Secondary Metabolism in *Trichoderma*: Chemo- and Geno-Diversity

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Abstract

Trichoderma species are filamentous ascomycetous fungi that have wide biotechnological applications in industry as well as agriculture. Having nearly 300 species, this genus represents one of the most diverse groups of fungi. Secondary metabolites are useful natural products having widespread applications in agriculture and medicine. *Trichoderma* species are prolific producers of secondary metabolites (natural products) with proven role in disease suppression. Genes for biosynthesis of these metabolites are often present as gene clusters, and one such cluster may be responsible for synthesis of a range of metabolites and intermediates. Depending on the chemical nature, these metabolites could be grouped as non-ribosomal peptides, polyketides, terpenes, steroids, etc. Three species of *Trichoderma* (*T. virens*, *T. atroviride*, and *T. reesei*) are well studied from genomics point of view, and this article focuses mainly on these three species. We discuss here the level of diversity with respect to secondary metabolite biosynthesis machinery at the genus, species, and strain level with genetic evidence where available. The article highlights the untapped potential of *Trichoderma* spp. as a source of a variety of secondary metabolites with potential applications in agriculture and medicine.

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Secondary metabolism · *Trichoderma* · Viridin · Gliotoxin · Gliovirin · Peptaibols · Genomics

17.1 Introduction

Trichoderma spp. are among the most diverse group of fungi with nearly 300 defined species (Robbertse et al. 2017). These fungi are widely used in industry as source of enzymes and in agriculture as biofungicides and plant growth promoters (Mukherjee et al. 2013a, b). Several formulation products for agricultural usage are available worldwide, and in India alone, a few hundred products are there in the market (Singh et al. 2017). *Trichoderma* species are strong mycoparasites and can kill other fungi in contact (Druzhinina et al. 2011). These fungi are prolific producers of secondary metabolites/natural products which can be broadly classified as high molecular weight (peptaibols) and low molecular weight (non-ribosomal peptides, polyketides, terpenes, steroids, etc.). There are more than 1000 peptaibols reported from *Trichoderma*, and similarly, more than 1000 small molecular weight compounds are known to be produced by this single genus (Zeilinger et al. 2016). Secondary metabolites play an important role in *Trichoderma*-fungus and *Trichoderma*-plant interactions (Mukherjee et al. 2012a). These compounds may be used to weaken the prey fungus before mycoparasitic attack generally mediated by hydrolytic enzymes like chitinases, beta-glucanases, and proteases. Some of these compounds exhibit strong antimicrobial properties (antibiosis), while others may be involved in inducing resistance response against plant pathogens (Viterbo et al. 2007; Mukherjee et al. 2012b). *Trichoderma* spp. also produce phytohormones that promote plant growth (Contreras-Cornejo et al. 2009). In short, secondary metabolites are important components of the package of benefits that *Trichoderma* spp. provide to plants. In this article, we discuss about the diversity of secondary metabolites that these fungi produce and, where information is available, provide genetic evidence for the origin of such vast chemo-diversity in these beneficial filamentous fungi. We focus on three species (*T. atroviride*, *T. virens*, and *T. reesei*) as these have been well characterized subsequent to the publication of the whole genome sequences.

17.2 Secondary Metabolites of *Trichoderma* spp. and Their Biosynthesis

Trichoderma species produce a plethora of secondary metabolites belonging to various classes like non-ribosomal peptides, polyketides, terpenes, etc. Table 17.1 presents some examples of secondary metabolites produced by the three well-characterized species of *Trichoderma*.

Table 17.1 Some examples of secondary metabolites produced by *T. reesei*, *T. atroviride*, and *T. virens*

Chemical category	Compound	Species	Biological role
Non-ribosomal peptides			
Peptaibols	Trichovirin II	*T. virens*	Induces resistance in cucumber plants against a bacterial pathogen
Peptaibols	Trichorzianins Atroviridins A–C	*T. atroviride*	Atroviridins A–C are associated with conidiation
Peptaibols	Paracelsin, hypojecorins A and B	*T. reesei*	Paracelsin is reported to be hemolytic
Siderophore (intracellular)	Ferricrocin	*T. atroviride*, *T. virens*, *T. reesei*	Intracellular storage of iron, involved in gliotoxin biosynthesis and ISR
Siderophores (extracellular)	Fusarinines A–B Dimerum acid Fusigen Coprogen	*T. virens*	Iron acquisition, competition
Diketopiperazine/ NRP	Gliotoxin	*T. virens*	Antiviral, antibacterial, fungistatic activity, anti-cancer and immuno-suppressive properties
Diketopiperazine	Gliovirin	*T. virens*	Antimicrobial compound against oomycetes and *Staphylococcus aureus*, antitumor
Dipeptide	Trichodermamide A, B	*T. virens*	Cytotoxicity
Polyketides			
Polyketides	Trichodermatides B–D	*T. reesei*	Cytotoxicity
Polyketide	Conidial pigment	*T. reesei*	Conidial pigmentation, stress tolerance
Polyketides	Trichorenins A–C	*T. virens*	Algicidal
Polyketide	Sorbicillin	*T. reesei*	Antiviral, anti-inflammatory, and antimicrobial activities
Terpenes and steroidal compounds			
Sesquiterpene	Heptelidic acid (koningic acid)	*T. virens*	Potential activity against the human malaria parasite *Plasmodium falciparum*, antimicrobial, anticancer
Sesquiterpene	*β*-Farnesene	*T. atroviride*	Acts as an alarm pheromone in aphids
Sesquiterpene	*β*-Caryophyllene	*T. virens*	Attracts nematodes that prey on insect larvae
Monoterpene	*β*-Myrcene	*T. virens*	Regulates the expression of genes related to abiotic and biotic stresses

(continued)

Table 17.1 (continued)

Chemical category	Compound	Species	Biological role
Monoterpenes	*Cis*- and *trans*-β-Ocimene	*T. virens*	Induces expression of JA defense response-related genes in *A. thaliana*
Steroidal compound	Viridin	*T. virens*	Antifungal metabolite that alters the spore germination of *Botrytis allii*, *Colletotrichum lini*, and *Fusarium caeruleum*
Other compounds			
Indolic compound	Indole-3-acetic acid (IAA)	*T. atroviride*, *T. virens*	Plant growth promotion
Indolic compound	Indole-3-acetaldehyde	*T. atroviride*, *T. virens*	Plant growth promotion
Indolic compound	Indole-3-carboxaldehyde	*T. atroviride*, *T. virens*	Induces adventitious root formation in *A. thaliana*
Carotanes	Trichocaranes A–D	*T. virens*	Inhibits the growth of etiolated wheat coleoptiles
Pyrone	6-Pentyl-2*H*-pyran-2-one	*T. atroviride*	Antifungal, antinematode, and plant growth-promoting activities in tomato and *A. thaliana*
Ketone	3-Octanone	*T. atroviride*	Induces conidiation
Alcohol	1-Octen-3-ol	*T. atroviride*	Induces conidiation and defense responses in plants through JA

Source: Reino et al. (2008), Ruiz et al. (2013) and Contreras-Cornejo et al. (2016)

17.2.1 Non-ribosomal Peptides (NRPs)

The non-ribosomal peptides contain both proteinogenic and non-proteinogenic amino acids and may exist in linear or cyclic form. They are synthesized by multi-modular non-ribosomal peptide synthetases. Each module includes adenylation, peptidyl carrier, and condensation domains. The important NRPs produced by *Trichoderma* spp. are peptaibols, epipolythiodioxopiperazines (ETPs), and siderophores.

17.2.1.1 Peptaibols

Peptaibols are short peptides containing α-aminoisobutyric acid (Aib) and a C-terminal alcohol. These are the most prominent NRPs produced by *Trichoderma* species. Peptaibols are reported to have antimicrobial property and cytotoxic activity, and these can induce systemic resistance in plants. The antibiotic property of peptaibols is majorly due to amphipathic nature of peptaibols which allow concentration-dependent membrane permeabilizing activity (Bortolus et al. 2013). Peptaibols are synthesized by peptaibol synthetases (NRPSs) consisting of different modules. Seven-, 14-, and 18–20-module peptaibol synthetases are present in *Trichoderma* genomes (Mukherjee et al. 2012b). The first peptaibol synthetase enzyme (Tex1) has been reported in *Trichoderma virens* (Wiest et al. 2002). Tex1 is an 18-module peptaibol synthetase and produces 18-residue trichovirin II type peptaibols. *T. virens* also possess 14-module peptaibol synthetase enzyme which

produces two classes of peptaibol (the 11- and 14-residue peptaibols) [Mukherjee et al. 2011]. *T. atroviride* is reported to produce 19-residue atroviridins which are produced by a 19-module peptaibol synthetase. Like *T. virens*, *T. atroviride* and *T. reesei* also produce 11- and 14-residue peptaibols catalyzed by a 14-module peptaibol synthetase (Degenkolb et al. 2012).

17.2.1.2 Epipolythiodioxopiperazines (ETPs)

Epipolythiodioxopiperazines (ETPs) are secondary metabolites with a characteristic cyclic peptide-derived diketopiperazine ring. Gliotoxin and gliovirin are members of the ETP class of peptides. Gliotoxin is produced by "Q" strains of *T. virens*, whereas gliovirin is produced by "P" strains of *T. virens* (Howell et al. 1993). Gliotoxin has antimicrobial activity and fungistatic property. In *Aspergillus fumigatus* which is a human pathogen, the gliotoxin acts as a virulence factor (Scharf et al. 2016). GliP is the NRPS dioxopiperazine synthetase enzyme involved in the biosynthesis of gliotoxin. The gliotoxin biosynthetic gene cluster in *A. fumigatus* consists of 12 genes inclusive of GliP gene, while in *T. virens*, the gliotoxin gene cluster consists of only eight genes (Fig. 17.1). In *T. virens*, the deletion of part of the *gliP* gene confirmed the role of it in gliotoxin production (Vargas et al. 2014). The gliotoxin biosynthetic gene cluster with only six genes was also identified in *T. reesei*, albeit this species is not reported to produce gliotoxin (Mukherjee et al. 2012a). The *T. virens* genome also contains a putative SirP gene cluster which is associated with the production of phytotoxin sirodesmin PL in the phytopathogen *Leptosphaeria maculans*. However, the product of SirP gene cluster is not known yet in *Trichoderma*. We have recently discovered the *glv* gene cluster responsible for gliovirin biosynthesis in "P" strains of *T. virens* (Sherkhane et al. 2017).

17.2.1.3 Siderophores

Siderophores are secondary metabolites that can bind, transport, and store iron. Siderophore-mediated iron acquisition is important for microbial competition, biocontrol, and in interactions with plants and other microbes. There are two types of siderophores, intracellular and extracellular. Ferricrocin is an intracellular siderophore and is reported to protect cells from oxidation-induced stress. The product of

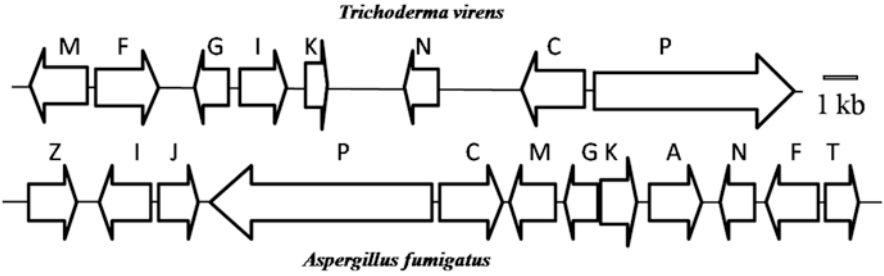

Fig. 17.1 The gliotoxin biosynthesis cluster of *Trichoderma virens* and *Aspergillus fumigatus* – P, non-ribosomal peptide synthetase (NRPS); G, glutathione-S-transferase; J, dipeptidase; N, N-methyl transferase; F and C, cytochrome P450; M, O-methyl transferase; I, C-S-bond lyase; K, gamma-glutamate cyclotransferase. (Adapted from Zeilinger et al. 2016)

NPS6 is an extracellular siderophore that acts as virulence factor in *Cochliobolus heterostrophus* as well as protects fungus from oxidative stress. In *Trichoderma* spp., three different NRPSs involved in siderophore biosynthesis have been identified. Intracellular ferricrocin-associated gene cluster has been found in all the three *Trichoderma* species, but the role for the gene for ferricrocin biosynthesis has been established only in *T. virens* (Mukherjee et al. 2018). NPS6 and SidD are the NRPSs reported to be involved in extracellular siderophore biosynthesis. A gene cluster with NPS6 as a core enzyme is found in all the three *Trichoderma* species, but the function is confirmed only in *T. virens* with gene deletion experiment (Mukherjee et al. 2013a). Another gene cluster with SidD as a core enzyme is found only in *T. virens* and *T. reesei* (Mukherjee et al. 2012b)

17.2.2 Polyketides

Polyketides are secondary metabolites, many of which are having antimicrobial and anti-cancer property. Some polyketides are important for competition for substrate and for interaction with other organisms. Polyketides are synthesized by the polyketide synthases, which are complex enzymes with ketoacyl synthase, an acyl transferase, and a phosphopantetheine attachment site domain. Few studies have been published on the biosynthesis and functional role of polyketides in *Trichoderma* species, although the genomes of *Trichoderma* species are rich in PKS-encoding genes. Orthologues of PKS genes associated with the conidial pigment biosynthesis cluster have been identified in all the three *Trichoderma* species (Baker et al. 2012). Additional 20 putative PKS gene clusters have also been reported in these three species (Bansal and Mukherjee 2016). The role of PKS genes in green pigmentation of conidia, teleomorphic structure, conidial cell wall stability, and antagonistic abilities has been confirmed in *T. reesei* by deletion of *pks4* gene which is an orthologue of pigment forming PKS in *Fusarium* spp.

17.2.3 PK/NRPs

Several PKS-NRPS hybrid enzymes are present in *Trichoderma* genomes. Functional study provided evidence for the role of one of the PKS-NRPS hybrid enzymes (Tex13) in inducing the defense-related *pal* gene in maize seedlings. The metabolite produced by Tex13 cluster is still not known (Mukherjee et al. 2012b).

17.2.4 Terpenoids

Terpenoids represent a diverse class of secondary metabolites produced by almost all the organisms including fungi. They are composed of five-carbon isoprene units (C_5H_8) producing hemiterpenes, monoterpenes, sesquiterpenes, diterpenes, sesterterpenes, triterpenes, tetraterpenes, or polyterpenes. Terpenoid biosynthetic gene clusters have terpene cyclase as the core enzyme. Only a few terpene cyclases may

be responsible for production of diverse form of terpenoids. *Trichoderma* species are reported to produce all forms of terpenoids like volatile terpenoids, diterpenes, sesquiterpenes, and triterpenes (Zeilinger et al. 2016).

In most organisms, the mevalonate pathway is responsible for the formation of isoprene units (Zeilinger et al. 2016). Hydroxy-methylglutaryl-CoA reductase (HMGR) encoded by *hmgR* gene is the first enzyme in the mevalonate pathway involved in conversion of hydroxy-methylglutaryl-CoA into mevalonate. Deletion of *hmgR* gene in *Trichoderma harzianum* showed reduction in antifungal activity against *Rhizoctonia solani* and *Fusarium oxysporum* and decrease in ergosterol levels. Ergosterol encoding gene (*erg1*) silencing also showed decrease in the ergosterol level in *T. harzianum*, whereas overexpression of erg1 gene increases the antifungal activity of *T. harzianum* (Cardoza et al. 2006, 2014). The genome analysis of *T. reesei*, *T. atroviride*, and *T. virens* revealed that *T. virens* (11) has the highest number of terpene cyclases followed by *T. atroviride* (7) and *T. reesei* (6), but the terpene cyclases associated with the biosynthetic gene cluster are six in *T. virens*, three in *T. atroviride*, and two in *T. reesei* (Bansal and Mukherjee 2016). The first terpenoid biosynthetic gene cluster was identified in *T. virens* using suppression subtractive hybridization technique (Mukherjee et al. 2006). The cluster was initially predicted to be associated with viridin production but later found to be responsible for biosynthesis of volatile sesquiterpenes (Crutcher et al. 2013). Deletion of terpene cyclase present in the cluster abolished the production of all the volatile sesquiterpene compounds. This cluster named as *vir* cluster was found to be present in *T. virens* and in few *Aspergillus* species but not in other species of *Trichoderma*. The reason for existence of the *vir* cluster in distantly related *Trichoderma* and *Aspergillus* species could be explained by horizontal gene transfer. Another terpene cyclase Tri5 is responsible for the production of a phytotoxic agent, trichodermin, in *Trichoderma brevicompactum*. Overexpression of Tri5 enhanced the production of trichodermin in *T. brevicompactum* (Tijerino et al. 2011a, b).

17.2.5 Steroids

Viridin is a triterpene steroidal metabolite produced by both "P" and "Q" strains of *T. virens*. It has antifungal and anticancer properties. The reduced form of viridin is known as viridiol. Viridiol has herbicidal properties (Jones and Hancock 1987). Both viridin and viridiol are produced abundantly by *T. virens*, and the *vdn* cluster for viridin biosynthesis has recently been discovered (Fig. 17.2). Interestingly, an orthologous gene cluster is also present in the bat white nose fungus *Pseudogymnoascus destructans* (Bansal et al. 2018).

17.2.6 6-Pentyl Pyrone (6-PP)

6-PP belongs to the volatile class of secondary metabolites. The characteristic "coconut aroma" produced by some *Trichoderma* species is due to 6-PP metabolite production. 6-PP has antifungal and plant growth-promoting property (Vinale et al.

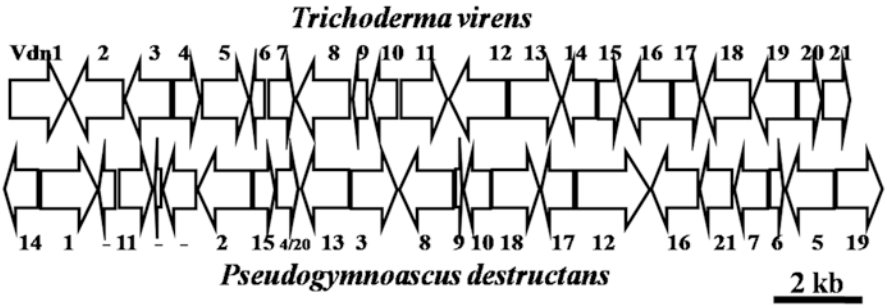

Fig. 17.2 The viridin-biosynthesis gene cluster of *Trichoderma virens* and its orthologue in *Pseudogymnoascus destructans.* (Adapted from Bansal et al. 2018). For details, please refer to Bansal et al. (2018)

Table 17.2 Secondary metabolism related core genes in the genomes of *T. reesei*, *T. atroviride*, and *T. virens*

Core genes	*T. reesei*	*T. atroviride*	*T. virens*
NRPS (with at least one complete module)	8	9	22
PKS	11	15	18
PKS/NRPS	2	1	4
Terpene cyclase	6	7	11

Source: Zeilinger et al. (2016)

2008). There is not much information available on the biosynthetic pathway for the 6-PP production, but a lipoxygenase enzyme has been predicted to be involved in the 6-PP biosynthesis as it is present exclusively in *T. atroviride*, but not in other *Trichoderma* species which are devoid of 6-PP production like *T. virens* and *T. reesei* (Kubicek et al. 2011).

17.3 Secondary Metabolism Genes and Gene Clusters: Diversity at the Genus Level

In order to have a comparative assessment of why *T. atroviride* and *T. virens* are strong mycoparasites compared to *T. reesei*, a comparative genomics analysis was performed, and it was found that *Trichoderma atroviride* and *Trichoderma virens*, the two aggressive mycoparasites, harbor more genes for hydrolytic enzymes like chitinases and glucanases (Kubicek et al. 2011). Interestingly, however, these two genomes also are richer in secondary metabolite biosynthesis genes (Table 17.2). Many of the secondary metabolite biosynthetic genes form gene clusters inclusive of core enzymes such as non-ribosomal peptide synthetases (NRPSs), polyketide synthases (PKSs), terpene synthases/cyclases, and others. These gene clusters harbor other genes as well like cytochrome P450s, oxidoreductases, methyl transferases, genes for transporters, and transcription factors (Bansal and Mukherjee 2016).

17.4 Intra-species Diversity in Secondary Metabolites

Trichoderma virens produces a plethora of secondary metabolites, some of which may be strain specific (Howell et al. 1993). Gliotoxin and gliovirin are the best examples showing intra-species diversity of secondary metabolites in *T. virens*. As discussed above, there are two strains of *T. virens*, P and Q. "Q" strains of *T. virens* produce gliotoxin, while "P" strains produce gliovirin (Fig. 17.3). Genome of *T. virens* "Q" strain Gv29-8 had already been sequenced (Kubicek et al. 2011), and by mining this genome, the gliotoxin biosynthesis gene cluster could easily be identified as information on gliotoxin gene cluster in *Aspergillus fumigatus* was already known (Mukherjee et al. 2012a). However, the gliovirin gene cluster remained elusive until the whole genome of a "P" strain (IMI 304061, a strain isolated from India) was sequenced by us. By comparative genome analysis, we were able to identify the whole cluster responsible for biosynthesis of gliovirin; its biosynthetic role was confirmed by gene knockout and LC-MS/MS analysis (Sherkhane et al. 2017). Gliotoxin gene cluster is absent in "P" strain. Interestingly, an orthologue of the gliovirin cluster is present in a distantly related fungus *Aspergillus udagawae* (Fig. 17.4).

17.5 One Gene Cluster: Many Metabolites

The diversity in secondary metabolites is also brought about by single gene cluster. In *T. virens*, the *vir* cluster has been reported to produce 22 volatile secondary metabolites. These include both monoterpenes and sesquiterpenes. The volatile compounds produced by the *vir* cluster have important roles. For example, beta-caryophyllene has anti-inflammatory and antimicrobial property, and germacrene D has antioxidant and antibacterial property. Interestingly, a glyceraldehyde 3 phosphate dehydrogenase (GAPDH) was found to be present in the *vir* cluster in both *Trichoderma* and *Aspergillus* species (Mukherjee et al. 2006). This GAPDH is isomer of the GAPDH involved in glycolysis in the genome of *Trichoderma* and *Aspergillus* species. Deletion of GAPDH in the *vir* cluster confirms its role in volatile sesquiterpene compound biosynthesis (Fig. 17.5) [Pachauri et al. 2018].

17.6 One Gene: Many Metabolites

Deletion of *tex2* encoding a 14-module non-ribosomal peptide synthetase (NRPS) resulted in abolition of two types of peptaibols, 14-residue peptaibols and 11-residue peptaibols. A total of 88 peptaibols were reported to be produced by Tex2 (53 14-residue peptaibols and 35 11-residue peptaibols). Module skipping and degeneracy gave rise to such diversity (Fig. 17.6).

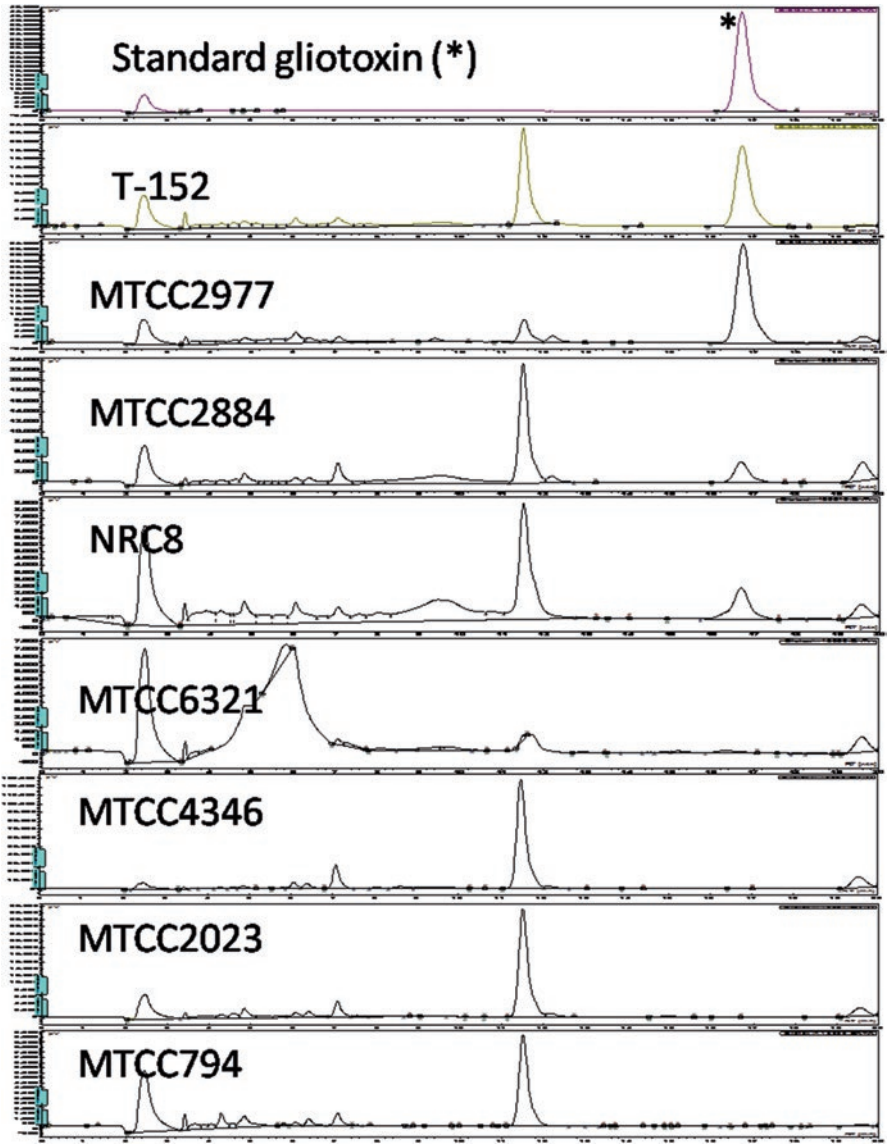

Fig. 17.3 HPLC analysis of filtrates of *T. virens* strains. Note the presence of gliotoxin in "Q" strains (T-152, MTCC 2977, MTCC 2884, NRC 8) and its absence in "P" strains

17.7 Regulation of Secondary Metabolism

Secondary metabolite diversity may also be regulated by environmental conditions including biotic and abiotic stresses. Though several gene clusters are present in a genome, only a few are expressed under standard laboratory cultivation conditions.

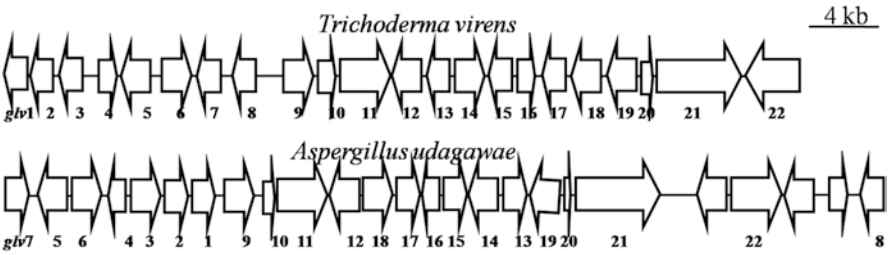

Fig. 17.4 The gliovirin gene cluster of *T. virens* and its orthologous cluster in *A. udagawae.* (Adapted from Sherkhane et al. 2017). Please refer to Sherkhane et al. (2017) for details

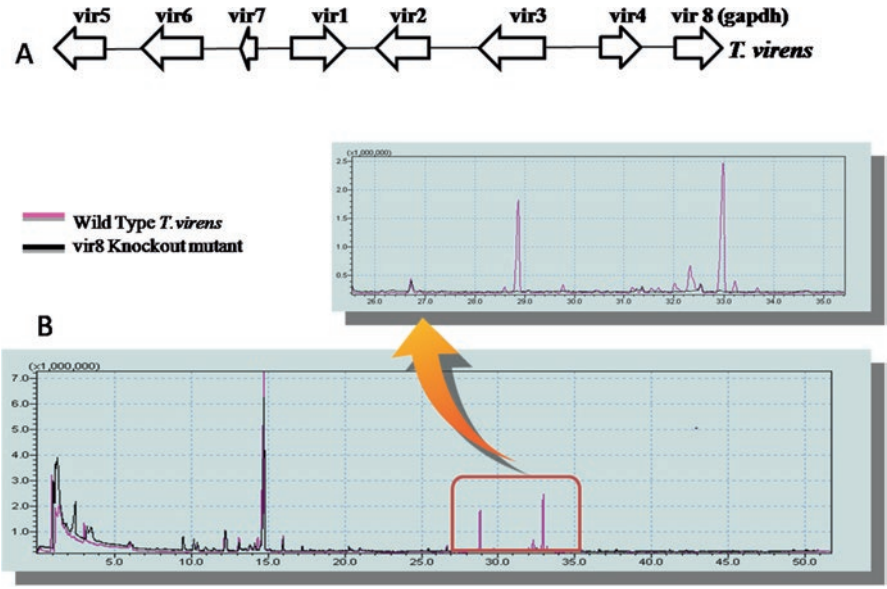

Fig. 17.5 (**a**) The *vir* cluster responsible for production of volatile sesquiterpene compounds in *Trichoderma virens*. (**b**) GC-MS profile of wild-type *T. virens* and *vir8* knockout mutant. No volatile sesquiterpene compounds are produced by *vir8* knockout mutant. (Adapted from Pachauri et al. 2018)

Inducing the expression of such gene clusters by using biological or genetic tools gives rise to chemo-diversity (Brakhage and Schroeckh 2011). Many secondary metabolite biosynthetic gene clusters possess putative transcription factors. Thctf1 is a transcription factor reported to be associated with the production of 6-PP in *T. harzianum*. Deletion of Thctf1 in *T. harzianum* resulted in decrease in the expression of two secondary metabolites derived from 6-PP and altered antimicrobial activity (Rubio et al. 2009). The secondary metabolites produced by *Trichoderma* species are reported to be influenced by other microorganisms, pH signaling, and the velvet-complex proteins. The interaction of *T. atroviride*, *T. virens*, and *T. reesei* with *Rhizoctonia solani* was studied using transcriptome analysis, and this analysis

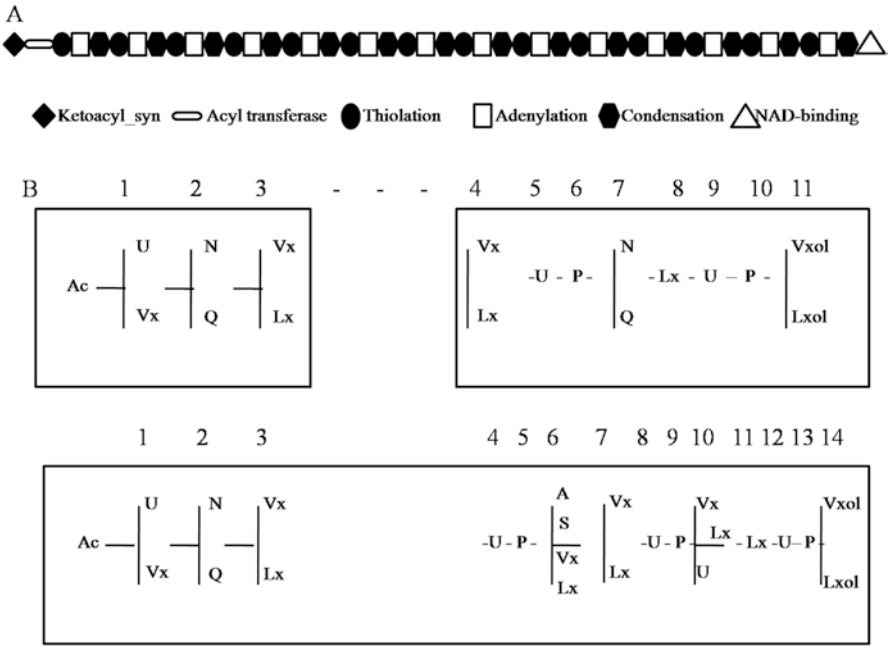

Fig. 17.6 (**a**) Modular organization of peptaibol synthetase Tex2. (**b**) sequence alignments of 11- and 14-residue peptaibols produced by *T. virens*. Sequences are given in standard single-letter code (Ac, acetyl-, U, Aib, Vx, Val/Iva, Lx, Leu/Ile, and ol represents the C-terminal amino-alcohol). The proposed module order is represented by numbers above each box. (Adapted from Mukherjee et al. 2011)

highlighted important genes influenced by the presence of *R. solani*. Interaction of *T. atroviride* and *T. reesei* with *R. solani* showed upregulation of two PKSs (Atanasova et al. 2013; Kubicek et al. 2011). In *T. atroviride*, the presence of *R. solani* also upregulates a 6-PP biosynthesis-related lipoxygenase gene (Kubicek*et al* 2011). In *T. virens*, the presence of *R. solani* upregulates expression of all the genes associated with the gliotoxin biosynthetic gene cluster (Atanasova et al. 2013). In another report, the presence of mycotoxin fusaric acid (FA) produced by *Fusarium* reduced the production of 6-PP and increased the 1-octen-3-ol biosynthesis. But in certain *Trichoderma* strains, the presence of fusaric acid does not alter the volatile profile; instead, 6-PP production inhibits FA production (Stoppacher et al. 2010). Change in the pH of the environment causes induction of a pH regulator PacC which further regulates expression of many genes in the fungus. For example, the deletion of a PacC orthologue in *T. virens* alters the expression of genes associated with secondary metabolite biosynthesis and iron transport, and the mutant also had decreased biocontrol activity (Mukherjee et al. 2012a). The velvet complex is best studied in *Aspergillus nidulans* and includes a methyltransferase LaeA and the two velvet proteins VeA and VelB. The velvet complex is responsible for coupling light response to the regulation of sexual development and secondary metabolite

biosynthesis. Vel1 is an orthologue of veA in *T. virens*, and deletion of vel1 gene in *T. virens* ceased the production of gliotoxin and downregulated many secondary metabolism-related genes (Mukherjee and Kenerley 2010). The *T. reesei* LaeA orthologue Lae1 is required for the expression of lignocellulose-degrading enzymes, and this enzyme is also found to be regulated epigenetically (Karimi-Aghcheh et al. 2013b). Lae1 gene deletion in *T. atroviride* resulted in the reduced expression of PKS-encoding genes and 6-PP-related lipoxygenase gene (Karimi-Aghcheh et al. 2013a). The mutant also displayed decreased mycoparasitic activity and reduction in the production of antifungal water-soluble metabolites and VOCs. The adenylyl cyclase-inhibiting Gα subunit is encoded by *tga1* gene, and the adenylyl cyclase-stimulating Gα subunit is encoded by *tga3* gene in *T. atroviride*. Deletion of *tga1* gene decreased the production of 6-PP, but the peptaibol production increased (Reithner et al. 2005), whereas deletion of *tga3* gene completely abolished the production of peptaibols, and its production was found to be regulated by two blue light regulators BLR1 and BLR2 in *T. atroviride* (Komon-Zelazowska et al. 2007). In *T. virens*, the role of *tac1* gene encoding adenylate cyclase has been identified (Mukherjee et al. 2007). MAPK-dependent signaling pathway was also shown to be involved in secondary metabolite biosynthesis and regulation. MAPK-encoding gene *tmk1* deletion in *T. atroviride* increased the production of peptaibols and 6-PP and also increased the antifungal activity (Reithner et al. 2007). But there was no change in secondary metabolites biosynthesis in *T. virens* in *tmk1* deletion mutants (Mendoza-Mendoza et al. 2003).

17.8 Conclusions

Trichoderma spp. are genetically diverse group of fungi which produce a plethora of secondary metabolites with known and unknown functions and applications. Tremendous amount of variability in secondary metabolite biosynthesis exists even within the same species. The genomes of these fungi are rich in secondary metabolism-related gene clusters, many of which are silent under standard laboratory culture conditions. With new genetic tools available, it is possible to induce the expression of such "silent" clusters which will add to the metabolic diversity of these fungi. Since many secondary metabolites are bioactive, it's possible to discover novel molecules from these "biocontrol fungi" that might actually find direct applications in agriculture and medical science.

References

Atanasova L, Le Crom S, Gruber S, Coulpier F, Seidl-Seiboth V, Kubicek CP, Druzhinina IS (2013) Comparative transcriptomics reveals different strategies of *Trichoderma* mycoparasitism. BMC Genomics 14:121

Baker SE, Perrone G, Richardson NM, Gallo A, Kubicek CP (2012) Phylogenomic analysis of polyketide synthase-encoding genes in *Trichoderma*. Microbiology (Reading, UK) 158:147–154

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

Bansal R, Sherkhane PD, Oulkar D, Khan Z, Banerjee K, Mukherjee PK (2018) The viridin biosynthesis gene cluster of *Trichoderma virens* and its conservancy in the bat white-nose fungus *Pseudogymnoascus destructans*. Chem Select 3(4):1289–1293

Bortolus M, De Zotti M, Formaggio F, Maniero AL (2013) Alamethicin in bicelles: orientation, aggregation, and bilayer modification as a function of peptide concentration. Biochim Biophys Acta 1828:2620–2627

Brakhage AA, Schroeckh V (2011) Fungal secondary metabolites – strategies to activate silent gene clusters. Fungal Genet Biol 48:15–22

Cardoza RE, Vizcaino JA, Hermosa MR, Sousa S, Gonzalez FJ, 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

Cardoza RE, Malmierca MG, 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

Contreras-Cornejo HA, Macías-Rodríguez L, Cortés-Penagos C, López-Bucio J (2009) *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. Plant Physiol 149(3):1579–1592

Contreras-Cornejo HA, Macías-Rodríguez L, del-Val E, Larsen J (2016) Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: interactions with plants. FEMS Microbiol Ecol 92(4):fiw036

Crutcher FK, Parich A, Schuhmacher R, Mukherjee PK, Zeilinger S, Kenerley CM (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

Degenkolb T, Karimi Aghcheh R, Dieckmann R, Neuhof T, Baker SE, Druzhinina IS, Kubicek CP, Brückner H, von Döhren H (2012) The production of multiple small Peptaibol families by single 14-module peptide Synthetases in *Trichoderma/Hypocrea*. Chem Biodivers 9(3):499–535

Druzhinina IS, Seidl-Seiboth V, Herrera-Estrella A, Horwitz BA, Kenerley CM, Monte E, Mukherjee PK, Zeilinger S, Grigoriev IV, Kubicek CP (2011) *Trichoderma*: the genomics of opportunistic success. Nat Rev Microbiol 9:749–759

Howell CR, Stipanovic RD, Lumsden RD (1993) Antibiotic production by strains of *Gliocladium virens* and its relation to the biocontrol of cotton seedling diseases. Biocontrol Sci Technol 3:435–441

Jones RW, Hancock JG (1987) Conversion of viridin to viridiol by viridin-producing fungi. Can J Microbiol 33:963–966

Karimi Aghcheh R, Druzhinina IS, Kubicek CP (2013a) The putative protein methyltransferase LAE1 of *Trichoderma atroviride* is a key regulator of asexual development and mycoparasitism. PLoS One 8:67144

Karimi-Aghcheh R, Bok JW, Phatale PA, Smith KM, Baker SE, Lichius A, Omann M, Zeilinger S, Seiboth B, Rhee C, Keller NP, Freitag M, Kubicek CP (2013b) Functional analyses of *Trichoderma reesei* LAE1 reveal conserved and contrasting roles of this regulator. G3 (Bethesda) 3:369–378

Komon-Zelazowska M, Neuhof T, Dieckmann R, von Dohren H, Herrera-Estrella A, Kubicek CP, Druzhinina IS (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

Kubicek CP, Herrera-Estrella A, Seidl-Seiboth V, Martinez DA, Druzhinina IS, Thon M, Zeilinger S, Casas-Flores S, Horwitz BA, Mukherjee PK, Mukherjee M et al (2011) Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of *Trichoderma*. Genome Biol 12:R40

Mendoza-Mendoza A, Pozo MJ, Grzegorski D, Martinez P, Garcia JM, 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 USA 100:15965–15970

Mukherjee PK, Kenerley CM (2010) Regulation of morphogenesis and biocontrol properties in *Trichoderma virens* by a VELVET protein, Vel1. Appl Environ Microbiol 76:2345–2352

Mukherjee M, Horwitz BA, Sherkhane PD, Hadar R, Mukherjee PK (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 PK, Kale SP (2007) cAMP signaling is involved in growth, germination, mycoparasitism and secondary metabolism in *Trichoderma virens*. Microbiology (Reading, UK) 153:1734–1742

Mukherjee PK, Wiest A, Ruiz N, Keightley A, Moran-Diez ME, McCluskey K, Pouchus YF, Kenerley CM (2011) Two classes of new peptaibols are synthesized by a single non-ribosomal peptide synthetase of *Trichoderma virens*. J Biol Chem 286:4544–4554

Mukherjee PK, Buensanteai N, Moran-Diez ME, Druzhinina IS, Kenerley CM (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, UK) 158:155–165

Mukherjee PK, Horwitz BA, Kenerley CM (2012b) Secondary metabolism in *Trichoderma* – a genomic perspective. Microbiology (Reading, UK) 158:35–45

Mukherjee PK, Horwitz BA, Herrera-Estrella A, Schmoll M, Kenerley CM (2013a) *Trichoderma* research in the genome era. Ann Rev Phytopathol 51:105–129

Mukherjee PK, Horwitz BA, Singh US, Mukherjee M, Schmoll M (2013b) *Trichoderma*: biology and applications. CABI, Wallingford

Mukherjee PK, Hurley JF, Taylor JT, Puckhaber L, Lehner S, Druzhinina I, Schumacher R, Kenerley CM (2018) Ferricrocin, the intracellular siderophore of *Trichoderma virens*, is involved in growth, conidiation, gliotoxin biosynthesis and induction of systemic resistance in maize. Biochem Biophys Res Commun. https://doi.org/10.1016/j.bbrc.2018.09.170

Pachauri S, Chatterjee S, Kumar V, Mukherjee PK (2018) A dedicated glyceraldehyde-3-phosphate dehydrogenase is involved in the biosynthesis of volatile sesquiterpenes in *Trichoderma virens*—evidence for the role of a fungal GAPDH in secondary metabolism. Curr Genet. https://doi.org/10.1007/s00294-018-0868-y

Reino JL, Guerrero RF, Hernandez-Galan R (2008) 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 42:749760

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

Robbertse B, Strope PK, Chaverri P, Gazis R, Ciufo S, Domrachev M, Schoch CL (2017) Improving taxonomic accuracy for fungi in public sequence databases: applying 'one name one species' in well-defined genera with *Trichoderma/Hypocrea* as a test case. DATABASE-OXFORD 2017: bax072

Rubio MB, Hermosa R, Reino JL, Collado IG, 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

Ruiz N, Roullier C, Petit K, Nemont CS, Grovel O, Pouchus YF (2013) Marine-derived *Trichoderma*: a source of new bioactive metabolites. In: Mukherjee PK, Horwitz BA, Singh US, Mukherjee M, Schmoll M (eds) *Trichoderma*: biology and applications. CABI, Wallingford

Scharf DH, Brakhage AA, Mukherjee PK (2016) Gliotoxin – bane or boon? Environ Microbiol 18:1096–1109

Sherkhane PD, Bansal R, Banerjee K, Chatterjee S, Oulkar D, Jain P, Rosenfelder L, Elgavish S, Horwitz BA, Mukherjee PK (2017) Genomics-Driven Discovery of the Gliovirin Biosynthesis Gene Cluster in the Plant Beneficial Fungus *Trichoderma virens*. Chem Select 2:3347–3352

Singh V, Ray S, Bisen K, Keswani C, Upadhyay RS, Sarma BK, Singh HB (2017) Unravelling the dual applications of *Trichoderma* spp. as biopesticide and biofertilizer. In: Singh HB, Sarma BK, Keswani C (eds) Advances in PGPR research. CABI, Wallingford

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

Tijerino A, Cardoza RE, Moraga J, Malmierca MG, Vicente F, Aleu J, Collado IG, 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 RE, Moraga J, Malmierca MG, Aleu J, Collado IG, 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

Vargas WA, Mukherjee PK, Laughlin D, Wiest A, Moran-Diez ME, Kenerley CM (2014) Role of gliotoxin in the symbiotic and pathogenic interactions of *Trichoderma virens*. Microbiology (Reading, UK) 160:2319–2330

Vinale F, Sivasithamparam K, Ghisalberti EL, Marra R, Barbetti MJ, Li H, Woo SL, 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

Wiest A, Grzegorski D, Xu BW, Goulard C, Rebuffat S, Ebbole DJ, Bodo B, Kenerley C (2002) Identification of peptaibols from *Trichoderma virens* and cloning of a peptaibol synthetase. J Biol Chem 277:20862–20868

Zeilinger S, Grubera S, Bansal R, Mukherjee PK (2016) Secondary metabolism in *Trichoderma* – chemistry meets genomics. Fungal Biol Rev 30:74–90