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REVIEW ARTICLE

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. secretes 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/Epl1, 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

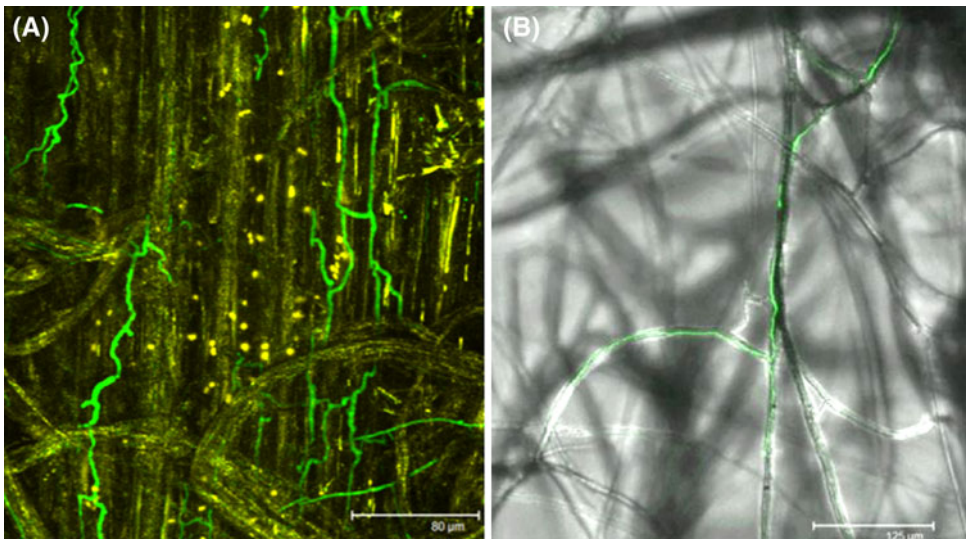

Fig. 1 Green fluorescent labeled *Trichoderma velutinum* G1/8 on sterile grown 2 weeks old sugar beet seedlings. Confocal laser scanning microscopy (CLSM) was performed with a Leica TCS SPE confocal microscope (Leica Microsystems, Mannheim, Germany). **a** Root surface (*yellow*) and *T. velutinum* hyphae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

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 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 Gα 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

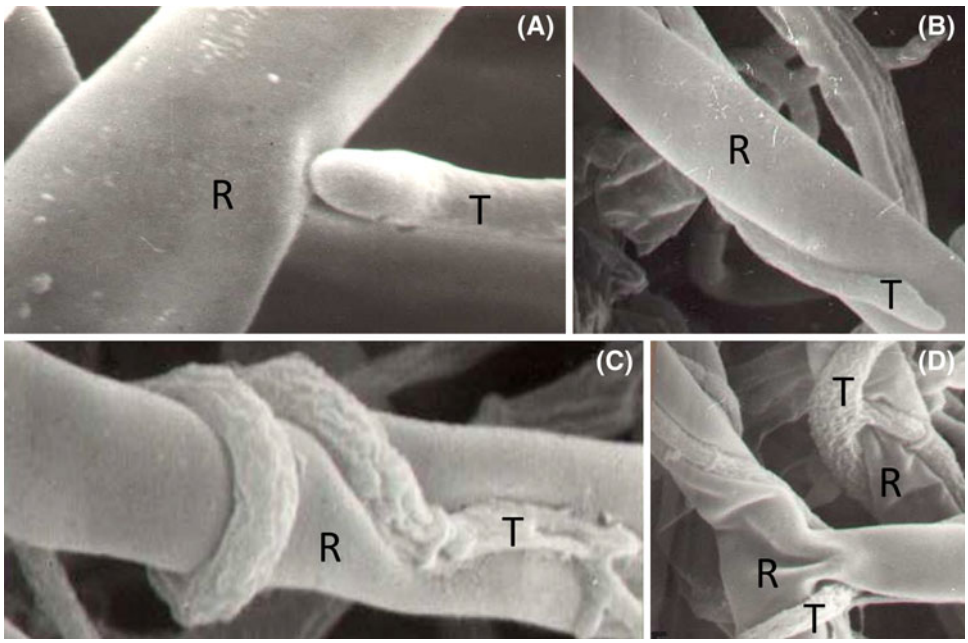

Fig. 2 Mycoparasitism of *Trichoderma virens* (*T*) on *Rhizoctonia solani* (*R*). **a** Attraction, **b** attachment, **c** coiling, **d** lysis of host hyphae [72]

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

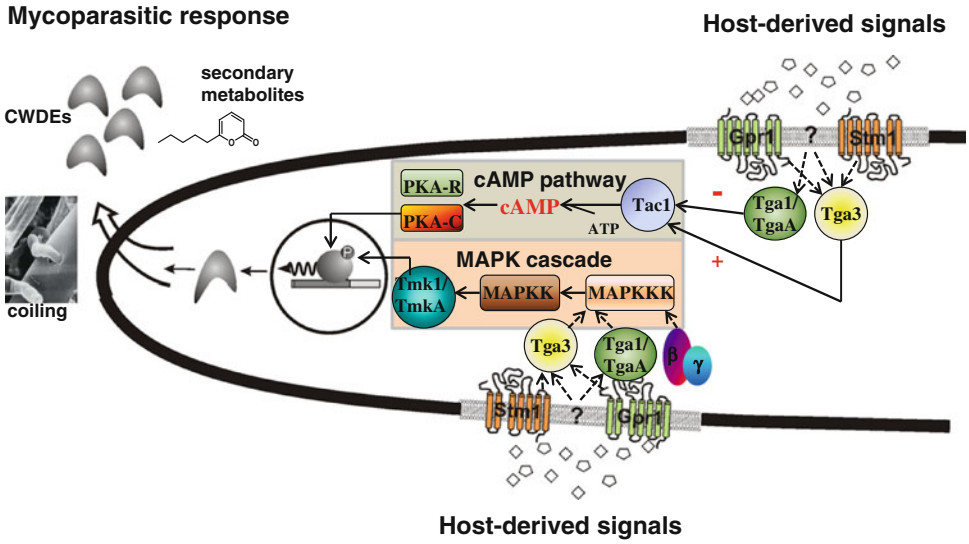

Fig. 3 Mycoparasitism-relevant signaling pathways of *Trichoderma atroviride*/*Trichoderma virens*. *Trichoderma* secretes cell wall-degrading enzymes (CWDEs) already before contact which release degradation products from the host's cell wall. These act as signals for host recognition in the mycoparasite.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