



Trichoderma specialized metabolites in biocontrol: gene–metabolite links, ecological functions, and translational bottlenecks

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ABSTRACT

Trichoderma spp. produce a diverse repertoire of metabolites with specific activities that contribute to biocontrol through direct antagonism, ecological signalling, and modulation of plant responses. However, current knowledge remains uneven: many metabolites are chemically described, whereas fewer are supported by robust gene–metabolite associations, experimentally validated ecological functions, and realistic translational evidence. Progress in this field will depend less on expanding compound catalogues than on integrating mechanistic, ecological, and translational evidence. This review examines the specialized metabolism of *Trichoderma* with emphasis on biosynthetic gene clusters, regulatory networks, ecological roles, and biosafety constraints relevant to biocontrol. Major metabolite classes, including polyketides, terpenoids, peptaibols, siderophores, diketopiperazines, and volatile organic compounds, are discussed together with representative case studies for which genetic and functional evidence is available. We further propose a translational framework to distinguish metabolites with mainly descriptive support from those approaching application readiness, based on four criteria: gene-level validation, demonstrated ecological role, manageable biosafety profile, and feasible delivery/stability. This perspective helps explain why metabolite inventories continue to expand faster than field translation. Recent advances in genomics, transcriptomics, metabolomics, genome editing, and formulation science are reshaping how *Trichoderma* metabolites are prioritized for future development.

1. Introduction

Fungal and oomycete diseases remain a major constraint for crop production, and their impact is being reinforced by climate variability, global trade, and the spread of invasive pathogens (Cacciola and Gulino, 2019; Fisher et al., 2012; Nazarov et al., 2020; Riolo et al., 2023). At the same time, concerns related to fungicide resistance, environmental impact, residue exposure, and the progressive restriction of active substances have intensified interest in alternative and complementary disease management strategies (Brauer et al., 2019; Brent and Hollomon, 2007; European Food Safety Authority (EFSA), 2024; Jabłońska-Trypuc et al., 2017; Wightwick et al., 2010). Among these, microbial biocontrol has gained renewed attention, not only because of its role in integrated crop protection, but also because it offers access to a broad and still incompletely resolved repertoire of bioactive microbial metabolites (Hammami et al., 2022; He et al., 2021; La Bella et al., 2024; Leonardi et al., 2023; Stracquadanio et al., 2020).

Among microbial biocontrol agents, species of *Trichoderma* occupy a particularly important position because they combine ecological

versatility with an unusually rich secondary metabolism. Members of this genus occur in soils and plant associated habitats and can express saprotrophic, endophytic, and mycoparasitic lifestyles, features that help explain both their ecological success and their agricultural relevance (Harman et al., 2004; Mukherjee et al., 2022; Sood et al., 2020). Although *Trichoderma* is widely known for strain based biocontrol products, its biological activity cannot be understood only in terms of colonisation ability or antagonistic growth. A substantial part of its functional repertoire depends on specialized metabolites that act as antimicrobials, signalling molecules, plant response modulators, and mediators of interactions within complex microbial communities (Vicente et al., 2022; Vinale et al., 2008; Zeilinger et al., 2016a, 2016b).

The specialized metabolites reported from *Trichoderma* include peptaibols, polyketides, terpenoids, non ribosomal peptides, siderophores, diketopiperazines, and diverse volatile organic compounds. Together, these molecules contribute to direct antagonism against plant pathogens, modulation of plant defence responses, nutrient and ion related competition, and inter organism signalling in the rhizosphere and other plant associated environments (Stracquadanio et al., 2020;

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Vicente et al., 2022; Vinale et al., 2008; Zeilinger et al., 2016a; Zhang et al., 2021). However, the field is still marked by a noticeable asymmetry. The number of metabolites described chemically continues to increase, whereas only a smaller subset is supported by robust evidence linking a specific metabolite to a biosynthetic determinant, a defined ecological function, and a realistic translational perspective. In recent years, comparative genomics, genome mining, transcriptomics, and metabolomics have substantially improved the resolution of this problem by allowing more reliable connections between biosynthetic gene clusters, metabolite production, and biological function (Kubicek et al., 2019; Rubio et al., 2023; Vicente et al., 2022; Zheng et al., 2025).

The regulation of specialized metabolism in fungi adds a further layer of complexity. In *Trichoderma*, metabolite output is shaped by global regulators, pathway specific factors, chromatin level control, and environmental signals such as nutrient availability, pH, and light (Aghcheh et al., 2013; Bok and Keller, 2004; Hou et al., 2024; Keller, 2019; Yu et al., 2023). This regulatory plasticity helps explain why metabolite detection alone is often insufficient to infer ecological relevance or application potential. It also explains the growing interest in approaches such as OSMAC, co culture systems, and genome editing to activate poorly expressed pathways and reveal hidden metabolic capacity (Brakhage and Schroeckh, 2011; Leal et al., 2024; Zheng et al., 2025).

From a translational perspective, an important gap remains between metabolite discovery and functional deployment. Much of the agronomic evidence still derives from living *Trichoderma* inocula, where metabolite effects are embedded within a broader biological context and cannot easily be separated from colonisation, competition, and host responses. By contrast, evidence based on purified metabolites, enriched fractions, or chemically defined preparations is still comparatively limited and often restricted to controlled systems. In parallel, biosafety, delivery, persistence, and reproducibility remain decisive constraints for evaluating which metabolites are realistic candidates for further development (European Commission, 2024; OECD, 2018; Vicente et al., 2022; Woo et al., 2023).

Taken together, the literature now allows a shift from descriptive inventories of *Trichoderma* metabolites to a more selective evaluation of their biological and translational relevance. In this review, we examine specialized metabolites produced by *Trichoderma* with emphasis on four connected dimensions: biosynthetic basis, regulatory context, ecological function, and translational constraints. Rather than treating all reported

metabolites as equally informative, we highlight the difference between compounds supported mainly by descriptive evidence and those for which gene level validation, functional evidence, biosafety considerations, and delivery feasibility begin to converge (Fig. 1). These four criteria are subsequently developed into an operational prioritization framework and applied comparatively to representative metabolites and metabolite classes. Evidence derived from living *Trichoderma* systems is discussed only when needed to interpret ecological function or to frame the gap between strain based activity and metabolite based translation.

1.1. Molecular characterization, taxonomy, and biocontrol-relevant species of *Trichoderma*

Taxonomic resolution is not a marginal issue in *Trichoderma*. A substantial part of the literature on metabolite production, ecological traits, and biosafety was generated before current species delimitation criteria became widely adopted, and this limits direct comparison across studies. Morphology based systems represented an essential historical framework, but they are not sufficient for reliable species level identification in a genus marked by cryptic speciation and phenotypic plasticity. This limitation is well illustrated by the *T. harzianum* complex, in which isolates once treated as a single taxon were later resolved into distinct phylogenetic species with different ecological properties and metabolite repertoires. The transition from morphology-based identification to molecular systematics has therefore been essential for interpreting the specialized metabolism of *Trichoderma* (Atanasova et al., 2013; Bissett, 1991; Chaverri et al., 2015; Chaverri and Samuels, 2003; Druzhinina and Kubicek, 2005; Rifai, 1969; Samuels, 2006).

The internal transcribed spacer region remains useful for preliminary placement, but its resolution within the genus is often insufficient. By contrast, protein coding loci such as *tef1* and *rpb2* provide stronger phylogenetic signal and are now central to species level assignment, especially when used in multilocus frameworks. More recently, phylogenomic approaches and whole genome sequencing have provided still higher resolution while also revealing biosynthetic gene clusters, regulatory traits, and lineage specific metabolic potential. These advances are relevant not only for taxonomy, but also for interpreting metabolite reports generated under different naming systems and for assessing whether apparently similar strains can reasonably be expected to share comparable biosynthetic repertoires (Cai and Druzhinina, 2021; Kubicek et al., 2019, 2011; Nascimento Brito et al., 2023; Qin and Zhuang,

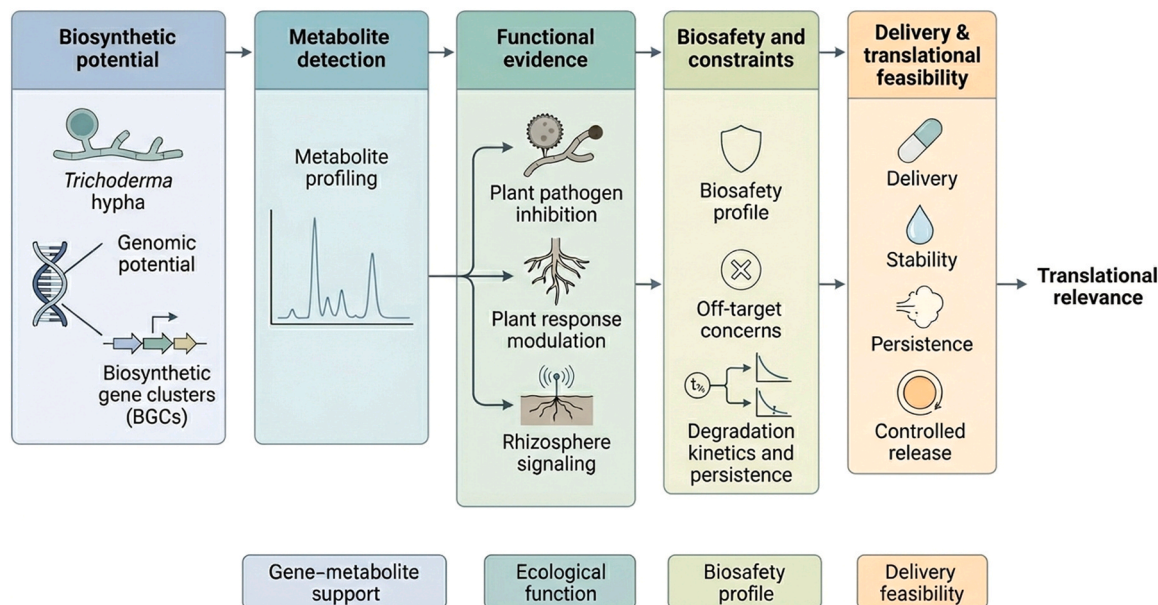


Fig. 1. Conceptual framework for translational prioritization of specialized metabolites of *Trichoderma*.

2016). A summary of the main markers and genome scale approaches used in *Trichoderma* taxonomy is provided in Table 1.

This point has direct consequences for the interpretation of the literature. Reports based on broad species names or low resolution identification may overestimate the reproducibility of metabolite profiles across strains and may obscure lineage specific traits that are relevant to antagonism, signalling, or biosafety. The problem is especially evident in older studies that attributed metabolites to broadly defined taxa such as the *T. harzianum* complex, where later phylogenetic resolution showed that chemically and ecologically distinct species had been grouped under the same name. The same issue affects attempts to compare biosynthetic gene clusters across studies or to infer biological properties from taxonomic labels alone. For this reason, metabolite reports supported by multilocus or genome informed identification provide a more reliable basis for evaluating gene metabolite relationships, ecological relevance, and translational potential (Atanasova et al., 2013; EFSA, 2024; Kubicek et al., 2019; Riolo et al., 2025).

1.2. Biocontrol-relevant species and representative commercial products

Among the many described species, a comparatively limited set of strain-defined lineages is repeatedly represented in biocontrol research and commercial formulations. *Trichoderma afroharzianum* includes strains that continue to be marketed under the legacy name *T. harzianum*, notably T-22, which is primarily used for root and rhizosphere colonisation, suppression of soilborne pathogens, and stimulation of plant defence and growth; Triatum-P and Triatum-G are representative formulations (Chaverri et al., 2015; Harman et al., 2004; Woo et al., 2023). *Trichoderma atroviride* strains SC1 and I-1237 are particularly effective colonisers of woody tissues and pruning wounds and are commercialised as Vintec and Esquive WP, respectively, especially for the management of grapevine trunk diseases (Pertot et al., 2016; Yacoub et al., 2024). *Trichoderma asperellum* T34 is mainly used for root protection against soilborne pathogens and is commercialised in formulations such as T34 Biocontrol and Asperello T34, whereas *T. asperellum* ICC012 is combined with *T. gamsii* ICC080 in Remedier for the prevention of soilborne and wood-associated fungal diseases (Di Marco et al., 2022; Leonardi et al., 2026b, 2026a; Segarra et al., 2010). *Trichoderma virens* remains an important experimental biocontrol model because of its strong mycoparasitic and antibiotic-mediated activity. These examples demonstrate that biocontrol performance cannot be inferred from the species name alone, because efficacy depends on the individual strain, target pathosystem, formulation, application site, and

environmental conditions. Product names are provided as representative examples, as authorisation and commercial availability vary among countries.

2. Secondary metabolites of *Trichoderma*

2.1. Classes of metabolites and ecological roles

Specialized metabolites reported from *Trichoderma* span several major biosynthetic classes, but the strength of evidence is uneven across them. Some metabolites are supported by relatively robust links between chemical identity, biosynthetic basis, and biological function, whereas others are known mainly from occurrence reports or from assays that do not yet resolve their ecological relevance. This distinction is important because chemical diversity alone does not indicate translational value, as summarized comparatively in Fig. 2. At present, the strongest evidence is available for a limited number of compounds or classes, particularly 6-pentyl- α -pyrone, selected peptaibols, some terpenoids, and a subset of volatile metabolites, whereas many additional compounds remain biologically poorly characterized despite growing metabolomic inventories (Contreras-Cornejo et al., 2016; Kubicek et al., 2019; Vicente et al., 2022; Zeilinger et al., 2016b).

2.2. Polyketides

Among polyketides, 6-pentyl- α -pyrone remains the clearest example of a metabolite for which chemical identity, biological activity, and mechanistic interpretation begin to converge. It shows antifungal activity against different plant pathogens and also modulates plant development and defence related signalling, including auxin and ethylene associated responses (Garnica-Vergara et al., 2016; Mendoza-Mendoza et al., 2024). Evidence for improved tolerance to salinity and oxidative stress further supports its broader ecological relevance at the plant interface (Wu et al., 2023). By contrast, other reported polyketides, including koniginins and trichodermatides, clearly expand the chemical repertoire of the genus but remain less integrated into a functional framework, because ecological roles, strain distribution, and translational potential are still less clearly resolved (Peng et al., 2023; Zhou et al., 2023). This imbalance illustrates a recurrent pattern in *Trichoderma* metabolite research, where compound discovery often progresses faster than functional validation.

Table 1
Molecular markers and genomic approaches for taxonomic resolution in *Trichoderma*.

Approach	Resolution	Advantages	Limitations	Applications	Typical relative cost / throughput	Reference
ITS (rDNA spacer)	Low	Standard barcode; large database	Poor resolution in <i>Trichoderma</i>	Preliminary screening	Low cost / high throughput	(Cai and Druzhinina, 2021; Samuels, 2006)
<i>tef1</i> (translation elongation factor 1- α)	Moderate-high	Strong phylogenetic signal	Less extensively represented in public databases than ITS	Species delimitation	Low-moderate cost / high throughput	(Atta et al., 2025; Chaverri et al., 2015)
<i>rpb2</i> (RNA polymerase II subunit)	Moderate-high	Complements <i>tef1</i> ; robust phylogenetics	Less represented in public databases compared to ITS	Resolution of species complexes	Low-moderate cost / high throughput	(Cai and Druzhinina, 2021; Nascimento Brito et al., 2023; Qin and Zhuang, 2016)
Multilocus Sequencing (ITS + <i>tef1</i> + <i>rpb2</i>)	Very high	High resolution for species	More costly and labor- intensive than single- locus analysis	Formal species descriptions	Moderate cost / moderate throughput	(Cai and Druzhinina, 2021; Chaverri et al., 2015; Ye et al., 2024)
Whole-Genome Sequencing (WGS)	Genome-wide	Definitive ID; reveals BGCs and traits	Cost and bioinformatics required	Regulatory approval; comparative genomics	High but decreasing cost / moderate throughput; bioinformatics dependent	(Kubicek et al., 2019, 2011)

Note: Cost and throughput categories are qualitative and relative. They may vary according to sequencing platform, sample number, multiplexing capacity, laboratory infrastructure, database availability, and bioinformatic requirements. WGS, whole-genome sequencing.

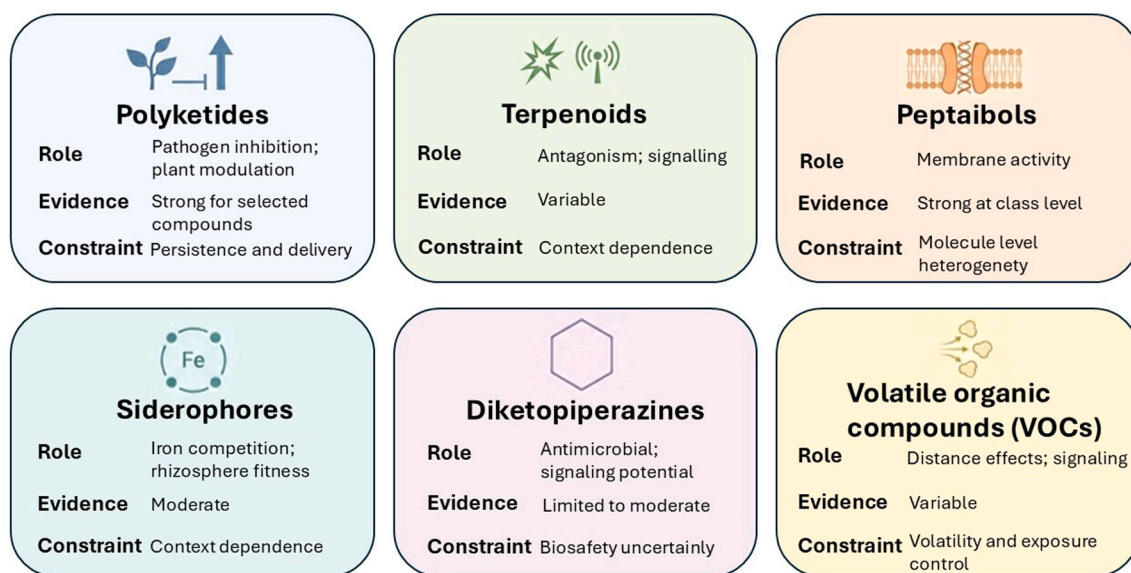


Fig. 2. Major classes of specialized metabolites of *Trichoderma* and their main evidence domains.

2.3. Tetramic acid derivatives

Harzianic acid is a tetramic acid derivative associated with anti-fungal and plant growth-promoting effects. More recent evidence has also identified acetohydroxyacid synthase as a molecular target, further strengthening its mechanistic characterization (Vinale et al., 2009; Xie et al., 2021).

2.4. Terpenoids

Terpenoid metabolism in *Trichoderma* includes metabolites with very different biological implications, ranging from compounds associated with antagonism or signalling to metabolites that raise biosafety concerns. Trichodiene is best viewed as the biosynthetic entry point into trichothecene production and therefore as part of a branch that requires careful biosafety consideration (Shentu et al., 2018; Tijerino et al., 2011b, 2011a). Sesquiterpenes also contribute to signalling functions in plant associated environments, and comparative analyses suggest that volatile terpenoid profiles can be strongly species specific, which is consistent with ecological specialization rather than generic activity across the genus (Gualtieri et al., 2022; Lee et al., 2016). Viridins add a further layer of complexity. Viridin has long been cited as a canonical antifungal metabolite of *Trichoderma*, yet its conversion to viridiol, a compound reported as phytotoxic, shows that metabolite fate and biological outcome cannot be inferred from nominal class assignment alone (Pakora et al., 2021; Vicente et al., 2022). Novel terpenoids such as trichobrasilenol confirm the biosynthetic breadth of the genus, but for many recently described compounds the ecological context remains less clear than the chemical novelty itself (Murai et al., 2019).

2.5. Nonribosomal peptides and related metabolites

Peptaibols are among the best supported metabolite classes in *Trichoderma* because they combine clear structural identity with a well established mode of action. Their membrane permeabilizing activity explains much of their antifungal effect, and recent evidence also supports additional functions, including antibacterial activity against Gram positive bacteria and stimulation of plant physiological processes such as root plasma membrane H⁺ ATPase activity (Bjørk et al., 2023; Marik et al., 2019; Szekeres et al., 2005; Tang et al., 2023; Zhang et al., 2022). At the same time, the class is chemically heterogeneous, and not all peptaibols can be treated as functionally equivalent. Siderophores are

also relevant because they support iron acquisition and competitive fitness in nutrient limited habitats such as the rhizosphere. Available metabolite profiling indicates substantial variability among species and strains, which is consistent with an ecological role in resource competition and root associated fitness (Lehner et al., 2013; Vicente et al., 2022). Diketopiperazines, including epithiodiketopiperazine related compounds reported from *Trichoderma*, are of interest because they may contribute to antimicrobial activity and signalling, but they are also important from a biosafety perspective given that some members of this broader chemical space show cytotoxicity. Here again, the literature is stronger in documenting occurrence than in resolving ecological function under realistic conditions (Vicente et al., 2022; Zhao et al., 2020).

2.6. Volatile organic compounds

Volatile metabolites deserve separate attention because their ecological behaviour differs fundamentally from that of diffusible nonvolatile compounds. In *Trichoderma*, VOC profiles are strongly species dependent and strain dependent, and they are shaped by nutritional conditions, microbial interaction, and other environmental variables (Gualtieri et al., 2022; Guo et al., 2019). Among the VOCs most frequently reported in *Trichoderma* profiles, C8 compounds such as 1-octen-3-ol and 3-octanone are recurrent products of fatty acid metabolism, whereas 6-pentyl- α -pyrone is one of the best-characterized and, in some strains, predominant volatile metabolites. Their relative abundance is not universal, however, and varies according to species, strain, growth medium, developmental stage, and interaction conditions (Gualtieri et al., 2022; Stoppacher et al., 2010). Under controlled conditions, *Trichoderma* VOCs can suppress plant pathogens, stimulate biomass accumulation, and alter chlorophyll related traits in host plants (Elsherbiny et al., 2025, 2020; Joo and Hussein, 2022; Rao et al., 2022). However, the biological interpretation of VOC data requires caution because emission, diffusion, adsorption, and degradation are highly context dependent in soil and rhizosphere systems, which means that activity observed in vitro or in closed systems cannot be transferred directly to open environments (Peñuelas et al., 2014; Tilocca et al., 2020; Werner et al., 2016). Closing this gap will require experimental designs that quantify VOC production, transport, and loss as distinct processes. Dynamic flow-through soil microcosms coupled with proton-transfer-reaction time-of-flight mass spectrometry or GC-MS can provide time-resolved flux measurements while maintaining controlled humidity and gas exchange and avoiding the artificial accumulation of

volatiles that occurs in sealed headspaces (Kim et al., 2021). Comparisons between natural soil and inert matrices, combined with controlled temperature and substrate exposure, can help distinguish physical retention from biological consumption; such approaches have shown that soils may act as net VOC sinks and that microbial uptake can exceed the contribution of physical adsorption for several compounds (Jiao et al., 2023). Stable-isotope probing with ^{13}C -labelled volatiles, coupled with GC-MS and DNA-SIP or related isotope-resolved measurements, can further trace VOC degradation and identify the microorganisms responsible for their consumption in soil (Larke-Mejía et al., 2019). Although these approaches have not yet been applied systematically to *Trichoderma* VOCs, their integration with rhizosphere-relevant microcosms would allow direct estimation of emission, retention, biological transformation, and effective exposure under more realistic conditions. VOCs therefore represent one of the most promising but also one of the most difficult metabolite classes to translate, precisely because ecological range, persistence, and delivery are harder to control than for nonvolatile metabolites. Multitrophic effects, including indirect modulation of plant volatile blends and insect behaviour, further support their ecological importance while also increasing interpretative complexity (Coppola et al., 2019). Representative chemical structures of selected non-volatile metabolites and VOCs discussed in this review are shown in Fig. 3.

2.7. Beyond pathogen suppression

A growing body of literature indicates that specialized metabolites of *Trichoderma* cannot be reduced to direct antagonism alone. At the plant interface, some metabolites modulate hormonal crosstalk, defence priming, and stress related physiology, with downstream effects on salinity tolerance, oxidative stress responses, and root development (Garnica-Vergara et al., 2016; Jiménez-Bremont et al., 2024; Wu et al., 2023). In contaminated environments, low molecular weight chelators and other diffusible metabolites may also contribute to bioremediation related processes by affecting metal availability or by reshaping microbial interactions linked to pollutant turnover (Elhamouly et al., 2022; Vicente et al., 2022). These broader roles are plausible and increasingly supported, but they remain highly dependent on strain identity,

environmental matrix, and experimental design. As a result, they should be interpreted as emerging functional dimensions rather than as uniformly established properties across the genus. This point is central to the evaluation of translational potential, because the metabolites most frequently reported are not necessarily those supported by the strongest ecological evidence.

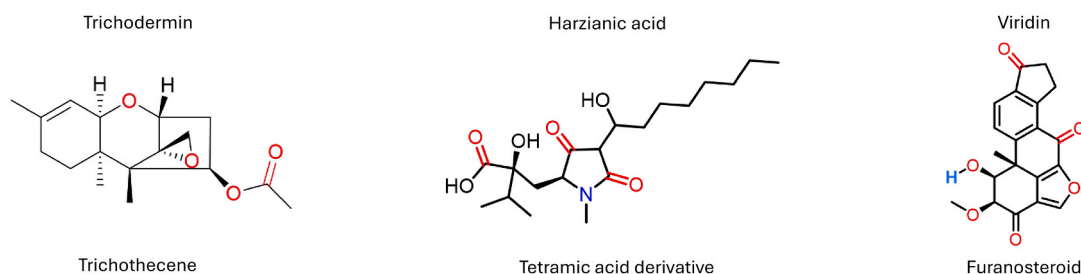
2.8. Gene-metabolite links

Linking defined metabolites to biosynthetic determinants has become one of the central criteria for interpreting the specialized metabolism of *Trichoderma*. This step is more informative than metabolite detection alone because it allows chemical observations to be connected with pathway architecture, regulatory control, and biological function. At the same time, the strength of evidence is not uniform across the literature. In some cases, gene disruption or targeted functional analysis provides a direct connection between a biosynthetic determinant and a defined metabolite. In many others, the relationship remains inferential and is supported mainly by genome mining, transcriptional association, or comparative annotation. Distinguishing between these levels of evidence is essential, because predicted biosynthetic capacity is not equivalent to demonstrated metabolite production or ecological relevance (Kubicek et al., 2019; Vicente et al., 2022; Zeilinger et al., 2016b). Representative examples of gene metabolite associations and their supporting evidence are summarized in Table 2.

Among the best supported examples, *tri5* remains a reference case. This gene encodes trichodiene synthase and catalyses the first committed step in trichothecene biosynthesis. In *Trichoderma*, it underpins the production of trichodermin and related metabolites, providing a clear example of how a biosynthetic gene can be connected to both antifungal activity and biosafety concerns. The importance of this pathway goes beyond a single metabolite because it illustrates how gene level evidence can clarify the dual biological profile of a compound that is active against pathogens but also potentially phytotoxic (Shentu et al., 2018; Tijerino et al., 2011b, 2011a).

A second strong example is represented by the polyketide synthase linked to 6-pentyl- α -pyrone. Functional disruption of *pks1* abolishes 6-pentyl- α -pyrone production, making this one of the clearest gene

A. Representative non-volatile specialized metabolites



B. Representative volatile organic compounds

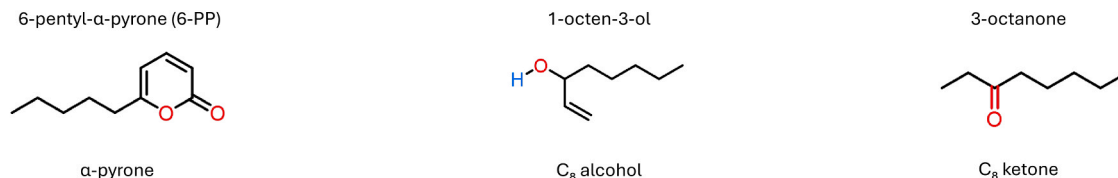


Fig. 3. Representative chemical structures of selected *Trichoderma* specialized metabolites. (A) Non-volatile metabolites: trichodermin, harzianic acid, and viridin. (B) Volatile organic compounds: 6-pentyl- α -pyrone (6-PP), 1-octen-3-ol, and 3-octanone. The selected compounds illustrate the chemical diversity of the specialized metabolites discussed in this review.

Table 2
Representative gene–metabolite associations in *Trichoderma*.

Gene/ Cluster	Metabolite/Class	Evidence type	Ecological or applied role	References
<i>tri5</i>	Trichodermin (trichothecene)	Gene overexpression; metabolite quantification; antimicrobial assays	Antifungal activity; phytotoxicity	(Tijerino et al., 2011b, 2011a)
<i>pks1</i>	6-pentyl- α -pyrone (polyketide)	Gene deletion; metabolite profiling	Antifungal activity; plant growth and defence-related effects	(Flatschacher et al., 2025; Garnica-Vergara et al., 2016; Mendoza-Mendoza et al., 2024)
<i>TaTS9</i>	TaTS9-dependent sesquiterpenes (β -cubenene-dominant profile)	Gene knockout; heterologous expression; enzyme characterization; VOC profiling	Antifungal activity associated with the sesquiterpene profile	(Zhang et al., 2025)
NRPS families	Peptaibols (NRPs, e.g. alamethicin)	Comparative genomics; biochemical assays	Membrane permeabilization; plant stimulation	(Björk et al., 2023; Marik et al., 2019; Szekeres et al., 2005)
<i>pksT-1/</i> <i>pksT-2</i>	Polyketides, pigments	Gene disruption, transcriptomics	Conidial pigmentation; ecological adaptation	(Yao et al., 2016)
<i>TriDTCs</i>	Novel terpenes (from GGPP)	Gene discovery; enzyme characterization	New terpenoids; potential ecological functions	(Murai et al., 2019)

metabolite associations available in the genus (Flatschacher et al., 2025). This is particularly important because 6-pentyl- α -pyrone is also among the few *Trichoderma* metabolites for which biosynthetic support, ecological function, and translational interest begin to converge. It combines antagonistic activity against plant pathogens with plant-related effects such as growth modulation and defence priming, which makes it more informative than compounds known only from occurrence reports (Mendoza-Mendoza et al., 2024; Wu et al., 2023).

Within terpenoid metabolism, *TaTS9* provides another well-supported case. Gene knockout, heterologous expression, enzyme characterization, and VOC profiling showed that *TaTS9* controls a sesquiterpene profile dominated by β -cubenene in *T. afroharzianum* (Zhang et al., 2025). Compared with many volatile metabolites that are known mainly from headspace profiling, the *TaTS9*-dependent sesquiterpene profile is therefore supported by a stronger mechanistic basis. This distinction matters because volatile-metabolite literature is often rich in descriptive profiles but poorer in direct gene-level validation. The *TaTS9* example shows that VOC-related functions can also be anchored to defined biosynthetic determinants when genetic evidence is available.

Peptaibols illustrate a different situation. Their biosynthesis is clearly associated with large nonribosomal peptide synthetases, and their membrane active properties are well established, but the literature often resolves the class better than the individual molecule. In practice, this means that the biological significance of peptaibols is well supported at class level, whereas one to one assignment between a given synthetase architecture and a single ecological function is not always equally precise. This is not a weakness of the field, but a reflection of the intrinsic complexity of modular NRPS systems and of the broad chemical diversity they generate. Even so, peptaibols remain among the metabolite groups for which structural, biochemical, and functional evidence is comparatively strong (Björk et al., 2023; Marik et al., 2019; Szekeres et al., 2005).

Beyond these better resolved examples, genome mining has revealed that sequenced *Trichoderma* genomes contain a much larger biosynthetic repertoire than the set of metabolites that has been functionally validated. Comparative analyses indicate both a conserved core and marked interspecific variation in predicted biosynthetic gene clusters, with mycoparasitic species such as *T. atroviride* and *T. virens* showing broader secondary metabolic potential than *T. reesei* (Bansal and Mukherjee, 2016; Kubicek et al., 2011). More recent studies have reinforced the same picture at strain level, showing that genomes can encode numerous PKS, NRPS, and terpene related clusters for which no direct chemical assignment is yet available. This gap is informative in itself. It indicates that the limiting step is no longer the recognition of biosynthetic potential, but the experimental resolution of which clusters are expressed, under which conditions, and with what ecological consequences (Al-Salihi and Alberti, 2023).

Regulatory and environmental context is equally important because the presence of a biosynthetic gene cluster does not by itself determine whether it is expressed. Specialized metabolism is influenced by global

and pathway-specific regulators, chromatin-level control, nutrient and pH signalling, light, and interaction-dependent cues (Aghcheh et al., 2013; Hou et al., 2024; Keller, 2019; Yu et al., 2023).

Within this broader network, LaeA and the Velvet complex represent important regulatory components, but they do not provide a complete explanation of pathway activation. Their effects interact with pathway-specific regulation and environmental signals that determine whether predicted biosynthetic capacity becomes chemically and ecologically detectable (Bok and Keller, 2004; Keller, 2019; Yu et al., 2023). Consequently, pathway assignment in *Trichoderma* is strongest when genome mining is integrated with transcriptional data, targeted genetics, and metabolomic validation, rather than inferred from sequence annotation alone.

Taken together, the most informative gene metabolite links in *Trichoderma* are not simply those supported by pathway prediction, but those in which genetic evidence, metabolite detection, and biological interpretation converge. This distinction is central to the present literature because it separates a rapidly expanding biosynthetic inventory from the smaller subset of metabolites that can already be discussed with confidence in functional and translational terms.

2.9. Recent discoveries and perspectives

Recent progress in the study of specialized metabolites of *Trichoderma* has not depended only on the description of new compounds. The main advance has been methodological. Comparative genomics, metabolomics, and targeted genetics are increasingly being combined to discriminate between broad biosynthetic potential and the smaller subset of metabolites that can be assigned a clearer biological significance. This shift is important because the literature is no longer limited by the detection of putative clusters, but by the ability to resolve which clusters are expressed, under which conditions, and with what ecological consequences.

A recurrent result across recent studies is that the biosynthetic capacity predicted from genomes exceeds the number of metabolites that have been chemically and functionally resolved. This pattern is evident in strain scale and species scale analyses. In *T. harzianum* T9, integrated genomic and metabolomic analysis identified substantial variation in genes associated with secondary metabolism, highlighted peptaibols as a major axis of predicted chemical diversification, and delineated pathways underlying several candidate metabolites linked to antagonistic potential. In *T. gamsii*, high quality genome analysis of agriculturally relevant strains identified 44 and 45 predicted biosynthetic gene clusters, again showing that genomic potential is broader than the set of metabolites currently validated experimentally. More broadly, recent phenogenomic analysis across *Trichoderma* species has shown pronounced ecophysiological diversification despite genomic cohesion, reinforcing the idea that metabolite related traits should be interpreted at strain or lineage level rather than generalized across the genus (Colou et al., 2026; Steindorff et al., 2026; Vargas-Gasca et al., 2026).

These developments also change how new metabolites should be prioritized. Chemical novelty alone is no longer sufficient. A newly reported compound becomes much more informative when it is placed within a biosynthetic context, supported by comparative cluster analysis, and linked to a measurable phenotype or ecological role. This is especially relevant for metabolite classes such as peptaibols and volatile terpenoids, where structural diversity is large but functional resolution often remains uneven. Recent studies using synteny based cluster comparisons, phylogenomic reconstruction of core biosynthetic genes, and integrated metabologenomics have begun to improve this situation by connecting chemical outputs with evolutionary and genetic frameworks (Vargas-Gasca et al., 2026).

Genome editing and pathway activation strategies remain central to this transition, but they should be framed realistically. In *Trichoderma*, CRISPR based approaches are valuable because they support the validation of candidate biosynthetic genes and improve the analysis of poorly resolved pathways, as shown by recent advances in marker free editing systems and multiplex editing platforms developed in *T. harzianum*, *T. atroviride*, and *T. reesei* (Cortes et al., 2023; Gründlinger et al., 2025; Zhang et al., 2024). However, the main bottleneck is not the availability of editing tools alone. It lies in the integration of genetics with informative cultivation conditions, comparative transcriptomics, and metabolomic readouts capable of revealing condition dependent expression. In this context, OSMAC strategies, co culture approaches, and targeted activation of silent or weakly expressed clusters remain useful not as generic discovery tools, but as ways to test specific biosynthetic hypotheses under biologically meaningful conditions (Brakhage and Schroeckh, 2011; Jin and Alberti, 2025; Wang and Mao, 2025).

The main perspective emerging from recent literature is therefore selective rather than expansive. The field does not primarily need longer metabolite inventories. It needs stronger criteria for identifying which metabolites justify deeper functional validation. Recent comparative and integrated studies indicate that the most informative candidates are not simply those newly detected, but those for which biosynthetic assignment, ecological function, and translational feasibility begin to align (Colou et al., 2026; Steindorff et al., 2026; Vargas-Gasca et al., 2026). This is also the level at which future studies are most likely to advance biocontrol, because specialized metabolism becomes interpretable not only as chemical diversity, but as a source of traits with defined biological and practical relevance (Vicente et al., 2022; Jin and Alberti, 2025).

2.10. Representative case studies

Only a limited number of metabolites reported from *Trichoderma* can be discussed with comparable confidence across biosynthetic basis, biological function, and application related relevance. The three examples examined below were selected to illustrate contrasting evidence profiles rather than as an exhaustive representation of the metabolic diversity of the genus. 6-pentyl- α -pyrone illustrates a metabolite for which genetic support, ecological function, and translational interest begin to converge. Trichodermin represents the opposite situation, where strong antifungal activity coexists with clear biosafety limitations. Peptaibols occupy an intermediate position, because they are among the best established metabolite classes in the genus, yet their functional interpretation often remains clearer at class level than for individual molecules (Björk et al., 2023; Marik et al., 2019; Mendoza-Mendoza et al., 2024; Tijerino et al., 2011b).

2.10.1. 6-pentyl- α -pyrone

Among the specialized metabolites reported from *Trichoderma*, 6-pentyl- α -pyrone is one of the clearest examples of convergence between biosynthetic support, ecological function, and translational interest. Its biosynthesis is linked to *pkS1*, and deletion of this gene abolishes 6-pentyl- α -pyrone production, providing one of the strongest

experimentally validated gene-metabolite associations currently available in the genus (Flatschacher et al., 2025).

The biological profile of 6-pentyl- α -pyrone is unusually broad but still relatively coherent. It inhibits the development of several plant pathogens, including *Fusarium oxysporum*, *Botrytis cinerea*, and *Peronosphythora litchii*, and available evidence indicates that its activity is not limited to a single target process (Garnica-Vergara et al., 2016; Wu et al., 2023). At the same time, it affects plant physiology through pathways associated with root architecture, defence priming, and stress related signalling, which makes it relevant not only as an antifungal compound but also as a mediator at the plant interface (Garnica-Vergara et al., 2016; Mendoza-Mendoza et al., 2024).

Its volatile nature further increases its ecological interest because it allows biological effects without direct contact, but it also introduces an important translational limitation. Emission, persistence, and exposure are much easier to document under controlled conditions than under open environmental settings. For this reason, 6-pentyl- α -pyrone is best viewed not as a fully mature application candidate, but as a benchmark metabolite that defines the level of evidence the field should seek more broadly. It is one of the few cases in which biosynthetic assignment, biological function, and delivery related considerations can already be discussed within the same interpretative frame (Chaparro-Rodríguez et al., 2023; Lobel et al., 2024; Mendoza-Mendoza et al., 2024).

2.10.2. Trichodermin

Trichodermin provides a useful counterexample to metabolites selected mainly for their application potential. It is one of the best known toxic metabolites reported in *Trichoderma* and illustrates how strong antifungal activity does not necessarily translate into practical suitability. Its biosynthesis is anchored to the trichothecene pathway initiated by *tri5*, and additional genes in the cluster, including *tri3*, *tri4*, and *tri11*, are required for production of the final metabolite (Shentu et al., 2018; Tijerino et al., 2011b, 2011a).

From a biological perspective, trichodermin is relevant because it contributes to antagonism against plant pathogens and has helped clarify how trichothecene related pathways operate in *Trichoderma*. At the same time, its phytotoxic profile sharply limits its translational value and makes it a key reference point in biosafety discussions. In this sense, trichodermin is informative not because it represents an ideal biocontrol candidate, but because it shows why metabolite prioritization cannot be based on antifungal potency alone (Barúa et al., 2019; Malmierca et al., 2012).

This case also has broader interpretative value. The uneven distribution of trichothecene biosynthetic clusters across filamentous fungi, including *Fusarium* and *Trichoderma*, indicates that toxic metabolic potential is lineage dependent rather than a generic feature of the genus (Proctor et al., 2009). For strain evaluation, this means that pathway detection and chemical screening remain essential whenever *Trichoderma* isolates are considered for agricultural use.

2.10.3. Peptaibols

Peptaibols represent one of the most robustly supported metabolite classes in *Trichoderma*. Their structural identity, membrane active properties, and antifungal effects are supported by a substantial body of biochemical and functional evidence, making them a strong reference class for discussions of specialized metabolism in the genus (Marik et al., 2019; Szekeres et al., 2005).

Their main strength, however, lies at class level more than at the level of single molecules. The diversity generated by modular NRPS systems means that structurally related peptaibols can differ in length, residue composition, and biological effect. As a result, broad functional statements about peptaibols are often better supported than one to one assignments between a specific molecule and a precisely defined ecological role. This does not weaken their importance. On the contrary, it shows that peptaibols are one of the clearest examples of how biosynthetic architecture shapes functional diversity in *Trichoderma*.

(Björk et al., 2023; Marik et al., 2019).

In ecological terms, peptaibols are relevant not only because they permeabilize fungal membranes, but also because some members of the class display antibacterial activity and can influence plant physiological responses. Their ability to stimulate root plasma membrane H⁺ ATPase activity further supports the view that they operate beyond simple antagonism and participate in a wider set of plant associated interactions (Björk et al., 2023; Tang et al., 2023; Zhang et al., 2022). For this reason, peptaibols are best interpreted as a chemically diverse but biologically coherent class with high explanatory value for the ecology of *Trichoderma*, even when the function of individual molecules remains only partially resolved.

2.11. Operational framework for translational prioritization

To make the proposed framework operational, each metabolite or metabolite class can be evaluated through four threshold questions: (i) has its biosynthetic origin been linked to a defined gene or biosynthetic gene cluster; (ii) has an ecological or biological role been demonstrated using a chemically defined metabolite under conditions relevant to the proposed function; (iii) has the hazard and exposure profile been sufficiently characterized to determine whether biosafety concerns are manageable, unresolved, potentially limiting, or limiting; and (iv) can an active and reproducible exposure be achieved and maintained in a defined use setting? These questions distinguish chemical detection and pathway prediction from mechanistic validation and application-oriented evidence (Flatschacher et al., 2025; Tijerino et al., 2011a; Vicente et al., 2022; Zeilinger et al., 2016a).

Gene-level support is considered strong when targeted genetic manipulation, including gene deletion, complementation, or over-expression associated with a corresponding change in metabolite output, heterologous expression, or direct enzyme characterization establishes a causal gene-metabolite relationship. It is considered intermediate when the association is supported by comparative genomics, transcriptional analysis, or a reproducible correlation between a candidate cluster and metabolite production, and limited when it relies mainly on sequence prediction or chemical occurrence. Ecological or functional evidence is considered strong when a purified or chemically defined metabolite produces a reproducible, preferably dose-dependent phenotype in a biologically relevant system. It is considered intermediate when activity is demonstrated mainly in controlled *in vitro* or closed systems, or through enriched fractions that allow only partial attribution to a defined compound; limited when function is inferred primarily from the producing strain, a crude extract, or metabolite occurrence alone; and not established when no direct functional evidence is available (Flatschacher et al., 2025; Garnica-Vergara et al., 2016; Lombardi et al., 2020; Pascale et al., 2017; Zhang et al., 2025).

Biosafety and delivery require a different interpretation because a large body of evidence does not necessarily indicate a favourable outcome. Biosafety is therefore classified as manageable when adequate hazard and exposure evidence identifies no relevant concern under the proposed conditions of use; unresolved when the available data are insufficient; potentially limiting when credible hazard signals have been identified but their relevance to the proposed exposure remains uncertain; and limiting when an established hazard materially restricts the intended use. Delivery and stability are classified as feasible, context-dependent, or not established according to chemical stability, persistence, dose control, release characteristics, and the availability of a realistic use scenario. These categories are intended for comparative research prioritization and do not replace the formal regulatory risk assessment applicable to microbial active substances or to separately developed metabolite-based products (European Commission, 2024; OECD, 2018). Delivery feasibility additionally depends on the ability to control exposure, persistence, and biological activity in the proposed use setting, particularly for volatile compounds (Elsherbiny et al., 2020; Razo-Belmán et al., 2023).

In the framework proposed here, integration of the four dimensions is sequential rather than additive. Strong evidence in one domain cannot compensate for a critical limitation in another. In particular, genetic and functional validation cannot overcome a limiting biosafety profile, while the absence of a realistic delivery strategy may prevent translation even when the mechanism of action is well supported. An advanced evidence profile is assigned when mechanistic and ecological support is comparatively strong, no potentially limiting or limiting biosafety concern has been identified, and at least one defined use scenario is supported. Candidates with substantial mechanistic or ecological support but unresolved biosafety or delivery constraints are classified as translation limited. Metabolites supported mainly by chemical detection, predicted biosynthetic capacity, or preliminary activity assays remain at an early evidence stage, whereas compounds presenting potentially limiting or limiting biosafety concerns are assigned low translational suitability. These categories indicate relative research and development priorities rather than application readiness. The comparative application of this framework to representative *Trichoderma* metabolites and metabolite classes is provided in Table 3, while Table 2 retains its specific focus on experimentally supported gene-metabolite associations.

3. Translational evidence and biosafety

3.1. Translational evidence beyond controlled systems

Most evidence beyond controlled systems still derives from living *Trichoderma* inocula rather than from chemically defined metabolite based treatments. This distinction is critical for interpretation. In strain based applications, disease suppression and plant responses reflect the combined effects of colonisation, competition, induction of host responses, and *in situ* metabolite production, so efficacy cannot be attributed directly to a specific metabolite even when metabolite mediated mechanisms are biologically plausible (Tyśkiewicz et al., 2022; Woo et al., 2023).

By contrast, translational evidence obtained with purified metabolites, enriched fractions, crude extracts, or volatile preparations remains comparatively limited and is concentrated mainly in controlled, confined, or postharvest systems. A few studies provide encouraging signals, including responses of grapevine and strawberry systems to metabolite based treatments, but these examples remain too few to support broad generalization across biological contexts (Lombardi et al., 2020; Pascale et al., 2017). The main conclusion from the available literature is therefore not that metabolite based strategies are ineffective, but that their translational evidence base remains much narrower than that of living inocula. This asymmetry has an important consequence for the field. It means that metabolite discovery continues to outpace application level validation, especially under open environmental conditions where persistence, exposure, and delivery become limiting variables (Elsherbiny et al., 2025; Razo-Belmán et al., 2023).

Accordingly, evidence from greenhouse and field studies is most informative when used as a boundary condition rather than as direct proof of metabolite function. It clarifies where translation is currently strongest, where it remains preliminary, and why the gap between mechanistic promise and practical use is still substantial.

3.2. Biosafety considerations

Biosafety evaluation of *Trichoderma* strains intended for agricultural use cannot be generalized at genus level and requires strain specific assessment. Current regulatory practice increasingly relies on precise taxonomic resolution, genome informed identification, and targeted evaluation of traits potentially relevant to safety, including genes associated with pathogenicity, antimicrobial resistance, or the biosynthesis of toxic metabolites (EFSA, 2022; European Commission, 2024; OECD, 2018).

Table 3

Comparative evidence matrix for the translational prioritization of representative *Trichoderma* metabolites and metabolite classes based on gene/BGC support, ecological and functional evidence, biosafety, delivery and stability, and overall translational status.

Metabolite or class	Gene/BGC support	Ecological or functional evidence	Biosafety	Delivery/stability	Translational status	References
6-Pentyl- α -pyrone	Strong	Strong	Unresolved	Context dependent	Advanced evidence profile	(Flatschacher et al., 2025; Garnica-Vergara et al., 2016; Mendoza-Mendoza et al., 2024; Pascale et al., 2017)
Trichodermin	Strong	Strong	Limiting	Not established	Low translational suitability	(Malmierca et al., 2012; Tijerino et al., 2011a, 2011b)
Peptaibols	Intermediate (class level)	Strong (class level)	Unresolved	Not established	Translation limited	(Björk et al., 2023; Marik et al., 2019; Zhang et al., 2022)
Harzianic acid	Strong	Intermediate	Unresolved	Not established	Translation limited	(Lombardi et al., 2020; Vinale et al., 2009; Xie et al., 2021)
TaTS9-dependent sesquiterpenes (β -cubenene-dominant profile)	Strong	Intermediate	Unresolved	Not established	Early evidence stage	(Zhang et al., 2025)
Viridin/viridiol	Strong	Intermediate	Potentially limiting	Not established	Low translational suitability	(Bansal et al., 2018; Pakora et al., 2021)
Koninginins	Limited	Intermediate	Unresolved	Not established	Early evidence stage	(Dunlop et al., 1989; Peng et al., 2023; Zhou et al., 2023)
Trichodermatides	Limited	Not established	Unresolved	Not established	Early evidence stage	(Sun et al., 2008; Zhou et al., 2023)
Siderophores	Intermediate (class level)	Intermediate	Unresolved	Not established	Translation limited	(Kubicek et al., 2011; Lehner et al., 2013)
Diketopiperazines and related epithiodiketopiperazines	Limited	Limited	Unresolved	Not established	Early evidence stage	(Vicente et al., 2022; Zhao et al., 2020)

Note: Evidence categories represent the Authors' qualitative appraisal of the type and strength of the available evidence and should not be interpreted as validated regulatory scores or quantitative efficacy rankings. Strong evidence indicates causal genetic validation or reproducible activity of chemically defined metabolites in biologically relevant systems; intermediate evidence indicates converging but incomplete support; limited evidence refers mainly to pathway prediction, chemical occurrence, strain-level inference, or preliminary controlled assays; and not established indicates that direct supporting evidence is currently lacking. Biosafety categories indicate whether concerns are unresolved, potentially limiting, or limiting, whereas delivery and stability categories reflect the availability of realistic exposure and application strategies. Parenthetical qualifications indicate that the assessment applies primarily at metabolite-class rather than individual-compound level. BGC, biosynthetic gene cluster.

Among specialized metabolites, trichothecenes produced by *T. brevicompactum*, including trichodermin, remain the clearest biosafety concern discussed in the literature (Shentu et al., 2018; Tijerino et al., 2011a). However, current European Union practice does not require the exhaustive untargeted characterization of every metabolite potentially produced by a candidate strain. Instead, it follows a stepwise weight-of-evidence approach combining literature on the strain and closely related taxa, toxicological and ecotoxicological findings, whole-genome sequence interrogation for known toxin and relevant antimicrobial determinants, and analytical testing of the microbial pest control agent as manufactured or of the formulated product when metabolites of potential concern are identified. If hazardous effects cannot be attributed to known compounds, the responsible unknown metabolite or metabolites should be identified using appropriate analytical approaches (European Commission, 2024; OECD, 2018). Within this framework, untargeted LC-HRMS may be considered as a complementary screening approach for novel or poorly characterized strains or when toxicological effects remain unexplained. However, current European Union guidance does not prescribe untargeted metabolomics as a universal requirement; instead, it follows a stepwise approach based on the identification and analytical characterization of metabolites of potential concern, with further identification required when hazardous effects cannot be attributed to known compounds (European Commission, 2024; OECD, 2018). Genome mining and chemical analysis should therefore be interpreted together, because the detection of a biosynthetic gene cluster indicates biosynthetic potential but does not establish metabolite production under manufacturing, storage, or representative use conditions (European Commission, 2024; Kubicek et al., 2019; Vicente et al., 2022). A similar caution applies to other potentially harmful metabolites reported in some *Trichoderma* strains, including gliotoxin in *T. virens* (European Commission, 2024; OECD, 2018; Vicente et al., 2022; Zaid et al., 2022).

An additional point that should not be overlooked is that *Trichoderma*

is not invariably beneficial in all biological contexts. Reports of ear rot associated with *T. afroharzianum* in maize in Europe and reports involving *T. asperellum* in China indicate that host range and opportunistic pathogenicity deserve explicit consideration in biosafety assessment, especially for strains intended for cereal systems (Pallavi et al., 2025; Pfordt et al., 2023, 2020; Trillas et al., 2024; Wang et al., 2025). These cases do not negate the value of *Trichoderma* in agriculture, but they show clearly that benefits cannot be extrapolated across strains or host systems without appropriate evidence.

At present, no specific metabolite, including any non-trichothecene compound, has been causally linked to the pathogenicity of *T. afroharzianum* on maize. Experimental studies have confirmed that aggressive isolates can infect maize ears without previous mechanical wounding, while a recent comparison of isolates from plants, soil, and commercial biocontrol products revealed marked strain-level heterogeneity and no clear separation between pathogenic and non-pathogenic isolates based on *tef1* and *rpb2* phylogeny (Pfordt et al., 2025, 2020). These findings indicate that species identity or the presence of a single known biosynthetic gene cluster cannot currently be used as a proxy for virulence. Secreted cell-wall-degrading enzymes, effector-like proteins, transport and detoxification systems, and condition-dependent specialized metabolites represent plausible candidate determinants based on the broader interaction biology of *Trichoderma*, but their contribution to maize infection remains unproven (Kubicek et al., 2011). Resolving this question will require comparative genomics and infection-stage transcriptomics and metabolomics of pathogenic and non-pathogenic isolates, followed by targeted genetic and chemical validation. Until such evidence becomes available, pathogenicity should be regarded as a strain-specific phenotype rather than attributed to a defined metabolite profile.

Within regulatory dossiers, the most relevant evidence usually includes strain identity confirmation, screening for relevant metabolites, mammalian toxicology and ecotoxicology data, and information on

environmental fate and persistence under representative conditions of use (EFSA, 2022; EPA, 2024; European Commission, 2024, 2023; OECD, 2018). Co formulants and carriers may further influence exposure profiles, which means that product safety cannot be inferred solely from the microorganism or metabolite considered in isolation (European Commission, 2021, 2024).

4. Production and delivery constraints for metabolite based applications

4.1. Delivery, stability, and quality control

For metabolite based applications, delivery and stability are among the main determinants of biological relevance outside controlled conditions. Unlike living propagules, purified metabolites, enriched fractions, and volatile preparations do not benefit from self renewal or adaptive establishment in the target environment. Their performance therefore depends directly on formulation, persistence, release profile, and exposure control (Chaparro-Rodríguez et al., 2023; Lee et al., 2017; Moo-Koh et al., 2022; Mutawila et al., 2016; Staropoli et al., 2023).

These constraints differ across product types. Culture filtrates and crude extracts pose challenges of compositional reproducibility, whereas purified metabolites offer clearer dose definition but may be limited by photodegradation, volatility, or rapid dissipation. VOC based strategies are especially sensitive to confinement, flux, and persistence. For this reason, downstream processing, encapsulation, and controlled release systems are not secondary technical details, but central translational variables. At the same time, robust field scale validation remains limited for most of these approaches. Table 4 summarizes the main metabolite oriented product types, delivery concepts, and quality-control readouts discussed in the literature (Chaparro-Rodríguez et al., 2023; Lee et al., 2017; Mutawila et al., 2016; Razo-Belmán et al., 2023).

4.2. Metabolite-based versus strain-based strategies: advantages and translational bottlenecks

Living *Trichoderma* inocula and metabolite-based products should not be treated as biologically equivalent strategies. Living strains can colonise plant-associated habitats, adapt to local conditions, renew their biomass, and combine multiple mechanisms, including competition, mycoparasitism, induction of host responses, and *in situ* metabolite production. These advantages are accompanied by dependence on successful establishment and by context-dependent performance associated with the strain, formulation, crop, resident microbiome, and environmental conditions (Tyśkiewicz et al., 2022; Woo et al., 2023).

Metabolite-based strategies offer different advantages. Chemically

defined preparations permit more precise dose standardisation, clearer attribution of biological effects, and quality control based on chemical identity, purity, concentration, and stability. They can also deliver biological activity without requiring the establishment of a living microorganism. However, these advantages do not automatically facilitate practical development, because purified metabolites, enriched fractions, and VOC preparations may be limited by production costs, compositional variability, photodegradation, volatility, rapid dissipation, and loss of effective exposure (Chaparro-Rodríguez et al., 2023; Lee et al., 2017; Razo-Belmán et al., 2023; Staropoli et al., 2023).

These bottlenecks can be addressed only by matching the physico-chemical properties of the candidate to the intended use scenario. Priority approaches include scalable fermentation and downstream processing, defined quality-control specifications, protective encapsulation or stabilising carriers, controlled-release systems, and application in settings where exposure can be managed more effectively, such as postharvest, protected, confined, or other targeted treatments. Development for open environmental conditions will additionally require direct measurement of persistence, release, effective dose, and biological activity under representative conditions. The relevant question is therefore not whether metabolites are intrinsically superior to living strains, but which product type is better suited to a defined biological and application context (Chaparro-Rodríguez et al., 2023; Elsherbiny et al., 2020; Lobel et al., 2024; Razo-Belmán et al., 2023; Staropoli et al., 2023).

5. Conclusions

The literature on specialized metabolites of *Trichoderma* has expanded rapidly, but this expansion has been uneven. Chemical diversity is increasing faster than the number of metabolites that can be interpreted with confidence in terms of biosynthetic origin, ecological role, biosafety profile, and realistic application potential. This asymmetry explains why metabolite catalogues continue to grow while translational progress remains more selective. It also indicates that metabolite discovery, by itself, is no longer the main limitation in the field.

A more informative framework emerges when metabolites are evaluated according to the level of convergence among four dimensions: gene metabolite support, functional evidence, biosafety considerations, and delivery feasibility. From this perspective, only a limited subset of *Trichoderma* metabolites currently approaches translational relevance. 6-pentyl- α -pyrone represents one of the clearest positive examples, whereas trichodermin illustrates why antifungal potency alone is not a sufficient criterion for prioritization. Peptaibols further show that high biological relevance may coexist with substantial internal complexity, so

Table 4
Metabolite based product types and delivery concepts for *Trichoderma* metabolites.

Product type	Typical downstream step(s)	Formulation goal / main constraint	Delivery concept / use setting	Quality-control readout	References
Culture filtrate (supernatant)	Biomass separation; filtration/ centrifugation.	Reduce matrix effects; ensure reproducible chemical profile	Spray/drench in controlled assays; sometimes postharvest	Chemical fingerprint (LC-MS/ GC-MS); marker peaks	(Lee et al., 2017; Mutawila et al., 2016)
Crude extract (solvent extract)	Solvent extraction; solvent removal	Stability and handling; standardize composition	Dip/spray; mainly postharvest or controlled assays	Targeted markers plus fingerprint	(Lee et al., 2017; Moo-Koh et al., 2022; Staropoli et al., 2023)
Enriched fraction (bioactive fraction)	Fractionation/ enrichment	Increase consistency; concentrate bioactivity; reduce variability	Controlled trials; niche applications	Fraction profile plus bioassay-guided consistency	(Moo-Koh et al., 2022; Mutawila et al., 2016)
Purified metabolite (e. g., 6-PP, peptaibols)	Purification (compound isolation)	Dose standardization; mitigate photodegradation or rapid dissipation	Encapsulation/controlled release; targeted uses	Purity, stability, and release profile	(Chaparro-Rodríguez et al., 2023; Lee et al., 2017)
VOCs (headspace/ mycofumigation)	In situ generation; headspace exposure (capture if needed)	High volatility; control exposure and persistence	Mycofumigation; sealed/ controlled atmosphere; slow-release concepts	VOC profile (HS- SPME/GC-MS); exposure time/flux	(Contreras-Cornejo et al., 2014; Elsherbiny et al., 2020; Razo-Belmán et al., 2023)

that interpretation is often stronger at class level than at the level of individual molecules.

This view also helps clarify the relationship between metabolite based and strain based strategies. Living *Trichoderma* inocula have progressed further in agriculture because they operate through ecologically integrated mechanisms in which metabolite production is embedded within colonisation, competition, and host responses. By contrast, metabolite based approaches offer clearer mechanistic interpretation and better dose definition, but they remain more constrained by persistence, release control, and exposure under open environmental conditions. For this reason, the most immediate opportunities for metabolite oriented applications are likely to arise in settings where delivery can be controlled more effectively, including postharvest, confined, or otherwise targeted use scenarios.

Overall, the field now needs stronger prioritization rather than broader enumeration. The most useful future studies will be those that connect biosynthetic gene clusters, condition dependent expression, metabolite detection, and ecologically meaningful phenotypes within the same experimental framework. In this way, specialized metabolism in *Trichoderma* can be assessed not simply as chemical diversity, but as a set of biologically interpretable traits with defined translational relevance.

Based on the evidence reviewed, five priorities should guide *Trichoderma* specialized metabolite research over the next five years: (i) establishing causal gene-metabolite relationships through targeted genetic and biochemical validation; (ii) confirming metabolite functions under ecologically realistic conditions; (iii) integrating strain-specific biosafety assessment at an early stage; (iv) evaluating formulation, delivery, stability, and exposure during candidate selection; and (v) standardizing the reporting of strain identity, production conditions, analytical confidence, active dose, experimental matrix, and negative results.

CRedit authorship contribution statement

Mario Riolo: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Santa Olga Cacciola:** Writing – review & editing, Validation, Resources, Project administration, Funding acquisition, Conceptualization.

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