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Mechanism of Mycoparasitism in *Trichoderma*spp.

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Abstract

In agriculture, *Trichoderma* spp. are frequently employed as biocontrol agents for plant diseases. An essential mechanism of lowering the pathogen inocula is mycoparasitism, an ancestral characteristic of *Trichoderma*. Mycoparasitism is a physiological process that is intricate and involves the creation of enzymes and secondary metabolites. It is best understood within the context of microbial competition. *Trichoderma* spp. have historically been thought of as necrotrophicmycoparasites, however there is evidence that, at least in some cases, they act as hemibiotrophs, inflicting only modest damage to the host cellwall and living for a considerable amount of time inside the host cell. We discuss a variety of topics about *Trichoderma* as mycoparasites in this study, including their evolution, genomes, and interactions with fungi that are not their intended targets.

Keywords: Mycoparasitism, ISR, Secondary metabolites, Mycotrophy

Introduction

As agents of biological control (biocontrol) for plant diseases, *Trichoderma* spp. (Hypocreales, Ascomycota) are commonly utilized. The discovery that these fungi can parasitize other (plant pathogenic) fungi historically spurred research on *Trichoderma*-mediated biocontrol (Weindling, 1932). To prove that *Trichoderma* can be employed in the field to inhibit a fungal plant disease, however, took an additional four decades (Wells *et al.*, 1972). Mycoparasitism has since been emphasized in a number of papers as a significant biological control mechanism (Lifshitz*et al.*, 1986; Inbar*et al.*, 1996; Howell, 2002; Steyaert*et al.*, 2003; Harman *et al.*, 2004b; Xu*et al.*, 2010; John *et al.*, 2010; Huang *et al.*, 2011).

Information about the method by which *Trichoderma* parasitize other fungus has been published. This method includes the use of fluorescence microscopy, scanning and transmission electron

microscopy, and a biomimetic system (Inbar and Chet, 1992). It has been determined that the main enzymes implicated in mycoparasitism are chitinases, b-glucanases, and proteases (Vazquez-Garciduenas*et al.*, 1998; Cortes *et al.*, 1998; Carsolio*et al.*, 1999). Subsequently, specific gene analyses—particularly those pertaining to signal transduction—were conducted, and ultimately, genome-wide research was conducted (Druzhinina*et al.*, 2011). The phenomenon known as induced systemic resistance (ISR) was first noticed in 1997 when it was discovered that *Trichoderma*colonization of roots could lessen the symptoms of a foliar pathogen. To include all types of induced resistance, both local and systemic, we prefer to refer to this phenomenon as induced defence response, or IDR.

Over the next two decades, IDR as a mechanism of plant disease control dominated research on *Trichoderma* (Harman *et al.*, 2004; Mendoza-Mendoza *et al.*, 2018). Aside from competition, mycoparasitism is the most effective of the three common modes of disease suppression (mycoparasitism, antibiosis, and IDR) in reducing pathogen inoculum load, particularly for soil-borne pathogens, against which *Trichoderma* spp. are widely used. The degree of disease suppression by mycoparasitic strains is stronger than that mediated by IDR or antibiosis (Zeilinger*et al.*, 2016b), though synergistic activities in these processes may occur. Here, we revisit mycoparasitism as a mechanism of biocontrol by *Trichoderma* spp. in addition to comprehending the physiological basis of mycoparasitism in detail.

Mycoparasitism vs. Mycotrophy

A mycoparasitic fungus attaches itself to another fungus, such as asclerotia that is in a resting state or an actively growing hypha, in order to establish itself as a parasite. As a parasite, the fungus is frequently referred to as either the host or the prey. Rather than the type of contact, mycotrophy describes the source of the nutrients. Live or dead fungi provide food for mycotrophs, albeit not all mycotrophic fungi are mycoparasites. According to Druzhinina*etal.* (2011), saprotrophy on fungal biomass may really be the source of specialized mycoparasitism. Plant infections can either kill the host tissue (necrotrophs) or have a close relationship with the host cells that continue to exist and provide nutrition (biotrophs).Similar to this, a mycoparasite

can exist as a necrotroph, which consumes dead biomass for nutrition, or as a biotroph, which gets nourishment from a living host (Barnett and Binder, 1973).

Necrotrophs, in contrast to biotrophs, frequently have a wide host range (avoid making too generalizations). *Ampelomycesquisqualis* (Pleosporales) (Haridas*et al.,* 2020), a biocontrol agent against powdery mildew fungus, is an illustration of a biotrophicmycoparasite. The mycoparasite uses the host's (*Podosphaeraxanthii*; Erysiphales) life cycle and dispersion mechanism to propagate its own pycnidiospores, as demonstrated by a GFP-tagged intracellular *Ampelomyces* strain. An additional facet of biotrophicmycoparasitism was discovered through an investigation into the three-way interaction between powdery mildew and its barley (*Hordeumvulgare*) host, the basidiomycete yeast *Anthracocystis*(*Pseudozyma*) *flocculosa* (Ustilaginales). *Blumeria* (Erysiphales) is a barley pathogen that is parasitized by *Anthracocystis*. According to Lauren et al. (2018), the mycoparasite first acts as a pathogen by consuming nutrients from barley and then gradually destroys *Blumeria.*

The elimination of the plant pathogen represents the overall equilibrium of the interaction. Despite the fact that certain *Trichoderma* species are classified as necrotrophs, there is evidence of intracellular parasitic growth (Rousseau *et al.,* 1996; refer to Fig. 2). By comparison with plant pathogens, this could be considered a hemibiotrophic stage. It is clear that the line separating biotrophs from necrotrophs is not entirely distinct, but the *Trichoderma*-host fungal interaction progresses rapidly from a stage in which intracellular growth is occasionally visible to host degradation, even in cases where the host cell wall is still intact (Fig. 2B, D, E).Most land plants, including crops, are involved in arbuscularmycorrhizal fungus (AMF) symbioses (Sawers*et al.,* 2008). It will be crucial to weigh the benefits of *Trichoderma* and AMF together against unfavorable interactions like the one seen in Fig. 2D, E. A recent study on rice (*Oryza sativa*) shown, for instance, that AMF can increase disease resistance in the host plant (Campo *et al.,* 2020). However, in soilborne pathogen mycoparasitism, AMF cannot replace *Trichoderma*.

Mycoparasitism in disease suppression

Mycoparasitism, antibiosis, IDR, and competitive exclusion all have an impact on *Trichoderma*-

mediated disease suppression (Sharma *et al.*, 2017). When the biocontrol fungi are administered to seeds or roots, *Trichoderma* spp. quickly colonize the spermosphere (seed zone) and rhizosphere (root zone), helping to keep out invasive pathogens. *Trichoderma* secretes enzymes or produces secondary metabolites that prevent pathogen germination or growth in direct antibiosis (Howell, 2003; Viterbo*et al.*, 2001). IDR, mycoparasitism, and competition may all be influenced by the metabolites (Zeilinger*et al.*, 2016a). In contrast to the abundance of research conducted in vitro, there are very few direct studies on the function of mycoparasitism in plant disease control since it is challenging to examine the phenomenon "in exclusion."

The mycoparasitic behavior of *T. virens* (formerly known as *T. lignorum*) on *Rhizoctoniasolani*(Cantharellales) was originally described in vivid detail by Weindling (1932): "coiling of hyphae, growth in straight or wavy lines, coagulation of protoplasts, and loss of vacuolated structures." Nevertheless, this *Trichoderma* strain's antagonistic activity was later linked to a "lethal principle," which was later found to be gliotoxin (Weindling, 1934; Weindling and Emerson, 1936). Additionally, pot studies showed that this strain was effective in suppressing *R. solani* (Weindling and Fawcett, 1936). The antibiotic activity of T. virens was responsible for the suppression of *R. solani* and *Globisporangium* (*Pythium*) *debaryanum* in cucumber (*Cucumissativus*) and peas (*Pisumsativum*) in pot experiments (Allen and Haenseler, 1935).

Numerous researchers (e.g., Hashioka, 1973; Ruano-Rosa *et al.*, 2016) used electron and fluorescence microscopy to examine the ultrastructure of hyphal parasitism. When *Trichodermahamatum* was used as a seed treatment, Harman et al. (1980) hypothesized that mycoparasitism was the mechanism of biocontrol of *Pythium* spp. (Pythiales) and *R. solani*.

The fact that *T. hamatum*, a potent mycoparasite, exhibited no antibiosis against these infections served as the basis for this. Applying *Trichoderma*harzianum isolate to soil [note that Chaverri et al. (2015) separated the old metaspecies*T. harzianum* into 14 phylogenetic species, and since then, multiple other species have been added (Cai and Druzhinina, 2021). We have kept the original species name for *R. solani* and *S. rolfsii* in this review, which is mycoparasitic on these

pathogens and suppresses illnesses produced by them in the field (Elad*et al.,* 1980). The authors did not address whether mycoparasitism alone was responsible for the biocontrol activity.In white beans (*Phaseolus vulgaris*), a mycoparasitic strain of *T. virens* inhibited *R. solani*-incited illness in a dose-dependent manner (Tu and Vaartaja, 1981). By parasitizing the sclerotia, *Trichoderma*koningii was able to decrease the inoculum levels of *Sclerotiniasclerotiorum* (Helotiales) in soil (Santos and Dhingra, 1982; Trutmann and Keane, 1990). Sclerotial parasitism was suggested as the main method of suppression of *S. rolfsii* and *R. solani* by a "P" (gliovirin-producing) strain of *T. virens* in soil, based on a comparative assessment of hyphal parasitism, sclerotial parasitism, and antibiosis (Mukherjee *et al.,* 1995).

We recently obtained a mutant (G2) by gamma-ray induced mutagenesis that produces more secondary metabolites and has elevated genes linked to mycoparasitism, secondary metabolism, and plant interactions (Mukherjee *et al.,* 2019). Compared to the wild type strain, this mutant demonstrated better biocontrol against *S. rolfsii* in greenhouse trials. Over the course of five years, both in "on-farm" experiments and in farmers' fields, excellent field control of collar rot (*S. rolfsii*) in chickpea (*Cicerarietinum*) and lentil (Lens culinaris) was demonstrated (Fig. 3). Howell (1987) produced *T. virens* UV-induced mutants that lacked *R. solani*hyphal parasitism; the mutants' ability for biocontrol was equal to that of the parental type.

Likewise, mutants lacking in the manufacture of gliotoxin demonstrated equivalent efficacy in biocontrol. Induced resistance was highlighted, and the significance of mycoparasitism and antibiosis was called into question (Howell, 1987, 2003). Nevertheless, Howell *et al*. (1987) did not take into account how the sclerotia's parasitism affects *T. virens* ability to be biocontrolled. In an early study, employing an assay using river sand, it was shown that *T. virens* (then known as *T. lignorum*) parasitized the sclerotia of *R. solani* (then known as *Corticiumsasakii*) and *Sclerotinialibertiana* (obsolete taxonomy, possibly *S. sclerotiorum*) (Hino and Endo, 1940). A *T. virens* isolate (formerly named *Gliocladiumvirens*) easily parasitized *S. sclerotiorum*'s hyphae and sclerotia. Extensive hyphae of the mycoparasite were seen inside the colonized sclerotia, but no conidia were present (Tu, 1980).

Role of cell wall-degrading enzymes

Since glucans, chitin, and proteins make up the cell walls of fungi (Gow*et al.*, 2017; Garcia-Rubio *et al.*, 2020; Ruiz-Herrera and Ortiz-Castellanos, 2019), it makes sense that the biopolymers in mycoparasitism would be broken down by glucanases, chitinases, and proteases. The most researched of these are those related to chitin breakdown.

Enzymes involved in chitin degradation

Trichoderma species have a higher concentration of chitinolytic genes in their genomes than other fungus, which indicates the significance of these enzymes in the mycoparasitic lifestyle (Ihrmark*et al.*, 2010; Kubicek*et al.*, 2011, 2019). On the other hand, only a small number of chitinases are found in fungi that are early diversers and that are connected to plants, animals, or the open environment. Examples of these fungi include the endoparasiteRozella, the rumen symbiontNeocallimastigomycota, and the Glomeralesarbuscularmycorrhiza fungi (Goughenour*et al.*, 2021). Chitinases found in fungi are members of the GH families 18 and 20. There are three subfamilies of GH18 chitinases: A, B, and C. According to detailed genome analyses conducted thus far, *T. virens*, *T. atroviride*, *T. harzianum*, *T. asperellum*, *T. gamsii*, and *T. atrobrunneum* have considerably larger GH18 families of chitinolytic enzymes (Kubicek*et al.*, 2011; Fanelli*et al.*, 2018).

Comparably, chitosanases (GH75) have more genes—at least five—than most other fungi, which only have one or two (Kubicek*et al.*, 2011; Karlsson*et al.*, 2017; Kappel*et al.*, 2020). The first attack on chitin in the host's cell wall is carried out by endochitinases (Klemsdal*et al.*, 2006; Boer *et al.*, 2007). In fact, a large number of these genes are either activated by fungal cell walls or expressed during mycoparasitic interactions (Garcıa *et al.*, 1994; Steyaert*et al.*, 2004; Seidl, 2008). The most researched endochitinase, ECH42, is the most abundant chitinase present under chitinase-inducing circumstances, along with CHT33 (Chit33/CHI18-12), CHT36 (Chit36/CHI18-15), and the GH20 N-acetyl-b-glucosaminidase NAG1 (Carsolio*et al.*, 1999).

Mutants with significantly reduced mycoparasitic abilities were produced by deletion of either cda1 or cda5, two of the six chitin deacetylase genes contained in the *T. atroviride* genome

(Kappel*et al.*, 2020). According to Delgado-Jarana et al. (2006), chitosan may scavenge reactive oxygen species produced by the parasitized fungi, and chitin deacetylases may help *Trichoderma* handle the weight of reactive oxygen. The function of *T. atroviride*'s putative exo- and endoactingchitosanases was also examined by Kappel et al. (2020).

All six chitosanaseencoding genes were upregulated after host contact and lysis, i.e., especially during the later stages of the mycoparasitic interaction and two (cho1 and cho3) also during contact of *Trichoderma* with itself, evidencing, similar to chitinases, a general role in cell wall remodelling (Kappel*et al.*, 2020). The same study further proved the involvement of chitin synthases in cell wall remodelling during *T. atroviride*mycoparasitism, of which CHS8 emerged as a candidate of particular interest. This enzyme not only shows similarity to chitin synthases but also to hyaluronan synthases. Such hybrid synthases may use both UDP-N-acetyl-glucosamine and UDP-D-glucuronate as substrates. The authors hence speculated that CHS8, in cooperation with CDA1, forms a chitin glycopolymer layer protecting the *Trichoderma* cell wall during the mycoparasitic interactions (Kappel*et al.*, 2020).

α- and β-glucanases

Members of the GH71 family, including fungal a-1,3-glucanases, are poorly understood in relation to *Trichoderma*. In the presence of Botrytis cinerea (Helotiales) cell walls but not chitin, *T. harzianum* CECT 2413 and *T. asperellum* CECT20539, respectively, explicitly generate the exo-a-1,3-glucanases AGN13.1 and AGN13.2 (AitLahsen*et al.*, 2001; Sanz*et al.*, 2004). AGN13.1 shown both antifungal efficacy and lytic capabilities against fungal cell walls (Ait-Lahsen*et al.*, 2001). After being isolated from *T. harzianum* OMZ779, an a-1,3-glucanase MUT1 (MutAp) with endo-hydrolytic activity was demonstrated to primarily release glucose following hydrolysis of crystalline 1,3-a-glucan (Guggenheim and Haller, 1972; Grun€ *et al.*, 2006).

On the other hand, there are no reports on its biological function or transcriptional regulation. b-1,3-glucanases are members of the GH families 16, 17, 55, 64, and 81. *Trichoderma*mycoparasites have more genes coding for members of the GH55 and GH64 families than other fungi (Kubicek*et al.*, 2011; Fanelli*et al.*, 2018). It has been proposed that b-

glucanases play a particularly significant role in the mycoparasitism of oomycete preys, whose cell walls are primarily composed of cellulose and b-1,3- and b-1,6-glucans. In soil infected with *Globisporangium* (*Pythium*) *ultimum* (Pythiales), *Trichodermalongibrachiatum*Rifai CECT2606 mutants expressing more EGL1 b-1,4-endoglucanase performed better in shielding cucumber seeds (Mighel*et al.*, 1998). Similar outcomes were observed with *T. virens*Gv29-8, where strains overexpressing bgn3 and the b-1,3-glucanase gene bgn2 displayed enhanced pathogen inhibition and protected cotton (*Gossypiumhirsutum*) plants against *G. ultimum*, and mutants overexpressing the b-1,6-glucanase encoding gene bgn3 showed enhanced antagonism of *G. ultimum* (Djonovic*et al.*, 2006b, 2007).

In contrast to these reports, glu31 (gluc31/encoding GH16 endo-b-1,3-glucanase), exhibited differential expression during interaction with specific host fungi; however, loss of the gene did not affect the mycoparasitic activity; rather, it affected the organization and remodelling of the cell wall, which in turn led to the differential expression of other glycosyl hydrolases belonging to the GH16 family (SurianiRibeiro*et al.*, 2019). There are few studies on the role of glucanases in fungal mycoparasites. Before the precise role of glucanases in *Trichoderma*mycoparasitism can be determined, more work must be done, including the (admittedly difficult) generation of multiple mutants, as these enzymes may be functionally redundant.

Proteases

Proteases, like CAZymes, are necessary for the breakdown of fungal cell walls and are therefore connected to *Trichoderma*mycoparasites' antagonistic activity, which compromises the cellular integrity of the host fungus and deactivates enzymes produced from it (Elad and Kapat, 1999). Although *Trichoderma* species have a similar amount of proteases encoded as other fungi (Kubicek*et al.*, 2019), some families of proteases, such as S8 subtilis, are more abundant in the well-studied mycoparasites*T. virens* and *T. atroviride* than in the weakly mycoparasitic*T. reesei* (Kubicek*et al.*, 2011). During mycoparasitism or growth on fungal cell walls, a number of protease genes are differently regulated (Seidl*et al.*, 2009; Geremia*et al.*, 1993; Viterbo*et al.*, 2004; Suarez *et al.*, 2007; Troian*et al.*, 2014).

The presence of other fungi and dead fungal biomass, respectively, stimulate the expression of the gene encoding the neutral metalloprotease NMP1 of *Trichoderma*guizhouense NJAU4742 and its orthologue in *T. harzianum* CECT2413 (Suarez *et al.,* 2004; Zhang *et al.,* 2016). Further research revealed that NMP1 is crucial for *T. guizhouense's* interaction (including parasitism, predation, and defense) with other fungi as well as for its antifungal activities (Zhang *et al.,* 2016). One of the best-characterized genes relevant to mycoparasitism is prb1, the protease-encoding gene of *T. atroviride's* S8 family (Geremia*et al.,* 1993). Plant protection against *R. solani* was enhanced by overexpressing *T. atroviride* (formerly *T. harzianum*) prb1 or its homologue from *T. virens* (Flores *et al.,* 1997; Pozo*et al.,* 2004).

Similar to this, *T. harzianum* T334 became a more potent antagonist of plant diseases due to protease overproduction produced from random UV mutagenesis (Szekeres*et al.,* 2004). Prb1 gene expression is induced prior to interaction with the fungal host, similar to ech42 (Cortes *et al.,* 1998). Subsequent investigations showed that prb1 transcription was activated in response to nitrogen limitation, which is consistent with possible binding sites in its promoter region for the ARE1 transcriptional activator of nitrogen catabolite-repressed genes (Olmedo-Monfil*et al.,* 2002). As a result, more recent transcriptome studies demonstrated that *T. atroviride's* response to a fungal host resembles the pattern of gene expression during a stress of nitrogen limitation and established the significance of proteases in mycoparasitism in *T. virens* and *T. reesei*(Seidl*et al.,* 2009; Atanasova*et al.,* 2013). These results led to the hypothesis that host-derived nitrogenous products produced by proteolytic enzymes during the early stages of the mycoparasitic interaction activate genes relevant to mycoparasitism by binding to the appropriate nitrogen sensors on the surface of *Trichoderma* cells (Druzhinina*et al.,* 2011).

Secondary metabolites and their role in mycoparasitism

Trichoderma species are efficient producers of secondary metabolites that are used in self-signalling, interactions with plants, and antagonism (antibiosis and mycoparasitic attack) as chemical weapons (Mukherjee *et al.,* 2012a; Zeilinger*et al.,* 2016a). Over the years, several secondary metabolites, both volatile and non-volatile, have been documented for *Trichoderma*

fungus. These include polyketides, non-ribosomal peptides, terpenoids, pyrones, etc (Zeilinger*et al.,* 2016a). Using 12 *Trichoderma* species under investigation, a recent comparative genomics study found 10e25 genes encoding for polyketide synthases (PKS), 12e34 genes for non-ribosomal polypeptide synthetases (NRPS), and 6e14 genes encoding terpenoid synthases (TS) (Kubicek*et al.,* 2019).These core genes are frequently found in metabolite gene clusters and encode backbone-generating enzymes that function in distinct biosynthesis pathways for particular secondary metabolites.

Species from the *Harzianum/Virens* clades contain the highest number of PKS genes (Kubicek*et al.,* 2019) of which, however, only two have been functionally characterized hitherto. Secondary metabolite production may be species- or even strain-specific as exemplified by 6-pentyl-2Hpyran-2-one/6-pentyl-a-pyrone (6-PP) and other volatile organic compounds (VOCs). Transcript levels of pks2 (pksT-2) were up regulated in *T. harzianum* 88 upon confrontation with host fungi, and disruption of the gene impacted the conidial pigmentation of the fungus (Yao *et al.,* 2016). Similarly, pks4 of *T. reesei* QM6a has been shown to provide pigmentation of conidia and teleomorph structures and impact the antagonistic activity of *T. reesei* by affecting the formation of inhibitory metabolites and the expression of other PKS-encoding genes (Atanasova*et al.,* 2013b).

Only the section Longibrachiatum contains the gene cluster consisting of two PKS (SOR1 and SOR2), which biosynthesizes sorbicillinoids, which are cyclic polyketides (Druzhinina*et al.,* 2016).When ppt1 was inactivated in *T. virens*, the result was mutants with non-pigmented conidia and a defect in the biosynthesis of non-ribosomal peptides. These mutants also lost their ability to induce plant defense responses and their *in vitro* antagonistic activity against phytopathogenic fungi (Velazquez-Robledo*et al.,* 2011). Among the principal non-ribosomal peptides produced by *Trichoderma* species are peptidaibols, siderophores, and epipolythiodioxopiperazines (ETPs). Peptaibols have the ability to permeate cell membranes and combine with enzymes that break down cell walls to cause disruption of the cell (Schirmbock*et al.,* 1994; Lorito*et al.,* 1996; Shi *et al.,* 2012).

It's interesting to note, nevertheless, that during the mycoparasitic contact with *R. solani* (Atanasova*et al.,* 2013b), peptaibolsynthetase genes are downregulated in *T. atroviride*, *T. virens*, and *T. reesei*, but elevated in *T. virens* during co-cultivation with plant roots (Viterbo*et al.,* 2007). However, as demonstrated by Holzlechner et al. (2016), even modest gene expression appears to be sufficient for sufficient peptaibol synthesis. A clear local release of peptaibols with chain lengths of 11, 18, and 20 residues was seen in both the pre-contact and contact stages of the interaction, according to imaging-based MALDI mass spectrometry monitoring of secondary metabolites directly in *T. atroviride* P1 e *R. solani* co-cultures (Holzlechner*et al.,* 2016).

Similar to this, *T. harzianum* CCF2714 was able to prevent the plant pathogen from producing the mycotoxinbeauvericin while also producing peptaibols with antifungal action when co-cultured with *Fusariumoxysporum* f. sp. *conglutinans (*Hypocreales) (Palyzova*et al.,* 2019). Mechanism of biocontrol mediated by *Trichoderma* 21 Microbes that generate effective extracellular siderophores benefit from the low bioavailability of iron in soil (Haas *et al.,* 2008). Thus, it has been proposed that iron competition inhibits the growth of other microorganisms, leading to *Trichoderma*mycoparasitism (Benıtez *et al.,* 2004). Although siderophores were found to accumulate in a co-culture of *Talaromycespinophilus* (Eurotiales) and *T. harzianum* M10 by Vinale*et al*. (2017), there isn't any direct experimental evidence to support siderophores' role in the mycoparasitic relationship. Gliotoxin is an ETPs family drug formed from NRPS GLI1 (GliP) that is produced by specific strains of *T. virens* known as "Q" strains. According to Kubicek et al. (2019), GLI1 has been found in all species belonging to the clades Longibrachiatum and Harzianum/Virens. *Trichoderma* species, including those that don't produce this metabolite, appear to have a truncated gliotoxin biosynthesis cluster rather frequently.

*T. virens*gliotoxin producers appear to employ a "predation by poisoning" tactic during antagonism, as evidenced by the fungistatic activity of gliotoxin and the up-regulation of the gliotoxin cluster genes during the mycoparasitic interaction with *R. solani* (Atanasova*et al.,* 2013a). Curiously, though, gli1 mutants lost their ability to mycoparasise*R. solani* but lost their

ability to mycoparasite*G. ultimum* and *S. sclerotiorum* (Vargas *et al.*, 2014) (Fig. 4). Secondary metabolites called volatile organic compounds (VOCs) may promote mycoparasitism by preventing fungal hosts from growing (Ait-Lahsen*et al.*, 2001; Cruz-Magalhaes, *et al.*, 2019; Moya*et al.*, 2018). One of *T. atroviride's* primary volatile secondary metabolites is 6-PP. According to Jenels et al. (2014), *Trichoderma*viridescens, *T. hamatum*, and *Trichodermacitrinoviride* were also identified as makers of 6-PP.

6-PP possesses antifungal and growth-modulating properties for plants. For many years, it has been believed that the first and limiting step in *T. atroviride's* production of 6-PP is the oxidation of linoleic acid to 13S-hydroperoxy-9Z,11E-octadecadienoic acid by a lipoxygenase (SerranoCarreon*et al.*, 1993). However, we recently challenged this assumption by demonstrating that LOX1 is dispensable for 6-PP production by deleting the single lipoxygenase-encoding gene lox1 present in the *T. atroviride* genome (Speckbacher*et al.*, 2020).

The culture supernatant of *R. solani* significantly increased 6-PP production of *T. atroviride* IMI 206040 (Flores *et al.*, 2019). A recent study revealed that 6-PP disturbs the homeostasis of amino acid metabolism and induces autophagy in the ginseng root pathogen *Ilyonectria*(*Cylindrocarpon*) *destructans*(Jin *et al.*, 2020). Besides, 6-PP can inhibit mycotoxin secretion by *F. graminearum* (Cooney *et al.*, 2001) and this volatile acts as an important self-signaling compound. 6-PP emerged as a highly potent chemoattractant and growth stimulating cue for *T. atroviride* P1 which, especially in the early stages of colony development, represents an important self-signal that advances expansion and density of the mycelia required for the mycoparasitic attack (Moreno-Ruiz *et al.*, 2020).

When *T. guizhouense* interacts with its host fungus, *Fusariumodoratissimum* (formerly known as *F. oxysporum* f. sp. *cubense* race 4), it creates an excessive amount of hydrogen peroxide (H2O2). (Zhang *et al.*, 2019; Pang *et al.*, 2020).

According to Zhang *et al*. (2019), interaction with a host fungus initiates the production of H2O2, which is required for mycoparasitic activity and is dependent on the NADPH oxidase NOX1 and its regulator NOR1. In reaction to the build-up of H2O2, *T. guizhouense* produces

polyketides, or azaphilones, which are essential for self-defense against the reactive oxygen species that *Trichoderma* releases when it interacts with *F. odoratissimum* (Pang *et al.*, 2020). Furthermore, in comparison to the parental strain, *T. harzianum* T34's overexpression of NOX1 led to higher protease, cellulase, and chitinase activities.

Remarkably, in confrontation assays against the oomycete*G. ultimum*, the overexpressing strains exhibited more antagonistic activity than the wild type, but this was not the case in assays against *B. cinerea* or *R. solani* (Montero-Barrientos*et al.*, 2011), indicating the possibility of distinct host protection mechanisms.

Role of small, secreted cysteine-rich proteins

CAZymes, small secreted cysteine-rich proteins (SSCPs), and proteins with an uncertain functional annotation account for over 60% of the proteins secreted by *Trichoderma* spp. (Druzhinina*et al.*, 2012; Mendoza-Mendoza *et al.*, 2018). Mycoparasitism and the induction of plant defenses are known to be facilitated by SSCPs in *Trichoderma* (Schmoll*et al.*, 2016; Guzman-Guzmán*et al.*, 2017; Mendoza-Mendoza *et al.*, 2018; Romero-Contreras *et al.*, 2019). Cerato-platanins (CPs) are SSCPs that lack any known enzymatic activity and typically include 105–134 amino acid residues. Four conserved cysteine residues that create two disulphide bridges are found in CPs (Gao*et al.*, 2020). CPs are also known as eliciting factors since they are released in the early phases of development and contribute to the induction of systemic resistance in plants (Djonovic*et al.*, 2006a).

Between three and four EPLs are found in *Trichoderma* spp., and EPL1, EPL2, and EPL3 are found in every genome that Gao et al. (2020) analyzed; EPL4 is lacking in certain species. According to Fischmann et al. (2013), the EPL1 from *T. atroviride* binds to different types of polymeric chitin and may act similarly to expansins in that it causes the opening of physical gaps in the fungal cell wall, such as those seen in chitin polymers. *T. harzianum* needs EPL1 in the *S. sclerotiorum* interaction for the production of genes linked to mycoparasitism and for coiling around the host (Gomes *et al.*, 2015). Furthermore, according to Gomes et al. (2017), *T. harzianum* EPL1 takes involvement in the downregulation of virulence genes (BcBOT) essential

for *B. cinerea'*sbotrydial biosynthesis.

EPL may function as a recognition molecule in *T. harzianum*mycoparasitism to recognize its host hyphae and to shield the *Trichoderma* cell wall from its secondary metabolites and enzymes that break down the cell wall (Gomes *et al.*, 2017). It has been proposed that the cell wall-bound protein QID74 from *T. harzianum*, the effector TAL6 from *T. atroviride* (Gruber and Seidl-Seiboth, 2012), and EPL1 from *T. harzianum* are involved in the self-protection of the cell wall during mycoparasitism.

Further thorough research is necessary to fully understand the self-protection mechanism, as it may also be applicable to other mycoparasitic species. According to a recent publication, T. guizhouensepolyketides called azaphilones are essential for self-defense against H2O2 hyperproduction that occurs during interaction with the host fungus (Pang *et al.*, 2020). Mycoparasitic activity in *Trichoderma* has also been linked to hydrophobins, which are linked to coating spores for dispersion and protecting filamentous hyphae (Guzman-Guzmán*et al.*, 2017). Interaction with other fungi has been linked to class II hydrophobins. The antagonistic action against *R. solani* AG2 in *T. virens* was enhanced by overexpressing the class II hydrophobin HYD2-1 (TVHYDII1) (Guzman-Guzmán*et al.*, 2017).

Trichoderma genetics

Filamentous fungi often have tiny genomes. Their nucleus is haploid. *Trichoderma* spp. have estimated genome sizes and chromosomal counts ranging from 31 to 39 Mb and 3 to 7, respectively. Each species has a different chromosomal size. Worldwide, conferences centered around*Trichoderma* are taking place. Molecular mechanisms and applications of biocontrol in agriculture was the topic of an October 2010 ARD (US–Israel Binational Research and Development Fund) workshop held at the Technion, Israel Institute of Technology, Haifa, Israel. Following the publication of the sequenced genomes of *T. atroviride* and T. virens, which along with *T. reesei* provided new insight into the evolution of mycoparasitism, this workshop was organized.

Genomics of *Trichoderma*

Out of all the *Trichoderma* species, *T. reesei* has the only fully sequenced genome. This strain is highly significant from an industrial standpoint because of its huge capacity to manufacture the enzymes cellulose and hemicellulose. *T. reesei* has seven chromosomes and a 33 Mb genome.

***Trichoderma*biocontrol genes and their function**

Worldwide, *Trichoderma* species are employed as biocontrol agents in artificial intelligence. According to Pratibha Sharma et al. (2011), this genus has multiple biocontrol genes that are engaged in the biocontrol mechanisms therein. Stress-tolerant genes, proteases, chitinases, glucanases, tubulins, and cell adhesion proteins are some of the main gene types that are engaged in biocontrol action. These genes are in charge of stress tolerance, hyphal development, cell wall disintegration, and parasitic activity. For instance, xylanase breaks down hemicellulose, chitinase breaks down glycosidic bonds, etc. Fungi are important in biology and biotechnology, and their genomes are currently being sequenced.

The genomes of about 500 fungi have been sequenced. The Genome Online Database GOLD (https://gold.jgi-psf.org/cgi-bin/GOLD/bin/gold.cgi) contains the data for every sequenced genome. Around 50 fungal genomes, mostly from the Ascomycota, are now available through the Broad Institute's Fungal Genome Initiative. These genomes include model organisms like Neurospora and Aspergillus species, as well as various basidiomycetes, chytrids, and at least one mucormycete. The goal of the mycorrhizal genome initiative is to sequence and examine the genomes of ascomycetes and basidiomycetes, which coexist peacefully with trees and other woody plants.

Following collection, this information will offer a framework for comprehending the biology of these species and investigating their potential uses in ecological biodiversity preservation and reforestation. The kingdom of fungi accounts for slightly more than 15% of the species richness present in the main class of microorganisms. The most effective method for figuring out fungal diversity is the 28s rRNA genome analysis.

Conclusion

The last several years have seen an accumulation of research data exploring numerous novel

applications of fungal species in the fields of agriculture and biotechnology. These fungi have the power to alter plant metabolism and confer resistance against both biotic and abiotic stresses. Numerous biocontrol activities have been reported for the genes identified in the *Trichoderma* species. Several enzymes that break down cell walls are secreted by this fungus.

To eliminate phytopathogens, transgenic plant cells use these enzymes. Liu et al. conducted an experiment wherein the genes responsible for degrading fungal cell walls were extracted from *Trichoderma*atroviride and subsequently inserted into the rice genome through Agrobacterium-mediated transformation. Specifically, endochitinase ech42, exochitinase nag70, and exo-1,3-glucanase gluc78 were excised from *Trichoderma*atroviride and utilized to create plasmids with various combinations. Plants that were injected with R. solani showed a high resistance to sheath blight in those that carried the ech42 gene. Worldwide recognition for the biocontrol capabilities of *Trichoderma* species is widespread. Because the particular genes involved in this mechanism have been identified and isolated, this biocontrol activity has gained widespread recognition.

In the agricultural sector, phytopathogens are a major source of loss. Consequently, growers employ dangerous pesticides to stop these phytopathogens. Because they leave toxic residues in the soil that reduce soil fertility, these chemical pesticides are extremely bad for the environment. This issue can be resolved by using biocontrol chemicals more frequently. It was discovered that the genes extracted from these biocontrol agents were crucial to the biocontrol action. For the benefit of future generations, even more genes should be found with the aid of genetic engineering.

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