

Trichoderma–Plant–Pathogen Interactions: Advances in Genetics of Biological Control

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Abstract *Trichoderma* spp. are widely used in agriculture as biofungicides. Induction of plant defense and mycoparasitism (killing of one fungus by another) are considered to be the most important mechanisms of *Trichoderma*-mediated biological control. Understanding these mechanisms at the molecular level would help in developing strains with superior biocontrol properties. In this article, we review our current understanding of the genetics of interactions of *Trichoderma* with plants and plant pathogens.

Keywords *Trichoderma* · Induced resistance · Biological control · Mycoparasitism · Genetics

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Introduction

Trichoderma spp. (teleomorph *Hypocrea*) are the most successful biofungicides used in today's agriculture with more than 60 % of the registered biofungicides world-wide being *Trichoderma*-based [1]. In India alone, about 250 products are available for field applications [2]. Despite this remarkable success, the share of biofungicides is only a fraction of the fungicides market, dominated by synthetic chemicals. The major limitations of microbe-based fungicides are their restricted efficacy and their inconsistency under field conditions. The origin of these difficulties is that microbes are slow to act, compared to chemicals, and are influenced by environmental factors. Here, “genetic intervention” to design strains that are more effective than the native ones might prove useful. This goal could be attained by gaining knowledge on the molecular mechanisms of interactions of these organisms with other biotic and abiotic factors. *Trichoderma* spp. have received a great deal of attention from the academia in the past, generating extensive data on their molecular genetics and physiology. This work culminated in whole genome sequencing of four mycoparasitic *Trichoderma* species [3; <http://genome.jgi.doe.gov/Triha1/Triha1.home.html>, <http://genome.jgi.doe.gov/Trias1/Trias1.home.html>]. We summarize here the recent findings on the genetics of interactions of *Trichoderma* with plants and pathogens.

Trichoderma–Plant Interactions

Many *Trichoderma* spp. grow in the rhizosphere and are capable of penetrating and internally colonizing plant roots [4]. This opportunistic/facultative symbiosis is driven by the ability of *Trichoderma* to derive sucrose or other

nutrients from plants, in return for boosting plant immunity against invading pathogens and improving photosynthetic abilities [5–7]. The presence of *Trichoderma* in the rhizosphere evokes a coordinated transcriptomic, proteomic and metabolomic response in the plant [5, 8–11]. This reprogramming of the plant is often beneficial, improving growth, yield and resistance to pathogens.

Root Colonization

Trichoderma spp. can colonize plant roots, both externally and internally (Fig. 1). As in other biological interactions, the attraction of *Trichoderma* to plant roots likely results from interplay of chemical signals from both partners. This primary step in the *Trichoderma*–plant interaction is rather poorly understood compared to those that follow, i.e., attachment, penetration and internal colonization of plant roots. *Trichoderma* spp. produce and modulate hormonal signals in order to facilitate the colonization of roots. The fungus produces auxins that promote root growth which, in turn, facilitates colonization by increasing the available surface area [12]. The role of *accd*, encoding ACC deaminase, in regulation of canola root growth by *T. asperellum* was demonstrated by gene knockout [13]. *Trichoderma* deploys small secreted cysteine-rich hydrophobin-like proteins to facilitate anchoring/attachment. Two such proteins have been found to facilitate attachment to the roots—TasHyd1 from *T. asperellum* and Qid74 of *T. harzianum* [14, 15]. *Trichoderma* spp. secrete expansin-like proteins with cellulose binding modules and endopolygalacturonase to facilitate root penetration [16, 17]. Once inside the roots, these fungi can grow inter-cellularly, albeit limited to epidermal layer and outer cortex. Initial suppression of plant defense may facilitate root invasion. *T. koningii*, for example, suppresses the production of phytoalexins during colonization of *Lotus japonicus* roots [18].

Induced Defense

Plants respond immediately to *Trichoderma* invasion by rapid ion fluxes and an oxidative burst, followed by deposition of callose and synthesis of polyphenols [19]. Subsequent events involve salicylate (SA) and jasmonate/ethylene (JA/ET)-signaling, which results in the entire plant acquiring varying degrees of tolerance to pathogen invasion [19]. This response has, most frequently, been described as JA/ET-mediated induced systemic resistance (ISR) and resembles the response triggered by plant growth-promoting rhizobacteria (PGPR). Recent findings, however, indicate that at higher inoculum doses *Trichoderma* can trigger a SA-mediated systemic acquired resistance (SAR) response, similar to that invoked by necrotrophic pathogens [20–23]. The signaling events leading to induced resistance are not

thoroughly understood. A hint comes from implication of a mitogen-activated protein kinase (MAPK) from cucumber and a MAPK from *T. virens* in the molecular cross talk between plant and *Trichoderma*, presumably triggering the downstream defense responses [24, 25].

Xylanase and peptaibols (peptaibiotics with high content of alpha amino isobutyric acid) like alamethicin and trichovirin II which are produced by *Trichoderma* spp. were shown to elicit an immune response in plants [26–29]. Recently, a PKS/NRPS hybrid enzyme involved in defense responses in maize was identified [30]. The best characterized elicitor produced by *Trichoderma* spp. is Sm1/Ep11, an abundantly secreted, small cysteine-rich hydrophobin-like protein of the cerato-platanin (CP) family [31, 32]. Deletion of this *Trichoderma* gene impairs elicitation of ISR in maize [33]. The monomeric form of Sm1 is in a glycosylated state which is essential for elicitation properties. It was suggested that the monomeric form in the non-glycosylated state is susceptible to oxidative-driven dimerization in plants rendering Sm1 inactive as inducer of ISR [34]. Recently, the 3-D structure of the *Ceratocystis platani* cerato-platanin has been resolved and the carbohydrate residue (an oligomer of *N*-acetyl glucosamine) that binds to it has been identified [35]. Since the CP protein family is highly conserved, its structure and carbohydrate-binding properties may suggest a mechanism for the elicitation properties of Sm1.

The Endophytic *Trichoderma*

Recent reports suggest that some *Trichoderma* spp. are not restricted to outer root tissues, but can also live in the plant as “true” endophytes [29]. Interestingly, most of the endophytic *Trichoderma* discovered are “new” species (e.g., *T. stromaticum*, *T. amazonicum*, *T. evansii*, *T. martiale*, *T. taxi* and *T. theobromicola*), different from those routinely isolated from soil/rhizosphere and a phylogenetic analysis revealed that these species are of recent evolutionary origin [29–40]. The endophytic *Trichoderma* species are reported to induce transcriptomic changes in plants and some are known to protect plants from diseases and abiotic stresses [41, 42]. Some of these endophytes preferentially colonize the surface of glandular trichomes and form appressoria-like structures [43]. This is one example where *Trichoderma* uses a “non-root” mode of entry into the plant.

Interactions with Plant Pathogens

Mycoparasitism is apparently an ancestral trait of *Trichoderma/Hypocrea* [3, 29]. The ability of *Trichoderma* to parasitize and kill other fungi has been the major driving force behind the commercial success of these fungi as biofungicides. In addition, some *Trichoderma* spp. can kill

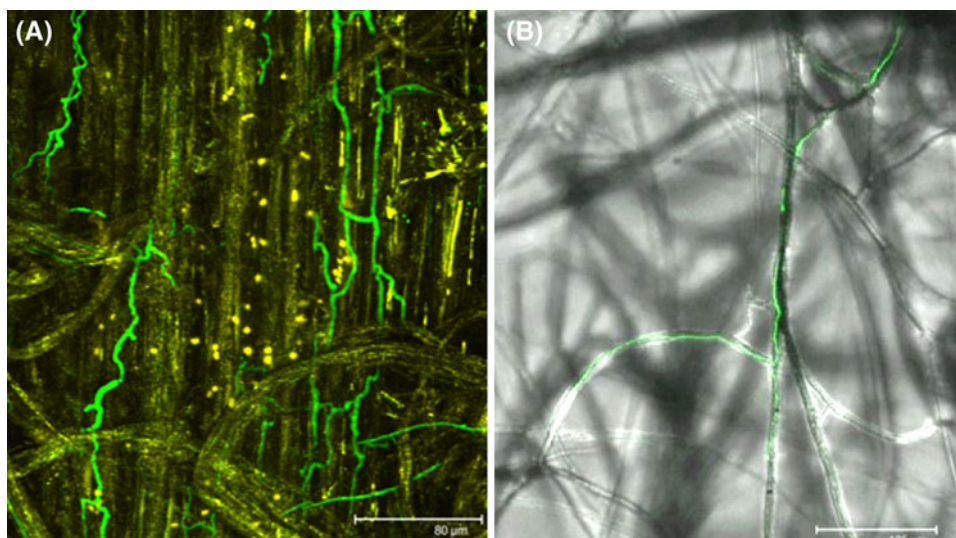


Fig. 1 Green fluorescent labeled *Trichoderma velutinum* G1/8 on sterile grown 2 weeks old sugar beet seedlings. Confocal laser scanning microscopy (CLSM) was performed with a Leica TCS SPE confocal microscope (Leica Microsystems, Mannheim, Germany). **a** Root surface (yellow) and *T. velutinum* hyphae (green). Hyphae

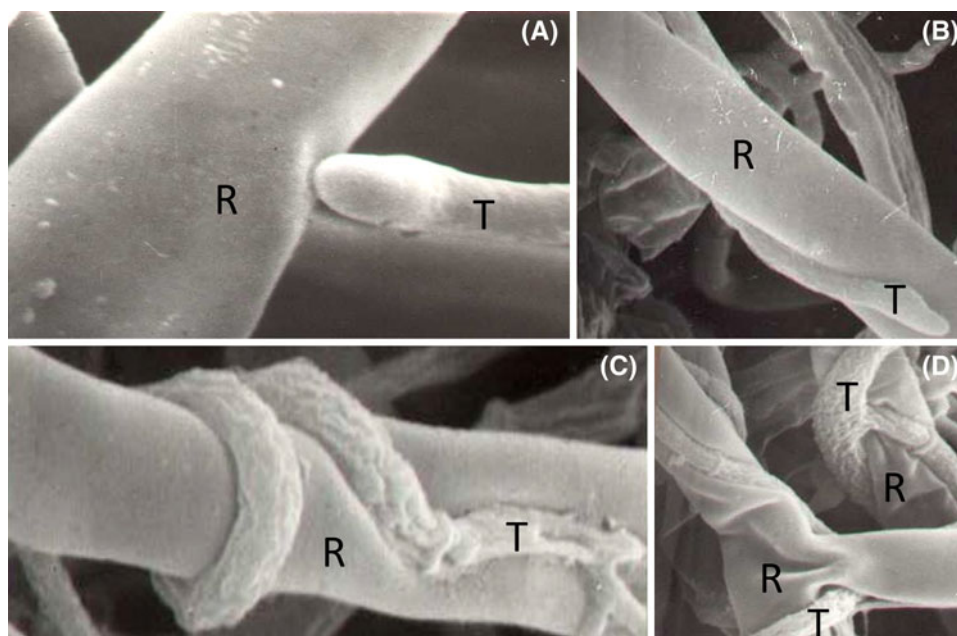
nematodes and hence have the potential for applications as bio-nematicides [44]. A typical mycoparasitic interaction involves sensing of the host/prey fungus, attraction, attachment, coiling around and lysis brought about by hydrolytic enzymes, in many cases, in conjunction with secondary metabolites (Fig. 2).

Environmental signaling plays an important role in cellular organisms. Understanding of the mechanisms of cell signaling in *Trichoderma* is limited compared to “model” fungi like *Magnaporthe grisea* and *Neurospora crassa*, but there has been significant progress based on

grow between the root cells and follow their cell shape and direction. **b** Differential interference contrast microscopy of the lateral roots and root hairs combined with CLSM of green *T. velutinum* hyphae following the growth direction of the root hairs. (Color figure online)

genetic approaches. The seven transmembrane G protein-coupled receptor Gpr1 is involved in sensing the fungal prey: silencing of the *gpr1* gene in *T. atroviride* rendered the mycoparasite unable to respond to the presence of the host fungus [45]. Binding of a ligand to such receptors leads to downstream signaling events via activation of G-protein cascades. Indeed, deletion of the Tga3 G α protein-encoding gene affected the mycoparasitic abilities of *T. atroviride* in a similar way to loss of Gpr1 [46]. Deletion of the adenylate cyclase gene *tac1* severely impaired growth and mycoparasitic abilities of *T. virens* [47]. Like

Fig. 2 Mycoparasitism of *Trichoderma virens* (T) on *Rhizoctonia solani* (R). **a** Attraction, **b** attachment, **c** coiling, **d** lysis of host hyphae [72]



most other filamentous fungi, *Trichoderma* spp. have three MAPK cascades comprising MAPKKK, MAPKK and MAPK [48] and MAPK pathways may act in mycoparasitism and biocontrol [49, 50]. These data imply important functions of signaling cascades in mycoparasitism and related biocontrol properties (Fig. 3).

Attachment to Host Fungi

Attachment to and attack of host fungi by mycoparasitic *Trichoderma* is accompanied by the formation of appressoria- or papillae-like structures and/or coiling around host hyphae [29]. The genetics underlying attachment of the mycoparasite to the host fungus are not well understood, although hydrophobins are possibly involved [29]. Though experimental evidence is lacking, indirect support for the involvement of hydrophobins comes from the finding that *T. virens* mutants in the transcriptional regulator of secondary metabolism and morphogenesis *Vel1*, which have decreased hydrophobin expression, were defective in both hydrophobicity and mycoparasitism [51].

Killing the Host: Production of Hydrolytic Enzymes and Antibiotics

Hydrolytic enzymes and antibiotics are among the most important members of the chemical arsenals deployed by

Trichoderma to kill other fungi. Not surprisingly, the genomes of the mycoparasitic *Trichoderma* spp. are rich in genes encoding enzymes like chitinases and glucanases, and those for secondary metabolism like NRPSs [3]. Earlier evidences suggested the involvement of chitinases in biocontrol though the effects of deletion of *chit42/ech42* were not very drastic, possibly because of a large reservoir of genes with a compensatory effect [29]. Glucanases are another group of cell wall-lytic enzymes with roles in mycoparasitism/biocontrol. Deletion of *tvbgn3* (β -1,6-glucanase-encoding) reduced the mycoparasitic and biocontrol potential of *T. virens* against *P. ultimum* [52]. Co-overexpression of two β -glucanases (*Bgn2* and *Bgn3*) resulted in improved biocontrol of *T. virens* against *R. solani*, *P. ultimum* and *Rhizopus oryzae* [53]. In addition to chitinases and glucanases, proteases like *Prb1/Sp1* are induced during mycoparasitism and play definitive roles in biocontrol [54]. In contrast to studies on hyphal parasitism, very little research has been done on the molecular mechanisms of parasitism of resting structures. One exception is the suggested role of a laccase in colonization of sclerotial structures by *T. virens* [55].

Trichoderma spp. are prolific producers of secondary metabolites and the genomes of the mycoparasitic *Trichoderma* spp. are especially enriched in genes for secondary metabolism [3, 56]. Nevertheless, genome analyses suggest that most of the secondary metabolism-related genes are

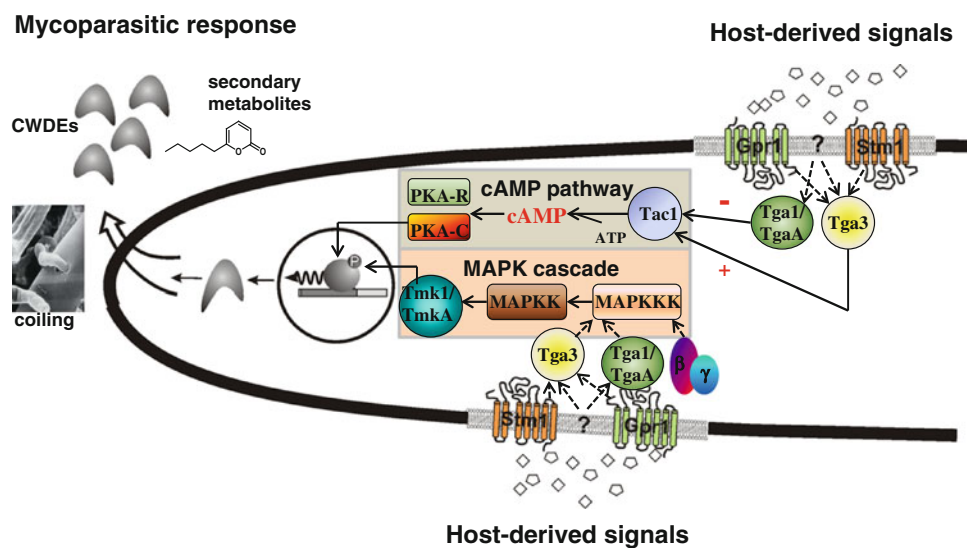


Fig. 3 Mycoparasitism-relevant signaling pathways of *Trichoderma atroviride/Trichoderma virens*. *Trichoderma* secretes cell wall-degrading enzymes (CWDEs) already before contact which release degradation products from the host's cell wall. These act as signals for host recognition in the mycoparasite. After activation of G protein signaling (Gpr1, Stm1 = GPCRs, Tga1/TgaA, Tga3 = G α proteins), MAPK (Tmk1/TmkA = MAPK) and cAMP pathways (Tac1 = adenylate

cyclase, PKA-R = regulatory subunit, PKA-C = catalytic subunit of cAMP-dependent protein kinase) act as downstream effectors. Via phosphorylation, respective targets are regulated resulting in full induction of CWDEs and secondary metabolism. *T. atroviride* Gpr1, Tga1, Tga3, Tmk1 and *T. virens* TgaA, TmkA, Tac1 were proven to regulate essential mycoparasitism-related processes. Involvement of Stm1 was deduced from a transcriptomic study [65]

not expressed under standard laboratory conditions [3, 57]. Roles of antimicrobial secondary metabolites such as gliotoxin and gliovirin in suppression of *R. solani* and *P. ultimum* have been suggested, although contradictory reports exist [58]. The non-ribosomal peptide synthetase Tex1 assembles an 18-residue peptaibol (trichovirin II) and by using $\Delta tex1$ mutants the trichovirin II type peptaibols were shown to trigger induced resistance in plants [27, 59]. Recently, genetic evidence has been provided for the assembly of 11- and 14-modules peptaibols by a single NRPS (Tex2 of *T. virens*; [60]). Given the fact that these peptaibiotics are strongly antimicrobial (by being able to form voltage-gated membrane channels), their role in fungus–fungus interactions cannot be ruled out. Accordingly, the *T. pseudokoningii* peptaibol trichokonin VI was shown to induce programmed cell death in *Fusarium oxysporum* [61]. Certain species like *T. atroviride* produce the volatile metabolite 6-pentyl-2H-pyran-2-one (6-PP) which plays an important role in *Trichoderma*–plant and *Trichoderma*–fungal interactions [62, 63]. Though the pathway is yet to be identified, a transcription factor, Thctf1, involved in the biosynthesis of 6-PP has been characterized [64].

Lessons from Genome Sequencing

At present, the genome sequences of five species, *T. reesei*, *T. atroviride*, *T. virens*, *T. harzianum* and *T. asperellum*, are available. The saprophyte *T. reesei* often is found on decaying wood and, because it can secrete large amounts of cellulases and hemicellulases, this species is of industrial importance. Compared to the mycoparasitic species *T. atroviride*, *T. virens*, *T. harzianum* and *T. asperellum*, *T. reesei* has the smallest genome (34.1 Mb, 9,129 gene models) probably resulting from a loss of mycoparasitism-specific genes [3, 29]. The genome sizes of the mycoparasites range from 36.1 Mb (*T. atroviride*, 11,863 gene models), 37.4 Mb (*T. asperellum*, 12,586 gene models), 38.8 Mb (*T. virens*, 12,427 gene models) to 40.98 Mb (*T. harzianum*, 14,095 gene models). In addition to being saprophytes found in soil, mycoparasitic *Trichoderma* species frequently live in association with plant roots and living or dead fungal biomass. *T. atroviride* and *T. asperellum* are phylogenetically ancestral species [3] and both are powerful antagonists of other fungi (necrotrophic mycoparasites). *T. virens* and *T. harzianum* are aggressive parasites of phytopathogenic fungi, too; in addition, these species are particularly effective in the stimulation of plant defense responses [29].

Comparative genome analysis between *T. atroviride*, *T. virens* and *T. reesei* revealed an expansion of several gene families in the mycoparasites relative to *T. reesei* or

other ascomycetes. These expansions comprise genes specific for mycoparasitism such as chitinases and some glucanases and those involved in secondary metabolite biosynthesis [3]. Many members of these families are expressed before and during contact with the host/prey fungus [65]. Recent secretome analysis further revealed that *Trichoderma* may have one of the largest sets of proteases among fungi. Subtilisin-like proteases of the S8 family, dipeptidyl and tripeptidyl peptidases are expanded in the mycoparasites [66]. These findings not only show the importance of these genes in attacking and killing the fungal prey but further support the adaptation of the mycoparasitic *Trichoderma* species to their antagonistic lifestyle.

Conclusions

Being biotechnologically important, mycoparasitic *Trichoderma* spp. are extensively researched for both field applications as well as basic biology. Even though there have been several studies on the genetic basis of interaction of *Trichoderma* with other organisms (notably fungi and plants), an in depth understanding of the mechanisms is lacking. The absence of high throughput studies in these organisms has been due to the lack of whole genome sequences. However, this scenario is now expected to change with the availability of five *Trichoderma* genomes. Some progress has already been made in this direction with genome-wide expression studies [65, 67–70]. An international initiative should be undertaken to elucidate the functions of each gene by high throughput gene knockouts as accomplished with *N. crassa* in an exemplary community effort [71]. This, together with transcriptome analyses under conditions of mycoparasitism and plant root colonization, would help in identifying novel candidate genes involved in the interactions of *Trichoderma* spp. with plants and plant pathogens. Once this is achieved, it should be possible to engineer tailor-made strains for optimal biocontrol and other biotechnological applications.

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References

1. Verma M, Brar SK, Tyagi RD, Surampalli RY, Val'ero JR (2007) Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. *Biochem Eng J* 37:1–20
2. Singh HB, Singh BN, Singh SP, Singh SR, Sarma BK (2009) Biological control of plant diseases: current status and future prospects. In: Johri JK (ed) *Recent advances in biopesticides: biotechnological applications*. New India Pub, New Delhi, p 322

3. Kubicek CP, Herrera-Estrella A, Seidl-Seiboth V, Martinez DA et al (2011) Comparative genome sequence analysis underscores mycoparasitism as the ancestral life style of *Trichoderma*. *Genome Biol* 12:R40
4. Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) *Trichoderma* species—opportunistic, avirulent plant symbionts. *Nat Rev Microbiol* 2:43–56
5. Shores M, Harman GE (2008) The molecular basis of shoot responses of maize seedlings to *Trichoderma harzianum* T22 inoculation of the root: a proteomic approach. *Plant Physiol* 147: 2147–2163
6. Vargas WA, Mandawe JC, Kenerley CM (2009) Plant-derived sucrose is a key element in the symbiotic association between *Trichoderma virens* and maize plants. *Plant Physiol* 151:792–808
7. Vargas WA, Crutcher FK, Kenerley CM (2011) Functional characterization of a plant-like sucrose transporter from the beneficial fungus *Trichoderma virens*. Regulation of the symbiotic association with plants by sucrose metabolism inside the fungal cells. *New Phytol* 189:777–789
8. Lorito M, Woo SL, Harman GE, Monte E (2010) Translational research on *Trichoderma*: from omics to the field. *Annu Rev Phytopathol* 48:234–246
9. Hermosa R, Woo SL, Lorito M, Monte E (2010) Proteomic approaches to understand *Trichoderma* biocontrol mechanisms and plant interactions. *Curr Proteomics* 7:298–305
10. Brotman Y, Lisec J, Meret M, Chet I, Willmitzer L, Viterbo A (2012) Transcript and metabolite analysis of the *Trichoderma* induced systemic resistance response to *Pseudomonas syringae* in *Arabidopsis thaliana*. *Microbiology* 158:139–146
11. Morán-Diez E, Rubio B, Domínguez S, Hermosa R, Monte E, Nicolás C (2012) Transcriptomic response of *Arabidopsis thaliana* after 24 h incubation with the biocontrol fungus *Trichoderma harzianum*. *J Plant Physiol* 169:614–620
12. 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* 149:1579–1592
13. Viterbo A, Landau U, Kim S, Chernin L, Chet I (2010) Characterization of ACC deaminase from the biocontrol and plant growth-promoting agent *Trichoderma asperellum* T203. *FEMS Microbiol Lett* 305:42–48
14. Viterbo A, Chet I (2006) *TasHyd1*, a new hydrophobin gene from the biocontrol agent *Trichoderma asperellum*, is involved in plant root colonization. *Mol Plant Pathol* 7:249–258
15. Samolski I, Rincon AM, Pinzon LM, Viterbo A, Monte E (2012) The *qid74* gene from *Trichoderma harzianum* has a role in root architecture and plant biofertilization. *Microbiology* 158:129–138
16. Brotman Y, Briff E, Viterbo A, Chet I (2008) Role of swollenin, an expansin-like protein from *Trichoderma*, in plant root colonization. *Plant Physiol* 147:779–789
17. Morán-Diez E, Hermosa R, Ambrosino P, Cardoza RE, Gutiérrez S, Lorito M, Monte E (2009) The ThPG1 endopolygalacturonase is required for the *Trichoderma harzianum*–plant beneficial interaction. *Mol Plant Microbe Interact* 22:1021–1031
18. Masunaka A, Hyakumachi M, Takenaka S (2011) Plant growth-promoting fungus *Trichoderma koningii* suppresses isoflavonoid phytoalexin vestitol production for colonization on/in the roots of *Lotus japonicus*. *Microbes Environ* 26:128–134
19. Shores M, Harman GE, Mastouri F (2010) Induced systemic resistance and plant responses to fungal biocontrol agents. *Annu Rev Phytopathol* 48:21–43
20. Segarra G, Casanova E, Bellido D, Odena MA, Oliveira E, Trillas I (2007) Proteome, salicylic acid, and jasmonic acid changes in cucumber plants inoculated with *Trichoderma asperellum* strain T34. *Proteomics* 7:3943–3952
21. Contreras-Cornejo HA, Macías-Rodríguez L, Beltrán-Pena E, Herrera-Estrella A, López-Bucio J (2011) *Trichoderma*-induced plant immunity likely involves both hormonal and camalexin dependent mechanisms in *Arabidopsis thaliana* and confers resistance against necrotrophic fungi *Botrytis cinerea*. *Plant Signal Behav* 6:1554–1563
22. Salas-Marina MA, Silva-Flores MA, Uresti-Rivera EE, Castro-Longoria E, Herrera-Estrella A, Casas-Flores S (2011) Colonization of *Arabidopsis* roots by *Trichoderma atroviride* promotes growth and enhances systemic disease resistance through jasmonic acid/ethylene and salicylic acid pathways. *Eur J Plant Pathol* 131:15–26
23. Yoshioka Y, Ichikawa H, Naznin HA, Kogure A, Hyakumachi M (2012) Systemic resistance induced in *Arabidopsis thaliana* by *Trichoderma asperellum* SKT-1, a microbial pesticide of seed-borne diseases of rice. *Pest Manag Sci* 68:60–66
24. Viterbo A, Harel M, Horwitz BA, Chet I, Mukherjee PK (2005) *Trichoderma* mitogen-activated protein kinase signaling is involved in induction of plant systemic resistance. *Appl Environ Microbiol* 71:6241–6246
25. Shores M, Gal-On A, Leibman D, Chet I (2006) Characterization of a mitogen-activated protein kinase gene from cucumber required for *Trichoderma*-conferred plant resistance. *Plant Physiol* 142:1169–1179
26. Leitgeb B, Szekeres A, Manczinger L, Vagvolgyi C, Kredics L (2007) The history of alamethicin: a review of most extensively studied peptaibol. *Chem Biodivers* 4:1027–1051
27. Viterbo A, Wiest A, Brotman Y, Chet I, Kenerley CM (2007) The 18mer peptaibols from *Trichoderma virens* elicit plant defence responses. *Mol Plant Pathol* 8:737–746
28. Luo Y, Zhang DD, Dong XW, Zhao PB, Chen LL, Song XY, Wang XJ, Chen XL, Shi M, Zhang YZ (2010) Antimicrobial peptaibols induce defense responses and systemic resistance in tobacco against tobacco mosaic virus. *FEMS Micro Lett* 313: 120–126
29. 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
30. Mukherjee PK, Buensanteai N, Moran-Diez ME, Druzhinina IS, Kenerley CM (2012) Functional analysis of non-ribosomal peptide synthetases (NRPSs) in *Trichoderma virens* reveals a polyketide synthase (PKS)/NRPS hybrid enzyme involved in induced systemic resistance response in maize. *Microbiology* 158: 155–165
31. Djonović S, Pozo MJ, Dangott LJ, Howell CR, Kenerley CM (2006) Sm1, a proteinaceous elicitor secreted by the biocontrol fungus *Trichoderma virens* induces plant defense responses and systemic resistance. *Mol Plant Microbe Interact* 19:838–853
32. Seidl V, Marchetti M, Schandl R, Allmaier G, Kubicek CP (2006) EPL1, the major secreted protein of *Hypocrea atroviridis* on glucose, is a member of a strongly conserved protein family comprising plant defense response elicitors. *FEBS J* 273: 4346–4359
33. Djonovic S, Vargas WA, Kolomiets MV, Horndeski M, Wiest A, Kenerley CM (2007) A proteinaceous elicitor Sm1 from the beneficial fungus *Trichoderma virens* is required for induced systemic resistance in maize. *Plant Physiol* 145:875–889
34. Vargas WA, Djonović S, Sukno SA, Kenerley CM (2008) Dimerization controls the activity of fungal elicitors that trigger systemic resistance in plants. *J Biol Chem* 283:19804–19815
35. de Oliveira AL, Gallo M, Pazzagli L, Benedetti CE, Cappugi G, Scala A, Pantera B, Spisni A, Pertinhez TA, Cicero DO (2011) The structure of the elicitor cerato-platanin (CP), the first member of the CP fungal protein family, reveals a double $\psi\beta$ -barrel fold and carbohydrate binding. *J Biol Chem* 286:17560–17568

36. Zhang CL, Liu SP, Lin FC, Kubicek CP, Druzhinina IS (2007) *Trichoderma taxi* sp. nov., an endophytic fungus from Chinese yew *Taxus mairei*. FEMS Microbiol Lett 270:90–96
37. Hanada RE, deJorge SouzaT, Pomella AW, Hebbbar KP, Pereira JO, Ismaiel A, Samuels GJ (2008) *Trichoderma martiale* sp. nov., a new endophyte from sapwood of *Theobroma cacao* with a potential for biological control. Mycol Res 112:1335–1343
38. Hanada RE, Pomella AW, Costa HS, Bezerra JL, Loguercio LL, Pereira JO (2010) Endophytic fungal diversity in *Theobroma cacao* (cacao) and *T. grandiflorum* (cupuaçu) trees and their potential for growth promotion and biocontrol of black-pod disease. Fungal Biol 114:901–910
39. Samuels GJ, Ismaiel A (2009) *Trichoderma evansii* and *T. lieckfeldtiae*: two new *T. hamatum*-like species. Mycologia 101:142–156
40. Chaverri P, Gazis RO, Samuels GJ (2011) *Trichoderma amazonicum*, a new endophytic species on *Hevea brasiliensis* and *H. guianensis* from the Amazon basin. Mycologia 103:139–151
41. Bailey BA, Bae H, Strem MD, Roberts DP, Thomas SE, Crozier J, Samuels GJ, Choi IY, Holmes KA (2006) Fungal and plant gene expression during the colonization of cacao seedlings by endophytic isolates of four *Trichoderma* spp. Planta 224:1449–1464
42. Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, MeNice RL, Bailey BA (2009) The beneficial endophyte *Trichoderma hamatum* isolate DS 219b promotes growth and delays the onset of the drought response in *Theobroma cacao*. J Exp Bot 60:3279–3295
43. Bailey BA, Stream MD, Wood D (2009) *Trichoderma* species form endophytic associations within *Theobroma cacao* trichomes. Mycol Res 113:1365–1376
44. Sharon E, Chet I, Spiegel Y (2011) *Trichoderma* as biological control agent. In: Davies K, Spiegel Y (eds) Biological control of plant parasitic nematodes: building coherence between microbial ecology and molecular mechanisms. Springer, Berlin, pp 183–202
45. Omann MR, Lehner S, Escobar Rodriguez C, Brunner K, Zeilinger S (2012) The seven-transmembrane receptor Gpr1 governs processes relevant for the antagonistic interaction of *Trichoderma atroviride* with its host. Microbiology 158:107–118
46. Zeilinger S, Reithner B, Scala V, Peiss I, Lorito M, Mach RL (2005) Signal transduction by Tga3, a novel G protein alpha subunit of *Trichoderma atroviride*. Appl Environ Microbiol 71:1591–1597
47. Mukherjee M, Mukherjee PK, Kale SP (2007) cAMP signalling is involved in growth, germination, mycoparasitism and secondary metabolism in *Trichoderma virens*. Microbiology 153:1734–1742
48. Schmoll M (2008) The information highways of a biotechnological workhorse—signal transduction in *Hypocrea jecorina*. BMC Genomics 9:430
49. Reithner B, Schuhmacher R, Stoppacher N, Pucher M, Brunner K, Zeilinger S (2007) Signaling via the *Trichoderma atroviride* mitogen-activated protein kinase Tmk1 differentially affects mycoparasitism and plant protection. Fungal Genet Biol 44:1123–1133
50. Kumar A, Scher K, Mukherjee M, Pardovitz-Kedmi E, Sible GV, Singh US, Kale SP, Mukherjee PK, Horwitz BA (2010) Overlapping and distinct functions of two *Trichoderma virens* MAP kinases in cell-wall integrity, antagonistic properties and repression of conidiation. Biochem Biophys Res Commun 398:765–770
51. Mukherjee PK, Kenerley CM (2010) Regulation of morphogenesis and biocontrol properties in *Trichoderma virens* by a VEL-VET protein, Vell1. Appl Environ Microbiol 76:2345–2352
52. Djonović S, Pozo MJ, Kenerley CM (2006) Tvbgn3, a beta-1,6-glucanase from the biocontrol fungus *Trichoderma virens*, is involved in mycoparasitism and control of *Pythium ultimum*. Appl Environ Microbiol 72:7661–7670
53. Djonović S, Vittone G, Mendoza-Herrera A, Kenerley CM (2007) Enhanced biocontrol activity of *Trichoderma virens* transformants constitutively coexpressing beta-1,3- and beta-1,6-glucanase genes. Mol Plant Pathol 8:469–480
54. Viterbo A, Horwitz BA (2010) Mycoparasitism. In: Borkovich KA, Ebbole DJ (eds) Cellular and molecular biology of filamentous fungi. ASM Press, Herndon, pp 676–694
55. Catalano V, Vergara M, Hauzenberger JR, Seiboth B, Sarrocco S, Vannacci G, Kubicek CP, Seidl-Seiboth V (2011) Use of a non-homologous end-joining-deficient strain (delta-ku70) of the biocontrol fungus *Trichoderma virens* to investigate the function of the laccase gene *lcc1* in sclerotia degradation. Curr Genet 57:13–23
56. Reino JL, Guerrero RF, Hernandez-Galan R, Collado IG (2008) Secondary metabolites from species of the biocontrol agent *Trichoderma*. Phytochem Rev 7:89–123
57. Mukherjee PK, Horwitz BA, Kenerley CM (2012) Secondary metabolism in *Trichoderma*—a genomic perspective. Microbiology 158:35–45
58. Howell CR (2006) Understanding the mechanisms employed by *Trichoderma virens* to effect biological control of cotton diseases. Phytopathology 96:178–180
59. Wiest A, Grzegorski D, Xu BW, Goulard C, Rebuffat S, Ebbole DJ, Bodo B, Kenerley CM (2002) Identification of peptaibols from *Trichoderma virens* and cloning of a peptaibol synthetase. J Biol Chem 277:20862–20868
60. 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
61. Shi M, Chen L, Wang XW, Zhang T, Zhao PB, Song XY, Sun CY, Chen XL, Zhou BC, Zhang YZ (2012) Antimicrobial peptaibols from *Trichoderma pseudokoningii* induce programmed cell death in plant fungal pathogens. Microbiology 158:166–175
62. 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
63. Vinale F, Ghisalberti EL, 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
64. 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
65. Seidl V, Song L, Lindquist E, Gruber S, Koptchinsky A, Zeilinger S, Schmoll M, Martinez P, Sun J, Grigoriev I, Herrera-Estrella H, Baker SE, Kubicek CP (2009) Transcriptomic response of the mycoparasitic fungus *Trichoderma atroviride* to the presence of fungal prey. BMC Genomics 10:567
66. Druzhinina IS, Shelest E, Kubicek CP (2012) Novel traits of *Trichoderma* predicted through the analysis of its secretome. FEMS Microbiol Lett. doi:10.1111/j.1574-6968.2012.02665.x
67. Samolski I, deLuis A, Vizcaino JA, Monte E, Suarez MB (2009) Gene expression analysis of the biocontrol fungus *Trichoderma harzianum* in the presence of tomato plants, chitin or glucose using a high-density oligonucleotide microarray. BMC Microbiol 9:217
68. Gruber S, Vaaje-Kolstad G, Matarese F, López-Mondéjar R, Kubicek CP, Seidl-Seiboth V (2011) Analysis of subgroup C of fungal chitinases containing chitin-binding and LysM modules in

- the mycoparasite *Trichoderma atroviride*. *Glycobiology* 21:122–133
69. Reithner B, Ibarra-Laclette E, Mach RL, Herrera-Estrella A (2011) Identification of mycoparasitism-related genes in *Trichoderma atroviride*. *Appl Environ Microbiol* 77:4361–4370
70. Rubio MB, Domínguez S, Monte E, Hermosa R (2012) Comparative study of *Trichoderma* gene expression in interactions with tomato plants using high-density oligonucleotide microarrays. *Microbiology* 158:119–128
71. Dunlap JC, Borkovich KA, Henn MR, Turner GE, Sachs MS, Glass NL, McCluskey K, Plamann M, Galagan JE, Birren BW, Weiss RL, Townsend JP, Loros JJ, Nelson MA, Lambregts R, Colot HV, Park G, Collopy P, Ringelberg C, Crew C, Litvinkova L, DeCaprio D, Hood HM, Curilla S, Shi M, Crawford M, Koerhsen M, Montgomery P, Larson L, Pearson M, Kasuga T, Tian C, Baştürkmen M, Altamirano L, Xu J (2007) Enabling a community to dissect an organism: overview of the *Neurospora* functional genomics project. *Adv Genet* 57:49–96
72. Mukherjee PK (2011) Genomics of biological control—whole genome sequencing of two mycoparasitic *Trichoderma* spp. *Curr Sci* 101:268