Specialty Journal of Pharmacognosy, Phytochemistry, and Biotechnology
ISSN: 3062-441X
2023, Volume 3, Issue 2, Page No: 62-71

Available online at: www.galaxypub.co/page/journals

Galaxy Publication

Who Actually Makes the Metabolite? A Critical Review of Plant–Endophyte Attribution, Reproducibility, and Biosynthetic Evidence in Medicinal-Plant Natural Products

Mark Anderson[1]*, Lisa Wong[2], Sarah Lee[1]

[1] Department of Plant–Endophyte Chemistry and Biosynthetic Attribution, College of Pharmacy, University of Florida, Gainesville, United States.

[2] Department of Reproducibility and Metabolite Origin, Faculty of Pharmacy, National University of Singapore, Singapore.

***E-mail** ✉ mark.anderson@ufl.edu

Received: 29 May 2023; **Revised:** 06 August 2023; **Accepted:** 08 August 2023

ABSTRACT

Endophytic fungi are repeatedly presented as alternative producers of medicinal-plant natural products, yet the evidential basis of producer attribution varies from culture-based chemical detection to isotope tracing, genome analysis, transcriptional association, and functional pathway testing. This critical review examines what these different observations can and cannot establish about metabolite origin. The central problem is that the same chemical phenotype may arise through independent microbial biosynthesis, persistence of plant-derived material, microbial transformation of supplied precursors, or endophyte-mediated alteration of host metabolism. Accordingly, chemical identity and biosynthetic provenance must be evaluated separately. The reviewed evidence shows that repeated axenic cultivation and stable-isotope incorporation can provide unusually strong support for microbial production, whereas biosynthetic-gene-cluster prediction, transcript abundance, metabolomic similarity, or repeated literature citation remain incomplete forms of attribution unless connected to product formation by orthogonal tests. It also shows why culture dependence, strain variation, analytical annotation limits, and alternative host-mediated mechanisms complicate reproducibility. We propose an attribution logic in which confidence rises through convergence among authenticated biological material, independently reproducible chemical evidence, biosynthetic plausibility, and functional or tracer-based validation, while explicit competing explanations are retained until experimentally excluded. This framework is intended as a critical interpretive aid rather than a universal scoring system. Its principal limitation is the heterogeneity of available studies: many high-profile plant-like metabolites still lack pathway-complete, independently replicated evidence in their reported endophytes

Keywords: Endophytic fungi, Metabolite attribution, Biosynthetic provenance, Medicinal plants, Secondary metabolism, Reproducibility

How to Cite This Article: Anderson M, Wong L, Lee S. Who Actually Makes the Metabolite? A Critical Review of Plant–Endophyte Attribution, Reproducibility, and Biosynthetic Evidence in Medicinal-Plant Natural Products. Spec J Pharmacogn Phytochem Biotechnol. 2023;3(2):62-71. https://doi.org/10.51847/c817QopHiS

Introduction

Endophytic fungi occupy an attractive position in medicinal-plant research because they can yield chemically diverse metabolites under cultivable conditions and are repeatedly discussed as potential alternative sources of pharmacologically interesting plant-associated compounds. The literature consequently contains many reports in which a metabolite known from a medicinal plant is also assigned to an endophytic isolate, but the depth of validation behind these assignments is uneven [1]. That heterogeneity matters because a claim about chemical recovery from a fungal culture is not equivalent to a claim about who encoded, assembled, and regulated the biosynthetic route. It also changes the translational meaning of the observation: a reproducible microbial source would imply a different development problem from a metabolite that appears only after plant–microbe interaction

or precursor transformation. For a critical review, the central question is therefore not simply whether a named compound has been reported from an endophyte, but what experimental observations justify calling that organism its producer and what alternative explanations remain viable after those observations.

This distinction has become more important as sequencing has exposed a persistent gap between chemical reports and genetically resolved biosynthesis. Reviews of endophytic-fungal genetics and genomics show that the biosynthetic basis of many reported products remains incomplete or uncharacterized despite continued bioprospecting [2]. Absence of such evidence does not by itself disprove microbial production: pathways may be novel, poorly annotated, conditionally expressed, or technically difficult to reconstruct. It does, however, limit the strength of producer attribution, particularly when a chemically famous plant metabolite is assigned to a fungus from a single analytical observation. The evidential task is therefore asymmetric. Positive chemical detection establishes that a signal or compound was recovered from the tested preparation, whereas biosynthetic attribution asks an additional causal question about origin. A defensible review must keep genomic potential, pathway nomination, pathway activity, and demonstrated product formation as separate propositions rather than allowing one to substitute automatically for another.

A further complication is biological interaction. Plant–endophyte systems can alter specialized-metabolite phenotypes through several mechanisms, including microbial metabolism, transformation of available substrates, and modulation of host biochemical responses [3]. This review therefore proposes a producer-attribution distinction among three broad causal states: independent microbial biosynthesis, microbial biotransformation of plant- or medium-derived substrates, and endophyte-associated stimulation or suppression of host biosynthesis. These states may coexist, and the current literature does not always discriminate among them. The distinction also prevents a common interpretive shortcut in which any increase of a host-associated molecule after inoculation is treated as evidence that the microorganism synthesized it. The purpose of the review is to evaluate attribution claims through their evidential reach, identify why apparently similar findings reproduce differently, and define what stronger biosynthetic evidence should look like. The analysis remains evidence-bounded: it does not presume that disputed reports are artefacts, nor that repeated detection is sufficient to establish an autonomous microbial pathway. It also treats metabolite identity, biological source, and biosynthetic mechanism as separate questions, because each requires a different class of control and each can fail independently.

The attribution problem

Fungal secondary metabolism is intrinsically conditional: biosynthetic gene clusters can be tightly regulated, silent under routine laboratory conditions, or responsive to environmental and developmental cues [4]. This creates a genuine reproducibility problem, because failure to recover a metabolite after subculture may reflect altered regulation rather than loss of biosynthetic capacity. Yet conditional expression cannot serve as an unfalsifiable explanation for every failed replication. Culture dependence becomes evidentially useful only when changes in metabolite production are linked experimentally to defined strain, medium, developmental, or regulatory conditions.

Attribution becomes stronger when chemical phenotype is connected to biosynthetic capacity through orthogonal evidence. Integrated genomics-to-metabolomics strategies can narrow the correspondence between candidate gene clusters and fungal secondary metabolites by asking whether genomic predictions and observed chemistry converge in the same biological material and condition [5]. Even such convergence remains an association until gene function or precursor incorporation is tested. We therefore treat multi-omic concordance as an evidential bridge between chemical observation and causal biosynthesis, not as the endpoint of attribution.

Metabolomics expands chemical coverage and can reveal reproducible differences among endophyte extracts, conditions, or interaction states, but annotation and provenance are different analytical questions. Endophyte-focused metabolomic work illustrates the value of profiling for detecting candidate bioactive chemistry while also emphasizing dependence on extraction, instrumentation, databases, and annotation quality [6]. A feature assigned tentatively to a known plant metabolite may justify targeted confirmation; it does not by itself establish definitive structure, exclude isomers, or identify which organism generated the carbon skeleton.

Feature-based molecular networking improves this comparison by retaining chromatographic feature information while organizing related tandem mass spectra in a reproducible analysis environment [7]. For attribution studies, its value lies in comparing chemical families across isolates, passages, media, or controls and in prioritizing features for confirmation. Its limitation is equally important: spectral relatedness is evidence about chemical

similarity, not biosynthetic authorship. Network position cannot distinguish de novo fungal production from transformed precursor, carry-over, or a chemically similar analogue without additional experiments.

Genomic homology presents a parallel problem. Curated repositories of experimentally characterized biosynthetic gene clusters make it possible to compare predicted fungal clusters with pathways of known function and thereby grade the plausibility of a candidate biosynthetic assignment [8]. Similarity to a characterized cluster is informative, particularly when gene organization and chemistry are coherent, but it remains constrained by sequence divergence, incomplete databases, assembly quality, and the possibility of altered enzymatic function. Thus neither chemical similarity nor cluster similarity should be converted into a binary producer label without an explicit validation step.

What has been claimed in the plant–endophyte literature

The claim landscape is broad. Recent reviews continue to catalogue endophytic fungi as sources of compounds also associated with medicinal plants, including prominent anticancer, antimicrobial, antioxidant, and other bioactive natural products [9]. Such catalogues are valuable for locating primary reports and showing the persistence of the alternative-source hypothesis, but repeated appearance of a compound name across reviews is not independent replication. The underlying claims may originate from very different evidential levels: a chromatographic or mass-spectral match, targeted isolation with stronger structural characterization, repeated recovery from culture, genomic plausibility, or direct pathway testing. They may also differ in whether the fungus was freshly isolated, repeatedly passaged, chemically compared with host tissue, or tested against sterile-media and extraction controls. Those distinctions affect what can be inferred even when the reported metabolite name is identical. Collapsing heterogeneous observations into a single inventory of “endophyte-produced” metabolites therefore overstates what the literature collectively establishes and obscures which claims are most amenable to decisive replication. It can also create a false impression of convergence when several secondary sources ultimately depend on one primary observation. For this reason, this review interprets the claim landscape by evidential characteristics rather than by counting how often a metabolite–fungus pairing is repeated.

Contemporary taxoid work illustrates this ambiguity. Endophytic fungi isolated from *Corylus avellana* showed taxoid-associated profiles whose composition and detectability varied with fungal isolate and culture medium [10]. The study therefore supports a chemically grounded proposition that particular cultured isolates can yield taxoid-associated signals under specified conditions, and it usefully demonstrates that the phenotype is not uniform across strains or media. That variability is itself scientifically informative: it makes strain identity and cultivation conditions part of the attribution statement rather than ancillary experimental detail. At the same time, culture-associated detection does not, by that observation alone, reconstruct a complete independent fungal paclitaxel pathway, show that all detected taxoids share the same origin, or exclude every alternative source for each signal. The critical review problem is consequently one of calibrated language. “Detected in a fungal culture,” “structurally confirmed from fungal biomass,” “reproducibly produced after serial passage,” and “biosynthetically demonstrated in the fungus” should not function as interchangeable descriptions simply because they concern the same named natural product. Each formulation implies a different evidential burden, and the strongest wording should be reserved for experiments that directly discriminate biosynthesis from persistence, transformation, or host-mediated production.

The mismatch between commonly used claim language and the actual evidential reach of each observation is summarized in **Table 1.**

Table 1. Evidence characteristics that distinguish a reported endophyte-associated metabolite from a biosynthetically attributed microbial product

Evidence characteristic	What the observation can support	What remains unresolved	Critical interpretation
Repeated appearance in review literature	Persistence and prominence of a reported association	Independent replication and producer identity	Citation recurrence is not new experimental evidence
Metabolite signal in isolated fungal culture	Chemical phenotype under stated culture conditions	De novo origin, carry-over, transformation, exact pathway	Culture detection is necessary evidence in many cases, but not sufficient attribution
Variation among isolates or media	Strain- and condition-specific expression of a chemical phenotype	Whether non-producing states reflect regulation or absence of capacity	Reproducibility must preserve strain and cultivation context

Metabolomic or spectral correspondence	Prioritized chemical annotation and cross-sample comparison	Definitive structure and biosynthetic provenance	Analytical similarity and producer identity remain separate
Biosynthetic-cluster similarity	Genomic plausibility for a candidate pathway	Exact product, pathway activity, causal gene function	Homology strengthens a hypothesis but requires functional linkage

Evidence supporting microbial production

The strongest attribution studies directly test alternatives to simple culture detection. In an *Alternaria* isolate from *Nothapodytes nimmoniana*, camptothecin production was examined after repeated axenic subculturing and by incorporation of carbon from ^13C-labelled glucose into the recovered compound [11]. Persistence across passages weakens a simple explanation based on residual host material, while labelled-carbon incorporation provides direct evidence that the cultured fungus contributes newly assimilated carbon to camptothecin under the tested conditions. This combination is substantially stronger than a one-time chromatographic match because it links continued production to an independent carbon source. It still does not provide a complete gene-by-gene reconstruction of the pathway or justify generalization to other isolates.

Genomics can add a different layer of plausibility. Characterization of the Huperzine-A-associated endophyte *Colletotrichum gloeosporioides* Cg01 identified candidate genomic machinery relevant to secondary metabolism and proposed genes potentially involved in Huperzine A biosynthesis [12]. Such evidence is valuable because it asks whether the microorganism contains an enzymatic repertoire consistent with the reported chemistry rather than relying on metabolite detection alone. Nevertheless, candidate assignment from annotation or homology remains provisional. Without functional perturbation, enzyme characterization, or pathway reconstruction, genomic compatibility supports biosynthetic plausibility rather than definitive identification of the complete route.

Genome and transcriptome evidence can also test evolutionary explanations attached to producer claims. Analysis of the camptothecin-producing endophyte *Alternaria burnsii* NCIM 1409 identified genomic and transcriptional features consistent with fungal biosynthetic capacity while failing to support a simple model in which the relevant capability was acquired wholesale from the host through horizontal gene transfer [13]. This is important because metabolite sharing between a plant and fungus does not establish gene sharing. The evidence is compatible with a fungal route and constrains one proposed origin story, but it does not prove the independent evolutionary origin or biochemical role of every pathway step.

Ultimately, stronger causal attribution requires experimental movement from candidate sequence to product formation. Functional perturbation of fungal biosynthetic genes can establish necessity within a pathway, while heterologous expression of defined fungal gene clusters can test whether a selected genetic unit is sufficient to generate a product or pathway intermediate [14, 15]. These are methodological benchmarks rather than direct validation of every medicinal-plant endophyte claim, and native regulation may not be reproduced in a heterologous host. Their importance is conceptual: they show how an attribution argument can progress beyond coincident chemistry and predicted capacity toward experimentally linked biosynthesis, while retaining the possibility that pathway behavior in the native endophyte depends on additional genes, regulation, or environmental context.

Evidence that weakens or contradicts attribution

The taxol literature provides the clearest demonstration that historically influential producer claims can be materially revised. Comparative-genomic reassessment of *Taxomyces andreanae* did not recover the expected complement of taxol-biosynthetic genes and also revised the organism's taxonomic placement [16]. This does not establish that fungal taxol biosynthesis is biologically impossible, because negative genomic inference depends on assembly completeness, pathway knowledge, and the possibility of unrecognized chemistry. It does show that a canonical attribution can weaken when the biological material is re-identified and its genome fails to support the pathway expected from the original claim. Negative evidence is therefore not merely absence of confirmation; when appropriately bounded, it can actively change the plausibility of an attribution.

Host-side evidence strengthens the need for competing explanations. The *Taxus* genome reveals substantial endogenous organization of paclitaxel biosynthesis, making plant production a well-supported source model for taxanes recovered from *Taxus*-associated systems [17]. Host capacity does not exclude independent fungal production in a particular isolate, but it removes any justification for treating chemical overlap as evidence that

biosynthesis is shared. A fungal claim must instead explain why the compound persists in axenic culture and how fungal biosynthetic evidence differs from plant carry-over or host-derived material.

Association-level molecular evidence can also be overread. Comparative transcriptomics of the reported taxol-producing *Aspergillus aculeatinus* Tax-6 and a mutant identified expression differences associated with the production phenotype [18]. Such contrasts nominate candidate processes, but differential expression can arise from pleiotropy, altered growth, stress, or other metabolic changes. Without gene-specific functional tests, expression correlation should remain intermediate evidence rather than pathway proof.

A recent critical assessment of fungal taxol biosynthesis likewise emphasizes unstable or low production phenotypes and incomplete molecular resolution across the field [19]. The appropriate inference is calibrated skepticism, not categorical rejection: some isolates may yet prove authentic producers, but repeated citation cannot repair missing replication or biosynthetic linkage.

A further alternative is host modulation. Endophyte or microbiota exposure can increase a medicinal-plant metabolite through host-pathway responses without demonstrating that the microorganism synthesized that metabolite [20, 21]. This route must remain distinct from both autonomous microbial biosynthesis and microbial biotransformation. **Figure 1** integrates these competing attribution states with the evidential tests needed to distinguish them.

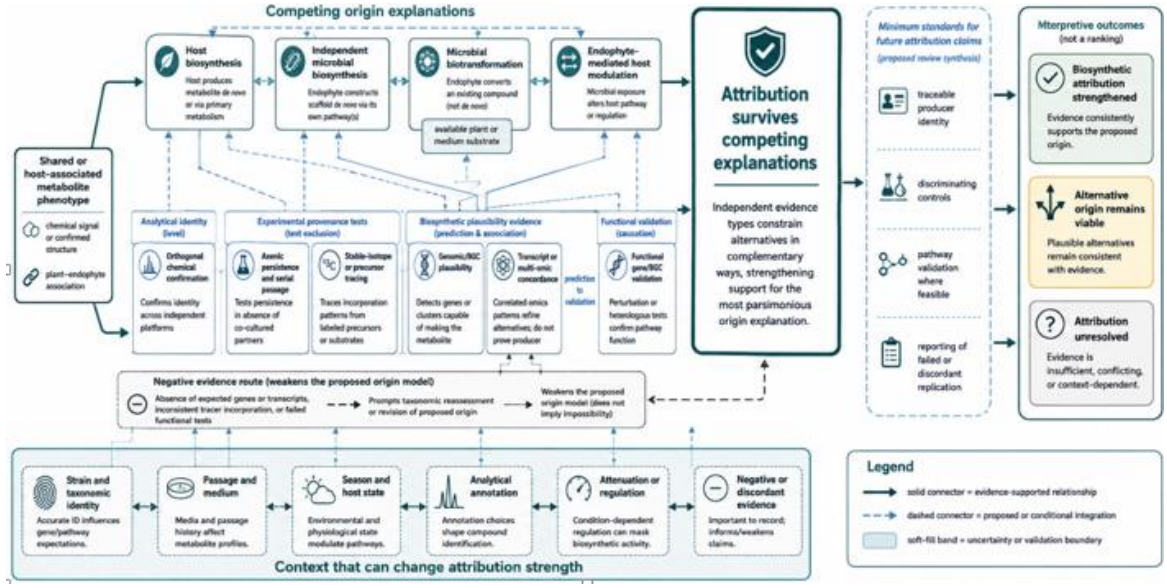

Figure 1. From shared chemical phenotype to defensible metabolite attribution in plant–endophyte systems

Reproducibility across isolation and cultivation studies

Reproducibility in this literature cannot be reduced to asking whether the same metabolite name appears twice. It requires reproduction of the biological material, cultivation context, analytical assignment, and causal state being claimed. In *Coleus forskohlii*, defined fungal co-inoculation with *Trichoderma viride* altered host responses associated with forskolin augmentation [22]. The observation demonstrates that microbial combination is part of the experimental context; it does not show that the inoculated fungi themselves synthesize forskolin. A replication using a different inoculum composition therefore tests a different biological system, even if the host species is unchanged.

Environmental context can similarly modify metabolite outcomes. An endophytic consortium applied to *Catharanthus roseus* produced season-dependent effects on key terpenoid indole alkaloids [23]. “Season” is not a single mechanism, because temperature, light, host developmental state, and other variables may covary, but the result shows why environmental metadata cannot be detached from a reproducibility claim. Repeating an inoculation under materially different host conditions may legitimately yield a different chemical phenotype without resolving who synthesized the compound.

Biotransformation provides another reason apparent reproduction can be misclassified. Endophytes isolated from *Panax notoginseng* converted supplied ginsenosides into other ginsenoside products [24]. That is a reproducible microbial biochemical capability, but it is categorically different from de novo construction of the ginsenoside

scaffold. Demonstrating substrate-to-product conversion should therefore strengthen a transformation claim while simultaneously preventing inflation into autonomous biosynthesis.

Finally, endophyte function is strain-level rather than a property that can safely be inferred from host association alone. Isolates obtained from *Alkanna tinctoria* roots showed substantial taxonomic and functional heterogeneity [25]. Culture-dependent collections also sample only part of the endosphere. Reproducibility consequently begins with traceable isolate identity and continues through matched culture and analytical conditions. **Table 2** compares experimental strategies by the specific attribution question each can reproduce or resolve.

Table 2. Experimental approaches for reproducing and discriminating metabolite-origin claims

Approach	Attribution question addressed	Main evidential value	Key interpretation boundary
Matched isolate and culture replication	Does the phenotype recur in the same biological context?	Tests strain- and condition-specific persistence	Failure may reflect regulation or an incorrect original claim
Serial axenic passage	Does production persist away from host tissue?	Weakens simple host carry-over explanations	Persistence alone does not identify the pathway
Stable-isotope or precursor tracing	Is new carbon or precursor incorporated into product?	Directly tests biosynthetic or transformation flux	Tracer design must distinguish de novo synthesis from conversion
Controlled substrate-conversion assay	Can the isolate transform a defined metabolite?	Establishes biotransformation capability	Does not establish de novo scaffold biosynthesis
Multi-condition or seasonal replication	Is the phenotype robust to environmental context?	Reveals conditionality and moderators	Context dependence is not itself a biosynthetic mechanism
Strain-resolved comparative testing	Is function conserved across isolates?	Tests biological heterogeneity and transferability	Host association cannot substitute for strain identity

Critical interpretation of biosynthetic evidence

The central interpretive task is not to accumulate more evidence types indiscriminately, but to ask whether independent layers constrain different alternative explanations. Combined transcriptomic and metabolomic approaches can refine hypotheses about plant–endophyte interactions by linking pathway-responsive expression with chemical change [26]. Yet concordant omics in mixed or interacting biological material does not identify the producer cell or establish causal metabolic flux. Strong inference therefore depends on complementarity: one evidence layer should answer a question that another leaves open.

For mixed plant–microbe material, this distinction is especially important because a correlated transcript and metabolite can originate from different partners or from a response cascade spanning both. Accordingly, this review treats cellular provenance, temporal ordering, and perturbational response as separate interpretive questions. Designs that physically separate partners or follow time-resolved responses can reduce ambiguity, but chemical and biosynthetic validation remain necessary before producer identity is assigned.

Genome mining illustrates this prediction–validation boundary. antiSMASH and related approaches can identify candidate biosynthetic gene clusters and compare them with characterized cluster families [27]. These capabilities are powerful for nominating loci, prioritizing genes, and evaluating biochemical plausibility, but a predicted cluster remains a hypothesis about product identity and function. Sequence novelty, uncertain cluster boundaries, incomplete assemblies, and divergent enzymology prevent a genomic prediction from serving as a terminal producer label. Where feasible, candidate loci should be connected experimentally to product formation through perturbation, enzyme testing, heterologous reconstruction, or other pathway-specific validation.

The same logic applies to pathway completeness. A plausible enzyme set may explain part of a scaffold while leaving precursor origin or tailoring unresolved. This review therefore distinguishes “capacity consistent with biosynthesis” from “pathway demonstrated”: the former is a genomic proposition; the latter requires evidence that nominated machinery contributes to the observed product in the relevant system.

Production attenuation presents the opposite interpretive hazard. Host signals, culture physiology, and epigenetic regulation have all been discussed as plausible contributors to variable fungal taxol phenotypes [28]. Such mechanisms could explain genuine condition-sensitive production, but they should be demonstrated in the focal strain rather than invoked after every failed replication. Otherwise “silencing” becomes an explanation that cannot

be falsified and shields weak original claims from revision. Loss and rescue of production should therefore be treated as testable phenotypes with defined molecular and culture correlates.

The taxol controversy makes the broader principle concrete: strong producer attribution should triangulate positive fungal observations against negative fungal evidence and independent host-side biosynthetic evidence before competing origins are dismissed [16, 17, 19]. This triangulation rule is proposed here as an interpretive framework, not as a validated universal scoring system. Different evidence streams have unequal scope and cannot be pooled numerically. Their value lies in whether, together, they narrow plausible origin models without erasing unresolved alternatives.

Minimum standards for future attribution claims

Future claims should begin with biological identity. Contemporary fungal taxonomic guidance emphasizes traceable naming, sequence-based characterization, and deposition practices that make the studied organism recoverable and re-evaluable [29]. For metabolite attribution, this is a necessary entry condition rather than biosynthetic proof. A producer claim tied only to a host plant, genus-level label, or unavailable isolate cannot be replicated rigorously when taxonomy changes or strain-level function differs.

Traceability should extend beyond a species name. Minimum reporting should preserve isolate designation, voucher or culture-collection information where available, sequence identifiers used for identification, passage history, host source, and the material used for chemistry and sequencing. These details do not make a biosynthetic claim true; they make it possible for another laboratory to test the same claim rather than a nominally similar organism.

Second, experiments should discriminate de novo biosynthesis from simpler alternatives. Serial axenic passage combined with stable-isotope incorporation provides one strong example because persistence and newly assimilated carbon directly challenge host carry-over [11]. The proposed minimum is not one mandatory tracer protocol, but an explicit control logic: sterile medium and extraction controls, authenticated host-free cultures, substrate-aware designs, and, where feasible, tracers or precursor experiments that separate new synthesis from transformation. Structural confirmation should match the specificity of the compound claim.

Analytical controls should follow the proposed causal alternative. If carry-over is plausible, passage and host-free culture matter; if transformation is plausible, substrate-free and defined-substrate controls matter; if host induction is plausible, fungus-only and host-only comparators matter. The purpose is discriminating design: each control should remove or retain a specific origin hypothesis.

Third, genomic evidence should be reported as nomination until linked to function. Genome-mining tools are appropriate for finding candidate biosynthetic loci, but predicted clusters should not be renamed as demonstrated pathways solely from similarity [27]. Stronger claims should, where technically feasible, connect candidate genes to product or intermediates through perturbation, biochemical testing, expression in a defined system, or convergent native multi-omic evidence. No single platform is universally required; the requirement is that the experiment address the competing explanation left open by prediction.

Finally, discordant results should remain part of the evidence record. The reassessment of *Taxomyces* shows how re-identification and negative genomic findings can materially revise a celebrated attribution [16]. Negative evidence must itself be bounded by assay sensitivity, genome quality, and verified material identity, but failed replication should not disappear behind post-hoc assumptions of silencing.

Reports should also distinguish failure to detect from evidence of absence. Detection limits, extraction coverage, sequencing completeness, and the conditions under which a pathway was sought should accompany negative conclusions. Conversely, apparent rescue after culture modification should be documented with matched controls rather than retrospectively treated as proof that earlier non-production was merely attenuation. We therefore propose that future attribution reports state the strongest supported claim explicitly—detection, transformation, host modulation, biosynthetic plausibility, or demonstrated microbial production—and report unresolved alternatives alongside it.

Conclusion

The question “who actually makes the metabolite?” cannot be answered reliably by chemical coincidence alone. Plant–endophyte systems permit several biologically distinct explanations for the same observed molecule: autonomous microbial biosynthesis, host biosynthesis, microbial transformation of available substrates, and

microbial modulation of host pathways. The critical error is not uncertainty itself, but allowing evidence that addresses one of these possibilities to be written as though it excluded the others.

The literature nevertheless provides a route toward stronger attribution. Repeated host-free cultivation, chemically specific confirmation, isotope or precursor tracing, credible genomic capacity, and functional pathway tests can progressively constrain alternative origins when they are applied to authenticated biological material. Conversely, taxonomic revision, absent expected pathway evidence, unstable production, strain heterogeneity, and failed replication are scientifically informative and should modify claim strength rather than being treated as inconvenient exceptions. Omics and genome mining are most useful when they nominate mechanisms that can subsequently be challenged, not when prediction is relabelled as proof.

This review therefore proposes a convergence-based attribution logic rather than a numerical hierarchy. Confidence should depend on whether independent evidence streams answer different causal questions and survive plausible competing explanations under reproducible conditions. The framework is intentionally bounded: it is not a consensus standard, does not imply that every historical fungal producer claim is false, and cannot eliminate uncertainty created by incomplete genomes, condition-sensitive metabolism, or technically inaccessible pathways.

This change in language also improves reproducibility: disagreements can be localized to identity, chemistry, culture dependence, pathway prediction, or functional evidence instead of being reduced to contradictory "producer" labels. It also allows convincing positive cases to become stronger precisely because they survive explicit alternatives rather than because they are repeated uncritically. The framework's practical value is therefore narrower but important—to replace the binary language of "producer" versus "non-producer" with experimentally discriminable claims about chemical identity, biosynthetic provenance, transformation, host modulation, and the evidence still required to distinguish them.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

References

1. Gupta S, Chaturvedi P, Kulkarni MG, Van Staden J. A critical review on exploiting the pharmaceutical potential of plant endophytic fungi. Biotechnol Adv. 2020;39:107462. doi:10.1016/j.biotechadv.2019.107462
2. Sagita R, Quax WJ, Haslinger K. Current state and future directions of genetics and genomics of endophytic fungi for bioprospecting efforts. Front Bioeng Biotechnol. 2021;9:649906. doi:10.3389/fbioe.2021.649906
3. Kumari P, Deepa N, Trivedi PK, Singh BK, Srivastava V, Singh A. Plants and endophytes interaction: A "secret wedlock" for sustainable biosynthesis of pharmaceutically important secondary metabolites. Microb Cell Fact. 2023;22(1):226. doi:10.1186/s12934-023-02234-8
4. Keller NP. Fungal secondary metabolism: regulation, function and drug discovery. Nat Rev Microbiol. 2019;17(3):167-80. doi:10.1038/s41579-018-0121-1
5. Hautbergue T, Jamin EL, Debrauwer L, Puel O, Oswald IP. From genomics to metabolomics, moving toward an integrated strategy for the discovery of fungal secondary metabolites. Nat Prod Rep. 2018;35(2):147-73. doi:10.1039/C7NP00032D
6. Mishra S, Priyanka, Sharma S. Metabolomic insights into endophyte-derived bioactive compounds. Front Microbiol. 2022;13:835931. doi:10.3389/fmicb.2022.835931
7. Nothias LF, Petras D, Schmid R, Dührkop K, Rainer J, Sarvepalli A, et al. Feature-based molecular networking in the GNPS analysis environment. Nat Methods. 2020;17(9):905-8. doi:10.1038/s41592-020-0933-6
8. Kautsar SA, Blin K, Shaw S, Navarro-Muñoz JC, Terlouw BR, van der Hooft JJJ, et al. MIBiG 2.0: A repository for biosynthetic gene clusters of known function. Nucleic Acids Res. 2020;48(D1):D454-58. doi:10.1093/nar/gkz882

9. Wen J, Okyere SK, Wang S, Wang J, Xie L, Ran Y, et al. Endophytic fungi: An effective alternative source of plant-derived bioactive compounds for pharmacological studies. J Fungi (Basel). 2022;8(2):205. doi:10.3390/jof8020205
10. Mohammadi Ballakuti N, Ghanati F, Zare-Maivan H, Alipour M, Moghaddam M, Abdolmaleki P. Taxoid profile in endophytic fungi isolated from Corylus avellana, introduces potential source for the production of Taxol in semi-synthetic approaches. Sci Rep. 2022;12(1):9390. doi:10.1038/s41598-022-13602-6
11. Mohinudeen IAHK, Kanumuri R, Soujanya KN, Shaanker RU, Rayala SK, Srivastava S. Sustainable production of camptothecin from an Alternaria sp. isolated from Nothapodytes nimmoniana. Sci Rep. 2021;11(1):1478. doi:10.1038/s41598-020-79239-5
12. Kang X, Liu C, Shen P, Hu L, Lin R, Ling J, et al. Genomic characterization provides new insights into the biosynthesis of the secondary metabolite Huperzine A in the endophyte Colletotrichum gloeosporioides Cg01. Front Microbiol. 2019;9:3237. doi:10.3389/fmicb.2018.03237
13. Natarajan S, Pucker B, Srivastava S. Genomic and transcriptomic analysis of camptothecin producing novel fungal endophyte: Alternaria burnsii NCIM 1409. Sci Rep. 2023;13(1):14614. doi:10.1038/s41598-023-41738-6
14. Luo F, Hong S, Chen B, Yin Y, Tang G, Hu F, et al. Unveiling of swainsonine biosynthesis via a multibranched pathway in fungi. ACS Chem Biol. 2020;15(9):2476-84. doi:10.1021/acschembio.0c00466
15. Harvey CJB, Tang M, Schlecht U, Horecka J, Fischer CR, Lin HC, et al. HEx: A heterologous expression platform for the discovery of fungal natural products. Sci Adv. 2018;4(4):eaar5459. doi:10.1126/sciadv.aar5459
16. Cheng T, Kolařík M, Quijada L, Stadler M. A re-assessment of Taxomyces andreanae, the alleged taxol-producing fungus, using comparative genomics. IMA Fungus. 2022;13(1):17. doi:10.1186/s43008-022-00103-4
17. Xiong X, Gou J, Liao Q, Li Y, Zhou Q, Bi G, et al. The Taxus genome provides insights into paclitaxel biosynthesis. Nat Plants. 2021;7(8):1026-36. doi:10.1038/s41477-021-00963-5
18. Qiao W, Tang T, Ling F. Comparative transcriptome analysis of a taxol-producing endophytic fungus, Aspergillus aculeatinus Tax-6, and its mutant strain. Sci Rep. 2020;10(1):10558. doi:10.1038/s41598-020-67614-1
19. Subban K, Kempken F. Insights into Taxol® biosynthesis by endophytic fungi. Appl Microbiol Biotechnol. 2023;107(20):6151-62. doi:10.1007/s00253-023-12713-y
20. Maggini V, De Leo M, Granchi C, Tuccinardi T, Mengoni A, Gallo ER, et al. The influence of Echinacea purpurea leaf microbiota on chicoric acid level. Sci Rep. 2019;9(1):10897. doi:10.1038/s41598-019-47329-8
21. Cao X, Xu L, Wang J, Dong M, Xu C, Kai G, et al. Endophytic fungus Pseudodidymocyrtis lobariellae KL27 promotes taxol biosynthesis and accumulation in Taxus chinensis. BMC Plant Biol. 2022;22(1):12. doi:10.1186/s12870-021-03396-6
22. Mastan A, Rane D, Dastager SG, Babu CSV. Molecular insights of fungal endophyte co-inoculation with Trichoderma viride for the augmentation of forskolin biosynthesis in Coleus forskohlii. Phytochemistry. 2021;184:112654. doi:10.1016/j.phytochem.2021.112654
23. Singh S, Pandey SS, Tiwari R, Pandey A, Shanker K, Kalra A. Endophytic consortium with growth-promoting and alkaloid enhancing capabilities enhance key terpenoid indole alkaloids of Catharanthus roseus in the winter and summer seasons. Ind Crops Prod. 2021;166:113437. doi:10.1016/j.indcrop.2021.113437
24. Wei G, Chen Z, Wang B, Wei F, Zhang G, Wang Y, et al. Endophytes isolated from Panax notoginseng converted ginsenosides. Microb Biotechnol. 2021;14(4):1730-46. doi:10.1111/1751-7915.13842
25. Rat A, Naranjo HD, Krigas N, Grigoriadou K, Maloupa E, Alonso AV, et al. Endophytic bacteria from the roots of the medicinal plant Alkanna tinctoria Tausch (Boraginaceae): Exploration of plant growth promoting properties and potential role in the production of plant secondary metabolites. Front Microbiol. 2021;12:633488. doi:10.3389/fmicb.2021.633488
26. Chen XL, Sun MC, Chong SL, Si JP, Wu LS. Transcriptomic and metabolomic approaches deepen our knowledge of plant-endophyte interactions. Front Plant Sci. 2022;12:700200. doi:10.3389/fpls.2021.700200
27. Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, Medema MH, et al. antiSMASH 6.0: Improving cluster detection and comparison capabilities. Nucleic Acids Res. 2021;49(W1):W29-35. doi:10.1093/nar/gkab335

28. Mohamed NZ, Shaban L, Safan S, El-Sayed ASA. Physiological and metabolic traits of Taxol biosynthesis of endophytic fungi inhabiting plants: Plant-microbial crosstalk, and epigenetic regulators. Microbiol Res. 2023;272:127385. doi:10.1016/j.micres.2023.127385
29. Aime MC, Miller AN, Aoki T, Bensch K, Cai L, Crous PW, et al. How to publish a new fungal species, or name, version 3.0. IMA Fungus. 2021;12(1):11. doi:10.1186/s43008-021-00063-1