploring biotechnological strategies to optimize this process for pharmaceutical and agricultural applications. By integrating endophyte-based strategies into metabolic engineering, researchers can develop innovative, eco-friendly solutions for the sustainable production of high-value plant-derived compounds.

2. Methodology

This review adopts a narrative approach to explore and synthesize current knowledge on the role of endophytes in enhancing plant secondary metabolite production. Relevant peer-reviewed articles were identified through comprehensive searches in electronic scientific databases including Scopus**,** PubMed**,** Web of Science, and Google Scholar. The search was conducted using combinations of keywords such as: "endophytes," "plant secondary metabolites," "biosynthetic pathways," "epigenetic modulation," "elicitors," "metabolic engineering," and "pharmaceutical applications of endophytes**."** Articles published between 2015 and 2025, written in English, and presenting experimental, mechanistic, or review data on endophytes' role in plant secondary metabolism were included. Key information was extracted from the selected studies, including the importance of endophyte species, their plant hosts, enhanced secondary metabolite types, mechanisms involved, and potential industrial or pharmaceutical applications. The findings were analyzed and discussed sequentially under different smaller categories. A representative table and figure was created to provide a concise overview of key studies, highlighting endophyte-plant-metabolite relationships and their underlying mechanisms. While this narrative review did not apply formal risk-of-bias tools, efforts were made to prioritize high-quality, peer-reviewed studies published in reputable journals. Emphasis was placed on studies with clear mechanistic insights and reproducibility of findings.

3. Mechanisms of endophyte-driven metabolite enhancement

Endophytes influence plant secondary metabolite production through multiple interconnected mechanisms, including the activation of biosynthetic pathways, the secretion of metabolic precursors and signal molecules, and the modulation of stress responses as illustrated in Fig. 1. These interactions result in enhanced metabolite accumulation, contributing to plant defense and adaptation while offering promising applications in biotechnology. Mechanistic reports cluster into BGC/epigenetic control, precursor/elicitor exchange, and stress-linked signaling. Juxtaposing them: BGC/epigenetic routes provide the clearest causal levers and reproducibility. Precursor/elicitor routes can spike flux past rate-limiting steps but depend on host uptake/partitioning. Stress priming generalizes across hosts but shows the weakest evidence

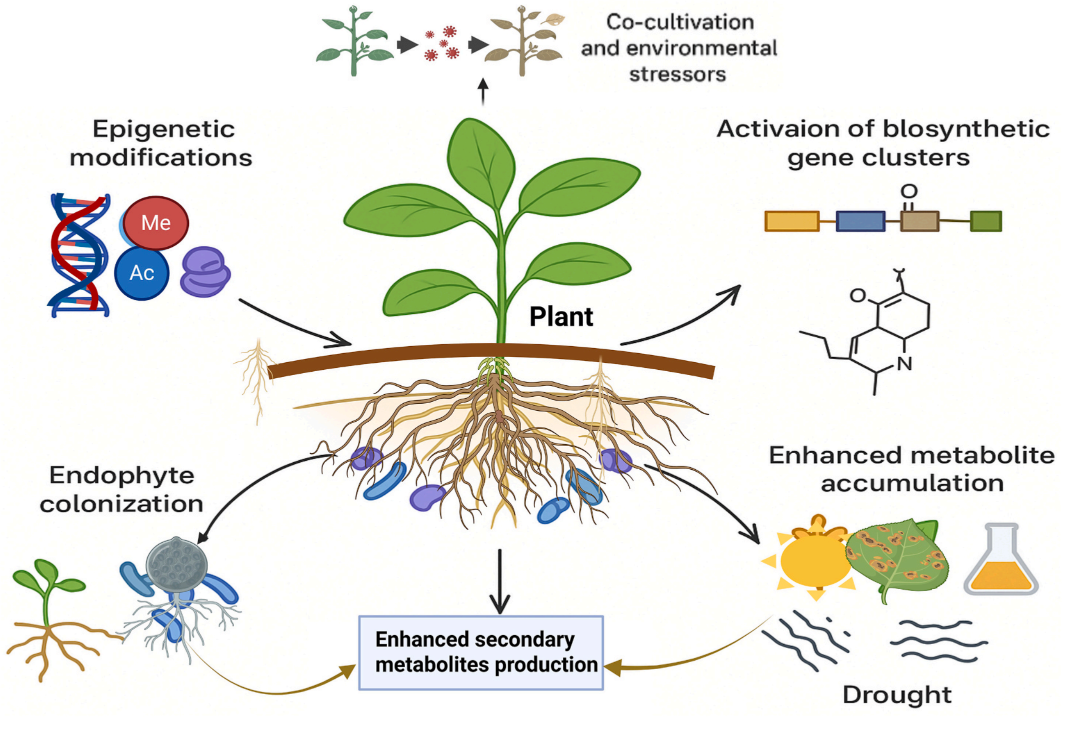

Fig. 1. Overview of molecular mechanisms involved in endophyte-mediated enhancement of plant secondary metabolites.

for *durability* and the highest risk of growth trade-offs. Collectively, this implies that platform choice should match the end use: tightly regulated drug precursors favor epigenetic/BGC routes; broad agronomic fortification may leverage stress/elicitor priming.

3.1. *Endophytes* ***activation of biosynthetic pathways***

Endophytes can stimulate plant secondary metabolism by upregulating BGCs responsible for metabolite synthesis. Many secondary metabolite pathways in plants are dormant or weakly expressed under normal conditions. Endophytes produce signaling molecules that trigger the expression of key metabolic genes. For example, studies have shown that endophytes associated with medicinal plants can induce the biosynthesis of alkaloids, flavonoids, and terpenoids by modulating transcription factors and epigenetic regulators [13]**.** For example, studies have shown that *Aspergillus niger* can induce the biosynthesis of vinblastine and vincristine in *Catharanthus roseus* by modulating transcription factors that control the expression of alkaloid biosynthesis genes [14]. Endophytes have been found to play a crucial role in epigenetic modification, which affects gene expression in host plants. For instance, *Talaromyces chrysogenum* enhances artemisinin production in *Artemisia annua* by modulating histone deacetylase and DNA methylation, leading to the activation of dormant BGCs [15]. Other studies on endophytes such as *Piriformospora indica* and *Fusarium oxysporum* highlight how these microorganisms influence chromatin remodeling to activate plant secondary metabolism [16]. Additionally, endophytes possess their own BGCs, which complement plant pathways, leading to the co-production of novel hybrid metabolites. Studies by Pillay et al. [17] and Verma et al. [18] have demonstrated how chemical compounds and epigenetic manipulation can activate dormant biosynthetic gene clusters. Evidence abound suggesting real in planta examples of endophyte-mediated secondary metabolite production. For instance, *Epichloë festucae*, an endophyte found in Ryegrass, has been found to stimulate alkaloid biosynthesis by upregulating BGCs [12]. Furthermore, Astragalus species produce swansonine (an alkaloid), a symbiont that triggers stress responses in plants, leading to metabolite accumulation [11]. Recent studies have identified azaphilones as a new secondary metabolite in *Aster tataricus*, a plant characterized by endophyte interactions [11].

Some techniques, like co-cultivation, epigenetic modification, and heterologous expression, have been used to improve the production of secondary metabolites [19]. Co-cultivation of endophytes process involves the interaction between microbial species to produce hybrid metabolites. For example, the co-cultivation of *Trichoderma harzianum* and *Ocimum basilicum* enhances the production of flavonoids and phenolic acids, demonstrating the benefit of microbial interaction in metabolite biosynthesis [11,20].

Inhibitors of DNA methyltransferase and histone deacetylase are essential for activating cryptic genes involved in metabolite synthesis [21]. Furthermore, CRISPR-Cas9-mediated genome editing has proven to be an efficient instrument for augmenting the production of targeted metabolites [21]. By editing the genomes of endophytes, researchers can target specific genes involved in metabolite synthesis and boost production levels [22]. For instance, genome editing in endophytes has been shown to enhance taxol production in *Taxus* spp. and vinblastine production in *Catharanthus roseus* [23]. Metabolomics profiling using chromatography linked with mass spectrometry enables the exploration of the complexity and chemical variety of bioactive substances [24,25]. The progress made in activating cryptic biosynthetic gene clusters and metabolomic profiling techniques opens up exciting chances to find new natural compounds that might be useful in medicine [26].

Reports of hybrid metabolite "co-production" via endophyte BGCs are promising, but in planta claims of endophyte-driven pathway activation remain sparser than culture-based demonstrations. Where alkaloid boosts are observed (for example, vinblastine/vincristine claims), several studies confound endophyte effects with host elicitation regimes or co-infections. Priority next steps are randomized, side-by-side designs that include endophyte-free but stress-matched controls and orthogonal readouts to isolate causal chains.

3.2. *Endophyte-derived precursors and signal molecules*

Endophytes also produce precursors and signal molecules that directly affect biosynthetic pathways in plants. For example, Shen and colleagues demonstrated that *Aspergillus niger* secretes phenolic acids, which serve as key precursors for the biosynthesis of flavonoids in pineapple [11]. Similarly, volatile organic compounds produced by endophytes play a critical role in inducing plant responses to abiotic stress and facilitating metabolite biosynthesis [17]. Signaling molecules facilitate the interaction between endophytes and host plants, controlling metabolite synthesis [22]. These microbial-derived compounds include indole derivatives for alkaloid production, phenolic acids and polyketides for flavonoid biosynthesis, and volatile organic compounds for plant growth and stress signaling. By supplying these precursors, endophytes bypass rate-limiting steps in plant biosynthetic pathways, leading to an increased yield of bioactive compounds [27]. Fungal elicitors can swiftly stimulate gene expression and activate particular metabolic pathways, resulting in the buildup of active compounds [24]. The symbiotic association between endophytes and host plants facilitates mutual growth and stimulates the manufacture of novel therapeutic substances. Through these signal molecule, endophytes modulate the production of a diverse array of metabolites, such as alkaloids, terpenoids, flavonoids, and steroids, which may have benefits in the pharmaceutical, agricultural, and cosmetic sectors [28]. Table 1 below summarizes some representative studies on endophyte-mediated enhancement of plant secondary metabolites, including species involved, host plants, and mechanisms. While *A. niger*–derived phenolic acids illustrate a clean supply-side mechanism, precursor-supplying or volatile-mediated gains vary widely across hosts and carbon status. Effects range from transient transcriptional surges to durable titer increases. Standardized reporting of precursor concentrations, uptake kinetics, and carbon partitioning is needed to judge portability beyond controlled conditions.

3.3. *Stress-induced secondary metabolism modulation*

Environmental stress conditions, such as drought, salinity, and pathogen attacks, are known to trigger secondary metabolite production in plants as a defense mechanism. Endophytes play a crucial role in this process by priming plant defense responses and modulating stress-related hormonal pathways (e.g., jasmonic acid and salicylic acid). This leads to increases in antioxidant phenolics and flavonoids accumulation, antimicrobial alkaloids and terpenoids production, while simultaneously upregulating genes associated with secondary metabolism. For instance, methyl jasmonate and reactive oxygen species (ROS) generation are key factors that stimulate defense responses in plants, leading to enhanced secondary metabolite production. By mitigating stress while simultaneously stimulating metabolite biosynthesis, endophytes act as natural elicitors that enhance the therapeutic potential of medicinal plants. ROS function as signaling molecules, stimulating transcription factors that govern antioxidant responses and secondary metabolism [32]. Methyl jasmonate, a phytohormone, stimulates reactive oxygen species generation and subsequent defence responses, resulting in enhanced secondary metabolite production in plant cells and organ cultures. Endophytes like *Trichoderma harzianum* exploit these stress responses by triggering the jasmonic acid and salicylic acid pathways, resulting in the synthesis of antioxidants and bioactive compounds [33]. In addition to jasmonic acid and salicylic acid signaling, abscisic acid (ABA) and ethylene Endophyte Activation of also play critical roles in regulating endophyte-induced secondary metabolite synthesis. Endophyte-induced ABA signaling activates MYB and WRKY transcription factors, modulating flavonoid and alkaloid

Table 1

Key studies on endophyte-mediated enhancement of plant secondary metabolites.

Endophyte Species	Host Plant	Metabolite Enhanced	Mechanism	Experimental system	Assay/readout	Reference
Piriformospora indica	*Curcuma longa* (Turmeric; rhizome)	Curcumin	Upregulation of biosynthetic gene clusters	in planta	HPLC/LC–MS titer	11
Aspergillus niger	*Catharanthus roseus* (leaves/cells)	Vinblastine, Vincristine	Secretion of elicitors and precursors	mixed	LC–MS/MS; pathway gene expression	27
Epichloë festucae	*Lolium perenne* (Ryegrass; foliar)	Alkaloids	Symbiotic stress response activation	in planta	GC/LC quant of alkaloids	12
Talaromyces chrysogenum	*Artemisia annua* (shoots)	Artemisinin	Epigenetic modulation, elicitor secretion	in planta	LC–MS; HDAC/DNMT activity	29
Fusarium oxysporum	*Taxus* spp. (Yew; callus/ cells; bioreactor/flask)	Paclitaxel (Taxol)	Co-production and activation of taxol biosynthesis genes	in vitro	HPLC; pathway gene expression	30
Colletotrichum gloeosporioides	*Camptotheca acuminata* (seedlings/cells)	Camptothecin	Induction under biotic stress	mixed	HPLC/LC–MS	31
Trichoderma harzianum & *Azotobacter*	*Ocimum basilicum* (Sweet Basil; (aerial parts)	Flavonoids, Phenolic Acids	Co-cultivation enhances production of flavonoids and phenolic acids	in planta	Spectrophotometric (TPC/ TFC), LC–MS	11
Aspergillus niger	*Ananas comosus* (Pineapple; tissue)	Flavonoids	Secretion of phenolic acids as precursors for flavonoid biosynthesis	mixed	LC–MS; precursor titers	11
Alternaria oxytropis	*Oxytropis* spp. (whole plant)	Swainsonine	Modulation of gene expression and activation of secondary metabolites	in planta	LC–MS; biosynthetic gene function	11
Tengochaeta bulbillosa	*Aster tataricus* (whole plant)	Azaphilones	Endophyte interactions leading to novel secondary metabolite production	in planta	LC–MS/NMR identification	11

List of abbreviations: DNMTs = DNA methyltransferases; HDACs=Histone deacetylases; GC = gas chromatography; LC–MS/MS = liquid chromatography–tandem mass spectrometry; NMR= Nuclear magnetic resonance spectroscopy; TPC/TFC = total phenolics/flavonoids content.

biosynthesis. Epigenetically, endophytes mediate chromatin accessibility via histone acetylation (e.g., H3K9ac) and methylation (e.g., H3K4me3), leading to the transcriptional upregulation of previously silent BGCs. Recent studies also highlight endophyte-triggered non-coding RNAs and miRNAs that fine-tune metabolite biosynthesis via post-transcriptional silencing of repressor genes. Similarly, UV-B radiation, drought, and high salt levels are all environmental stressors that make it easier for reactive oxygen species to form and for secondary metabolites to build up [34]. Some fungi, like *Aspergillus* species, have transcription factors that control the expression of antioxidant genes and genes involved in secondary metabolism [35]. Understanding these pathways is important for making it easier for plants and fungi to make useful secondary metabolites that can be used in many industrial settings. JA/SA/ABA priming reliably elevates antioxidant phenolics, yet growth penalties and resource reallocation are rarely quantified. Some stress regimens risk activating undesirable secondary metabolites. For instance, temperature/UV-linked fungal responses may activate undesirable mycotoxins in some species. Thus, stress-centric strategies should include benefit–cost curves (metabolite gain vs. biomass/fitness) and routine toxin surveillance.

4. Strategies for enhancing the production of secondary metabolites

Metabolic engineering, genome editing, synthetic biology, and co-cultivation, play crucial roles in enhancing the ability of endophytes to produce secondary metabolites. By modifying the genetic and metabolic pathways in endophytes, these techniques offer innovative, eco-friendly, and scalable methods for the production of high-value natural products in both the pharmaceutical and agricultural sectors. These strategies are complementary, and when applied together, they can accelerate the development of novel therapeutics and sustainable biotechnological applications.

4.1. Metabolic engineering

Metabolic engineering is a biotechnology discipline that focuses on the modification of cellular pathways to enhance the production of desired metabolites. The goal is to optimize the natural metabolic pathways or introduce new pathways to increase the yield of bioactive compounds, such as secondary metabolites, in host organisms like plants or microorganisms [36]. In the context of endophytes, metabolic engineering can be used to enhance their ability to produce plant secondary metabolites. Endophytes, being symbionts within plant tissues, provide a unique platform for sustainable production of high-value metabolites due to their ability to interact directly with host plants and modulate their metabolism.

Applications to Endophytic Microorganisms.

i. *Aspergillus niger* and Vinblastine Production: *Aspergillus niger*, an endophytic fungus associated with *Catharanthus roseus*, has been genetically engineered to increase the production of vinblastine, a potent anti-cancer agent. Through metabolic engineering, key enzymes involved in the terpenoid indole alkaloid (TIA) pathway were overexpressed in *A. niger*, leading to a significant increase in vinblastine yield [37]. Moreover, the use of CRISPR-Cas9 technology enabled the activation of cryptic BGCs within the endophyte, revealing its untapped potential for producing secondary metabolites [38].

ii. *Fusarium oxysporum* and Paclitaxel Production: The endophyte *Fusarium oxysporum*, which naturally resides in *Taxus* species, has been engineered to enhance the production of paclitaxel (taxol), a widely used anticancer drug. By inserting specific genes from *Taxus* into *F. oxysporum*, researchers successfully activated the paclitaxel biosynthetic pathway. However, this approach faced challenges in gene stability, as the introduced genes exhibited variable expression levels over time. Overcoming this required the development of stable expression systems in which regulatory mechanisms, such as the use of synthetic promoters and epigenetic modifiers, were optimized [39].

iii. *Piriformospora indica* and Curcumin Production: The root endophyte *Piriformospora indica* has been shown to colonize *Curcuma longa* (turmeric) and enhance its curcumin content and yield [40]. Independently, metabolic engineering approaches have been applied to boost curcumin biosynthesis via up-regulation of key curcuminoid synthases and manipulation of polyketide/phenylpropanoid pathways [41]. This study demonstrates that co-cultivation strategies, where the endophyte is grown alongside the plant, can synergistically enhance the metabolite yield.

Technical Challenges in Engineering Endophytes.

While these advancements in metabolic engineering have shown promise, several technical issues remain that hinder the widespread industrial application of engineered endophytes.

i. Gene Stability: One of the major challenges in engineering endophytes is maintaining gene stability over multiple generations. Unlike genetically modified plants, which can be propagated through traditional means, endophytic microorganisms often suffer from genomic drift when subcultured in laboratory settings. This results in the silencing or loss of engineered traits, thereby reducing the consistency and efficiency of metabolite production. Strategies such as plasmid-based expression systems and genomic integration of target genes are being explored to address this issue, although the long-term stability of these modifications remains a concern [42].
ii. Regulation of BGC Expression: The BGCs responsible for secondary metabolite production in endophytes are often silenced or expressed at low levels under natural conditions. Engineering the expression of these BGCs is critical to unlocking the full potential of endophytes as biosynthetic factories. Researchers are using epigenetic modifications (e.g., histone deacetylase inhibitors) and synthetic biology tools (like CRISPR interference) to enhance the expression of these cryptic BGCs [43]. However, achieving precise regulation ensuring that the genes are expressed in a controlled and reproducible manner remains a key challenge.
iii. Industrial Viability: The industrial scalability of engineered endophytes is another critical challenge. While laboratory-scale studies have demonstrated the potential for increased metabolite production, translating these findings to large-scale fermentation systems is not straightforward. Issues such as bioreactor design, nutrient optimization, and the cost-effectiveness of cultivating endophytes at a commercial scale need to be addressed [44]. Additionally, the regulatory landscape for genetically modified microorganisms in industrial applications is still evolving, and overcoming regulatory hurdles will be essential for the commercial viability of these engineered endophytes.

To overcome these challenges, future research should concentrate on creating stable expression systems for consistent metabolite production, employing synthetic biology to accurately regulate BGCs, and enhancing endophyte cultivation methods to achieve high yield and cost-effectiveness for industrial applications.

Mechanisms of Metabolic engineering.

i. ***Pathway Optimization***: Metabolic engineering often involves overexpressing key enzymes in metabolic pathways responsible for secondary metabolite biosynthesis. For example, in the case of vinblastine production in *Catharanthus roseus*, engineers can manipulate the terpenoid indole alkaloid biosynthetic pathway by overexpressing enzymes like tryptophan decarboxylase and serotonin N-acetyltransferase to increase alkaloid yield [45].
ii. ***Pathway Redirection***: Metabolic engineering can also include redirecting metabolic flux from the host plant or microbe's central metabolism towards the production of specific metabolites. This can be achieved by knocking down competing pathways or introducing novel enzymes that channel carbon or precursors into the desired biosynthetic pathway [46].

***Outcomes of** Metabolic engineering.*

i. ***Increased Yield:*** By enhancing the activity of rate-limiting enzymes or introducing new metabolic routes, metabolic engineering can substantially increase the yield of secondary metabolites. For instance, high-level artemisinin production has been achieved through metabolic engineering of yeast and *E. coli* by overexpressing key genes from *Artemisia annua* [29].
ii. ***Novel Compounds***: Metabolic engineering may lead to the production of novel bioactive compounds by combining genes from different organisms to create hybrid metabolites. A notable example is the production of taxol by manipulating endophytic fungi to enhance its biosynthetic pathways [47].

4.2. Genome editing

Genome editing technologies, especially CRISPR-Cas9, allow precise manipulation of the genome of both host plants and endophytes to enhance metabolite production. Genome editing is particularly useful for activating silent genes**,** introducing novel biosynthetic pathways**,** or modifying key genes involved in secondary metabolite production [48].

Mechanisms of Genome Editing.

i. ***Gene Activation***: One of the most powerful applications of genome editing in endophytes is the activation of cryptic BGCs**.** Many endophytes possess genes for producing secondary metabolites that are silent or under-expressed under standard growth conditions. CRISPR-Cas9 can be used to activate these genes by modifying epigenetic regulators or introducing transcriptional activators that enhance the expression of these BGCs [17]. For example, *Aspergillus niger* can be genetically edited to increase the production of metabolites such as vinblastine [49].
ii. ***Precise Gene Knockout***: Genome editing can also be used to knock out competing pathways or non-essential genes, enabling the organism to focus its metabolic resources on the production of the desired compound [50]. For instance, knocking out certain biosynthetic genes in endophytes can increase the yield of secondary metabolites by minimizing the diversion of precursors into unwanted compounds [17].

Outcomes of Genome Editing.

i. ***Enhanced Production***: CRISPR-Cas9-based genome editing has led to the optimized production of compounds like artemisinin in genetically engineered yeast and vinblastine in *Catharanthus roseus* [51].
ii. ***Creation of Novel Metabolites***: Genome editing enables the creation of hybrid metabolites by integrating genes from multiple species. This has vast potential in drug discovery and the development of novel therapeutic agents [52].

4.3. Synthetic biology

Synthetic biology is an interdisciplinary field that combines engineering principles with biology to design and construct new biological parts, devices, and systems. In the context of endophytes and secondary metabolites, synthetic biology focuses on the creation of artificial biosynthetic pathways and the modular assembly of genes to improve the efficiency of metabolite production [53]. Beyond gene integration, synthetic biology strategies now include orthogonal pathway design to minimize metabolic crosstalk, use of synthetic promoters and ribosome-binding sites to fine-tune expression, and integration of biosensors to dynamically regulate production in response to metabolite levels [54]. Modular design using standardized bioparts (e.g., BioBricks) facilitates assembly of plug-and-play systems tailored to produce specific metabolites in yeast, *E. coli*, or fungal chassis [55]. Engineering minimal endophyte genomes (reduced metabolic burden) has further enabled efficient metabolite factory development.

Mechanisms of Synthetic Biology.

i. ***Synthetic Pathway Construction***: By designing modular biosynthetic pathways, synthetic biology allows the integration of multiple genes from different organisms to form a new, efficient pathway for metabolite production. This includes inserting foreign genes that encode for enzymes involved in metabolite biosynthesis, thus enabling endophytes to produce compounds they would not naturally produce [56].
ii. ***Biosynthetic Pathway Optimization***: Synthetic biology also uses design-build-test-learn cycles to optimize gene expression levels in endophytes to improve the efficiency of biosynthetic pathways [57]. Additionally, by incorporating biosensors into the synthetic pathways, the production process can be fine-tuned for maximum yield and efficiency.

Outcomes of Synthetic Biology.

i. ***Novel Metabolite Production***: Synthetic biology has enabled the creation of novel bioactive compounds, which are not found in nature, by combining biosynthetic pathways from different species. For example, synthetic biology has been employed to produce artemisinin in yeast, a process previously only achievable in plants [58].
ii. ***Scalable Production:*** Synthetic biology has improved the scalability of secondary metabolite production by engineering microbial factories to produce large quantities of bioactive compounds at an industrial scale.

4.4. Co-cultivation strategies

Co-cultivation is a method where two or more microorganisms, such as endophytes and plant cells or fungi, are cultured together to stimulate synergistic metabolite production. Co-cultivation strategies can enhance metabolite yields by facilitating interactions between microbes that lead to the production of hybrid metabolites or the activation of dormant biosynthetic pathways [59].

Mechanisms of Co-Cultivation

i. ***Microbial Interactions***: In co-cultivation, metabolite exchange between species occurs. Endophytes can produce signaling molecules, such as volatiles or elicitors, that activate the plant's defense pathways, leading to enhanced production of secondary metabolites.
ii. ***Cross-kingdom Interactions***: Co-cultivating fungal endophytes with plant tissue cultures or other microorganisms can lead to synergistic production of secondary metabolites, as different species bring their own unique biosynthetic pathways into the interaction. For example, co-cultivating *Aspergillus* fungi with *Ocimum basilicum* leads to enhanced flavonoid and phenolic acid production.

Outcomes of Co-Cultivation.

i. ***Increased Yields***: Co-cultivation has been shown to increase secondary metabolite production in plants and fungi by stimulating cross-species interaction. For example, co-cultivating endophytes with medicinal plants has enhanced the production of alkaloids, terpenoids, and other bioactive compounds.
ii. ***Novel Metabolites:*** Co-cultivation strategies can lead to the discovery of novel metabolites not produced by either microorganism alone. For example, *Aspergillus* species and *Ocimum basilicum* produce novel flavonoids when grown together, which are not produced in mono-cultures.

5. Biotechnological and industrial applications

The integration of endophytes into biotechnology has significant implications for pharmaceuticals, sustainable agriculture, and industrial production. Harnessing endophyte-mediated secondary metabolite biosynthesis offers eco-friendly and cost-effective alternatives to conventional production methods. For instance, *Fusarium oxysporum*, an endophyte of *Taxus* spp., has been genetically manipulated to overexpress rate-limiting genes in the taxol biosynthetic pathway, leading to significantly elevated paclitaxel yield in vitro. Similarly, *Talaromyces chrysogenum* enhances artemisinin synthesis in *Artemisia annua* through epigenetic modulation and elicitor secretion. These examples underscore the translational potential of endophyte-host specificity in industrial metabolite biosynthesis. Drug-oriented case studies (like taxol, artemisinin) demonstrate clearer genotype-to-phenotype causality and quality control pathways; agronomic deployments are dominated by environmental variance and microbiome drift. Claims of field robustness should therefore report effect sizes across sites/seasons. Below are key areas where endophyte-driven secondary metabolite biosynthesis is making an impact.

i. ***Endophyte-Based Metabolic Engineering:*** Genetic engineering and synthetic biology techniques enable scientists to manipulate endophytes for optimized metabolite production. These include genome editing, heterologous expression systems, and synthetic co-cultures. For example, CRISPR-Cas9-mediated genome editing has shown promise in enhancing metabolite yields by targeting key biosynthetic genes [9]. These methods enhance the production of medicinal compounds like taxol, vinblastine, and artemisinin [9].
ii. ***Pharmaceutical Applications:*** Endophytes are being studied for their potential in the bioproduction of high-value pharmaceuticals, including anticancer compounds like paclitaxel (Taxol), antimicrobial metabolites, and neuroprotective agents for neurodegenerative diseases [17]. By integrating metabolomic profiling and high-throughput screening, researchers can identify promising endophyte strains for large-scale drug discovery and production [33].
iii. ***Agricultural and Environmental Sustainability***: Endophytes are sustainable solutions for improving crop health and resilience, reducing reliance on synthetic agrochemicals. Endophytes can be used as biofertilizers, biopesticides, and stress tolerance agents, improving crop resilience and reducing chemical inputs. Additionally, they can be used in phytoremediation to degrade environmental pollutants, making them valuable in bioremediation efforts [17].

6. Sustainability in endophyte-mediated metabolite production

The integration of endophytes into plant secondary metabolite production offers significant sustainability benefits compared to traditional methods of biosynthesis. Conventional approaches, such as chemical synthesis or large-scale agricultural cultivation, often result in high costs, environmental degradation, and unsustainable resource usage. In contrast, endophyte-based strategies provide eco-friendlier, cost-effective, and scalable alternative that can help mitigate these challenges while offering enhanced metabolite yields. By reducing chemical inputs, enhancing crop productivity, and contributing to environmental sustainability, endophyte-based strategies offer substantial advantages over traditional methods of secondary metabolite production. Reduced chemical inputs are plausible only if colonization is stable and re-inoculation frequency is low. Otherwise, repeated applications erode life-cycle gains. Including simple life cycle assessment sidebars would substantiate sustainability claims. As these techniques evolve, industrial applications will become more scalable, providing a crucial step towards meeting global demands for natural products in an environmentally responsible manner.

6.1. Eco-friendly production

Endophytes offer a biological and environmentally gentle method for enhancing secondary metabolite production. These microbial symbionts naturally reside in plant tissues and facilitate the production of metabolites without the need for extensive land cultivation, synthetic chemicals, or harmful agrochemicals [60]. By enhancing the natural defense mechanisms of plants, endophytes help reduce the reliance on pesticides and chemical fertilizers, which are often harmful to the environment and human health. Moreover, endophyte-based applications in sustainable agriculture can help reduce soil degradation, water consumption, and the carbon footprint of traditional farming practices [17]. Through the natural modulation of plant stress responses, endophytes also promote increased crop resilience to environmental stressors such as drought, salinity, and pathogen attacks, reducing the need for external interventions and improving overall food security [61].

6.2. Reduced chemical inputs

One of the major challenges in conventional farming and large-scale cultivation of plants for secondary metabolite production is the heavy dependence on synthetic chemicals. The use of chemical fertilizers, pesticides, and herbicides can lead to soil erosion, water contamination, and loss of biodiversity [62]. Endophytes mitigate this issue by offering natural solutions that not only enhance the production of desired bioactive metabolites but also contribute to soil health and sustainability. For example, endophytes like *Trichoderma harzianum* and *Aspergillus niger* produce natural elicitors and growth-promoting substances that enhance plant growth and stress tolerance, reducing the need for chemical interventions [61]. By facilitating biofertilizer applications and promoting biocontrol of pathogens, endophytes offer a more sustainable alternative to chemical-based agricultural practices [63].

6.3. Enhancing crop productivity and resilience

The application of endophytes in agriculture extends beyond secondary metabolite production, offering a holistic approach to improving crop yield and quality. Through the enhancement of photosynthesis, nutrient uptake, and stress tolerance, endophytes help optimize crop productivity. This leads to reduced resource usage, including water, land, and energy, all of which contribute to sustainable farming systems [64]. Additionally, the symbiotic relationship between plants and endophytes ensures that crops remain resilient to changing climatic conditions, such as extreme temperatures, droughts, and salinity, without the need for extensive irrigation or external interventions [65]. This climate-resilient agriculture can support the growing global demand for food and bio-based products while preserving environmental resources [62].

6.4. Scalable and cost-effective production

Endophyte-based production systems are inherently scalable, providing opportunities for large-scale biotechnological applications with relatively low operational costs. Unlike traditional methods that require vast expanses of land and resources, the cultivation of endophytes in bioreactors or through co-cultivation strategies can be implemented in controlled environments, offering flexibility and higher yields of bioactive metabolites [17]. Moreover, endophyte-driven metabolite production can help reduce production costs, which often limit the commercialization of plant-derived bioactive compounds [66]. By integrating endophytes into industrial biotechnology, companies can harness natural processes that are both economically viable and environmentally sustainable.

6.5. Promoting bioremediation and environmental sustainability

Endophytes also play a crucial role in bioremediation, an area of environmental sustainability that involves the use of organisms to degrade pollutants and toxins. Endophytes, particularly those with high tolerance to heavy metals and other contaminants, can be used to enhance soil decontamination and pollutant degradation [67]. For example, some endophytes are capable of phytoremediation, where they interact with plants to enhance the uptake and detoxification of harmful substances from contaminated environments [68]. By integrating endophytes into bioremediation projects, we can not only clean up polluted sites but also promote the growth of healthy plants capable of producing high-value metabolites.

7. Challenges and strategies for the commercial utilization of endophytes

Despite the growing recognition of endophytes as sustainable bioresources, several challenges hinder their large-scale application in enhancing plant secondary metabolite production. These challenges span biological variability, scalability, regulatory barriers, and technological limitations. However, targeted strategies can be implemented to mitigate these barriers and accelerate industrial adoption as summarized in Table 2.

7.1. Ensuring stability and compatibility in industrial settings

One major bottleneck in industrial application is the inconsistency in endophyte performance across batches due to strain instability or host incompatibility. Long-term subculturing may lead to genomic drift, metabolic attenuation, or loss of symbiotic efficacy [76]. To address this, genome-stabilization techniques using CRISPR-Cas9 for plasmid integration, adaptive evolution in bioreactors, and synthetic microbial consortia have been proposed. Compatibility screening using multi-omics tools and root-colonization assays can help match endophytes with a wider range of host genotypes. Cryopreservation and

Table 2
Challenges in the commercial utilization of endophytes and corresponding mitigation strategies.

S/N	Challenges	Description	Strategies to Overcome
1	Strain Stability and Reproducibility	Endophyte performance can vary across plant species, environments, and passages [69]	- Select and engineer stable strains - Use omics tools to monitor functional consistency
2	Low Yield or Variability in Metabolite Production	Metabolite production may be inconsistent or suboptimal under lab/field conditions [70]	- Optimize culture conditions - Apply elicitors and metabolic precursors
3	Host Specificity and Limited Compatibility	Some endophytes exhibit narrow host ranges or require specific plant genotypes [71]	- Screen broad-spectrum endophytes - Use synthetic biology to enhance host compatibility
4	Scalability of Cultivation Systems	Difficulty in translating lab findings to industrial-scale bioreactors [72]	- Develop optimized bioreactor designs - Implement co-culture and fermentation platforms
5	Regulatory and Biosafety Concerns	Lack of standardized protocols for approval and safe deployment [73]	- Develop global safety frameworks and guidelines - Conduct ecological risk assessments
6	Limited Genetic Tools for Endophyte Engineering	Insufficient tools for precise genome editing or pathway manipulation [74]	- Expand use of CRISPR/Cas and synthetic biology - Integrate metabolomics and proteomics
7	Knowledge Gaps in Mechanisms	Incomplete understanding of molecular interactions and biosynthesis pathways [75]	- Invest in fundamental research - Promote interdisciplinary collaboration

encapsulation techniques also improve long-term viability and reproducibility [77].

8. Future directions

Integrating endophytes into biotechnology offers a promising avenue for the **sustainable and large-scale production of plant secondary metabolites.** Future research should focus on providing answers to some actionable questions (i-ii) as well as make some advancements (iii-iv) in this field.

i. Does epigenetic/BGC activation outperform stress priming for *stability* of yields across sites/seasons for the same metabolite (e.g., artemisinin vs. JA/SA priming)?
ii. Can precursor-supplying endophytes be engineered with flux sensors to auto-adjust output without host growth penalties?
iii. Establishing high-throughput screening platforms to identify novel endophytes with pharmaceutical and agricultural applications.
iv. Strengthening regulatory policies and commercial strategies to facilitate large-scale production and industrial adoption.

9. Recommendations for enhancing endophyte-mediated plant secondary metabolite production

When properly managed, endophyte-based metabolite production can move from basic research to real-world uses, providing long-term answers for biotechnology, agriculture, and pharmaceuticals. To advance research and applications of endophytes in enhancing plant secondary metabolite production, the following recommendations are proposed (Table 3).

Table 3
Recommendations for enhancing endophyte-mediated plant secondary metabolite production.

S/N	Recommendation	Action Strategy	Expected Outcomes
1	Expand Interdisciplinary Research	Foster collaboration among microbiologists, plant biotechnologists, chemists, and bioengineers	Improved mechanistic understanding of endophyte-plant interactions and enhanced metabolite biosynthesis pathways
2	Develop Engineered Endophytes	Apply synthetic biology, CRISPR-Cas systems, and genome editing for strain improvement	Creation of high-yielding endophyte strains with targeted biosynthetic capabilities
3	Establish High-Throughput Screening Platforms	Utilize metabolomic and genomic screening tools for rapid identification of productive endophyte strains	Accelerated discovery of novel endophytes with pharmaceutical and agricultural relevance
4	Optimize Industrial-Scale Production Systems	Design bioreactors, fermentation systems, and co-culture strategies for large-scale cultivation	Sustainable and cost-effective production of plant secondary metabolites for commercial applications
5	Integrate Endophytes into Sustainable Agriculture	Promote the use of endophytes as biofertilizers, biopesticides, and stress tolerance agents	Enhanced crop resilience, reduced chemical inputs, and improved agricultural productivity
6	Develop Regulatory Frameworks	Formulate standardized biosafety protocols and regulatory guidelines for commercialization	Safe and ethical deployment of endophyte-based products in pharmaceutical and agricultural industries
7	Explore Personalized Medicine Applications	Investigate the role of endophytes in modulating metabolites for precision medicine	Tailored natural product-based therapies for specific health conditions
8	Encourage Open-Access Data Sharing	Establish global databases on endophytes and their associated metabolites	Facilitated knowledge exchange, collaborative research, and accelerated innovation

10. Conclusion

Endophytes play a pivotal role in enhancing plant secondary metabolite production by activating dormant biosynthetic pathways, regulating gene expression, and mediating stress responses. Their unique ability to interact symbiotically with host plants provides a sustainable and eco-friendly approach to the biosynthesis of high-value metabolites. BGC/epigenetic activation offers the strongest causal levers for drug-grade targets; precursor/elicitor routes provide pathway shortcuts with context limits; stress priming is agronomically viable yet lacks substantial evidence regarding durability—three approaches that warrant comparative evaluation under standardised, multi-site protocols.

With advancements in metabolic engineering, genome editing, and co-cultivation strategies, endophytes offer significant potential for large-scale, cost-effective production of bioactive compounds for pharmaceutical, agricultural, and industrial applications. However, challenges such as gene stability, regulatory control of biosynthetic gene clusters, and industrial scalability must be addressed to fully realize their potential.

Harnessing the full capacity of endophytes for sustainable metabolite production represents a promising frontier in biotechnology, offering environmentally responsible alternatives to traditional production methods.

Funding

None.

Ethics approval: Not applicable.

Consent to participate: Not applicable.

Clinical trial date of registration**:** Not applicable.

Clinical trial registration **number:** Not applicable.

Clinical trial registry: Not applicable.

Consent to Publish declaration**:** Not applicable.

Data sharing statement: All data are utilized in the manuscript.

CRediT authorship contribution statement

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors are grateful to Kampala International University for its support.

Data availability

Data will be made available on request.

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