Secondary metabolism in *Trichoderma* – a genomic perspective

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*Trichoderma* spp. are a rich source of secondary metabolites (SMs). The recent publication of the genome sequences of three *Trichoderma* spp. has revealed a vast repertoire of genes putatively involved in the biosynthesis of SMs, such as non-ribosomal peptides, polyketides, terpenoids and pyrones. Interestingly, the genomes of the mycoparasitic species *Trichoderma virens* and *Trichoderma atroviride* are enriched in secondary metabolism-related genes compared with the biomass-degrading *Trichoderma reesei*: 18 and 18 polyketide synthases compared with 11; 28 and 16 non-ribosomal peptide synthetases compared with 10, respectively. All three species produce a special class of non-ribosomally synthesized peptides known as peptaibols, containing non-proteinogenic amino acids (particularly *α*-aminoisobutyric acid). In common with other filamentous ascomycetes, *Trichoderma* spp. may require siderophores (also produced by non-ribosomal peptide synthetases) to grow in iron-poor conditions and to compete with their hosts for available iron. Two generalizations can be made about fungal SM genes: they are often found in clusters, and many are not expressed under standard laboratory conditions. This has made it difficult to identify the compounds. *Trichoderma*, in particular, interacts with other microbes in the soil and with plant roots in the rhizosphere. A detailed metabolomic–genomic study would eventually unravel the roles of many of these SMs in natural ecosystems. Novel genetic tools developed recently, combined with biological understanding of the function of SMs as toxins or signals, should lead to ‘awakening’ of these ‘silent’ clusters. Knowledge of the SM repertoire should precede application of *Trichoderma* strains for biocontrol: some metabolites could be toxic to plants and their consumers, and thus should be avoided. Others could be beneficial, antagonizing pathogens or inducing resistance in crop plants.

Introduction

Fungi produce a wide range of secondary metabolites (SMs), small molecules that are not directly essential for growth yet have important roles in signalling, development and interaction with other organisms (Hoffmeister & Keller, 2007; Keller *et al.*, 2005; Osbourn, 2010). In some cases, a ‘secondary’ metabolite may be essential for survival under particular environmental conditions; for example, siderophores, which are needed for growth at low iron concentrations. More often, though, genes for biosynthesis are found in the genome, frequently in clusters, although the putative products are unknown. One reason is that under standard laboratory cultivation conditions, many of these gene clusters are not expressed (Brakhage & Schroechk, 2011). One hypothesis is that SM production may have evolved for communication with, or defence against, other microbes or multicellular organisms (Brakhage & Schroechk, 2011; Chiang *et al.*, 2011; O’Brien & Wright, 2011). The ancestral mycoparasitic lifestyle of *Trichoderma* (Kubicek *et al.*, 2011; Druzhinina *et al.*, 2011) may explain the unusually diverse repertoire of biosynthetic clusters, particularly in *Trichoderma atroviride* (*Hypocrea atroviridis*) and *Trichoderma virens* (*Hypocrea virens*), as a large array of compounds would be necessary in the attack against other microbes. These two species are aggressive mycoparasites, while the weakly mycoparasitic, biomass-degrading *Trichoderma reesei* (*Hypocrea jecorina*) has less diversity (Kubicek *et al.*, 2011). Mycoparasitism represents the culmination of competition for resources between *Trichoderma* and its host fungi. Based on the idea that the evolution of SM production was indeed driven by competition between species, a recent proposal is that the many silent biosynthetic clusters could be activated by competition or by conditions that simulate the normal route of activation (Osbourn, 2010; Brakhage & Schroechk,
2011). For example, co-culture of Aspergillus nidulans with actinomycetes that share the same niche triggers expression of fungal polyketide biosynthesis genes (Schroeckh et al., 2009).

Some of these fungal metabolites also play important roles in interactions with plants (Osbourm, 2010). A variety of plant pathogens owe their virulence (partially or fully) to host-specific toxins, which can be small secreted proteins [for example Stagonospora nodorum on wheat (Liu et al., 2009)] or SMs [for example HC-toxin of Cochliobolus carbonum (Sindhu et al., 2008; Walton, 2006)]. The ACE1 gene cluster [containing a hybrid polyketide synthase-non-ribosomal peptide synthetase (PKS-NRPS)] of rice blast fungus is specifically expressed at the plant penetration stage, and produces a metabolite recognized as an avirulence (AVR) determinant in a gene-for-gene manner, although not essential for virulence (Böhnhert et al., 2004; Collemare et al., 2008). Like the more extensively studied AVR factors that are proteins, the ACE1 product probably has a plant cellular virulence target, though not yet identified. Not all fungal metabolites are used as weapons against the plant. Indeed, specific fungal metabolites are necessary to establish arbuscular mycorrhizae (AM), which against the plant. Indeed, specific fungal metabolites are necessary to establish arbuscular mycorrhizae (AM), which are central to the life of land plants (Bonfante & Requena, 2011; Bucher et al., 2009).

Based upon analytical reports, Trichoderma spp. are prolific producers of SMs (natural products), with the structures of more than 100 compounds reported (Reino et al., 2008; Sivasithamparam & Ghisalberti, 1998). These include low-molecular-mass non-polar compounds such as pyrones, terpenoids, steroids and polyketides. Similar to other ascomycetes, Trichoderma spp. produce non-ribosomal peptides, for example the epipolythiodioxopiperazines (ETPs) and siderophores. Members of the genus are prominent producers of a subgroup of peptaibiotics known as peptaibols (short peptides of non-ribosomal origin characterized by the presence of high levels of non-standard amino acids). More than 700 peptaibol sequences are known to date, mostly of Trichoderma Hypocrea origin (Degenkolb et al., 2008). This vast potential of Trichoderma spp. to produce numerous types of metabolites is reflected in the genomes of the three species (http://genome.jgi-psf.org/). For example, the genome of T. virens contains 440 genes which have been classified (Eukaryotic Orthologous Groups; KOG) as related to SM biosynthesis, Transport and catabolism. The totals for T. atroviride and T. reesei are 349 and 262, respectively. Most of the SM genes present in T. reesei are also found in T. virens and T. atroviride (Kubicek et al., 2011). Furthermore, in a comparative genome study, it was reported that about half of the genes lacking obvious orthologues in T. reesei are unique to either T. virens or T. atroviride, and are located on non-syntenic islands on the genome; the functional analysis of specific genes based on this finding will provide a fascinating insight into the evolution of SM biosynthesis genes. Several of the SMs have been reported to be antimicrobial in vitro. Evidence for the role of these compounds in Trichoderma–plant pathogen interactions and the biocontrol of plant pathogens is still very incomplete. Of relevance for biocontrol, SMs and enzymes act in synergy when Trichoderma attacks host fungi (Lorito et al., 1994). If the principle that the evolution of SM production is driven by interactions between species can be generalized to fungal–fungal interactions, we may be able to gain an insight into the evolution of SM biosynthesis genes through the study of these genes in the genomes of mycoparasites. Our discussion of these SM genes of Trichoderma is organized around the signature genes encoding NRPSs, PKSs and terpene synthases, which define the biosynthetic pathways and clusters (Osbourm, 2010).

NRPSs

NRPSs arguably account for the most diverse and functionally important range of fungal SMs (von Döhren, 2009; Evans et al., 2011; Strieker et al., 2010). Genes encoding NRPSs are among the signature SM genes with respect to defining the clusters in which they reside. NRPS enzymes consist of a series of modules that act like an assembly line, each incorporating one monomer into the peptide (Strieker et al., 2010). The core of each module includes adenylation, peptidyl carrier and condensation domains. Additional domains are present in some modules; for example, the thioesterase in the last module, responsible for releasing the peptide. NRPSs produce a large variety of compounds composed of monomers that are from a much wider range than the 20 proteinogenic amino acids. The monomers may be non-proteinogenic amino acids (peptaibols are a notable example of this in Trichoderma, see below), or even compounds that are not amino acids at all. The peptides may be linear or cyclic, and often undergo extensive chemical modifications (Bushley & Turgeon, 2010; Stack et al., 2007; Strieker et al., 2010). Several classes of peptides are synthesized by these enzymes in Trichoderma spp. The mycoparasitic T. virens and T. atroviride have an expansion of NRPS-encoding genes (28 and 16, respectively) compared with the non-mycoparasitic T. reesei, whose genome contains only 10. A full phylogeny of NRPS genes in the three species emphasizes the particular expansion in T. virens (Supplementary Fig. S1 in Kubicek et al., 2011). The mere presence of these genes in mycoparasitic species, of course, does not prove function. This can be now tested by gene knockout experiments. An approach that asked this question simultaneously for a large class of metabolites was the construction of a mutant lacking 4-phosphopantetheinyltransferase (PPT) (Velázquez-Robledo et al., 2011). PPT is essential for activation of NRPS and PKS, among other enzymes. The ppt mutants are lysine auxotrophs, defective in their ability to antagonize fungi, and in the induction of some, but not all, plant systemic defence responses (Velázquez-Robledo et al., 2011).
Gliotoxin and gliovirin

Gliotoxin and gliovirin belong to the ETP class of peptides (Patron et al., 2007) (Fig. 1). Gliotoxin was the first metabolite described from *Trichoderma* (initially *Trichoderma lignorum*, later assigned to *Gliocladium virens*, and currently *Hypocrea virens*, and currently *Hypocrea virens*). Gliotoxin was discovered over the same time as aflatoxin was identified for its dramatic impact on humans.

The gliotoxin gene cluster is also known from the opportunistic human pathogen *Aspergillus fumigatus*. Gliotoxin may be a virulence factor in this pathogen (Dagenais & Keller, 2009). In line with the hypothesis discussed above, the antifungal activity of gliotoxin may provide an advantage to *A. fumigatus* in soil environments (see Giles et al., 2011). The 'Q' strains of *T. virens* produce copious amounts of gliotoxin within 16 h of growth in liquid culture (Wilhite & Straney, 1996), and the compound can be detected in the rhizosphere (Lumsden et al., 1992). Gliotoxin has received much attention for its role in the biocontrol of soil-borne fungal pathogens (Howell, 2006). There are, however, contradictory reports on its importance in biocontrol under controlled cultural and environmental conditions (Howell et al., 1993; Howell & Stipanovic, 1995; Wilhite et al., 1994). Nevertheless, being strongly antimicrobial, the role of gliotoxin in microbial competition under natural conditions cannot be dismissed. A gene deletion experiment has confirmed the role of the GliP NRPS in gliotoxin biosynthesis (C. M. Kenerley, unpublished data). Of interest is that gliotoxin is produced only by *T. virens* 'Q' strains and is absent in *T. atroviride* and 'P' strains of *T. virens*.

A GliP (the gliotoxin synthesis NRPS) cluster is present in *T. reesei* even though this species does not produce gliotoxin (Fig. 1). Compared with the *A. fumigatus* GliP cluster, *T. virens* contains only eight genes that are closely related to the *A. fumigatus* genes (Table 1). Since the scaffold (47) which harbours this cluster is small (about 30 kb), the possibility remains that the sequence is incomplete, and poor coverage of this region in the genome might account for the missing genes in this cluster. Indeed, some of these genes (the missing ones are highlighted in Fig. 1) likely remain to be identified in the *T. virens* 'Q' strain genome: for example *gliZ, gliI, gliA* and *gliT* (putatively encoding a transcription factor, dipeptidase, transporter and thioredoxin reductase, respectively). The even smaller size of the *T. reesei* cluster could be due to deletion. This would explain why this species is not known to produce gliotoxin. Unlike the *T. virens* GliP cluster, in which genes are induced during confrontation with its prey, *Rhizoctonia solani*, the genes of the GliP cluster in *T. reesei* are not expressed (C. P. Kubicek, personal communication). In addition to the GliP cluster, the *T. virens* genome harbours a putative SirP gene cluster which is well conserved (Fig. 1). This is in addition to another SirP-like gene that is present in all three *Trichoderma* genomes sequenced, but not as a part of a secondary metabolism cluster. The SirP gene cluster is functional in the phytopathogen *Leptosphaeria maculans* and produces the phytotoxin sirodesmin PL (Patron et al., 2007). However, none of the SirP gene cluster members is expressed during mycoparasitism in any species of *Trichoderma* (C. P. Kubicek, personal communication). Although the 'P' strains of *T. virens* do not produce gliotoxin, they do...
produce gliovirin instead (Fig. 1). Gliovirin is another ETP compound, with potent antimicrobial properties (especially against oomycetes) (Howell et al., 1993). A role for this compound in biocontrol of oomycete pathogens was proposed based on mutational analysis (Howell & Stipanovic, 1983). The gliotoxin-producing ‘Q’ strains, on the other hand, are more effective against isolates of R. solani (Howell, 2006). No gliotoxin gene cluster has been identified in the genome of T. atroviride. Whole-genome sequencing may provide an explanation for the production of gliovirin but not gliotoxin by ‘P’ strains of T. virens.

Environmental factors, including the growth substrate, regulate production of gliotoxin (Park et al., 1998). In T. virens, Mukherjee & Kenerley (2010) studied the role of the vel1 gene by construction of knockout mutants. The deletion of vel1 impaired production of conidia on solid medium and chlamydospores in rich medium; vel1 is also involved in the regulation of the gliotoxin biosynthesis gene gliP and other secondary metabolism genes in this species.

Peptaibols

Peptaibols [peptides containing α-aminoisobutyric acid (aib) and a C-terminal 1,2-amino alcohol] are produced largely by members of Trichoderma/Hypocrea (Degenkolb et al., 2008). They are ecologically and commercially important for their antimicrobial and anti-cancer properties as well as their ability to induce systemic resistance in plants against microbial invasion. There are two peptaibol synthetases (of 18 and 14 modules) in Trichoderma genomes. Even though there are more than 700 described peptaibol sequences (Degenkolb et al., 2008), no genetic studies on their synthesis have been conducted except in T. virens Gv29-8. Using gene disruptions, the 18-module peptaibol synthetase Tex1 has been shown to be responsible for the production of the trichovirin II-type 18-residue peptaibols, while the 14-module enzyme assembles both the 14-residue and the 11-residue peptaibols in T. virens (Mukherjee et al., 2011; Viterbo et al., 2007; Wiest et al., 2002). Of particular interest is the ability of a single synthetase to produce a large number of peptaibols. For example, the 14-module NRPS of T. virens produces at least 88 non-ribosomal peptides of two classes (the 11- and 14-residue classes) (Mukherjee et al., 2011; Fig. 2). Many of these peptides were found to be novel forms, indicating the enormous diversity in their biosynthesis as well as the tremendous opportunities for their commercial exploitation as novel compounds in agricultural and clinical applications. The peptaibols are amphipathic in nature and self-assemble to form voltage-dependent ion channels in membranes. This ability is largely responsible for the antibiotic properties of these compounds. However, the only experimental evidence to implicate these compounds in the biocontrol abilities of Trichoderma spp. has been a demonstration of the role of peptaibols such as alamethicin and trichovirin II in the induction of resistance in plants (Viterbo et al., 2007; Leitgeb et al., 2007). Alamethicin, produced by T. viride, is a mixture of at least 12 compounds, each containing 20 amino acid residues.

Table 1. Similarity of T. virens and T. reesei gliotoxin gene cluster members to those of A. fumigatus

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<th>Gene</th>
<th>Trichoderma protein ID</th>
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Table 1. Similarity of T. virens and T. reesei gliotoxin gene cluster members to those of A. fumigatus
This peptaibol has been shown to elicit the biosynthesis of homoterpenes and methyl salicylate, volatile compounds from lima bean (Engelberth et al., 2001). Recently, the application of alamethicin was shown to induce local and long-distance electrical signals in plants (Maischak et al., 2010) and defence-like responses in Arabidopsis that were similar to the hypersensitive reaction to pathogen attack (cell death, deposition of callose, transcription of defence genes and production of phenolic compounds) (Rippa et al., 2010). The role of a trichovirin II peptaibol (product of Tex1 NRPS) from T. virens in symbiotic interactions with cucumber was demonstrated through gene expression, mutants disrupted in the encoding gene (tex1), and the application of synthetic forms of the peptide to plants. The transcript levels of tex1 were higher in the presence of plant roots than when the wild-type was grown alone. Cucumber plants grown with mutants disrupted in tex1 showed a significantly reduced systemic resistance response to a foliar pathogen and produced lower levels of phenolic compounds. Additionally, two synthetic isoforms were capable of inducing systemic protection and the upregulation of defence genes (Viterbo et al., 2007). These compounds can act synergistically with secreted hydrolytic enzymes to promote ingress into pathogen structures, suggesting a role in the antagonism of plant pathogens (Schirmböck et al., 1994).

**Siderophores**

Iron acquisition is an important component of microbial competition, especially in the rhizosphere, where microbial activities are intense. The intracellular siderophore ferricrocin is responsible for storage of iron and is involved in the protection of cells from oxidative stress (Wallner et al., 2009). The extracellular siderophore produced by NPS6 in Cochliobus heterostrophus is a virulence factor, and also contributes to protecting the fungus from oxidative stress (Oide et al., 2007). All three Trichoderma spp. whose genomes have been sequenced have a single gene for ferricrocin synthesis, belonging to a secondary metabolism gene cluster (Kubicek et al., 2011). Preliminary investigations revealed that this gene is indeed involved in the synthesis of ferricrocin and protection against oxidative stress in T. virens (P. K. Mukherjee & C. M. Kenerley, unpublished data). Trichoderma spp. are known to produce extracellular siderophores of the fusigen and coprogen family ( Jalal et al., 1986). T. virens and T. reesei each have two putative gene clusters containing an NRPS as the core member, whose orthologues (SidD and NPS6) are known to be involved in siderophore synthesis (Kubicek et al., 2011; Fig. 3). The T. atroviride genome, however, harbours only the NPS6 orthologue. A genetic study of the role of these extracellular siderophores in biocontrol properties would shed light on the relevance of siderophore-mediated iron acquisition in microbial competition and biocontrol. Pathogens and their hosts compete for iron, so that siderophores can be virulence factors (Haas et al., 2008; Schrettl et al., 2010; Turgeon et al., 2008). This model may be extended to microbe–microbe (fungal–fungal) competition, e.g. via experiments on Trichoderma–fungal interactions. The competition for iron was indeed shown to be important for control of Fusarium wilt of tomato by Trichoderma asperellum (Segarra et al., 2010).

**Fig. 2.** Modular structure and synthesis of 14-residue (14-res) and 11-residue (11-res) peptaibols by the Tex2 NRPS. Ac, acetyl; U, Aib; Vx, Val/isovalerate (iva); Lx, Leu/Ile; ol, C-terminal amino alcohol (adapted from Mukherjee et al., 2011).
Polyketides

The polyketides are a group of SMs produced by many organisms, including filamentous fungi. Many polyketides are clinically important, with antimicrobial, anti-cancer and immunosuppressive properties. Moreover, they are important in the producing organisms, facilitating competition for substrates and communication between organisms (Khosla, 2009). Trichoderma genomes are rich in PKSs: 18 for T. virens and T. atroviride, and 11 for T. reesei (for a full phylogeny and other details, see Baker et al., 2012 in this issue). No genetic data are available on the role of these enzymes in the physiology and biocontrol properties of Trichoderma spp., although some of the PKSs are expressed during growth on synthetic medium (Khosla & Kenerley, 2009). Like gliP and other secondary metabolism-related genes, PKSs in T. virens are regulated by the velvet complex protein Vel1 (Mukherjee & Kenerley, 2010). Two PKS genes in T. atroviride are expressed during confrontation with R. solani, indicating a possible role in mycoparasitism (C. P. Kubicek, personal communication). A PKS belonging to the non-reducing fungal clade I defines a likely conidial pigment biosynthesis cluster. The T. virens orthologue (protein ID 77826), for example, has 39% identity to A. fumigatus PksP/Alb1 and is found in a cluster whose arrangement is conserved across the three species (Fig. 3 in Baker et al., 2012 in this issue). Conidial pigmentation is important in protection from short-wavelength UV radiation, as is evident from the greatly increased sensitivity of the conidia of non-pigmented mutants of T. atroviride (Sametz-Baron et al., 1997). The genes and pathway for conidial pigmentation have not yet been characterized experimentally, however. Similar to other SM-related genes, the T. virens genome has the greatest number of PKS/NRPS hybrid enzymes among the three genomes, four, compared with one in T. atroviride and two in T. reesei. The domain structures of these genes are conserved among these species, except in one of the two thiolation domains (Fig. 4). A functional analysis revealed that one of the PKS/NRPS hybrids is involved in induction of maize pal, but not of aos, another defence-related gene (Mukherjee et al., 2012 in this issue). Interestingly, this PKS/NRPS gene (protein ID 53833) is phylogenetically distinct from ACE1, the PKS/NRPS of Magnaporthe grisea involved in interactions with rice plants (Collemare et al., 2008). This indicates the diverse

![Polyketides Diagram](image.png)
functions of evolutionarily conserved SMs. The products of the Magnaporthe ACE1 cluster and Trichoderma Tex13 cluster, however, remain unknown.

**Terpenoids/steroids**

Another versatile and diverse class of compounds is synthesized from five-carbon isopentenyl units. In clusters responsible for terpenoid synthesis, the signature gene encodes a terpene cyclase; for example, the trichodiene synthase of Fusarium (Kimura et al., 2007). An examination of the genomes of the three Trichoderma spp. indicates that the T. virens genome has an expansion of terpene cyclases compared with the other two species. Apart from the oxidosqualene-lanosterol cyclase, the oxidosqualene-lanosterol cyclase, the T. atroviride and T. reesei have three each (Table 2). The fungistatic and antitumor steroidal compound viridin is widely produced by both ‘P’ and ‘Q’ strains of T. virens (Howell et al., 1993). Experimental evidence suggests that viridins, a group of furanosteroids, have a triterpene/sterol rather than diterpene origin (Sivasithamparam & Ghisalberti, 1998). Viridin is reduced to viridiol by T. virens, the producing organism; viridiol has herbicidal properties (Jones & Hancock, 1987). Using suppression subtractive hybridization, followed by a cosmid library screen, Mukherjee et al. (2006) identified a T. virens gene cluster that includes genes encoding a putative terpene cyclase and cytochrome P450s (Fig. 5). This cluster is present in Aspergillus oryzae, but absent in T. atroviride and T. reesei. Since the expression of T. virens, the producing organism; viridiol has herbicidal properties. Experimental evidence, however, suggests that this cluster is not involved in the production of viridin, but rather is responsible for the synthesis of volatile sesquiterpenes (C. M. Kenerley, unpublished results). Thus the question of which terpene cyclase is responsible for viridin biosynthesis remains open, and is being addressed by directed mutagenesis of each of the predicted cyclase genes.

In addition to terpenoids, several other volatile metabolites were detected by solid-phase microextraction combined with GC-MS from the headspace of T. atroviride cultures (Stoppacher et al., 2010). Some of the compounds were produced at defined culture times, and their accumulation was delayed by the Fusarium mycotoxin fusaric acid, suggesting that their role in interactions with fungal hosts and plants in the rhizosphere will be a promising area for study (Stoppacher et al., 2010). Trichodermin is a highly fungitoxic and also phytotoxic terpenoid trichothecene-type toxin produced by Trichoderma brevicompactum. Overexpression of the trichodiene synthase Tri5 resulted in overproduction of trichodermin in T. brevicompactum (Tijerino et al., 2011).

**Pyrones**

6-Pentyl pyrone (6-PP), the ‘coconut aroma’ volatile compound produced by Trichoderma spp., is one of the best-studied SMs from a biocontrol perspective (Bonnamme et al., 1997; Cooney et al., 2001; Reithner et al., 2005, 2007; Vinale et al., 2008). This compound is reported to have antifungal and plant growth-promoting activities. The biosynthetic pathway for the production of 6-PP has not been elucidated, but a lipooxygenase gene (Triat1: 33350) unique to T. atroviride may be involved (Kubicik et al., 2011). 6-PP biosynthesis is regulated by the G protein Tga1 in T. atroviride; other metabolites, however, are overproduced in tga1 mutants (Reithner et al., 2005). Recently, by gene disruption, Rubio et al. (2009) demonstrated the involvement of a transcription factor, Thctf1, in the 6-PP production and antifungal activity of T. harzianum. Elucidation of the biosynthetic pathways of these interesting groups of compounds would aid in genetically improving biocontrol organisms.

**Conclusions**

The abundance and diversity of SMs depends on which biosynthetic genes are present in the genome and on the conditions that induce their expression. SM biosynthesis

**Fig. 4.** PKS/NRPS hybrid proteins of Trichoderma spp. and their gene structures. Abbreviations: Trive, T. virens; Triat, T. atroviride; Trie, T. reesei; KS, ketoacyl synthase; AT, acyltransferase; MT12, methyltransferase 12; KR, ketoreductase; T, thiolation domain; C, condensation domain; A, adenylation domain; NB4, NAD-binding. The domains were identified by using the Pfam domain search algorithm on http://pfam.sanger.ac.uk/search.

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</table>

Table 2. Terpene cyclases of Trichoderma spp.
can be switched on in response to simple intrinsic or external factors. Examples are siderophore production in response to lack of iron, or the co-regulation of sporulation and toxin synthesis in Aspergillus in response to light. Other metabolites may be produced in response to interaction with other organisms. This pattern could be of great relevance for biocontrol, as Trichoderma interacts with fungal hosts and plant roots in the rhizosphere. Conserved eukaryotic signalling pathways [heterotrimeric G protein, mitogen activated protein kinase (MAPK)] modulate SM levels in T. atroviride (Reithner et al., 2005, 2007). The MAPK Tmk1 regulates different metabolites in opposite directions (Reithner et al., 2007).

From the genomic point of view, a comparison of biosynthesis genes among species is a good starting point. As in other filamentous ascomycetes, the SM genes of Trichoderma often belong to clusters. Walton (2000) proposed that the evolutionary pressure keeping clusters together is that the entire cluster confers new biosynthetic capability, and that horizontal transfer allows clusters to propagate: a pathway would ‘survive’ horizontal transfer if transferred in a cluster. A recent model states that one of the evolutionary forces keeping clusters together is that metabolic gene clustering allows an entire pathway to be gained or lost, preventing partial reactions that would lead to the accumulation of toxic intermediates (Slot & Rokas, 2011). The example of gliotoxin provides an interesting extension of this hypothesis: a member of the gliotoxin cluster, gliT, encodes a reductase that confers resistance to exogenous gliotoxin (Davis et al., 2011).

The study of Trichoderma metabolites began in the 1930s, yet not even the newest strategies have identified all the genes and corresponding metabolites. Approaches developed for other sequenced fungal species include: transgenic lines in which global regulators are expressed or repressed, epigenome manipulation, and co-culturing of species to simulate competition (Bok et al., 2009; Brakhage & Schroechk, 2011; Chiang et al., 2011; Cicewicz, 2010; Giles et al., 2011; Lee et al., 2009). These approaches can be applied to Trichoderma. Such experiments would be the first such efforts for fungal–fungal interactions and are therefore likely to yield novel results. Compounds produced by the activation of cryptic biosynthesis pathways in A. nidulans indeed have antimicrobial activity against A. fumigatus, Aspergillus flavus, Candida albicans, Bacillus cereus and Micrococcus luteus (Giles et al., 2011).

Antibiotics represent just one of the activities deployed by Trichoderma against host fungi, in synergism with cell wall-degrading enzymes (Lorito et al., 1994; Schirmböck et al., 1994). Some metabolites may be recognized by plants in the three-way Trichoderma–plant–host fungal interaction (Vinale et al., 2008). Studying SM biosynthesis is thus central to developing Trichoderma for better biocontrol. A. oryzae was domesticated for fermentation at least two millennia before the molecular reason that it cannot make aflatoxin was discovered (Rokas, 2009; Kiyota et al., 2011). For Trichoderma, the genomic and biochemical tools are available, in advance of the commercialization of new strains. In the worst case, could the use of Trichoderma in agriculture pose a hazard just like chemical fungicides, by imposing uncharacterized and perhaps toxic metabolites? In the best case, could secondary metabolites improve plant growth, defend against pathogens, and induce systemic resistance in crop plants? The reality is probably somewhere between these extremes, making it imperative to characterize genes and products in biocontrol strains, by carrying out genetic and metabolite profiling of strains before using them in agriculture.

References


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