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Chapter 3

Secondary Metabolites of Mycoparasitic Fungi

Verena Speckbacher and Susanne Zeilinger

Additional information is available at the end of the chapter

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Abstract

Mycoparasitic fungi, fungi preying on other fungal species, are prolific producers of volatile and non-volatile secondary metabolites. Several secondary metabolites are produced during mycoparasitism to weaken the host and support attack and parasitism. Further, evidence accumulated that some secondary metabolites also act as communication molecules. Besides their antagonistic activity, several fungal mycoparasites exhibit beneficial effects on plants and some of their secondary metabolites have plant growth-promoting and defense stimulating activities. As many secondary metabolism-associated gene clusters remain silent under standard laboratory conditions, the full variety as well as the underlying biosynthetic pathways employed by fungal mycoparasites for secondary metabolite production still await clarification. Nonetheless, the variety of currently known secondary metabolites and their range of activities is impressive already and they exhibit a great potential for agriculture, pharmacology and other industrial applications.

Keywords: secondary metabolites, volatile organic compounds (VOCs), peptaibiotics, mycoparasitism, biocontrol

1. Introduction

Mycoparasitic relationships, where a predatory fungal species gains nutrients on the expense of a host fungus, are widespread within the fungal kingdom. By the modalities of this non-mutual relationship, biotrophic and necrotrophic mycoparasitic fungi with different gradations within this classification (contact, invasive or intracellular necrotrophic; haustorial or fusion biotrophic) can be distinguished [1]. Biotrophic mycoparasites co-exist and nourish on their living host in a balanced way and are specifically adapted to one or few host species. In contrast, necrotrophic fungi destructively invade and kill a broad range of hosts to gain

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nutrients from the remains of their prey [2]. Mycoparasitic fungi are prolific producers of a plethora of volatile and non-volatile secondary metabolites, favoring their ecological fitness and survival under certain environmental conditions. For example, the excretion of siderophores – affecting high affinity iron chelation – is strongly up-regulated under iron-limiting conditions [3] and several antimicrobial metabolites empower the successful perseverance within the ecological niche [4]. The mycoparasitic lifestyle obviously substantiates the over-representation of secondary metabolism-associated genes and the extensive excretion of a variety of secondary metabolites [2] enabling the fungus´ successful access to its prey as well as its thriving persistence in or assassination of the host. Furthermore, selected fungal secondary metabolites are known to exhibit beneficial effects on plants: They may promote vitality and growth of roots and shoots, enhance the resilience against abiotic stress factors and prime the plants immune system (induced systemic resistance; ISR) thereby enhancing its resistance and survival in case of prospective infections with pathogens [5]. In recent times, evidence accumulated that some secondary metabolites also act as communication molecules over species boundaries [6, 7].

A great diversity of mycoparasitic species exists in the fungal kingdom, especially within the order *Hypocreales* [8]. In this aspect *Trichoderma* (teleomorph *Hypocrea*), a worldwide abundant, diverse fungal genus, is one of the best-studied examples [2]. *Trichoderma* comprises necrotrophic mycoparasitic species like *Trichoderma atroviride* or *Trichoderma virens*, which are successfully applied in agriculture as biocontrol agents against plant pathogenic fungi of crop plants. Further they are reported to promote plant growth, vitality and systemic resistance via priming the plants' immune system. The genomes of several *Trichoderma* species have been sequenced and analyzed revealing the ancestral mycoparasitic lifestyle of these fungi [9, 10]. The second largest lineage of mycorparasites within the *Hypocreales* is the genus *Tolypocladium*. *Tolypocladium* comprises, besides of some entomopathogenic species like *Tolypocladium inflatum,* mostly mycoparasitic species like the widespread on northern hemisphere *Tolypocladium ophioglossoides* which mycoparasitizes with a narrow host range on truffles of the genus *Elaphomyces* [11, 12]. In contrast to *Trichoderma*, the genus *Tolypocladium* exhibits an ancestral entomopathogenic lifestyle and developed to a mycoparasite by host jumping. The genomes of *Tolypocladium* species are rich in secondary metabolite gene clusters of which some, like the clusters for the production of peptaibiotics, seem to be exclusive to mycoparisitic lineages [12]. A further well-investigated mycoparasitic fungus is *Escovopsis weberi*. *E. weberi* is a contact necrotrophic mycoparasite on *Leucoagaricus* sp. in leaf-cutting ant agriculture [13]. As foraging for leaves causes considerable economic damages in neotropic agriculture by defoliation of a wide variety of crop plants, *E. weberi* would be a suitable biocontrol agent as it causes a breakdown of the fungal feeding structures, thereby starving out the ant colony leading to a collapse of the whole system [14]. Like other very specialized mycoparasites, the *E. weberi* genome exhibits a reduced size and content, but very unique secondary metabolite clusters for host attack, facilitating the excretion of fungicidal substances which can break down the host mycelia even without contact [15, 16]. Further examples of secondary metabolite analysis on mycoparasitic species include *Stachybotrys elegans,* a potential biocontrol agent against plant

pathogenic *Rhizoctonia solani* [17] as well as *Coniothyrium minitans* and *Microsphaeropsis ochracea* co-culture antagonizing the plant pathogenic *Sclerotinia sclerotiorum* [18].

A characteristic trait of filamentous fungi is that their secondary metabolism-associated genes are mostly situated within subtelomeric regions of the chromosomes in large biosynthetic gene clusters present in the genomes in significantly greater numbers than secondary metabolites currently identified [19]. The unique and often uncommon biosynthetic pathways are mostly characterized by signature enzymes, often also transcription factors and transporters present in the respective gene clusters, which enable the secondary metabolite synthesis starting from simple precursors gained from primary metabolism like amino acids and acetyl-CoA [20]. Most common core enzymes are non-ribosomal peptide synthases (NRPSs), polyketide synthases (PKSs) and terpene-synthases or -cyclases [4]. In necrotrophic mycoparasitic species like *T. atroviride* and *T. virens,* genes for the biosynthesis of secondary metabolites are enriched compared to the only weakly mycoparasitic relative *Trichoderma reesei*. Within the two mentioned strong mycoparasites nearly half of all secondary metabolism-associated genes are positioned on non-syntenic regions in the genome and do not exhibit orthologs in the respective other species [21]. Depending on the species, environmental conditions, and even the strain, a plethora of different compounds is derived by the genus *Trichoderma* [2, 4], awakening hope on the detection of new substances. Since resistance development to the currently applied substances constitutes an increasing problem in agriculture and medicine, the need for environmentally sustainable biological control of pathogens and the discovery of novel antagonistic substances is essential. Secondary metabolites of mycoparasitic fungi could contribute to the solution.

2. Non-ribosomal peptides

Non-ribosomal peptides (NRPs) are synthesized by NRPSs, enzymes that characteristically consist of multiple domains synthesizing the peptide in one by one steps. Characteristic for NRPSs are the core domains for adenylation, thiolation and condensation. The generated NRPs are very diverse: they mostly comprise of proteinogenic and non-proteinogenic amino acids, can be linear or branched to cyclic with a varying length. After their synthesis outside of the ribosome, they frequently pass extensive secondary modifications. Many fungal NRPs have high economic and/or ecologic value like β-lactam antibiotics, the immunosuppressant cyclosporine A but also mycotoxins like gliotoxin.

The occurrence of NRPS genes is enriched within the genome of mycoparasitic *T. atroviride* for 60% and in *T. virens* for 180% to the wood-degrading *T. reesei* [21]. Further, the functional involvement of NRPS and PKS in the mycoparasitic interaction was supported by deletion experiments of the *T. virens* 4-phosphopanteteinyl transferase-encoding gene (*ppt*) – which is essential for NRPs and PKSs activation – resulting in mutant strains defective in mycoparasitism and induction of systemic resistance (ISR) in plants [22]. The main NRPSs derived metabolites in *Trichoderma* species are peptaibiotics, epipolythiodioxypiperazines and siderophores.

2.1. Peptaibiotics

Peptaibiotics are mostly linear to rarely cyclic polypeptides, with a size of 0.5–2.1 kDa consisting of 4–21 residues. Characteristic for peptaibiotics is the inclusion of the non-proteinogenic amino acid α-aminobutyric acid (Aib). By module-skipping one NRPS is frequently capable of synthesizing a whole set of peptaibiotics [23, 24]. According to their sequence alignment and structure, peptaibiotics can be divided into several sub-clades: peptaibols, lipopeptaibols, lipoaminopeptides, cyclic peptaibiotics and two very special, small categories [25]. Because of their unusual synthesis and appearance, they are not included in regular protein databases, but in the "Comprehensive Peptaibiotics Database" [25].

Peptaibols are solely described for filamentous fungi exhibiting a mycoparasitic lifestyle, with a high abundance of over 80% of all known substances being derived from *Trichoderma* species [25]. Peptaibols are linear peptides, which besides of Aib include a characteristic acetylation of the N-terminus and a 1.2-amino-alcohol at the C-terminus. The first peptaibols, suzukacillin and alamethicin, have been described in the 1960s [26]. As demonstrated for alamethicin, the amphipathic character of peptaibols allows the voltage-dependent formation of helical structures acting as ion channels, thus spanning and permeabilizing the cell membrane and leading to cytoplasmic leakage and cellular breakdown [27].

Whereas all *Trichoderma* strains produce peptaibols, some substances are synthesized in a species- or even strain-specific manner [25]. For instance, in five different biocontrol agents containing species from the *Trichoderma harzianum* complex, peptaibols were the dominant secondary metabolites including three new and recurrent major groups present in all formulations [28]. Peptaibols play an important role in the mycoparasitic interactions as well as in induction of ISR in plants via up-regulation of the jasmonic acid and salicylic acid synthesis [29, 30]. In *T. harzianum*, peptaibols synergistically act together with hydrolytic, cell wall degrading enzymes on the cell wall destruction of the host fungus [31, 32]. Other mycoparasites such as *T. ophioglossoides* and *E. weberi* also comprise peptaibiotics-associated gene clusters, which are absent in plant- and entomopathogenic lineages of *Hypocreales,* suggesting the restriction of these genes to mycoparasitic species, further indicating their importance in the mycoparasitic interaction [11, 15]. The antifungal activity of the peptaibol trichokonin from *Trichoderma pseudokongii* caused extensive apoptosis by loss of the mitochondrial transmembrane potential resulting in apoptotic cell death in *Fusarium oxysporum* [33]. Similar evidences suggest a major involvement of peptaibiotics in mycoparasitism, substantiated by reports of antifungal action of peptaibols secreted by *Clonostachys rosea* against *S. sclerotiorum* [34], or *Sepedonium tulasneanum* against *Botrytis cinerea* and *Phytophthora infestans* [35].

2.2. Epipolythiodioxypiperazines

Epipolythiodioxypiperazines (ETPs) are characterized by the presence of an inter- or intramolecular disulfide bridge and a diketopiperazine core. The toxicity of ETPs lies in the disulfide bridging which is facilitating the inactivation of proteins by conjugation and the elicitation of reactive oxygen species (ROS) [36]. The best known substance of this class is gliotoxin derived

from *T. virens* Q-strains [37]. *T. virens* P-strains antagonize *Pythium ultimum* and do not produce gliotoxin but the terpenoid gliovirin, whereas Q-strains affect *R. solani* but not vice versa [38]. *C. rosea* also produces ETPs like verticillin A and gliocladines involved in the antagonism on nematodes [39] and glioperazine exhibiting antibacterial properties [40]. Whereas the role of gliotoxin (cluster comprising of 12 genes) as a virulence factor in human *Aspergillus fumigatus* infections and the self-protection via the *gliT* gene product of the biosynthetic gliotoxin cluster is well investigated, there is little and partially adverse information on the role of ETPs in biocontrol [36].

The weak mycoparasitic *T. reesei* exhibits an incomplete gliotoxin cluster whose genes were not expressed during confrontation with *R. solani* [4], whereas highly mycoparasitic *T. atroviride* does not contain a gliotoxin cluster [2]. The gliotoxin gene cluster of *T. virens* Q-strains consists of eight genes encoding the core enzyme *gliP* – an NRPS dioxypiperazine synthase – whose expression was induced during confrontation with *R. solani* [4]. Deletion of *gliP* resulted in gliotoxin production-deficient mutants, going hand in hand with a significantly reduced induction of ISR in cotton seedlings and antagonistic action against *P. ultimum* and *S. sclerotiorum*. Adversely, the mutants' antifungal activity against *R. solani* remained unaltered [41]. The involvement of ETPs in mycoparasitic interactions stays unresolved and seems to depend on the combination of several – largely unknown – factors like synergistic interactions with other metabolites or enzymes [42], environmental conditions, the species, strain and even the host organism.

2.3. Siderophores

Siderophores of fungal origin are high affinity iron chelating, linear to cylic oligomeric secondary metabolites mostly characterized by a N5-acyl-N5-hydroxyornithine basic unit [3]. Several siderophores are derived by one NRPS and post-synthetic subsequent modification [43]. As bio-available iron is rare in natural habitats, but an essential trace element to most organisms, efficient chelation, uptake and storage mechanisms for iron play an important role in competition and perseverance, especially within dense microbial communities like in soil [44]. Siderophores are important metabolites in the response against oxidative stress in several fungi like *Aspergillus nidulans*, *A. fumigatus*, *Cochliobolus heterostrophus*, *Gibberella zeae* and *T. virens;* furthermore, they play a role in conidial germination and sexual development [4, 45–47].

Evidences accumulate that siderophores act in biocontrol as virulence factors against other microbes during iron competition. Further, they promote plant growth by the reduction of oxidative stress: in biocontrol of *Fusarium* wilt disease by *Trichoderma asperellum* strain T34, the tomato plants exhibited reduced numbers of infected roots and a decrease in iron-associated abiotic stress [48]. The over-representation of siderophores in *Trichoderma hamatum* strain GD12 was reported to beneficially influence the biocontrol of *S. sclerotiorum* and plant growth promotion in lettuce [49]. More direct evidence for an involvement of siderophores in mycoparasitic interactions exists in *C. minitans:* the expression of *CmSIT1,* a gene-mediating siderophore-iron transport, not only enhanced antifungal abilities but also reduced growth [50].

3. Polyketides (PKs)

Polyketides (PKs) are derived from simple building blocks like acetyl-CoA or malonyl-CoA via consecutive PKS-mediated decarboxylative condensation and subsequent post-synthetic modification. Fungal PKSs are complex multi-modular enzymes, which obligatory include a characteristic ketoacyl-CoA-synthase (KS), an acyltransferase (AT) and an acyl-carrier (ACP) domain [20]. The structurally diverse PKs are the main class of secondary metabolites derived from fungi. The spectrum of substances ranges from spore pigments over antibiotics to toxins [2].

The *T. virens* and *T. atroviride* genomes are enriched for about 60% in PKS genes compared to *T. reesei* [21]. The *C. rosea* genome even exceeds this number with a total of 31 PKS genes [51], whereas the *T. ophioglossoides* genome comprises 15 PKSs [11]. The TMC-151 type PKs derived from *C. rosea* exhibits antibacterial properties [52], whereas *T. ophioglossoides* produces two antifungal and antibacterial substances: the polyketide ophiocordin and the NRPS-PKS hybrid enzyme-derived ophiosetin [11, 53, 54]. The deletion of *pks4* – encoding an orthologue of the aurofusarin and bikerfusarin forming PKSs of *Fusarium* spp. – in *T. reesei* caused extensive changes in morphology as well as physiology and metabolism. In Δ*pks4* mutants, the pigmentation of conidia and the generation of teleomorph structures were inhibited, and the stability of the conidial cell wall was reduced. *Pks4* deletion decreased *T. reesei*'s antagonistic abilities in confrontation assays, lowered its antifungal effect mediated by water soluble and volatile metabolites and altered the expression pattern of other PKSs [55]. It seems that also within this metabolite class, the effects are more diverse and global, than hitherto expected.

4. Terpenoids

Terpenoids are synthetized from the acetyl CoA-derived C-5-isopentenyl-diphosphate intermediates isopentenyl- and dimethylallyl-diphosphate. The C-5 units are subsequently processed via head-to-tail condensation by prenyl synthases and are post-synthetically modified by various enzymes resulting in different terpenoids originating from very few C-5 precursors [56]. Terpenoid biosynthetic clusters are characterized by the presence of a terpene cyclase gene [4]. Terpenoids are volatile to non-volatile substances constituting the highest abundant natural products on earth [37]. Terpenoids of fungal origin comprise phytohormones, mycotoxins as well as antibiotics and antitumor substances.

The *C. rosea* genome contains eight terpene synthase genes [51] and *E. weberi* comprises an expansion of six genes for terpene synthases in its genome, of which three lie within unique biosynthetic secondary metabolite clusters [15]. The majority of secondary metabolites of *S. elegans* secreted during mycoparasitizing *R. solani* are trichothezenes and atranones belong to the terpenoid class of secondary metabolites [17]. The *T. virens* genome comprises an enrichment of terpene cyclase genes compared to *T. atroviride* and *T. reesei* [57]. The production of several terpenoids was proven for *Trichoderma* species [58, 59], whereas their biosynthetic pathways mostly still remain obscure. The putative terpene cyclase *vir4* is well-researched

and only present in *T. virens,* but not in *T. reesei* or *T. atroviride*. Analysis of a mutant which exhibited defects in antibiotic production, a lack of viridin and viridiol synthesis and an under-expression of most of the genes of the *vir4* cluster evidenced that the cluster is involved in viridin biosynthesis [60]. Generation of a *vir4* deletion mutant and metabolic screening validated its involvement in terpene biosynthesis; the terpene cyclase gene *vir4*, however, turned out not to be involved in viridin or viridiol biosynthesis but in the synthesis of more than 20 volatile sesquiterpenes [61]. The involvement of terpenes in mycoparasitism relies unresolved, but there are hints: it is probable that genes underlying the mevalonate pathway also influence terpene synthesis. The *hmgR* gene codes for the glycoprotein 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, which processes HMG-CoA to mevalonic acid. Accordingly *hmgR*-silenced mutants of *T. harzianum* exhibited decreased antifungal abilities [62]. Deletion of the trichodiene synthase genes *tri4* and *tri5* in *Trichoderma arundinaceum* resulted in a loss of harzianum A production, a reduced antagonism against host fungi and a decreased ISR in tomato plants [63, 64]. Expression of the *T. arundinaceum tri4* and/or *tri5* genes in *T. harzianum* mainly influenced plant wealth and defense by induced production of trichodiene and 12, 13-epoxytrichothec-9-ene (EPT) [65, 66], whereas *tri5* overexpression in *Trichoderma brevicompactum* boosted the excretion of antifungal trichodermin [67, 68].

5. Ecology and regulation of secondary metabolism in mycoparasitic fungi

Ancestral and recent lifestyles fundamentally influence the existence as well as the expression of secondary metabolite genes and clusters up to the species or even strain level. The transcriptional responses of *T. reesei, T. atroviride* and *T. virens* – which all share an ancestral mycoparasitic lifestyle – to the confrontation with *R. solani* illustrates this fact very well. All three species exhibit few common metabolic responses but autonomous and specific strategies in defeating their opponent. Both potent mycoparasites, *T. atroviride* and *T. virens*, attack their hosts in the stage before physical contact, but with distinct strategies of antibiosis. *T. virens* offends mostly via the NRP biosynthetic pathway for gliotoxin synthesis, whereas *T. atroviride* combats mainly via the PKS biosynthetic pathway, as well as the excretion of 6-pentyl-α-pyrone (6-PP) – a volatile organic compound (VOC) with antifungal and plant growth-promoting properties [2]. Conversely, the transcriptional response of the only slightly mycoparasitic *T. reesei* is more defense-related and targeted on the excretion of cellulases, hemicellulases and ribosomal proteins before hyphal contact [69]. The long-term specialized lifestyle and co-evolution of *E. weberi* with its host in its relatively demarcated ecological niche facilitated the loss of a manifold of genes leading to a more or less obligatory mycoparasitism with limited growth and viability without the presence of the host. Hence, the *E. weberi* genome demonstrates a high degree of specialization, with a unique secretome containing an unusually high content of over 50% of proteins with unknown function. Further, the genome contains only 20% homologs with the closely related *T. atroviride, T. virens* and *T. reesei* and only 12% of the 1066 unique genes exhibit homology with proteins in the whole subdivision of *Pezizomycotina* [15].

Several environmental cues like temperature, light, carbon, nitrogen, pH and competing or synergistic organisms are known to influence the transcriptional regulation of secondary metabolism-associated gene clusters (**Figure 1**). Suboptimal environmental conditions thereby often facilitate and promote transcriptional activation or transcriptional reprogramming events [70]. In media containing chitin or *B. cinerea* cell walls, the predicted cutinase transcription factor 1 encoding gene of *T. harzianum* (*Thctf1*) was up-regulated. *Thctf1* deletion mutants exhibited reduced antagonistic and antifungal ability, and the mutant strain did not synthesize two 6-PP derivatives, indicating a role of *Thctf1* in secondary metabolism of *T. harzianum* [71]. Furthermore, the overexpression of the gene encoding multiprotein bridging factor 1 (*Thmbf1*) of *T. harzianum* – a transcriptional co-activator of *Thctf1* – negatively regulated the antifungal abilities, as well as the expression of VOCs [72].

Like known for the production of mycotoxins in non-mycoparasitic species [73], secondary metabolite production in mycoparasitic fungi is governed by heterotrimeric G protein signaling and the associated cAMP-pathway, as well as mitogen-activated protein kinase (MAPK) cascades [74, 75]. *T. atroviride* mutants, lacking the MAPK-encoding gene *tmk1* showed an enhanced production of peptaibols and of 6-PP [74]. First evidence for a positive regulation of the secondary metabolism by cAMP signaling came from *T. virens* Δ*tac1* mutants bearing a deletion of the adenylate cyclase-encoding gene. The mutants were unable to offend *Sclerotium rolfsii* and *R. solani,* but showed a clear inhibition zone in direct confrontation with *Pythium* sp., pointing to a host-dependent expression of secondary metabolism-associated

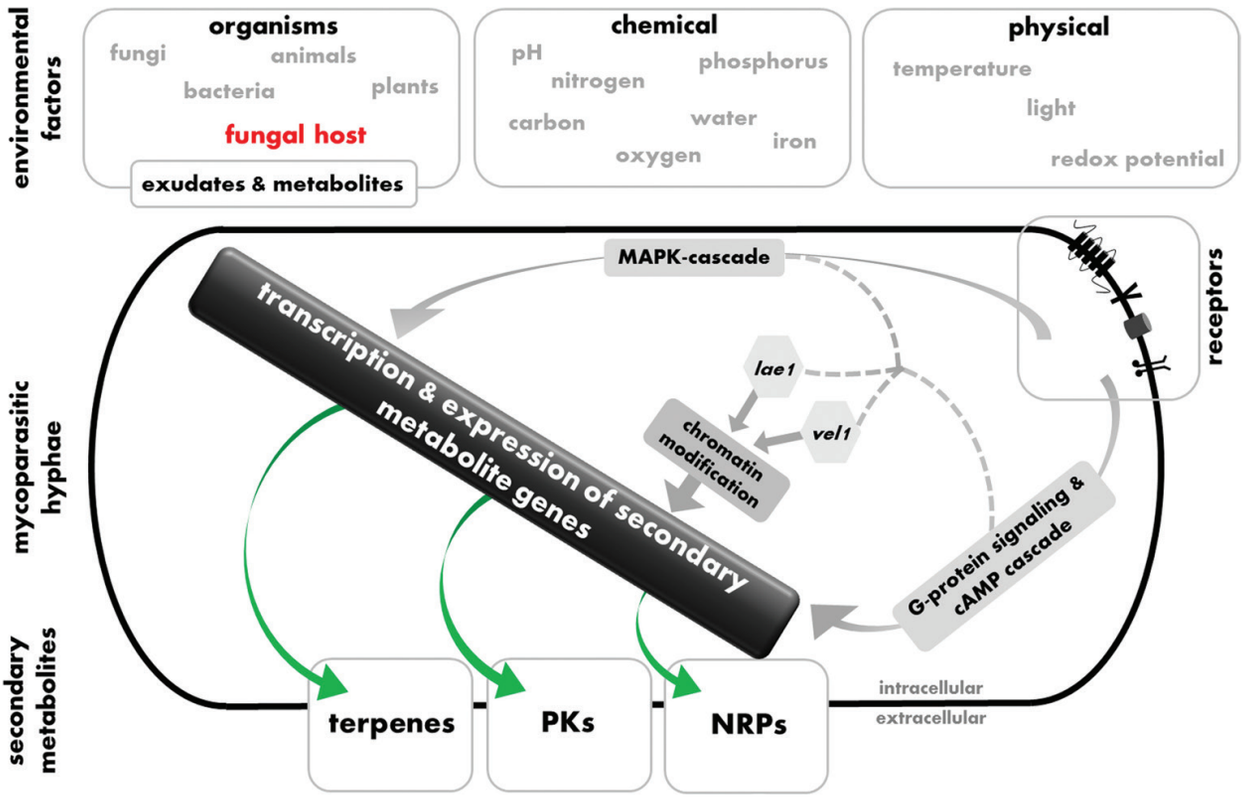


Figure 1. Overview on mycoparasitism-influencing factors and pathways in secondary metabolite biosynthesis of mycoparasitic fungi.

genes. Further, the mutant exhibited a diminished production of secondary metabolites like viridiol and a reduced expression of secondary metabolism-associated genes [75]. Prior to that, similar results were obtained for *T. atroviride* Δ*tga1* mutants missing the subfamily I G*α* protein-encoding gene. Deletion of *tga1* led to a complete loss of overgrowth and mycoparasitism of different preys during direct confrontation and a decrease of 6-PP and sesquiterpene production as well as chitinase gene transcription. Despite the reduction in chitinase and 6-PP accumulation, the Δ*tga1* mutant caused a strong growth inhibition of prey fungi in the interaction zone mediated by yet unidentified low molecular weight antifungal metabolites, thereby evidencing opposite roles of *tga1* in regulating the biosynthesis of different antifungal substances in *T. atroviride* [76]. Similar to Δ*tga1* mutants, transformants bearing a deletion of the subfamily III G*α* protein-encoding gene *tga3* were unable to overgrow and lyse prey fungi. However, absence of the adenylyl cyclase-stimulating Tga3 protein led to significantly reduced antifungal activity [77]. The global regulation of secondary metabolism and morphogenesis by the heterotrimeric VELVET protein complex, consisting of the S-adenosylmethionine-dependent methyltransferase LaeA and the velvet proteins VeA and VelB, was first described in *A. nidulans* [78]. Deletion of the *laeA* orthologue *lae1* in *T. atroviride* led to a loss of mycoparasitic abilities in direct confrontation and a major reduction in the synthesis of 6-PP and water-soluble secondary metabolites. Further, the expression of eight mycoparasitism-related genes was decreased in the mutant. The deletion of *vel1* – the *veA* orthologue – in *T. virens* caused defects in overgrowth and offense against the host in direct confrontation as well as in bioprotective plant interaction, accompanied by a decrease in the expression of several secondary metabolism-associated genes [79, 80].

6. Cross-talk by and response to secondary metabolites in mycoparasitic interactions

In bacteria, it has been shown that at sub-inhibitory concentrations antibiotics serve as mediators of microbial communication and interaction with one of the outcomes being the production of cryptic metabolites [81]. Accordingly, the interaction with other fungi may shape the secondary metabolite profile of a specific fungus, making co-cultures a valuable tool for eliciting the activation of silent secondary metabolism-associated gene clusters.

Studies on the mutual effects of secondary metabolites produced during mycoparasitic interactions are rare however. *Trichoderma*-derived 6-PP was shown to suppress the synthesis of the *Fusarium* mycotoxins fusaric acid and deoxynivalenol (DON) [82–85], suggesting that 6-PP acts as communication molecule that elicits biological responses in the interaction partners. On the other hand, fusaric acid and DON modulate 6-PP production as well as chitinase gene expression in *T. atroviride* and recent studies provided evidence that *Fusarium* mycotoxins induce defense mechanisms in mycoparasites such as *T. atroviride* and *C. rosea* which results in mycotoxin detoxification [59, 86]. *C. rosea* was shown to open the ring structure of zearalenone (ZEN), while *Trichoderma* spp. seem to convert ZEN into its reduced and sulfated forms and metabolize DON to deoxynivalenol-3-glucoside, a detoxification product of DON previously identified in plants [87, 88]. In the interaction of the mycoparasite *T. arundinaceum*

with *B. cinerea, Botrytis*-derived mycotoxins botrydial and botcinins attenuated trichothecene biosynthesis gene expression in *Trichoderma* while botrydial production was repressed by *Trichoderma*-derived harzianum A and aspinolide [89–91].

Co-culturing of mycoparasites with prey fungi simulates the conditions occurring during the mycoparasitic interaction in natural or agricultural systems and could hence encourage the production of secondary metabolites via communication and signaling molecules. Accordingly, pairwise interactions of *Aspergillus niger, Fusarium verticilliodes* and *C. rosea* led to metabolites that occurred in single cultures but were suppressed in dual cultures, and many new metabolites not present in single cultures were found in dual cultures [92]. Similar results were obtained in co-culturing experiments of *T. harzianum* and *Talaromyces pinophilus* with the accumulation of siderophores being induced in both interaction partners and the production of *Talaromyces*-derived 3-*O*-methylfunicone and herquline B being reduced. In addition, the novel substance harziaphilic acid was detected in the co-culture only [93].

Based on these studies, it is evident that secondary metabolites contribute to mycoparasitic interactions in various ways including inhibition of the activity or synthesis of mycoparasitism-relevant enzymes and other substances, by eliciting defense and detoxification responses or by triggering the production of cryptic metabolites. In most cases, however, information on the spatial distribution of the secreted substances is lacking and it is hence difficult to assign novel secondary metabolites specifically induced during the co-cultivation to its actual producer. Recently, mass spectrometry-based imaging (MSI) turned out as a valuable tool for in situ visualizing the dynamics and localization of small molecules released during microbial interactions [94]; however, reports on its application to mycoparasitic fungus-fungus interactions are still rare. By applying matrix-assisted laser desorption/ionization (MALDI)-based MSI for visualization and identification of secondary metabolites being exchanged during the mycoparasitic interaction of *T. atroviride* with *R. solani* [95], the diffusion of *Trichoderma*-derived peptaibols toward the prey and the accumulation of *Rhizoctonia*-derived substances at the borders of fungal interaction was tracked. Monitoring of the *T. harzianum* interaction with the fungal phytopathogen of cacao plants *Moniliophthora roreri* by MSI lead to the detection of T39 butenolide, harzianolide, sorbicillinol and an unknown substance specifically produced in the co-culture with a spatial localization in the interaction and overgrowth zones [96].

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Author details

Verena Speckbacher and Susanne Zeilinger*

*Address all correspondence to: susanne.zeilinger@uibk.ac.at

Department of Microbiology, University of Innsbruck, Innsbruck, Austria

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