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Review

Secondary metabolism in *Trichoderma* – Chemistry meets genomics

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ABSTRACT

Species of the genus *Trichoderma* inhabit diverse environments and undergo a variety of interactions with different other organisms. Mycoparasitic *Trichoderma* species are successfully applied as bio-fungicides due to their plant-protecting abilities and they are prolific producers of secondary metabolites which comes along with an enrichment in secondary metabolism-associated genes in their genomes. The objective of this article is to review the secondary metabolites produced by *Trichoderma* spp. along with a comprehensive overview of their genomic content for genes and gene clusters being involved in secondary metabolite biosynthesis. We discuss the bioactivity of selected *Trichoderma*-derived secondary metabolites with a focus on their roles in the interactions of *Trichoderma* with plants and fungal preys and give an overview on methods for secondary metabolite profiling.

1. Introduction

Fungi of the genus *Trichoderma* are outstanding among filamentous ascomycetes due to their high adaptability to various ecological conditions and variety of lifestyles. They live in soil and saprophytically grow on wood, bark and many other substrates, and interact with animals and plants. The ability to parasitically antagonize and kill other fungi (a process called mycoparasitism), however, is widespread among *Trichoderma* spp. and is the ancestral lifestyle of these fungi (Atanasova *et al.*, 2013b; Holzlechner *et al.*, 2016; Kubicek *et al.*, 2011). Owing to their ability to parasitize plant pathogenic fungi and to boost plant defense against invading pathogens even at sites away from the point of application, *Trichoderma* species are widely used in today's agriculture as commercial biofungicides (Mukherjee *et al.*, 2013a). Apart from this, several *Trichoderma* strains are known to promote plant growth and vigor as well as to impart tolerance to abiotic stresses like drought (Mukherjee *et al.*, 2013b).

There are direct and indirect evidences in favor of involvement of secondary metabolites (natural products) in the antagonistic activity of *Trichoderma* against a considerable number of bacteria, yeasts, and fungi as well as in the beneficial effects *Trichoderma* exerts on crop plants. Though not

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clinically used, some of the secondary metabolites produced by *Trichoderma* have potential as drugs and one compound (6-pentyl-α-pyrone) is used as a food flavoring agent. Beginning with the discovery of gliotoxin in the early 1930s (Weindling and Emerson, 1936), a plethora of substances has been reported from *Trichoderma* over the years with far more than 1000 compounds being estimated to be produced by fungi of this genus (Hermosa *et al.*, 2014). The genetics of secondary metabolism in these species however sorely lagged behind. As on today, biosynthetic genes for only a handful of compounds (notably the 18/20-residue and 11/14-residue peptaibols, certain volatile sesquiterpenes, gliotoxin and a couple of trichothecenes) have been identified. However, this scenario is expected to change rapidly as several genomes have been sequenced and many being in the pipeline and as functional genomics studies in *Trichoderma* advance. As more and more biosynthetic genes/gene clusters are identified, we can hope for the elucidation of pathways of the known metabolites as well as discovery of novel substances.

2. Secondary metabolites and biosynthesis genes of *Trichoderma*

The publication of the first whole genome sequence of the industrial enzyme producer *Trichoderma reesei* was soon followed by two mycoparasitic species *Trichoderma atroviride* and *Trichoderma virens* (Kubicek *et al.*, 2011; Martinez *et al.*, 2008). A comparative analysis of these three species yielded interesting information on the enrichment of genes related to anti-fungal properties of *Trichoderma*, including genes for secondary metabolism, in the mycoparasites (Druzhinina *et al.*, 2011; Mukherjee *et al.*, 2012b). Many of these genes are part of large biosynthetic gene clusters comprising core enzymes such as non-ribosomal peptide synthetases (NRPSs), polyketide synthases (PKSs), or terpene synthases/cyclases, accessory enzymes (like cytochrome P450s, oxidoreductases, methyl transferases, etc.), and, in some cases, genes for transporters and transcription factors (Mukherjee *et al.*, 2012a, 2012b; Bansal and Mukherjee, 2016a; Bansal and Mukherjee, 2016b; Mukherjee *et al.*, 2013a). Six more genomes (the mycotrophic *Trichoderma asperellum*, *Trichoderma harzianum*, *Trichoderma parareesei*, *Trichoderma gamsii*, and the opportunistic human pathogens *Trichoderma longibrachiatum* and *Trichoderma citrinoviride*) were subsequently added to the public databases (Baroncelli *et al.*, 2016; Mukherjee *et al.*, 2013a; Yang *et al.*, 2015) which are awaiting detailed analysis. We discuss here the main secondary metabolites produced by *Trichoderma* spp. and some of the advancements that have been made in the area of secondary metabolism research in *Trichoderma* that are largely based on the genome sequencing and analysis. Overall numbers of the core secondary metabolism-related genes and whether these are part of putative gene clusters in the *Trichoderma* genomes are presented in Table 1.

The biosynthesis of fungal secondary metabolites often involves unique and unusual biochemical pathways. These may be slightly varied to yield a high diversity of substances from only a few key precursors derived from primary metabolism, such as acetyl-CoA, mevalonate and amino acids that build up their backbones (Demain and Fang, 2000; Keller *et al.*, 2005).*Trichoderma*-derived secondary metabolites comprise non-ribosomal peptides such as peptaibiotics, siderophores and diketopiperazines-like gliotoxin and gliovirin, polyketides, terpenes, pyrones, and isocyane metabolites. However, it has to be borne in mind that the production of these substances is species- and even strain-dependent and not the whole repertoire will be biosynthesized by a given fungus under laboratory conditions as specific triggering stimuli may be required.

Non-ribosomal peptides

Non-ribosomal peptides (NRPs) constitute a large group of economically and ecologically important secondary metabolites that result from fusion of two or more amino acids by multi-modular mega-enzymes, known as non-ribosomal peptide synthetases (NRPSs) outside the ribosome, followed by, in many cases, secondary modifications. NRPs may be of various lengths, usually containing both proteinogenic and non-proteinogenic amino acids, may be linear or cyclic and comprise pharmacologically relevant fungus-derived substances such as the tripeptide beta-lactam antibiotics, the immunosuppressant cyclosporine, and the antitumor peptide terrequinone (Evans *et al.*, 2011; Strieker *et al.*, 2010). The major groups of NRPs from *Trichoderma* spp. are peptaibiotics, epidithiodioxopiperazines (ETPs), and siderophores with the former being the most prominent NRPs produced by *Trichoderma* species.

Peptaibiotics

Peptaibiotics are a large group of linear or, in a few cases, cyclic peptides with a length of 4–21 residues and a molecular weight of 500–2100 Da. Peptaibiotics contain the non-proteinogenic marker amino acid α-aminoisobutyric acid

Table 1 – Secondary metabolism genes and gene clusters in the genomes of *T. reesei*, *T. atroviride* and *T. virens*.

Core genes	*T. reesei*		*T. atroviride*		*T. virens*	
	Total number	Part of gene cluster	Total number	Part of gene cluster	Total number	Part of gene cluster
NRPS[a]	8	5	9	7	22	17
PKS	11	6	15	9	18	8
PKS/NRPS	2	2	1	1	4	4
Terpene cyclase	6	2	7	3	11	7

a With at least one complete module.

(Aib) and often also other α,α-dialkylated amino acids such as isovaline. More than 1000 different peptaibiotics that have been identified so far are categorized into several groups based on their chemical structures and include peptaibols, lipopeptaibols, lipoaminopeptides, and cyclic peptaibiotics (Neumann et al., 2015). Peptaibols (peptides containing α-aminoisobutyric acid and a C-terminal alcohol) constitute the largest group which is characterized by an acylated N-terminus and an amide-bound amino alcohol at the C-terminus (e.g. phenylalaninol, valinol, tryptophanol, leucinol, isoleucinol). Although their biological roles have only partially been elucidated, antimicrobial and cytotoxic activities have been reported for several peptaibols which is due to their concentration-dependent channel-forming and membrane-permeabilizing activity (Bortolus et al., 2013).

Since the isolation of the first peptaibols, suzukacillin and alamethicin, around fifty years ago (Meyer and Reusser, 1967; Ooka et al., 1966), the interest in these bioactive substances and their fungal producers has continuously grown. *Trichoderma* species are generally regarded as the richest source of peptaibols and over 80 % of the entries in the "Comprehensive Peptaibiotics Database" can be assigned to this fungal genus with *Trichoderma viride*, *Trichoderma brevicompactum*, *T. virens*, *Trichoderma parceramosum/ Trichoderma ghanense* and *T. harzianum* being the most intensively studied species (Neumann et al., 2015; Stoppacher et al., 2013). A comprehensive characterization of the peptaibome of *T. atroviride* strain P1 revealed 20 trichorzianines with 19 amino acids and 15 members of a novel family with 7–9 amino acids (trichoatrokontins) (Stoppacher et al., 2007, 2008). Hitherto unknown 11- and 14-residue peptaibols, a new family of 17-residue peptaibols, and new trichorzianines were reported from other *T. atroviride* strains supporting the variety of substances produced by a single species and their strain-specific variation. Analysis of the peptaibome of 28 different *Trichoderma* species belonging to different phylogenetic clades showed that all tested strains produced peptaibols; however, certain peptaibol groups were found to be restricted to particular clades or sections (Neuhof et al., 2007).*Trichoderma* species exhibiting strong mycoparasitic and plant growth-promoting activities, such as *T. harzianum*, are established commercial biocontrol agents (BCA). A screen of five BCA products formulated with strains of the *T. harzianum* complex for the presence of peptaibiotics revealed new and recurrent peptaibols belonging to three major groups (i.e. substances with 18, 14 and 11 residues) in every formulation (Degenkolb et al., 2015).

As with all NRPs, peptaibol biosynthesis is mediated by NRPS. These large enzymes consist of different modules with each module catalysing the addition of a single amino acid (Marahiel, 2009).The first full-length peptaibol synthetase gene having been cloned already before the *Trichoderma* genome sequences had been published is *T. virens tex1* (Wiest et al., 2002). The continuous ORF of more than 60 kb comprises 18-modules (each module consisting of adenylation, thiolation and condensation domains) and codes for the biosynthesis of 18-residue trichovirin II type peptaibols. Subsequently, with the availability of the genome sequence, the 19-module peptaibol synthetase of *T. atroviride* was identified that catalyzes the biosynthesis of 19-residue atroviridins (Komon-Zelazowska et al., 2007). Eleven-residue peptaibols are the most common and widely distributed in *Trichoderma* (Degenkolb et al., 2012). However, a survey of the three *Trichoderma* genomes revealed the presence of only 7-, 14- and 18–20 module peptaibol synthetases (Degenkolb et al., 2012; Mukherjee et al., 2011). The disruption of the 14-module peptaibol synthetase (*tex2*) in *T. virens*, *T. reesei* and *T. atroviride* abolished the biosynthesis of both 11- and 14-residue peptaibols (Degenkolb et al., 2012; Mukherjee et al., 2011). Module skipping has been proposed to be the mechanism behind the biosynthesis of two classes of peptaibols by a single, 14-module NRPS (Degenkolb et al., 2012; Mukherjee et al., 2011) suggesting that the limited set of peptaibol synthetases encoded in the *Trichoderma* genomes is responsible for the biosynthesis of a variety of products.

Epipolythiodioxopiperazines

Epipolythiodioxopiperazines (ETPs) are highly reactive fungal secondary metabolites characterized by a diketopiperazine ring that is derived from a cyclic peptide. Their toxicity is due to the presence of a disulphide bridge which can inactivate proteins by binding to thiol groups and by generating reactive oxygen species by redox cycling (Gardiner et al., 2005b). The best-studied ETP is gliotoxin whose name is derived from the fungus from which it was originally identified, *Gliocladium fimbriatum* (which was later on re-identified as *T. virens*) (Weindling and Emerson, 1936). Gliotoxin is immunosuppressive and a virulence factor of the human pathogen *Aspergillus fumigatus* but is also important in biocontrol of some plant pathogens by *T. virens* due to its antimicrobial activity (Scharf et al., 2016; Vey et al., 2001). It is produced by certain strains (so-called "Q" strains) of the plant beneficial fungus *T. virens*, whereas another ETP, gliovirin, is exclusively produced by "P" strains of *T. virens* (reviewed in Mukherjee et al., 2012b).

The *A. fumigatus* gliotoxin gene cluster comprises 12 genes including the core enzyme GliP (the NRPS dioxopiperazine synthetase), its auxiliary biosynthetic enzymes and the cluster-specific regulator GliZ (Gardiner et al., 2005a). With the publication of the genome sequence, a gene cluster similar to the gliotoxin cluster of *A. fumigatus* was found to be present in the *T. virens* genome (Mukherjee et al., 2012b). In *T. virens*, the cluster, however, consists of 8 genes only (Fig. 1) but its association with gliotoxin production was confirmed by deletion of part of the *gliP* open reading frame (Vargas et al., 2014).The gliotoxin-non producing mutants had an enhanced vegetative growth rate, but a reduced ability to suppress the plant pathogen *Pythium ultimum*. While much of the biosynthetic pathway has been elucidated in *A. fumigatus* (Scharf et al., 2016), it is not known if the biosynthesis follows the same route in *T. virens*. Unexpectedly, *gliP* and another six genes of the *gli* cluster were found in the *T. reesei* genome (Fig. 1), even though this species is not reported to produce gliotoxin. Another gene cluster, very similar to that responsible for the biosynthesis of sirodesmin PL in the phytopathogen *Leptosphaeria maculans*, is present in *T. virens* (Mukherjee et al., 2012b). However, the metabolite to which this cluster is related in *Trichoderma* remains elusive as it is not expressed under standard laboratory cultivation conditions.

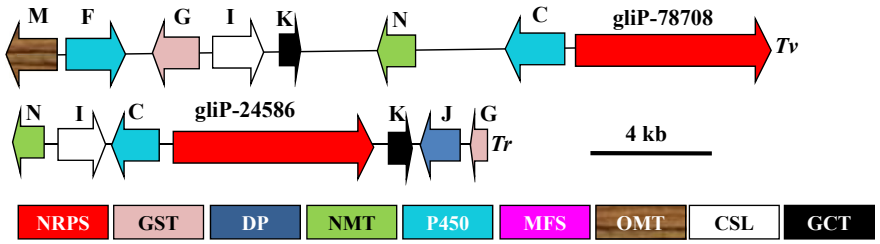

Fig. 1 – The gliotoxin biosynthesis gene clusters of *T. reesei* (Tr) and *T. virens* (Tv). NRPS: Non-ribosomal peptide synthetase; GST: Glutathione-S-transferase; DP: Dipeptidase; NMT: N-methyl transferase; P450: Cytochrome P450; OMT: O-methyl transferase; CSL: C-S-bond lyase; GCT: γ-glutamate cyclotransferase. (Adapted from (Mukherjee *et al.*, 2012b)).

Siderophores

Siderophores are iron-chelating secondary metabolites that are important in microbial interactions with plants and with other microbes. Fungal siderophores mainly are fusarinines, coprogens, and ferrichromes that all belong to the group of hydroxamate siderophores that share the structural unit N5-acyl-N5-hydroxyornithine (Renshaw *et al.*, 2002). Coprogen, coprogen B, and ferricrocin were excreted under iron-deficient conditions by all of the six *Trichoderma* species tested by Anke *et al.* (1991). A recent study further reported on the detection of an average 12–14 siderophores by isotope-assisted screening of *T. atroviride*, *T. asperellum*, *T. gamsii*, *Trichoderma hamatum*, *T. virens*, *T. harzianum*, *Trichoderma polysporum* and *T. reesei* with dimerum acid, coprogren, fusigen, fusarinine A and the intracellular siderophore ferricrocin being produced by all examined species (Lehner *et al.*, 2013). The biosynthesis of fungal siderophores is catalyzed by NRPS mostly from L-ornithine derived N5-acyl-N5-hydroxy-L-ornithine with different possible acyl groups whereby the NRPS covalently links these units *via* ester or peptide bonds to linear or cyclic oligomers which subsequently can be further modified to yield a diversity of different siderophores (Lehner *et al.*, 2013; Renshaw *et al.*, 2002).

Three NRPSs linked to siderophore biosynthesis have been identified in *Trichoderma* spp. (Mukherjee *et al.*, 2012b, 2013a). All species examined have a conserved gene cluster for the biosynthesis of intracellular ferricrocin (Fig. 2) whose role in ferricrocin biosynthesis has been confirmed in *T. virens* (unpublished results, cited in Mukherjee *et al.* 2012b). In addition, they encode a functional cluster with NPS6 as core enzyme for extracellular siderophore production (Mukherjee *et al.*, 2013a) (Fig. 3). Gene deletion experiments confirmed the role of the NPS6 cluster in the biosynthesis of 10 of the 12 siderophores excreted by *T. virens* (unpublished results, cited in Mukherjee *et al.*, 2013a). In the genomes of *T. virens* and *T. reesei* an additional putative gene cluster for siderophore biosynthesis is present (Mukherjee *et al.*, 2012b).

Vinale *et al.* (2009) described from *T. harzianum* the isolation of harzianic acid, a N-heterocyclic compound derived from tetramic acid (which arises from the assembly of an amino acid and an activated acyl entity) (Vinale *et al.*, 2009). Harzianic acid shows plant growth-promoting activity and was described as a novel siderophore due to its ability to bind with a good affinity essential metal ions such as Fe^{3+} (Vinale *et al.*, 2013). However, nothing is known on the genes or enzymes involved in its biosynthesis.

Metabolites produced by "NRPS-like" gene clusters

In addition to the NRPSs with at least one complete module comprising of adenylation (A), thiolation (T) and condensation (C) domains, fungal genomes also harbor genes for so called "NRPS-like" enzymes. These enzymes generally have an adenylation domain, a thiolation domain and a thioester reductase (R) domain, but lack a condensation domain. The biosynthetic potential of these enzymes, catalysing the generation of metabolites like isoquinoline alkaloids, aryl-aldehydes, dioxolanones, piperazines and furanones, has only recently been realized in filamentous fungi (Baccile *et al.* 2016; Forseth *et al.*,

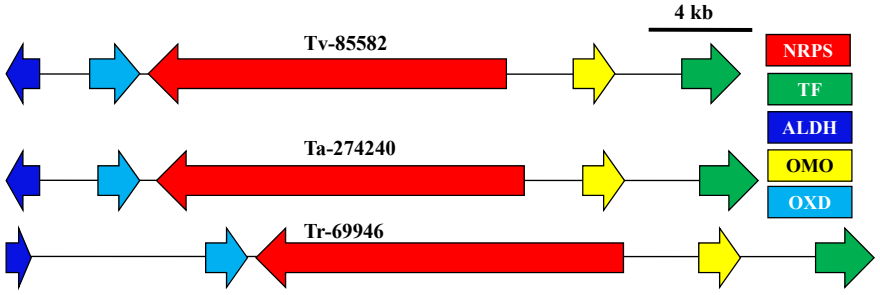

Fig. 2 – The ferrichrome biosynthesis gene clusters of *Trichoderma* spp. NRPS: Non-ribosomal peptide synthetase; TF: Transcription factor; ALDH: Aldehyde dehydrogenase; OMO: Orntithine monooxygenase; OXD: Oxidoreductase. (Adapted in part from (Mukherjee *et al.*, 2012b)).

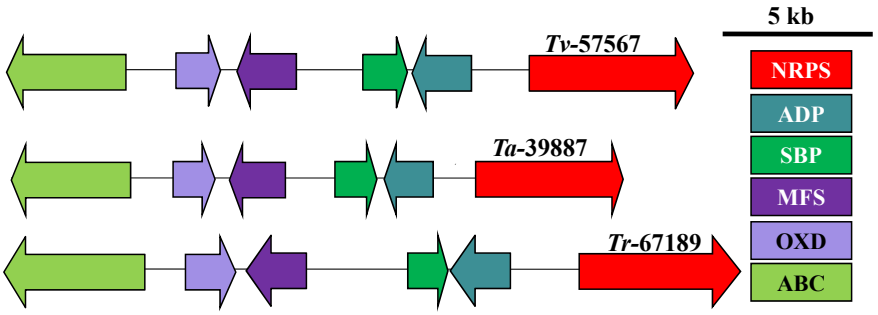

Fig. 3 – The siderophore biosynthesis gene clusters of *T. virens*. NRPS: Non-ribosomal peptide synthetase; ABC: ABC transporter; SBP: Siderophore biosynthesis protein; OXD: Oxidoreductase; MFS: MFS transporter; ADP: Adenylation domain protein. (Adapted from (Mukherjee *et al.*, 2012b)).

2013; Li *et al.*, 2016; Sun *et al.*, 2016; Yeh *et al.*, 2012). So far, the biosynthetic roles of none of these NRPS-like enzymes has been studied in any *Trichoderma* species. Our survey revealed the presence of three such genes in *T. reesei* (IDs 68204, 81014 and 103032). However, none of these are part of a gene cluster. *T. atroviride* has nine genes encoding NRPS-like enzymes (IDs 40037, 52932, 54835, 54918, 84122, 131513, 156569, 224197 and 290109), of which four are part of putative gene clusters (Fig. 4). *T. virens* harbors five NRPS-like genes (IDs 32741, 34076, 52700, 55364 and 60181), of which ID 60181 is part of a PKS/NRPS gene cluster (Mukherjee *et al.* 2012a). As expected, most of the NRPS-like enzymes consist of "A", "T" and "R" domains, except Tr68204 ("A" domain and a "NOX" domain) and Ta40037 ("A" domain and a ferridoxin reductase domain). Ta 40037, 52932 and 84122 comprise of "A" domain and "R" domain, but no "T" domain. Tr81014 has an aldehyde dehydrogenase domain and Ta54835 has a shikimate kinase domain, in addition to "A", "T" and "R" domains (R. Bansal and P.K. Mukherjee, unpublished).

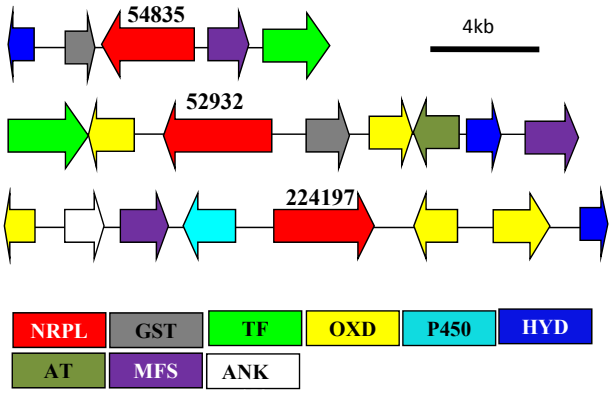

Fig. 4 – The putative "NRPS-like" gene clusters of *T. atroviride*. NRPL: NRPS-like enzyme; GST: Glutathione S-transferase; TF: Transcription factor; OXD: Oxidoreductase; P450: Cytochrome P450; HYD: Hydrolase; AT: Acetyl-transferase; MFS: MFS Transporter; ANK: Ankyrin-repeat protein. ID 84122 is part of a polyketide synthase gene cluster described earlier (Bansal and Mukherjee, 2016a) and hence not included here.

Polyketides

Polyketides are natural products with immense clinical and economical significance and are implicated in the competition and communication between organisms. This structurally diverse group of secondary metabolites produced by bacteria and fungi includes substances with antibiotic activity such as tetracyclines and macrolides, the mycotoxins aflatoxin, fusaric acid and fumonisin, the pigments bikaverin and fusarubin, as well as the cholesterol-lowering statins such as lovastatin and compactin (Zeilinger *et al.*, 2015). Polyketides are synthesized from simple units like acetyl-CoA and malonyl-CoA by polyketide synthases (PKS) which perform successive decarboxylative condensation reactions. PKSs are complex enzymes with the fungal PKS being composed of at least a ketoacyl synthase, an acyl transferase, and a phosphopantetheine attachment site domain (Keller *et al.*, 2005).

Although *Trichoderma* genomes are rich in PKS-encoding genes, not much work has been done on the biosynthesis and genetics of polyketides in these fungi. Baker *et al.* (2012) presented a detailed phylogenomic analysis of the PKS genes of *T. reesei*, *T. virens*, and *T. atroviride* revealing that most of the PKSs belong to the lovastatin/citrinine or fumonisins clades and are present as orthologues in all three species (Baker *et al.*, 2012). These PKS genes are frequently clustered with genes encoding cytochrome P450 monooxygenases, short-chain dehydrogenases/reductases, or epimerases (Schmoll *et al.*, 2016). Apart from the conidial pigment PKS gene clusters, 20 new putative PKS gene clusters have been identified in the genomes of these three species (Bansal and Mukherjee, 2016a).

Deletion of *T. reesei pks4*, an orthologue of the pigment-forming PKSs involved in synthesis of aurofusarin and bikaverin in *Fusarium* spp., provided direct evidence for the respective enzyme being responsible for the green pigmentation of conidia, teleomorph structure, conidial cell wall stability, and antagonistic abilities of *T. reesei* against other fungi. Interestingly, deletion of *pks4* influenced the expression of other PKS genes of the fungus as well (Atanasova *et al.*, 2013a). The organization of putative conidial pigment PKS gene clusters in *Trichoderma* species is presented in Fig. 5.

Several fungal secondary metabolites of interest are produced by PKS-NRPS hybrid enzymes that consist of a PKS

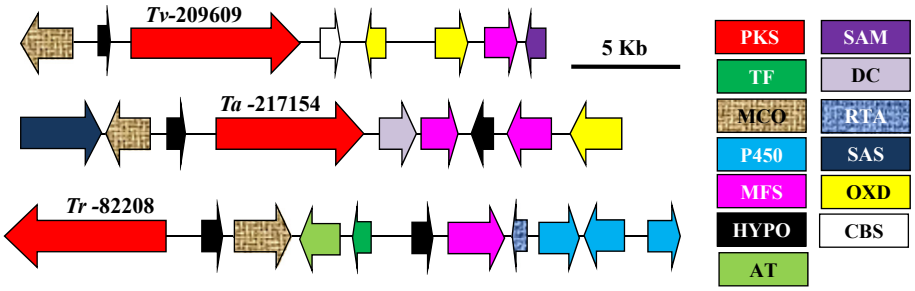

Fig. 5 – The putative conidial pigment PKS gene clusters of *Trichoderma* spp. PKS: Polyketide synthase; TF: Transcription factor; OXD: Oxidoreductase; P450: Cytochrome P450; MCO: Multicopper oxidase; MFS: MFS Transporter; HYPO: Hypothetical protein; SAS: Salicylic acid synthase; RTA- RTA1-like protein; SAM: S-adenosylmethyl transferase; AT: Acetyl-transferase; DC- Decarboxylase; CBS-Cystathionine β synthase.

fused to a single, in some cases truncated NRPS module (Fisch, 2013). Similar to other fungi, corresponding genes have been identified in the *Trichoderma* genomes with *T. virens* having the highest number (Table 1). Functional characterization of one of the four *T. virens* PKS/NRPS hybrids (Tex13), whose expression is induced during interaction with plant roots, revealed an involvement of the resulting still unidentified metabolite in the induction of the defence-related *pal* gene in maize seedlings (Mukherjee *et al.*, 2012a).

Terpenoids

Terpenoids are the most abundant natural products on earth and comprise a variety of volatile and non-volatile secondary metabolites. These are produced by most life forms, including fungi. The assembly of multiple activated forms of the isoprene (C_5H_8) unit results in either hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, tetraterpenes, or polyterpenes. Terpenoids identified from *Trichoderma* spp. include volatile terpenes, the tetracyclic diterpene harziandione, sesquiterpenes such as the trichothecenes trichodermin and harzianum A, and the triterpene viridin (Cardoza *et al.*, 2011; Ghisalberti *et al.*, 1992; Howell *et al.*, 1993; Stoppacher *et al.*, 2010).

The isoprene building block is derived from the mevalonate pathway in most organisms and in *Trichoderma* the involvement of a hydroxy-methylglutaryl-CoA reductase (HMGR) encoded by the *hmgR* gene has been shown. HMGR is the first enzyme in the mevalonate pathway and catalyzes the conversion of hydroxy-methylglutaryl-CoA into mevalonate. *T. harzianum hmgR*-silenced mutants showed a decrease in their antifungal activity against *Rhizoctonia solani* and *Fusarium oxysporum* and had reduced ergosterol levels (Cardoza *et al.*, 2007). A reduction in the production of ergosterol was also observed in *T. harzianum* transformants in which the *erg1* gene, encoding a squalene epoxidase implicated in the triterpene biosynthetic pathway, has been silenced. On the other hand, *erg1* over-expression increased the antifungal activity of *T. harzianum* against *Botrytis cinerea* and reduced lesion size in plant experiments (Cardoza *et al.*, 2006, 2014).

The key enzymes in terpene biosynthesis are terpene synthases/terpene cyclases. However, the diversity of terpene structures frequently exceeds the number of respective enzymes encoded in the genomes as many terpene synthases produce a mixture of closely related compounds instead of a single terpene (Degenhardt *et al.*, 2009). A recent survey indicated that 6 terpene synthases/cyclases are encoded in the *T. reesei* genome, 7 in *T. atroviride*, and 11 in *T. virens*, of which two, three and six are part of biosynthetic gene clusters (Bansal and Mukherjee 2016b). However, little is known hitherto on the products resulting from these enzymes. The first secondary metabolism-related gene cluster having been discovered in *Trichoderma* happens to be a terpenoid biosynthesis cluster (Mukherjee *et al.*, 2006). This cluster present in *T. virens* but absent from the genomes of *T. reesei* and *T. atroviride*, was initially predicted to be a viridin biosynthetic cluster but subsequently turned out to be responsible for the biosynthesis of more than 20 volatile sesquiterpenes (Crutcher *et al.*, 2013).

The sesquiterpenes trichothecenes are a large group of toxic secondary metabolites produced by a few fungal species mainly belonging to the genus *Fusarium* (Woloshuk and Shim, 2013).The *TRI* gene cluster for trichothecene biosynthesis has earlier been described in *Trichoderma arundinaceum* and *T. brevicompactum* and consists of orthologues of seven genes present in the *Fusarium TRI* cluster (Cardoza *et al.*, 2011; Fig. 6). An orthologue of the terpene cyclase Tri5 is present in *T. asperellum* and *T. gamsii* genomes (Table 2). However, no trichothecenes have been discovered yet in these two species which are used commercially as biofungicides. Interestingly, *T. gamsii* has been found to reduce the production of the trichothecene deoxynivalenol (DON) by *Fusarium graminearum* and *Fusarium culmorum* and spike application of *T. gamsii* in wheat resulted in control of *Fusarium* head blight under field conditions (Sarrocco *et al.*, 2013).

Cloning and over-expression of the trichodiene synthase-encoding gene *tri5* in *T. brevicompactum* resulted in enhanced production of trichodermin, an antifungal and phytotoxic agent (Tijerino *et al.*, 2011a, 2011b). Disruption of *tri4*, encoding a cytochrome P450 monooxygenase, or *tri5* in *T. arundinaceum* resulted in abolished biosynthesis of the non-phytotoxic trichothecene harzianum A, reduced antifungal activity against *B. cinerea* and *R. solani*, and a reduced ability to induce defense responses in tomato (Malmierca *et al.*, 2012, 2013). Expression of *tri5* in a biocontrol strain of *T. harzianum* resulted in the biosynthesis of the volatile compound

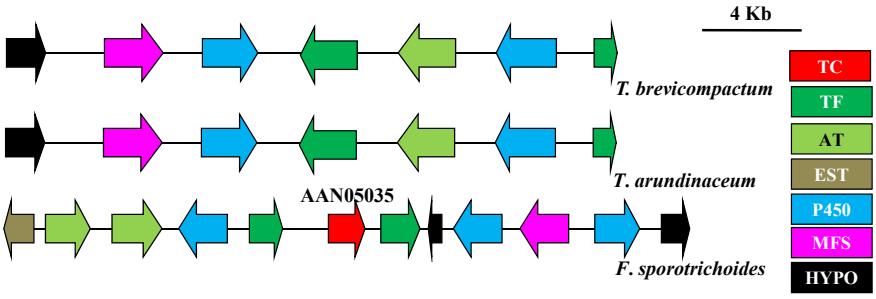

Fig. 6 – The "Tri" clusters of *T. brevicompactum* and *T. arundinaceum* vis-a-vis that of *Fusarium sporotrichoides*. TC: Terpene cyclase; TF: Transcription factor; AT: Acetyltransferase; EST: Esterase; P450: Cytochrome P450; MFS: MFS transporter; HYPO: Hypothetical protein. Adapted from (Mousa and Raizada, 2015). Please note that the *tri5* genes of *T. brevicompactum* and *T. arundinaceum* are located elsewhere in the genome and are not part of the gene cluster.

Table 2 – *tri5* genes of *T. gamsii* and *T. asperellum*.

Protein Id/accession no.	Homologue	Query coverage	E value	Identity	GenBank accession no.
Trichoderma gamsii (KUF04589)	Trichodiene synthase of *Trichoderma brevicompactum*	99 %	0.0	87 %	CAX94841
	Trichodiene synthase of *Fusarium sporotrichoides*	90 %	6e-150	57 %	AAN05035
Trichoderma asperellum (448313)	Trichodiene synthase of *Trichoderma brevicompactum*	98 %	0.0	83 %	CAX94841
	Trichodiene synthase of *Fusarium sporotrichoides*	93 %	3e-130	54 %	AAN05035

trichodiene which induced the expression of tomato defense-related genes (Malmierca *et al.*, 2015).However, co-expression of *T. arundinaceum tri4* and *tri5* in *T. harzianum* resulted in the production of 12,13-epoxytrichothec-9-ene (EPT) in the transformed strain. EPT production by *T. harzianum* down-regulated plant genes involved in fungal root colonization and plant defence (Cardoza *et al.*, 2015). The whole genome sequences of neither *T. arundinaceum* nor of *T. brevicompactum* are available so far.

6-Pentyl pyrone

6-Pentyl pyrone (6-PP), an unsaturated lactone, is responsible for the characteristic "coconut aroma" of certain *Trichoderma* species and has been reported to have anti-fungal and plant growth-promoting activities (Vinale *et al.*, 2008). 6-PP belongs to the chemically diverse group of low-molecular weight metabolites having a high vapor pressure at room temperature and low water solubility classified as volatile organic compounds (VOCs). Besides pyrones and the above described terpenes, fungal-derived VOCs mainly comprise alcohols, ketones, alkanes, and alkenes which are produced as intermediate or end products of the fungus' metabolism (Korpi *et al.*, 2009). The biosynthesis of fungal VOCs is dependent on growth conditions (nutrient availability, pH of the medium, temperature, light) and is species- or even strain-specific. VOC production often coincides with certain developmental stages such as spore formation and certain VOCs are produced in association with mycotoxins (e.g. Wilkins *et al.*, 2003); in addition, the VOC profile frequently changes during antagonistic interaction with other (micro)-organisms or substances derived thereof (Stoppacher *et al.*, 2010; Wheatley, 2002). For details on VOCs produced by *Trichoderma* spp., their biosynthesis and bioactivity, the reader is referred to recent reviews e.g. (Siddiquee, 2014; Zeilinger and Schuhmacher, 2013).

Pyrones are derived from fatty acids and the biosynthesis of 6-PP has been studied in *T. atroviride* IMI206040 (formerly classified as *T. harzianum*) by using [U-^{14}C] and [1-^{14}C] linoleic acid. It was proposed that linoleic acid is oxidized to 13-hydroperoxide-diene (13-HPOD) followed by the formation of 5-hydroxy-2,4-decenioc acid by beta-oxidation and isomerization. A final esterification then results in 6-PP (Serrano-Carreon *et al.*, 1993) which hence is a so-called oxylipin. While the lipoxygenase pathway leading to the formation of conjugated 13-HPOD intermediates of linoleic acid is well known in plants, the enzymes and underlying genes for 6-PP production in *Trichoderma* still remain obscure. However, comparative genome analysis revealed a lipoxygenase gene (ID 33350) in *T. atroviride* but not in *T. virens* and *T. reesei* that is up-regulated during the interaction of *T. atroviride* with *R. solani* (Atanasova *et al.*, 2013b; Kubicek *et al.*, 2011). *T. atroviride* ID 33350 contains a C-terminal isoleucine and the conserved WRYAK motif characteristic for the Ile-group of fungal lipoxygenases (Heshof *et al.*, 2014). The involvement of ID 33350 in 6-PP biosynthesis in *T. atroviride* is substantiated by the fact that 6-PP is formed by *T. atroviride* but not the other two species (Reino *et al.*, 2007). However, a gene deletion experiment to confirm the role of this gene in 6-PP biosynthesis is yet to be performed.

3. Regulation of secondary metabolism in *Trichoderma*

Fungal secondary metabolism is a tightly regulated cellular process and during recent years, several studies on the influence of

environmental conditions and regulatory factors significantly advanced our understanding on its regulation. Similar to other fungi, the expression of secondary metabolism-related genes in *Trichoderma* spp. is known to be controlled by interactions with other (micro-) organisms, pH signaling, and the velvet-complex proteins (Atanasova *et al.*, 2013b; Bazafkan *et al.*, 2015; Fekete *et al.*, 2014; Karimi-Aghcheh *et al.*, 2013; Malmierca *et al.*, 2015; Mukherjee and Kenerley, 2010; Trushina *et al.*, 2013) (Fig. 7). Atanasova *et al.* (2013b) studied the transcriptomic responses of *T. atroviride*, *T. virens* and *T. reesei* to the presence of *R. solani*. Two PKSs were among the genes induced in *T. atroviride*—*R. solani* and *T. reesei*—*R. solani* interactions, while in *T. virens* all genes in the gliotoxin biosynthesis cluster were up-regulated. *T. atroviride* further showed an up-regulation of the lipoxygenase gene thought to be involved in 6-PP biosynthesis (Kubicek *et al.*, 2011) and in *T. arundinaceum*, growth in co-culture with *B. cinerea* led to enhanced expression levels of the "*tri*" biosynthetic genes (Malmierca *et al.*, 2015). However, botrydial, a secondary metabolite and virulence factor of *B. cinerea*, attenuated *tri* gene expression and the production of harzianum A in *T. arundinaceum* in the interaction zone between the two fungi (Malmierca *et al.*, 2015). The fact that prey fungi and their secondary metabolites do not always have a stimulatory role on *Trichoderma* secondary metabolite production further is corroborated by profiling the production of VOCs in *T. atroviride* in the presence of the *Fusarium* mycotoxin fusaric acid (FA). FA was found to alter the expression pattern of *T. atroviride* VOCs, i.e. suppressed 6-PP production, while the production of sporulation-associated metabolites such as 1-octen-3-ol was increased in the presence of FA (Stoppacher *et al.*, 2010). In return, at least certain *Trichoderma* strains are capable of degrading FA and inhibiting *Fusarium moniliforme* FA production due to the secretion of 6-PP (El-Hasan *et al.*, 2008). These transcriptomics and metabolomics studies corroborate the species-specific production of secondary metabolites with only a subset of genes being expressed at a given condition and the regulatory role these substances may exert on the interaction partner.

As described, several secondary metabolism-related gene clusters present in the *Trichoderma* genomes harbor specific, although currently underexplored transcription factors. In

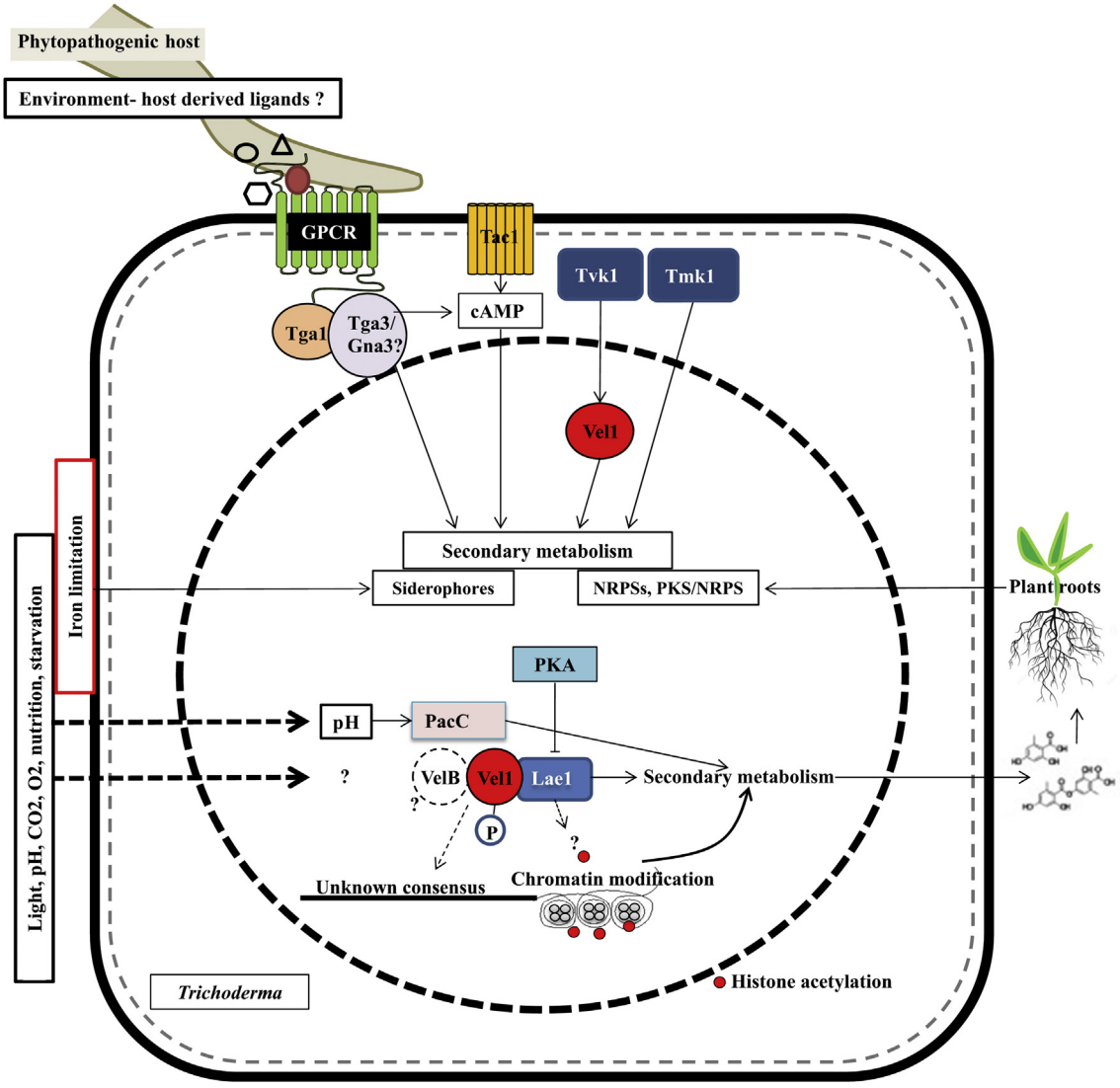

Fig. 7 – Model representing the regulators of secondary metabolite biosynthesis in *Trichoderma*. For details, see text.

addition to these in-cluster regulators, more global key players contribute to the regulation of fungal secondary metabolite biosynthesis (Fig. 7). These include the major pH regulator PacC which impacts a range of fungal genes in response to environmental pH (Knox and Keller, 2015; Trushina et al., 2013). The *T. virens* PacC orthologue governs secondary metabolite biosynthesis and iron transport as the expression of genes encoding the NRPS Tex15 (Mukherjee et al., 2012a), a neighboring cytochrome P450, as well as siderophore-related biosynthesis enzymes and transporters was altered in Δ*pacC* mutants. In addition, *T. virens* Δ*pacC* mutants displayed decreased biocontrol activity, which may be due to their inability to adapt to alkaline pH. Fungal secondary metabolite production further is under regulation of the heterotrimeric velvet complex consisting of the methyltransferase LaeA and the two velvet proteins VeA and VelB. The Vel complex synchronizes sexual development and secondary metabolism in response to light and its mechanism is best studied in *Aspergillus nidulans*, where a completely assembled Vel complex is only localized in the nucleus under dark conditions (Bayram et al., 2008). The *T. virens* veA ortholog Vel1 is involved in the regulation of secondary metabolic gene clusters as *vel1* gene disruption abolished gliotoxin biosynthesis and silenced several secondary metabolism-related genes including three NRPSs, two PKSs, one O-methyl transferase and a cytochrome P450 monooxygenase tested (Mukherjee and Kenerley, 2010). Recently, the impact of Vel1 on secondary metabolism and mating partner sensing in *T. reesei* has been shown (Bazafkan et al., 2015). A similar role can be attributed to Lae1, the *T. reesei* LaeA orthologue, which in addition is essential for the expression of lignocellulose-degrading enzymes (Karimi-Aghcheh et al., 2013; Seiboth et al., 2012). Exceptionally, cellulose-, hemicellulose- and other carbohydrate-active enzyme-encoding genes are accumulated in discrete clusters together with genes for secondary metabolic enzymes in the *T. reesei* genome (Martinez et al., 2008). Their Lae1-dependent expression pattern correlated with changes in histone marks which indicates epigenetic regulation (Karimi-Aghcheh et al., 2013). Deletion of *lae1* in *T. atroviride* abolished the mycoparasitic ability of the fungus. This correlated with a significantly decreased expression of PKS-encoding genes and the proposed 6-PP-related lipoxygenase gene as well as with a reduced production of antifungal water-soluble metabolites and VOCs. The dependence of 6-PP production on Lae1 is further corroborated by an enhanced 6-PP level being secreted by *lae1* over-expressing strains in antagonism experiments (Karimi Aghcheh et al., 2013). In *T. harzianum*, 6-PP biosynthesis is associated with the transcription factor Thctf1. Thctf1 shows considerable sequence similarity with the cutinase transcription factor 1 alpha (CTF1) of *Fusarium solani* f. sp. *pisi* and *Thctf1* deletion correlated with the absence of two secondary metabolites derived from 6-PP and altered antimicrobial activity of *T. harzianum* (Rubio et al., 2009).

The sensing and transfer of environmental cues impacting fungal secondary metabolism is achieved by membrane-bound receptors such as GPCRs (G protein-coupled receptors) and associated intracellular signaling pathways. Evidence accumulated for the biosynthesis of secondary metabolites in *T. atroviride* being governed by G protein signaling and the associated cAMP pathway (Omann and Zeilinger, 2010; Reithner et al., 2005; Zeilinger et al., 2005). Deletion of *tga1*, which encodes the adenylyl cyclase-inhibiting Gα subunit of *T. atroviride*, resulted in decreased 6-PP but enhanced peptaibol production (Reithner et al., 2005). In contrast, *T. atroviride* Δ*tga3* mutants, missing the adenylyl cyclase-stimulating Gα subunit, were fully impaired in the production of peptaibols (Komon-Zelazowska et al., 2007; Zeilinger et al., 2005) and peptaibol biosynthesis further depended on the function of the two blue light regulators BLR1 and BLR2 under certain conditions (Komon-Zelazowska et al., 2007). Unfortunately, no data on the exact roles of the adenylate cyclase (AC) are available for *T. atroviride*. However, cAMP signaling positively regulates secondary metabolism in *T. virens* and the AC-encoding *tac1* gene is involved in growth, germination, mycoparasitism and secondary metabolite biosynthesis in this species (Mukherjee et al., 2007). cAMP activates cAMP-dependent protein kinase (PKA) that consists of both regulatory and catalytic subunits with the latter (PKA1) having been reported as a negative regulator of *lae1* expression in *T. reesei*. Besides the cAMP pathway, MAPK (mitogen-activated protein kinase) signaling cascades are important downstream effectors of G proteins in fungi. Deletion of the *tmk1* MAPK-encoding gene resulted in *T. atroviride* mutants with increased antifungal activity and enhanced peptaibol and 6-PP levels (Reithner et al., 2007), whereas *T. virens* mutants missing the respective MAPK homolog TmkA/Tvk1 had an unaltered production of secondary metabolites (Mendoza-Mendoza et al., 2003; Mukherjee et al., 2003).

4. Bioactivity and biological role of *Trichoderma* secondary metabolites

Due to their mycotrophic lifestyle and the rhizosphere competence of several species, *Trichoderma* fungi are closely associated with other fungi, with plant roots and with a variety of prokaryotic soil microbes in their natural environments. As already pointed out, secondary metabolites usually are produced as families of related compounds during a limited stage of the cell cycle or a specific stage of morphological differentiation. Hence, they are not directly involved in normal growth but serve survival functions by supporting the fungus' abilities in competition, symbiosis, and self-protection and certain compounds may act as pathogenicity factors as well (Demain and Fang, 2000).

The success of *Trichoderma* as biocontrol agent is at least partly due to the ability of these fungi to produce a plethora of secondary metabolites. Gliotoxin was the first *Trichoderma*-derived compound for which an antifungal activity and a role in the antagonistic activity of *T. virens* has been reported (Weindling, 1943). *Trichoderma* mycoparasites antagonize and kill their fungal preys by a combined action of secondary metabolites and hydrolytic enzymes which lyse the cell wall of the prey fungus (Druzhinina et al., 2011). For gliotoxin, synergism with an endochitinase has been shown for inhibition of *B. cinerea* spore germination (Lorito et al., 1994). Furthermore, the absence of gliotoxin production in *T. virens gliP* deletion mutants resulted in attenuation of mycoparasitism of the oomycete pathogen *P. ultimum* and the soybean pathogen *Sclerotinia sclerotiorum*; however, the

mycoparasitic activity against *R. solani* seems to be *gliP*-independent (Vargas *et al.*, 2014). Besides its role in the antagonism of certain preys, phytotoxic activity of gliotoxin has been reported (Haraguchi *et al.*, 1996; Furuta *et al.*, 1984; Wright, 1951) which, however, was not evident in biocontrol trials (Howell, 2006).

Besides being mycoparasites, several *Trichoderma* species are associated with plant roots with which they can establish robust and long-lasting interactions. *Trichoderma* fungi, similar to other plant beneficial microbes, trigger localized or systemic resistance responses in the plant by releasing elicitor-like substances (Harman *et al.*, 2004). Several secondary metabolites like peptaibols, harzianolides, and certain VOCs are known both for their antifungal as well as plant growth-promoting activities (Fig. 8). 6-PP for example has antifungal activity as it reduces mycelial growth of phytopathogens such as *B. cinerea*, *R. solani* and *F. oxysporum*, as well as plant growth regulating and systemic defense-inducing effects by possibly acting as auxin-like compound (Vinale *et al.*, 2008). Recently, the positive effect of 6-PP treatment on tomato plants was correlated to changes in the composition of the tomato leave metabolome such as significantly enhanced acetylcholine and gamma-aminobutyric acid contents (Mazzei *et al.*, 2016). Likewise, there are several reports on oxylipins from other fungi mediating communication between plants and fungi. Examples include *Aspergillus flavus*, where a single lipoxygenase produces oxylipins that influence responses in maize and peanut host plants (Fischer and Keller, 2016) and the endophyte *Fusarium incarnatum* that produces archetypal plant defense oxylipins (Ding *et al.*, 2012). Activities on pea, canola and tomato plants were also reported for *Trichoderma*-derived harzianolide (Vinale *et al.*, 2008).

While, due to their volatility and ability to diffuse across cellular membranes, VOCs are ideal communication molecules even over long distances, water soluble polar secondary metabolites such as peptaibols will remain more closely associated with the producing fungus and hence will exert a short-distance effect. Similar to 6-PP, however, multi-functional roles have been reported for peptaibols. They promote antagonism of plant pathogens by inhibiting their β-glucan synthase activity, which, due to synergism with cell wall-degrading enzymes secreted by *Trichoderma*, contributes to prey attack by preventing the reconstruction of the pathogens' cell wall (Lorito *et al.*, 1996; Schirmbock *et al.*, 1994). Trichokonin VI, a *Trichoderma pseudokoningii*-derived peptaibol, further was effective against fungal plant pathogens by inducing extensive apoptotic programmed cell death and against the bacterium *Bacillus subtilis* where its application led to cell collapse and leakage of intracellular materials (Shi *et al.*, 2012; Su *et al.*, 2012). Antifungal activities were reported also for alamethicin, trichotoxins, trichopolins, paracelsin, trichorzins, harzianins, and trichokonins (Brückner *et al.*, 1983; Hou *et al.*, 1972; Leitgeb *et al.*, 2007; Rebuffat *et al.*, 1992; Xiao-Yan *et al.*, 2006). Besides their direct antibiotic action on competing bacteria and fungi, peptaibols also induce plant defense responses. *T. virens* Δ*tex1* mutants, devoid of the capacity to produce 18 residue peptaibols, lost the ability to induce systemic resistance responses in cucumber (Viterbo *et al.*, 2007). Similarly, application of the 20mer peptaibol alamethicin from *T. viride* elicited salicylic acid and jasmonic acid mediated defense responses in lima bean (Engelberth *et al.*, 2001). Low concentrations of trichokonins were further reported to induce defense responses and systemic resistance against tobacco mosaic virus in tobacco plants (Luo *et al.*, 2010). While the antimicrobial effect of peptaibols can be attributed to their ion channel-forming activity and an inhibitory action on membrane-associated enzymes involved in cell wall synthesis, the basis for their plant beneficial action is less clear. Alamethicin induced the formation of pores in the plasma membrane, the inner mitochondrial membrane and the plastid inner envelop of plant cells (Aidemark *et al.*, 2009; Johansson *et al.*, 2004; Matic *et al.*, 2005). However, this negative effect could be counteracted by treatment with *Trichoderma*-derived cellulase as this resulted in altered membrane properties and concurrent alamethicin resistance (Aidemark *et al.*, 2010).

Trichoderma species are fast growing fungi and hence successful competitors for space and nutrients. The siderophores secreted by *Trichoderma* scavenge iron from the environment thus making it unavailable for competing microorganisms. Competition for iron has been described as one of the key factors in the antagonism of *T. asperellum* against *F. oxysporum* f.sp. *lycopersici* (Segarra *et al.*, 2010) and *Trichoderma* siderophores may as well be beneficial for plants due to their iron solubilizing activity. The latter has recently been reported for the novel siderophore harzianic acid, which, besides being antifungal, improved the growth of tomato seedlings even in iron-deficient conditions. This promotion of plant growth was attributed to the Fe^{3+} – binding affinity of harzianic acid, as Fe^{3+} – siderophore complexes can be recognized and taken up by several plant species (Vinale *et al.*, 2013).

Auto-regulatory substances regulate morphogenetic transitions and hence are involved in germination, colony morphogenesis and sexual and asexual development. Secondary metabolites acting as auto-regulators involved in asexual sporulation have been described for *Trichoderma*. 1-octen-3-ol inhibits germination and colony growth but enhances the conidiation response in *T. atroviride* (Nemcovic *et al.*, 2008). A similar function of this C8-oxylipin has been described in *A. nidulans* (Herrero-Garcia *et al.*, 2011). In *T. virens*, the sesquiterpenoid carot-4-en9,10-diol has a stress-related conidiation-

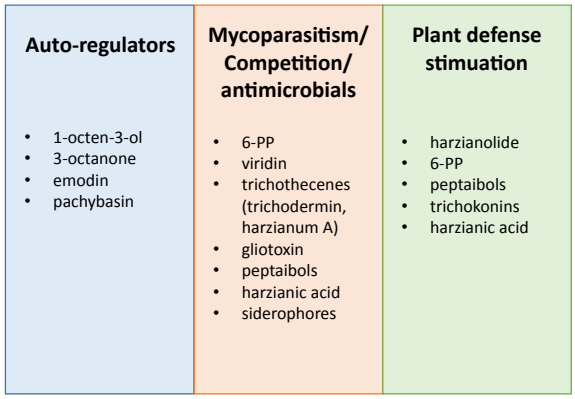

Fig. 8 – Examples of *Trichoderma*-derived secondary metabolites with roles in auto-regulatory processes, in mycoparasitism/competition and interaction with the plant.

inducing role (Wang et al., 2013). Coiling of *Trichoderma* around prey hyphae is a mycoparasitism-associated morphological change, which, together with the formation of penetration structures, aids in prey attack (Lu et al., 2004). Lin et al. (2012) reported on an involvement of pachybasin and emodin in self-regulation of coiling in *T. harzianum*. Addition of both of these *T. harzianum*-derived anthraquinone secondary metabolites increased the number of coils of the mycoparasite around *R. solani* hyphae and this effect seems to be due to a stimulation of cAMP synthesis.

5. Methods and approaches for profiling of *Trichoderma* secondary metabolites

The secondary metabolome usually is a result of many enzymes and their respective genes and there is not always a one-to-one relationship between a gene and a metabolite (Smedsgaard and Nielsen, 2005) making obvious the need for complementing genomic and transcriptomic approaches by metabolomics. In addition, the sequencing of fungal genomes revealed that secondary metabolism-associated gene clusters exceed the number of secondary metabolites that can be identified from a given fungus under laboratory conditions, i.e. several of the predicted gene clusters remain silent (Hertweck, 2009). Approaches for activating these silent clusters include the modification of factors involved in regulating their expression such as over-expressing relevant (cluster-specific) transcription factors and (epi)-genetic engineering (Brakhage and Schroeckh, 2011; Strauss and Reyes-Dominguez, 2011). Recent developments aimed at heterologous expression of secondary metabolism-associated gene clusters or biosynthetic pathways in model systems or cell factories such as *Aspergillus* or yeast combined with natural product discovery (Anyaogu and Mortensen, 2015; Bok et al., 2015). Expression in the heterologous host *A. nidulans* of intact *Aspergillus terreus* secondary metabolite clusters captured in self-replicating fungal artificial chromosomes led to the discovery of the *A. terreus* astechrome biosynthetic machinery (Bok et al., 2015). This example nicely shows that metabolomics combined with efforts to activate the expression of silent clusters can contribute to the identification and development of novel secondary metabolites even from less amenable, "exotic" fungi.

Metabolomics, the determination of the entity of all low-molecular weight metabolites of a given organism, comprises targeted as well as untargeted approaches. While a targeted approach aims at the identification (and quantification) of predefined already known metabolites in a given sample, untargeted profiling is a holistic approach trying to generate a snapshot of the biological sample by searching for all, even unknown, detectable compounds. Liquid or gas chromatography coupled with mass spectrometry (LC-MS, GC–MS) usually are the methods of choice for the specific and sensitive analysis of metabolites in complex biological samples as they allow the simultaneous detection of a great number of metabolites. The application of GC–MS, which is suitable for the detection of both volatile and polar small metabolites, and LC-MS, which is applied for the study of mid- to non-polar substances, for secondary metabolite profiling of filamentous fungi has recently been reviewed in (Kluger, 2015).

LC coupled to tandem mass spectrometry (MS/MS) is usually used for the detection of peptaibiotics in extracted samples from fungal cultures whereby mass differences of $\Delta m/z$ 85 indicate the peptaibiotic-specific amino acid Aib (Degenkolb et al., 2003). Based on the amino acid sequences obtained by MS/MS analysis, known structures can be identified by comparison to entries in respective databases such as the "*Comprehensive Peptaibiotics Database*" (Neumann et al., 2015). An example is the comprehensive characterization of the *T. atroviride* peptaibiome by an LC-MS/MS-based screening of culture filtrates by Stoppacher et al. (2008) whose outcome is described in more detail in Section 2.1.1. In addition, intact cell MALDI-TOF (matrix-assisted laser desorption/ionization-time of flight) mass spectrometry was used by Neuhof et al. (2007) for the detection of peptaibol production profiles of 28 different *Trichoderma/Hypocrea* species. This approach has the advantage of using small samples (microgram amounts of fungal mycelia grown on plates), requiring only minimal sample preparation and being very rapid; however, it only partially identifies the detected peptaibols (Neuhof et al., 2007).

Due to their volatility, VOCs can be determined by GS-MS without chemical derivatization from liquid culture extracts or by directly probing the headspace above fungal samples (Kluger, 2015). Extraction with organic solvents has for example been applied for the investigation of VOC production in *T. harzianum* and *T. viride* cultures (Claydon et al., 1987; Zeppa et al., 1990). Other reports describe the analysis of the VOC profile of *T. atroviride* directly from the headspace of the living fungus growing on solid media in headspace vials using non-invasive solid phase microextraction (SPME) followed by GS-MS (Kluger et al., 2013; Stoppacher et al., 2010). However, due to the diverse properties of VOCs, it can be assumed that a complete profile of a given sample may only be obtained by a combination of analytical methods.

GC–MS is also suitable for the determination of polar, non-volatile metabolites, although chemical derivatization is required that renders the metabolites less polar and hence more volatile for proper separation on a GC column. While this method is usually applied for primary metabolite analysis, the technique of choice for the profiling of non-volatile fungal secondary metabolites is reverse phase (RP) LC-MS with LC-ESI (electrospray ionization)-HRMS (high resolution MS) currently enabling the most comprehensive, unbiased coverage of the secondary metabolome (Kluger et al., 2015).

In untargeted profiling, data processing and reliable metabolite identification are critical steps as they are complicated by noise and background ions. Novel stable-isotope labeling (SIL)-assisted techniques, which are based on the incorporation of stable isotopes from precursor substrates into metabolites, are excellently suited to solve this problem (Kluger, 2015). Heavy stable isotopes of carbon (^{13}C), nitrogen (^{25}N) or sulphur (^{34}S) can be used as the mass spectrometer can reliably separate isotopically labeled compounds based on mass difference assisting the determination of elemental composition and the structural identification of metabolites (Chokkathukalam et al., 2014).

Imaging mass spectrometry (IMS) is another recent advancement in metabolomics that is optimally suited for

the direct analysis of living fungi. IMS usually is associated with MALDI, which is then coupled to a mass spectrometer, for which images can be produced depicting the spatial organization of natural products (e.g. Bouslimani *et al.*, 2014; Watrous and Dorrestein, 2011; Netzker *et al.*, 2015; Shih *et al.*, 2014). MALDI-IMS has repeatedly been used for metabolic profiling of living bacterial communities and of interkingdom interactions between bacteria and fungi directly from the petri dish (Moree *et al.*, 2012; Traxler *et al.*, 2013; Watrous *et al.*, 2012). Recently, this technique has also been established for the visualization of secondary metabolites in the mycoparasitic interaction between *T. atroviride* and *R. solani*. The two fungi were cultivated in a miniaturized confrontation assay directly on MALDI targets covered by a thin layer of agar and several signals could be assigned to *Trichoderma*-derived peptaibols (Holzlechner *et al.*, 2016). While MALDI requires the deposition of a matrix, electrospray ionization (DESI) or nanospray desorption electrospray ionization (nano-DESI)-MSI has little or no sample preparation requirements making it well suited for the analysis of co-cultivations (Fang and Dorrestein, 2014). Imprint DESI-MS imaging based monitoring of secondary metabolites during the antagonistic interaction of *T. harzianum* and the fungal phytopathogen of cacao plants *Moniliophtora roreri* has recently been reported (Tata *et al.*, 2015). Four phytopathogen-dependent secondary metabolites (T39 butenolide, harzianolide, sorbicillinol, and an unknown substance), that were specifically produced in the co-culture but not in monocultures of the two fungi, were identified and spatially localized in the interaction and overgrowth zones. T39 butenolide and harzianolide produced by *T. harzianum* have antifungal activity; sorbicillinol is a key intermediate in bisorbicillinoid biosynthesis, a family of *Trichoderma*-derived secondary metabolites (Abe *et al.*, 2001).

6. Conclusions and outlook

Even though species of the genus *Trichoderma* have an enormous capacity to produce secondary metabolites and *Trichoderma*-derived bioactive substances are known since the 1930s, our understanding of the underlying genes, biosynthesis pathways and their regulation is still limited. Recent genome-wide analyses led to the location of a multitude of secondary metabolism-associated genes and gene clusters in the sequenced *Trichoderma* genomes; however, the products of most of these remain obscure and await their discovery. Secondary metabolites not only are important for *Trichoderma* as communication and defense molecules in its natural, highly competitive environment, but also bear great potential for a variety of applications. These include the inhibition of mycotoxigenic fungi during crop storage by *Trichoderma* VOCs (Mejía Agüero *et al.*, 2008), the application of massoialactone as fungicidal ingredient for the control of fungal pathogens (Hill *et al.*, 2000), the potential of the lipopeptaibol trichogin GA IV as selective antibiotic against *Staphylococcus aureus* and methicillin-resistant strains thereof (De Zotti *et al.*, 2009) and the treatment of hepatocellular carcinoma cells with trichokonin VI (Shi *et al.*, 2010) to mention just a few examples. Detailed knowledge on the biosynthetic machinery and the biotic and abiotic factors triggering secondary metabolite production in *Trichoderma* will allow a tailor-made application of these fungi in biocontrol and will lead to the generation of strains producing adequate amounts of pharmaceutically and biotechnologically interesting substances.

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REFERENCES

Abe, N., Sugimoto, O., Arakawa, T., Tanji, K., Hirota, A., 2001. Sorbicillinol, a key intermediate of bisorbicillinoid biosynthesis in *Trichoderma* sp. USF-2690. Biosci. Biotechnol. Biochem. 65, 2271–2279.

Aidemark, M., Andersson, C.-J., Rasmusson, A.G., Widell, S., 2009. Regulation of callose synthase activity in situ in alamethicin-permeabilized Arabidopsisand tobacco suspension cells. BMC Plant Biol. 9, 1–13.

Aidemark, M., Tjellström, H., Sandelius, A.S., Stålbrand, H., Andreasson, E., Rasmusson, A.G., Widell, S., 2010. *Trichoderma viride* cellulase induces resistance to the antibiotic pore-forming peptide alamethicin associated with changes in the plasma membrane lipid composition of tobacco BY-2 cells. BMC Plant Biol. 10, 1–13.

Anke, H., Kinn, J., Bergqusit, K.-E., Sterner, O., 1991. Production of siderophores by strains of the genus Trichoderma. Bio Metals. 4, 176–180.

Anyaogu, D.C., Mortensen, U.H., 2015. Heterologous production of fungal secondary metabolites in Aspergilli. Front. Microbiol. 6, 77.

Atanasova, L., Knox, B.P., Kubicek, C.P., Druzhinina, I.S., Baker, S.E., 2013a. The polyketide synthase gene pks4 of *Trichoderma reesei* provides pigmentation and stress resistance. Eukaryot. Cell 12, 1499–1508.

Atanasova, L., Le Crom, S., Gruber, S., Coulpier, F., Seidl-Seiboth, V., Kubicek, C.P., Druzhinina, I.S., 2013b. Comparative transcriptomics reveals different strategies of *Trichoderma mycoparasitism*. BMC Genomics 14, 121.

Baccile, J.A., Spraker, J.E., Le, H.H., Brandenburger, E., Gomez, C., Bok, J.W., Macheleidt, J., Brakhage, A.A., Hoffmeister, D., Keller, N.P., Schroeder, F.C., 2016 Apr 11. Plant-like biosynthesis of isoquinoline alkaloids in *Aspergillus fumigatus*. Nat. Chem. Biol.. http://dx.doi.org/10.1038/nchembio.2061 (advance online publication).

Baker, S.E., Perrone, G., Richardson, N.M., Gallo, A., Kubicek, C.P., 2012. Phylogenomic analysis of polyketide synthase-encoding genes in *Trichoderma*. Microbiology (Reading, England) 158, 147–154.

Bansal, R., Mukherjee, P., 2016a. Identification of novel gene clusters for secondary metabolism in *Trichoderma* genomes. Microbiology 85 (2), 185–190.

Bansal, R., Mukherjee, P., 2016b. The terpenoid biosynthesis toolkit of *Trichoderma*. Nat. Prod. Commun. 11, 431–434.

Baroncelli, R., Zapparata, A., Piaggeschi, G., Sarrocco, S., Vannacci, G., 2016. Draft whole-genome sequence of *Trichoderma gamsii* T6085, a promising biocontrol agent

of fusarium head blight on wheat. Genome Announc. 4 e01747-01715.
Bayram, O., Krappmann, S., Ni, M., Bok, J.W., Helmstaedt, K., Valerius, O., Braus-Stromeyer, S., Kwon, N.J., Keller, N.P., Yu, J.H., Braus, G.H., 2008. VelB/VeA/LaeA complex coordinates light signal with fungal development and secondary metabolism. Science 320, 1504–1506.
Bazafkan, H., Dattenbock, C., Bohmdorfer, S., Tisch, D., Stappler, E., Schmoll, M., 2015. Mating type-dependent partner sensing as mediated by VEL1 in *Trichoderma reesei*. Mol. Microbiol. 96, 1103–1118.
Bok, J.W., Ye, R., Clevenger, K.D., Mead, D., Wagner, M., Krerowicz, A., Albright, J.C., Goering, A.W., Thomas, P.M., Kelleher, N.L., Keller, N.P., Wu, C.C., 2015. Fungal artificial chromosomes for mining of the fungal secondary metabolome. BMC Genomics 16, 343.
Bortolus, M., De Zotti, M., Formaggio, F., Maniero, A.L., 2013. Alamethicin in bicelles: orientation, aggregation, and bilayer modification as a function of peptide concentration. Biochim. Biophys. Acta 1828, 2620–2627.
Bouslimani, A., Sanchez, L.M., Garg, N., Dorrestein, P.C., 2014. Mass spectrometry of natural products: current, emerging and future technologies. Nat. Prod. Rep. 31, 718–729.
Brakhage, A.A., Schroeckh, V., 2011. Fungal secondary metabolites – strategies to activate silent gene clusters. Fungal Genet. Biol. 48, 15–22.
Brückner, H., Jung, G., Przybylski, M., 1983. Chromatographic and mass spectrometric characterization of the structures of the polypeptide antibiotics samarosporin and stilbellin and identity with emerimicin. Chromatographia 17, 679–685.
Cardoza, R.E., Hermosa, M.R., Vizcaino, J.A., Gonzalez, F., Llobell, A., Monte, E., Gutierrez, S., 2007. Partial silencing of a hydroxy-methylglutaryl-CoA reductase-encoding gene in *Trichoderma harzianum* CECT 2413 results in a lower level of resistance to lovastatin and lower antifungal activity. Fungal Genet. Biol. 44, 269–283.
Cardoza, R.E., Malmierca, M.G., Gutierrez, S., 2014. Overexpression of erg1 gene in *Trichoderma harzianum* CECT 2413: effect on the induction of tomato defence-related genes. J. Appl. Microbiol. 117, 812–823.
Cardoza, R.E., Malmierca, M.G., Hermosa, M.R., Alexander, N.J., McCormick, S.P., Proctor, R.H., Tijerino, A.M., Rumbero, A., Monte, E., Gutierrez, S., 2011. Identification of loci and functional characterization of trichothecene biosynthesis genes in filamentous fungi of the genus *Trichoderma*. Appl. Environ. Microbiol. 77, 4867–4877.
Cardoza, R.E., McCormick, S.P., Malmierca, M.G., Olivera, E.R., Alexander, N.J., Monte, E., Gutierrez, S., 2015. Effects of trichothecene production on the plant defense response and fungal physiology: overexpression of the *Trichoderma arundinaceum* tri4 Gene in *T. harzianum*. Appl. Environ. Microbiol. 81, 6355–6366.
Cardoza, R.E., Vizcaino, J.A., Hermosa, M.R., Sousa, S., Gonzalez, F.J., Llobell, A., Monte, E., Gutierrez, S., 2006. Cloning and characterization of the erg1 gene of *Trichoderma harzianum*: effect of the erg1 silencing on ergosterol biosynthesis and resistance to terbinafine. Fungal Genet. Biol. 43, 164–178.
Chokkathukalam, A., Kim, D.H., Barrett, M.P., Breitling, R., Creek, D.J., 2014. Stable isotope-labeling studies in metabolomics: new insights into structure and dynamics of metabolic networks. Bioanalysis 6, 511–524.
Claydon, N., Allan, M., Hanson, J.R., Avent, A.G., 1987. Antifungal alkyl pyrones of *Trichoderma harzianum*. Trans. Br. Mycol. Soc. 88, 503–513.
Crutcher, F.K., Parich, A., Schuhmacher, R., Mukherjee, P.K., Zeilinger, S., Kenerley, C.M., 2013. A putative terpene cyclase, vir4, is responsible for the biosynthesis of volatile terpene compounds in the biocontrol fungus *Trichoderma virens*. Fungal Genet. Biol. 56, 67–77.
De Zotti, M., Biondi, B., Formaggio, F., Toniolo, C., Stella, L., Park, Y., Hahm, K.S., 2009. Trichogin GA IV: an antibacterial and protease-resistant peptide. J. Pept. Sci. 15, 615–619.
Degenhardt, J., Kollner, T.G., Gershenzon, J., 2009. Monoterpene and sesquiterpene synthases and the origin of terpene skeletal diversity in plants. Phytochemistry 70, 1621–1637.
Degenkolb, T., Berg, A., Gams, W., Schlegel, B., Grafe, U., 2003. The occurrence of peptaibols and structurally related peptaibiotics in fungi and their mass spectrometric identification via diagnostic fragment ions. J. Pept. Sci. 9, 666–678.
Degenkolb, T., Fog Nielsen, K., Dieckmann, R., Branco-Rocha, F., Chaverri, P., Samuels, G.J., Thrane, U., von Dohren, H., Vilcinskas, A., Bruckner, H., 2015. Peptaibol, secondary-metabolite, and hydrophobin pattern of commercial biocontrol agents formulated with species of the *Trichoderma harzianum* complex. Chem. Biodivers. 12, 662–684.
Degenkolb, T., Karimi Aghcheh, R., Dieckmann, R., Neuhof, T., Baker, S.E., Druzhinina, I.S., Kubicek, C.P., Bruckner, H., von Dohren, H., 2012. The production of multiple small peptaibol families by single 14-module peptide synthetases in *Trichoderma/Hypocrea*. Chem. Biodivers. 9, 499–535.
Demain, A.L., Fang, A., 2000. The natural functions of secondary metabolites. Adv. Biochem. Eng. Biotechnol. 69, 1–39.
Ding, L., Peschel, G., Hertweck, C., 2012. Biosynthesis of archetypal plant self-defensive oxylipins by an endophytic fungus residing in mangrove embryos. Chembiochem 13, 2661–2664.
Druzhinina, I.S., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B.A., Kenerley, C.M., Monte, E., Mukherjee, P.K., Zeilinger, S., Grigoriev, I.V., Kubicek, C.P., 2011. *Trichoderma*: the genomics of opportunistic success. Nat. Rev. Microbiol. 9, 749–759.
El-Hasan, A., Walker, F., Buchenauer, H., 2008. *Trichoderma harzianum* and its metabolite 6-pentyl-alpha-pyrone suppress fusaric acid produced by *Fusarium moniliforme*. J. Phytopathol. 156, 79–87.
Engelberth, J., Koch, T., Schuler, G., Bachmann, N., Rechtenbach, J., Boland, W., 2001. Ion channel-forming alamethicin is a potent elicitor of volatile biosynthesis and tendril coiling. Cross talk between jasmonate and salicylate signaling in lima bean. Plant Physiol. 125, 369–377.
Evans, B.S., Robinson, S.J., Kelleher, N.L., 2011. Surveys of non-ribosomal peptide and polyketide assembly lines in fungi and prospects for their analysis in vitro and in vivo. Fungal Genet. Biol. 48, 49–61.
Fang, J., Dorrestein, P.C., 2014. Emerging mass spectrometry techniques for the direct analysis of microbial colonies. Curr. Opin. Microbiol. 19, 120–129.
Fekete, E., Karaffa, L., Karimi Aghcheh, R., Nemeth, Z., Fekete, E., Orosz, A., Paholcsek, M., Stagel, A., Kubicek, C.P., 2014. The transcriptome of lae1 mutants of *Trichoderma reesei* cultivated at constant growth rates reveals new targets of LAE1 function. BMC Genomics 15, 447.
Fisch, K.M., 2013. Biosynthesis of natural products by microbial iterative hybrid PKS-NRPS. RSC Adv. 3, 18228–18247.
Fischer, G.J., Keller, N.P., 2016. Production of cross-kingdom oxylipins by pathogenic fungi: an update on their role in development and pathogenicity. J. Microbiol 54 (3), 254–264.
Forseth, R.R., Amaike, S., Schwenk, D., Affeldt, K.J., Hoffmeister, D., Schroeder, F.C., Keller, N.P., 2013. Homologous NRPS-like gene clusters mediate redundant small-molecule biosynthesis in *Aspergillus flavus*. Angew. Chem. Int. Ed. Engl. 52, 1590–1594.
Furuta, T., Kigane, T., Suganoya, Y., 1984. Isolation of gliotoxin as lettuce seedling growth inhibit substance from the culture broth of *Aspergillus fumigatus* Fres. Bull. Fac. Agric. Tamagawa Univ. 24, 16–25.

Gardiner, D.M., Jarvis, R.S., Howlett, B.J., 2005a. The ABC transporter gene in the sirodesmin biosynthetic gene cluster of *Leptosphaeria maculans* is not essential for sirodesmin production but facilitates self-protection. Fungal Genet. Biol. 42, 257–263.

Gardiner, D.M., Waring, P., Howlett, B.J., 2005b. The epipolythiodioxopiperazine (ETP) class of fungal toxins: distribution, mode of action, functions and biosynthesis. Microbiology (Reading, England) 151, 1021–1032.

Ghisalberti, E.L., Hockless, D.C.R., Rowland, C., White, A.H., 1992. Harziandione, a new class of diterpene from *Trichoderma harzianum*. J. Nat. Prod. 55, 1690–1694.

Haraguchi, H., Hamatani, Y., Hamada, M., Fujii-Tachino, A., 1996. Effect of gliotoxin on growth and branched-chain amino acid biosynthesis in plants. Phytochemistry 42, 645–648.

Harman, G.E., Howell, C.R., Viterbo, A., Chet, I., Lorito, M., 2004. *Trichoderma* species–opportunistic, avirulent plant symbionts. Nat. Rev. Microbiol. 2, 43–56.

Hermosa, R., Cardoza, R.E., Rubio, M.B., Gutiérrez, S., Monte, E., 2014. Chapter 10-secondary metabolism and antimicrobial metabolites of *Trichoderma* A2-Gupta, Vijai K. In: Herrera-Estrella, M.S., Druzhinina, R.S.U., Tuohy, M.G. (Eds.), Biotechnology and Biology of *Trichoderma*. Elsevier, Amsterdam, pp. 125–137.

Herrero-Garcia, E., Garzia, A., Cordobes, S., Espeso, E.A., Ugalde, U., 2011. 8-Carbon oxylipins inhibit germination and growth, and stimulate aerial conidiation in *Aspergillus nidulans*. Fungal Biol. 115, 393–400.

Hertweck, C., 2009. Hidden biosynthetic treasures brought to light. Nat. Chem. Biol. 5, 450–452.

Heshof, R., Jylha, S., Haarmann, T., Jorgensen, A.L., Dalsgaard, T.K., de Graaff, L.H., 2014. A novel class of fungal lipoxygenases. Appl. Microbiol. Biotechnol. 98, 1261–1270.

Hill, R.A., Cutler, H.G., Parker, S.R., 2000. Use of massoialactone for inhibition of fungal growth. Google Patents.

Holzlechner, M., Reitschmidt, S., Gruber, S., Zeilinger, S., Marchetti-Deschmann, M., 2016. Visualizing fungal metabolites during mycoparasitic interaction by MALDI mass spectrometry imaging. Proteomics. http://dx.doi.org/10.1002/pmic.20150051.

Hou, C.T., Ciegler, A., Hesseltine, C.W., 1972. New mycotoxin, trichotoxin A, from *Trichoderma viride* isolated from southern leaf blight-infected corn. Appl. Microbiol. 23, 183–185.

Howell, C.R., 2006. Understanding the mechanisms employed by *Trichoderma virens* to effect biological control of cotton diseases. Phytopathology 96, 178–180.

Howell, C.R., Stipanovic, R.D., Lumsden, R.D., 1993. Antibiotic production by strains of *Gliocladium virens* and its relation to the biocontrol of cotton seedling diseases. Biocontrol Sci. Technol. 3, 435–441.

Johansson, F.I., Michalecka, A.M., Moller, I.M., Rasmusson, A.G., 2004. Oxidation and reduction of pyridine nucleotides in alamethicin-permeabilized plant mitochondria. Biochem. J. 380, 193–202.

Karimi-Aghcheh, R., Bok, J.W., Phatale, P.A., Smith, K.M., Baker, S.E., Lichius, A., Omann, M., Zeilinger, S., Seiboth, B., Rhee, C., Keller, N.P., Freitag, M., Kubicek, C.P., 2013. Functional analyses of *Trichoderma reesei* LAE1 reveal conserved and contrasting roles of this regulator. G3 (Bethesda) 3, 369–378.

Karimi Aghcheh, R., Druzhinina, I.S., Kubicek, C.P., 2013. The putative protein methyltransferase LAE1 of *Trichoderma atroviride* is a key regulator of asexual development and mycoparasitism. PLoS One 8, e67144.

Keller, N.P., Turner, G., Bennett, J.W., 2005. Fungal secondary metabolism – from biochemistry to genomics. Nat. Rev. Microbiol. 3, 937–947.

Kluger, B., 2015. Biosynthesis and Molecular Genetics of Fungal Secondary Metabolites, vol. II. Springer, ISBN 978-1-4939-2530-8.

Kluger, B., Bueschl, C., Lemmens, M., Michlmayr, H., Malachova, A., Koutnik, A., Maloku, I., Berthiller, F., Adam, G., Krska, R., Schuhmacher, R., 2015. Biotransformation of the mycotoxin deoxynivalenol in fusarium resistant and susceptible near isogenic wheat lines. PLoS One 10, e0119656.

Kluger, B., Zeilinger, S., Wiesenberger, G., Schöfbeck, D., Schuhmacher, R., 2013. Detection and identification of fungal microbial volatile organic compounds by HS-SPME-GC–MS. In: Gupta, K.V., Tuohy, G.M., Ayyachamy, M., Turner, M.K., O'Donovan, A. (Eds.), Laboratory Protocols in Fungal Biology: Current Methods in Fungal Biology. Springer New York, New York, NY, pp. 455–465.

Knox, P.B., Keller, P.N., 2015. Key players in the regulation of fungal secondary metabolism. In: Zeilinger, S., Martín, J.-F., García-Estrada, C. (Eds.), Biosynthesis and Molecular Genetics of Fungal Secondary Metabolites, vol. 2. Springer New York, New York, NY, pp. 13–28.

Komon-Zelazowska, M., Neuhof, T., Dieckmann, R., von Dohren, H., Herrera-Estrella, A., Kubicek, C.P., Druzhinina, I.S., 2007. Formation of atroviridin by *Hypocrea atroviridis* is conidiation associated and positively regulated by blue light and the G protein GNA3. Eukaryot. Cell 6, 2332–2342.

Korpi, A., Jarnberg, J., Pasanen, A.L., 2009. Microbial volatile organic compounds. Crit. Rev. Toxicol. 39, 139–193.

Kubicek, C.P., Herrera-Estrella, A., Seidl-Seiboth, V., Martinez, D.A., Druzhinina, I.S., Thon, M., Zeilinger, S., Casas-Flores, S., Horwitz, B.A., Mukherjee, P.K., Mukherjee, M., Kredics, L., Alcaraz, L.D., Aerts, A., Antal, Z., Atanasova, L., Cervantes-Badillo, M.G., Challacombe, J., Chertkov, O., McCluskey, K., Coulpier, F., Deshpande, N., von Dohren, H., Ebbole, D.J., Esquivel-Naranjo, E.U., Fekete, E., Flipphi, M., Glaser, F., Gomez-Rodriguez, E.Y., Gruber, S., Han, C., Henrissat, B., Hermosa, R., Hernandez-Onate, M., Karaffa, L., Kosti, I., Le Crom, S., Lindquist, E., Lucas, S., Lubeck, M., Lubeck, P.S., Margeot, A., Metz, B., Misra, M., Nevalainen, H., Omann, M., Packer, N., Perrone, G., Uresti-Rivera, E.E., Salamov, A., Schmoll, M., Seiboth, B., Shapiro, H., Sukno, S., Tamayo-Ramos, J.A., Tisch, D., Wiest, A., Wilkinson, H.H., Zhang, M., Coutinho, P.M., Kenerley, C.M., Monte, E., Baker, S.E., Grigoriev, I.V., 2011. Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of *Trichoderma*. Genome Biol. 12, R40.

Lehner, S.M., Atanasova, L., Neumann, N.K., Krska, R., Lemmens, M., Druzhinina, I.S., Schuhmacher, R., 2013. Isotope-assisted screening for iron-containing metabolites reveals a high degree of diversity among known and unknown siderophores produced by *Trichoderma* spp. Appl. Environ. Microbiol. 79, 18–31.

Leitgeb, B., Szekeres, A., Manczinger, L., Vagvolgyi, C., Kredics, L., 2007. The history of alamethicin: a review of the most extensively studied peptaibol. Chem. Biodivers. 4, 1027–1051.

Li, C., Matsuda, Y., Gao, H., Hu, D., Yao, X.S., Abe, I., 2016. Biosynthesis of LL-Z1272beta: discovery of a new member of NRPS-like enzymes for aryl-aldehyde formation. Chembiochem. http://dx.doi.org/10.1002/cbic.201600087.

Lin, Y.R., Lo, C.T., Liu, S.Y., Peng, K.C., 2012. Involvement of pachybasin and emodin in self-regulation of *Trichoderma harzianum* mycoparasitic coiling. J. Agric. Food Chem. 60, 2123–2128.

Lorito, M., Farkas, V., Rebuffat, S., Bodo, B., Kubicek, C.P., 1996. Cell wall synthesis is a major target of mycoparasitic antagonism by *Trichoderma harzianum*. J. Bacteriol. 178, 6382–6385.

Lorito, M., Hayes, C.K., Zoina, A., Scala, F., Del Sorbo, G., Woo, S.L., Harman, G.E., 1994. Potential of genes and gene products from *Trichoderma* sp. and *Gliocladium* sp. for the development of biological pesticides. Mol. Biotechnol. 2, 209–217.

Lu, Z., Tombolini, R., Woo, S., Zeilinger, S., Lorito, M., Jansson, J.K., 2004. In vivo study of trichoderma-pathogen-plant interactions, using constitutive and inducible green fluorescent protein reporter systems. Appl. Environ. Microbiol. 70, 3073–3081.

Luo, Y., Zhang, D.D., Dong, X.W., Zhao, P.B., Chen, L.L., Song, X.Y., Wang, X.J., Chen, X.L., Shi, M., Zhang, Y.Z., 2010. Antimicrobial peptaibols induce defense responses and systemic resistance in tobacco against tobacco mosaic virus. FEMS Microbiol. Lett. 313, 120–126.

Malmierca, M.G., Cardoza, R.E., Alexander, N.J., McCormick, S.P., Collado, I.G., Hermosa, R., Monte, E., Gutierrez, S., 2013. Relevance of trichothecenes in fungal physiology: disruption of tri5 in *Trichoderma arundinaceum*. Fungal Genet. Biol. 53, 22–33.

Malmierca, M.G., Cardoza, R.E., Alexander, N.J., McCormick, S.P., Hermosa, R., Monte, E., Gutierrez, S., 2012. Involvement of *Trichoderma trichothecenes* in the biocontrol activity and induction of plant defense-related genes. Appl. Environ. Microbiol. 78, 4856–4868.

Malmierca, M.G., McCormick, S.P., Cardoza, R.E., Alexander, N.J., Monte, E., Gutierrez, S., 2015. Production of trichodiene by *Trichoderma harzianum* alters the perception of this biocontrol strain by plants and antagonized fungi. Environ. Microbiol. 17, 2628–2646.

Marahiel, M.A., 2009. Working outside the protein-synthesis rules: insights into non-ribosomal peptide synthesis. J. Pept. Sci. 15, 799–807.

Martinez, D., Berka, R.M., Henrissat, B., Saloheimo, M., Arvas, M., Baker, S.E., Chapman, J., Chertkov, O., Coutinho, P.M., Cullen, D., Danchin, E.G., Grigoriev, I.V., Harris, P., Jackson, M., Kubicek, C.P., Han, C.S., Ho, I., Larrondo, L.F., de Leon, A.L., Magnuson, J.K., Merino, S., Misra, M., Nelson, B., Putnam, N., Robbertse, B., Salamov, A.A., Schmoll, M., Terry, A., Thayer, N., Westerholm-Parvinen, A., Schoch, C.L., Yao, J., Barabote, R., Nelson, M.A., Detter, C., Bruce, D., Kuske, C.R., Xie, G., Richardson, P., Rokhsar, D.S., Lucas, S.M., Rubin, E.M., Dunn-Coleman, N., Ward, M., Brettin, T.S., 2008. Genome sequencing and analysis of the biomass-degrading fungus *Trichoderma reesei* (syn. *Hypocrea jecorina*). Nat. Biotechnol. 26, 553–560.

Matic, S., Geisler, D.A., Moller, I.M., Widell, S., Rasmusson, A.G., 2005. Alamethicin permeabilizes the plasma membrane and mitochondria but not the tonoplast in tobacco (*Nicotiana tabacum* L. cv Bright Yellow) suspension cells. Biochem. J. 389, 695–704.

Mazzei, P., Vinale, F., Woo, S.L., Pascale, A., Lorito, M., Piccolo, A., 2016. Metabolomics by H-HRMAS-NMR of tomato plants treated with two secondary metabolites isolated from *Trichoderma*. J. Agric. Food Chem.. http://dx.doi.org/10.1021/acs.jafc.6b00801.

Mejía Agüero, L.E., Alvarado, R., Martínez, A., Dorta, B., 2008. Inhibition of *Aspergillus flavus* growth and aflatoxin b1 production in stored maize grains exposed to volatile compounds of *Trichoderma harzianum* Rifai. Interciência 33, 219–222.

Mendoza-Mendoza, A., Pozo, M.J., Grzegorski, D., Martinez, P., Garcia, J.M., Olmedo-Monfil, V., Cortes, C., Kenerley, C., Herrera-Estrella, A., 2003. Enhanced biocontrol activity of *Trichoderma* through inactivation of a mitogen-activated protein kinase. Proc. Natl. Acad. Sci. U. S. A. 100, 15965–15970.

Meyer, C.E., Reusser, F., 1967. A polypeptide antibacterial agent isolated from *Trichoderma viride*. Experientia 23, 85–86.

Moree, W.J., Phelan, V.V., Wu, C.H., Bandeira, N., Cornett, D.S., Duggan, B.M., Dorrestein, P.C., 2012. Interkingdom metabolic transformations captured by microbial imaging mass spectrometry. Proc. Natl. Acad. Sci. U. S. A. 109, 13811–13816.

Mousa, W.K., Raizada, M.N., 2015. Biodiversity of genes encoding anti-microbial traits within plant associated microbes. Front. Plant Sci. 6, 231.

Mukherjee, M., Horwitz, B.A., Sherkhane, P.D., Hadar, R., Mukherjee, P.K., 2006. A secondary metabolite biosynthesis cluster in *Trichoderma virens*: evidence from analysis of genes underexpressed in a mutant defective in morphogenesis and antibiotic production. Curr. Genet. 50, 193–202.

Mukherjee, M., Mukherjee, P.K., Kale, S.P., 2007. cAMP signalling is involved in growth, germination, mycoparasitism and secondary metabolism in *Trichoderma virens*. Microbiology (Reading, England) 153, 1734–1742.

Mukherjee, P.K., Buensanteai, N., Moran-Diez, M.E., Druzhinina, I.S., Kenerley, C.M., 2012a. Functional analysis of non-ribosomal peptide synthetases (NRPSs) in *Trichoderma virens* reveals a polyketide synthase (PKS)/NRPS hybrid enzyme involved in the induced systemic resistance response in maize. Microbiology (Reading, England) 158, 155–165.

Mukherjee, P.K., Horwitz, B.A., Herrera-Estrella, A., Schmoll, M., Kenerley, C.M., 2013a. *Trichoderma* research in the genome era. Annu. Rev. Phytopathol. 51, 105–129.

Mukherjee, P.K., Horwitz, B.A., Kenerley, C.M., 2012b. Secondary metabolism in *Trichoderma*–a genomic perspective. Microbiology (Reading, England) 158, 35–45.

Mukherjee, P.K., Horwitz, B.A., Singh, U.S., Mukherjee, M., Schmoll, M., 2013b. *Trichoderma*: Biology and Applications.

Mukherjee, P.K., Kenerley, C.M., 2010. Regulation of morphogenesis and biocontrol properties in *Trichoderma virens* by a VELVET protein, Vel1. Appl. Environ. Microbiol. 76, 2345–2352.

Mukherjee, P.K., Latha, J., Hadar, R., Horwitz, B.A., 2003. TmkA, a mitogen-activated protein kinase of *Trichoderma virens*, is involved in biocontrol properties and repression of conidiation in the dark. Eukaryot. Cell 2, 446–455.

Mukherjee, P.K., Wiest, A., Ruiz, N., Keightley, A., Moran-Diez, M.E., McCluskey, K., Pouchus, Y.F., Kenerley, C.M., 2011. Two classes of new peptaibols are synthesized by a single non-ribosomal peptide synthetase of *Trichoderma virens*. J. Biol. Chem. 286, 4544–4554.

Nemcovic, M., Jakubikova, L., Viden, I., Farkas, V., 2008. Induction of conidiation by endogenous volatile compounds in *Trichoderma* spp. FEMS Microbiol. Lett. 284, 231–236.

Netzker, T., Fischer, J., Weber, J., Mattern, D.J., Konig, C.C., Valiante, V., Schroeckh, V., Brakhage, A.A., 2015. Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters. Front. Microbiol. 6, 299.

Neuhof, T., Dieckmann, R., Druzhinina, I.S., Kubicek, C.P., von Dohren, H., 2007. Intact-cell MALDI-TOF mass spectrometry analysis of peptaibol formation by the genus *Trichoderma/Hypocrea*: can molecular phylogeny of species predict peptaibol structures? Microbiology (Reading, England) 153, 3417–3437.

Neumann, N.K., Stoppacher, N., Zeilinger, S., Degenkolb, T., Bruckner, H., Schuhmacher, R., 2015. The peptaibiotics database–a comprehensive online resource. Chem. Biodivers. 12, 743–751.

Omann, M., Zeilinger, S., 2010. How a mycoparasite employs g-protein signaling: using the example of trichoderma. J. Signal Transduct. 2010, 123126.

Ooka, T., Shimojima, Y., Akimoto, T., Takeda, I., Senoh, S., Abe, J., 1966. A new antibacterial peptide "Suzukacillin". Agric. Biol. Chem. 30, 700–702.

Rebuffat, S., Duclohier, H., Auvin-Guette, C., Molle, G., Spach, G., Bodo, B., 1992. Membrane-modifying properties of the pore-forming peptaibols saturnisporin SA IV and harzianin HA V. FEMS Microbiol. Immunol. 5, 151–160.

Reino, J.L., Guerrero, R.F., Hernández-Galán, R., Collado, I.G., 2007. Secondary metabolites from species of the biocontrol agent *Trichoderma*. Phytochem. Rev. 7, 89–123.

Reithner, B., Brunner, K., Schuhmacher, R., Peissl, I., Seidl, V., Krska, R., Zeilinger, S., 2005. The G protein alpha subunit Tga1 of *Trichoderma atroviride* is involved in chitinase formation and differential production of antifungal metabolites. Fungal Genet. Biol. 42, 749–760.

Reithner, B., Schuhmacher, R., Stoppacher, N., Pucher, M., Brunner, K., Zeilinger, S., 2007. Signaling via the *Trichoderma atroviride* mitogen-activated protein kinase Tmk 1 differentially affects mycoparasitism and plant protection. Fungal Genet. Biol. 44, 1123–1133.

Renshaw, J.C., Robson, G.D., Trinci, A.P.J., Wiebe, M.G., Livens, F.R., Collison, D., Taylor, R.J., 2002. Fungal siderophores: structures, functions and applications. Mycol. Res. 106, 1123–1142.

Rubio, M.B., Hermosa, R., Reino, J.L., Collado, I.G., Monte, E., 2009. Thctf1 transcription factor of *Trichoderma harzianum* is involved in 6-pentyl-2H-pyran-2-one production and antifungal activity. Fungal Genet. Biol. 46, 17–27.

Sarrocco, S., Moretti, A., Vannacci, G., 2013. Biological control of Fusarium Head Blight under field conditions biocontrol of plant pathogens in sustainable agriculture. IOBC/wprs Bull. 86 (2013), 95–100.

Scharf, D.H., Brakhage, A.A., Mukherjee, P.K., 2016. Gliotoxin – bane or boon? Environ. Microbiol. 18, 1096–1109.

Schirmbock, M., Lorito, M., Wang, Y.L., Hayes, C.K., Arisan-Atac, I., Scala, F., Harman, G.E., Kubicek, C.P., 1994. Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics, molecular mechanisms involved in the antagonistic action of *Trichoderma harzianum* against phytopathogenic fungi. Appl. Environ. Microbiol. 60, 4364–4370.

Schmoll, M., Dattenbock, C., Carreras-Villasenor, N., Mendoza-Mendoza, A., Tisch, D., Aleman, M.I., Baker, S.E., Brown, C., Cervantes-Badillo, M.G., Cetz-Chel, J., Cristobal-Mondragon, G.R., Delaye, L., Esquivel-Naranjo, E.U., Frischmann, A., Gallardo-Negrete Jde, J., Garcia-Esquivel, M., Gomez-Rodriguez, E.Y., Greenwood, D.R., Hernandez-Onate, M., Kruszewska, J.S., Lawry, R., Mora-Montes, H.M., Munoz-Centeno, T., Nieto-Jacobo, M.F., Nogueira Lopez, G., Olmedo-Monfil, V., Osorio-Concepcion, M., Pilsyk, S., Pomraning, K.R., Rodriguez-Iglesias, A., Rosales-Saavedra, M.T., Sanchez-Arreguin, J.A., Seidl-Seiboth, V., Stewart, A., Uresti-Rivera, E.E., Wang, C.L., Wang, T.F., Zeilinger, S., Casas-Flores, S., Herrera-Estrella, A., 2016. The Genomes of three uneven siblings: footprints of the lifestyles of three *Trichoderma* Species. Microbiol. Mol. Biol. Rev. 80, 205–327.

Segarra, G., Casanova, E., Aviles, M., Trillas, I., 2010. *Trichoderma asperellum* strain T34 controls Fusarium wilt disease in tomato plants in soilless culture through competition for iron. Microb. Ecol. 59, 141–149.

Seiboth, B., Karimi, R.A., Phatale, P.A., Linke, R., Hartl, L., Sauer, D.G., Smith, K.M., Baker, S.E., Freitag, M., Kubicek, C.P., 2012. The putative protein methyltransferase LAE1 controls cellulase gene expression in *Trichoderma reesei*. Mol. Microbiol. 84, 1150–1164.

Serrano-Carreon, L., Hathout, Y., Bensoussan, M., Belin, J.-M., 1993. Production of 6-pentyl-α-pyrone by *Trichoderma harzianum* from 18:n fatty acid methyl esters. Biotechnol. Lett. 14, 1019–1024.

Shi, M., Chen, L., Wang, X.W., Zhang, T., Zhao, P.B., Song, X.Y., Sun, C.Y., Chen, X.L., Zhou, B.C., Zhang, Y.Z., 2012. Antimicrobial peptaibols from *Trichoderma pseudokoningii* induce programmed cell death in plant fungal pathogens. Microbiology (Reading, England) 158, 166–175.

Shi, M., Wang, H.N., Xie, S.T., Luo, Y., Sun, C.Y., Chen, X.L., Zhang, Y.Z., 2010. Antimicrobial peptaibols, novel suppressors of tumor cells, targeted calcium-mediated apoptosis and autophagy in human hepatocellular carcinoma cells. Mol. Cancer 9, 26.

Shih, C.J., Chen, P.Y., Liaw, C.C., Lai, Y.M., Yang, Y.L., 2014. Bringing microbial interactions to light using imaging mass spectrometry. Nat. Prod. Rep. 31, 739–755.

Siddiquee, S., 2014. Chapter 11-Recent Advancements on the Role and Analysis of Volatile Compounds (VOCs) from *Trichoderma*, Biotechnology and Biology of *Trichoderma*. Elsevier, Amsterdam, pp. 139–175.

Smedsgaard, J., Nielsen, J., 2005. Metabolite profiling of fungi and yeast: from phenotype to metabolome by MS and informatics. J. Exp. Bot. 56, 273–286.

Stoppacher, N., Kluger, B., Zeilinger, S., Krska, R., Schuhmacher, R., 2010. Identification and profiling of volatile metabolites of the biocontrol fungus *Trichoderma atroviride* by HS-SPME-GC-MS. J. Microbiol. Methods 81, 187–193.

Stoppacher, N., Neumann, N.K.N., Burgstaller, L., Zeilinger, S., Degenkolb, T., Brückner, H., Schuhmacher, R., 2013. The comprehensive peptaibiotics database. Chem. Biodivers. 10, 734–743.

Stoppacher, N., Reithner, B., Omann, M., Zeilinger, S., Krska, R., Schuhmacher, R., 2007. Profiling of trichorzianines in culture samples of *Trichoderma atroviride* by liquid chromatography/-tandem mass spectrometry. Rapid Commun. Mass Spectrom. 21, 3963–3970.

Stoppacher, N., Zeilinger, S., Omann, M., Lassahn, P.G., Roitinger, A., Krska, R., Schuhmacher, R., 2008. Characterisation of the peptaibiome of the biocontrol fungus *Trichoderma atroviride* by liquid chromatography/tandem mass spectrometry. Rapid Commun. Mass Spectrom. 22, 1889–1898.

Strauss, J., Reyes-Dominguez, Y., 2011. Regulation of secondary metabolism by chromatin structure and epigenetic codes. Fungal Genet. Biol. 48, 62–69.

Strieker, M., Tanovic, A., Marahiel, M.A., 2010. Nonribosomal peptide synthetases: structures and dynamics. Curr. Opin. Struct. Biol. 20, 234–240.

Su, H.N., Chen, Z.H., Song, X.Y., Chen, X.L., Shi, M., Zhou, B.C., Zhao, X., Zhang, Y.Z., 2012. Antimicrobial peptide trichokonin VI-induced alterations in the morphological and nanomechanical properties of *Bacillus subtilis*. PLoS One 7, e45818.

Sun, W.W., Guo, C.J., Wang, C.C., 2016. Characterization of the product of a nonribosomal peptide synthetase-like (NRPS-like) gene using the doxycycline dependent Tet-on system in *Aspergillus terreus*. Fungal Genet. Biol. 89, 84–88.

Tata, A., Perez, C., Campos, M.L., Bayfield, M.A., Eberlin, M.N., Ifa, D.R., 2015. Imprint desorption electrospray ionization mass spectrometry imaging for monitoring secondary metabolites production during antagonistic interaction of fungi. Anal. Chem. 87, 12298–12305.

Tijerino, A., Cardoza, R.E., Moraga, J., Malmierca, M.G., Vicente, F., Aleu, J., Collado, I.G., Gutierrez, S., Monte, E., Hermosa, R., 2011a. Overexpression of the trichodiene synthase gene tri5 increases trichodermin production and antimicrobial activity in *Trichoderma brevicompactum*. Fungal Genet. Biol. 48, 285–296.

Tijerino, A., Hermosa, R., Cardoza, R.E., Moraga, J., Malmierca, M.G., Aleu, J., Collado, I.G., Monte, E., Gutierrez, S., 2011b. Overexpression of the *Trichoderma brevicompactum* tri5 gene: effect on the expression of the trichodermin biosynthetic genes and on tomato seedlings. Toxins 3, 1220–1232.

Traxler, M.F., Watrous, J.D., Alexandrov, T., Dorrestein, P.C., Kolter, R., 2013. Interspecies interactions stimulate diversification of the *Streptomyces coelicolor* secreted metabolome. mBio 4 e00459-00413.

Trushina, N., Levin, M., Mukherjee, P.K., Horwitz, B.A., 2013. PacC and pH–dependent transcriptome of the mycotrophic fungus *Trichoderma virens*. BMC Genomics 14, 1–21.

Vargas, W.A., Mukherjee, P.K., Laughlin, D., Wiest, A., Moran-Diez, M.E., Kenerley, C.M., 2014. Role of gliotoxin in the symbiotic and pathogenic interactions of *Trichoderma virens*. Microbiology (Reading, England) 160, 2319–2330.

Vey, A., Hoagland, R., Butt, T., 2001. Toxic Metabolites of Fungal Biocontrol Agents. Fungi as Biocontrol Agents: Progress,

Problems and Potential. CAB International, Bristol, pp. 311–346.
Vinale, F., Ghisalberti, E.L., Sivasithamparam, K., Marra, R., Ritieni, A., Ferracane, R., Woo, S., Lorito, M., 2009. Factors affecting the production of *Trichoderma harzianum* secondary metabolites during the interaction with different plant pathogens. Lett. Appl. Microbiol. 48, 705–711.
Vinale, F., Nigro, M., Sivasithamparam, K., Flematti, G., Ghisalberti, E.L., Ruocco, M., Varlese, R., Marra, R., Lanzuise, S., Eid, A., Woo, S.L., Lorito, M., 2013. Harzianic acid: a novel siderophore from *Trichoderma harzianum*. FEMS Microbiol. Lett. 347, 123–129.
Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Barbetti, M.J., Li, H., Woo, S.L., Lorito, M., 2008. A novel role for *Trichoderma* secondary metabolites in the interactions with plants. Physiol. Mol. Plant Pathol. 72, 80–86.
Viterbo, A., Wiest, A., Brotman, Y., Chet, I., Kenerley, C., 2007. The 18mer peptaibols from *Trichoderma virens* elicit plant defence responses. Mol. Plant Pathol. 8, 737–746.
Wang, M., Hashimoto, M., Hashidoko, Y., 2013. Carot-4-en-9,10-diol, a conidiation-inducing sesquiterpene diol produced by *Trichoderma virens* PS1-7 upon exposure to chemical stress from highly active iron chelators. Appl. Environ. Microbiol. 79, 1906–1914.
Watrous, J., Roach, P., Alexandrov, T., Heath, B.S., Yang, J.Y., Kersten, R.D., van der Voort, M., Pogliano, K., Gross, H., Raaijmakers, J.M., Moore, B.S., Laskin, J., Bandeira, N., Dorrestein, P.C., 2012. Mass spectral molecular networking of living microbial colonies. Proc. Natl. Acad. Sci. U. S. A. 109, E1743–E1752.
Watrous, J.D., Dorrestein, P.C., 2011. Imaging mass spectrometry in microbiology. Nat. Rev. Microbiol. 9, 683–694.
Weindling, Emerson, 1936. The isolation of a toxic substance from the culture fiItrate of *Trichoderma*. Phytopathology 26, 1068–1070.
Weindling, R., 1943. Studies on a lethal principle effective in the parasitic action of *Trichoderma lignorum* on *Rhizoctonia solani* and other soil fungi. Phytopathology 24, 1153–1179.
Wheatley, R.E., 2002. The consequences of volatile organic compound mediated bacterial and fungal interactions. Antonie Van Leeuwenhoek 81, 357–364.
Wiest, A., Grzegorski, D., Xu, B.W., Goulard, C., Rebuffat, S., Ebbole, D.J., Bodo, B., Kenerley, C., 2002. Identification of peptaibols from *Trichoderma virens* and cloning of a peptaibol synthetase. J. Biol. Chem. 277, 20862–20868.
Wilkins, K., Larsen, K., Simkus, M., 2003. Volatile metabolites from indoor molds grown on media containing wood constituents. Environ. Sci. Pollut. Res. Int. 10, 206–208.
Woloshuk, C.P., Shim, W.B., 2013. Aflatoxins, fumonisins, and trichothecenes: a convergence of knowledge. FEMS Microbiol. Rev. 37, 94–109.
Wright, J.M., 1951. Phytotoxic effects of some antibiotics. Ann. Bot. 15, 493–499.
Xiao-Yan, S., Qing-Tao, S., Shu-Tao, X., Xiu-Lan, C., Cai-Yun, S., Yu-Zhong, Z., 2006. Broad-spectrum antimicrobial activity and high stability of Trichokonins from *Trichoderma koningii* SMF2 against plant pathogens. FEMS Microbiol. Lett. 260, 119–125.
Yang, D., Pomraning, K., Kopchinskiy, A., Karimi Aghcheh, R., Atanasova, L., Chenthamara, K., Baker, S.E., Zhang, R., Shen, Q., Freitag, M., Kubicek, C.P., Druzhinina, I.S., 2015. Genome sequence and annotation of *Trichoderma parareesei*, the ancestor of the cellulase producer *Trichoderma reesei*. Genome Announc. 3 e00885-00815.
Yeh, H.H., Chiang, Y.M., Entwistle, R., Ahuja, M., Lee, K.H., Bruno, K.S., Wu, T.K., Oakley, B.R., Wang, C.C., 2012. Molecular genetic analysis reveals that a nonribosomal peptide synthetase-like (NRPS-like) gene in *Aspergillus nidulans* is responsible for microperfuranone biosynthesis. Appl. Microbiol. Biotechnol. 96, 739–748.
Zeilinger, S., García-Estrada, C., Martín, J.-F., 2015. Fungal secondary metabolites in the “OMICS” era. In: Zeilinger, S., Martín, J.-F., García-Estrada, C. (Eds.), Biosynthesis and Molecular Genetics of Fungal Secondary Metabolites, vol. 2. Springer New York, New York, NY, pp. 1–12.
Zeilinger, S., Reithner, B., Scala, V., Peissl, I., Lorito, M., Mach, R.L., 2005. Signal transduction by Tga3, a novel G protein alpha subunit of *Trichoderma atroviride*. Appl. Environ. Microbiol. 71, 1591–1597.
Zeilinger, S., Schuhmacher, R., 2013. Volatile Organic Metabolites of *Trichoderma* spp.: Biosynthesis, Biology and Analytics. CABI, Wallingford, pp. 110–127.
Zeppa, G., Allegrone, G., Barbeni, M., Guarda, P.A., 1990. Variability in the production of volatile metabolites by *Trichoderma viride*. Ann. Microbiol. Enzimol. ISSN: 0003-4649 90 (2), 171–176.