Chapter 8
Trichoderma as a Biological Control Agent

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Abstract *Trichoderma* species are free-living fungi that are common in soil and root ecosystems. Some strains establish root colonization and enhance growth and development, crop productivity, resistance to abiotic stresses and uptake and use of nutrients. *Trichoderma* species can antagonize and control a wide range of economically important plant pathogenic fungi, viruses, bacteria and nematodes. Root-knot nematodes, *Meloidogyne* spp., are sedentary, obligatory root endoparasites of great economic importance, and polyphagous species, such as *M. javanica* and *M. incognita* are among the major limiting factors of crops production worldwide. Therefore, these nematodes have been the main target for nematode biocontrol by *Trichoderma*. Several *Trichoderma* species and isolates have been evaluated as biocontrol agents against the nematodes with various crops and experimental conditions. Significant results of nematode control and plants growth were achieved. Aiming to improve the biocontrol process, modes of action of the fungus against the root-knot nematodes have been investigated and are described in this chapter. Mechanisms such as parasitism, enzymatic lysis, antibiosis and induced resistance were studied. Understanding the fungus-nematode-plant interactions and the mechanisms of the biocontrol process might contribute to improve the implementation of this biocontrol agent.

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Abbreviations

CF   culture filtrate
gm   gelatinous matrix
GFP   green fluorescent protein
J2   second-stage juvenile
MAb   monoclonal antibody
PAb   polyclonal antibody
RKN   root-knot nematode
SC   surface coat

8.1   Introduction

Trichoderma species are free-living fungi that are common in soil and root ecosystems; some strains are known as opportunistic, avirulent plant symbionts and can establish robust and long-lasting colonizations of root surfaces and penetrate into the epidermis and a few cells below this level. Root colonization by *Trichoderma* spp. frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and uptake and use of nutrients (Yedidia et al. 2001; Harman et al. 2004). *Trichoderma* species can antagonize and control a wide range of economically important plant-pathogenic fungi and have been known as biocontrol agents against soil-borne, foliar and postharvest phytopathogenic fungal pathogens and can control also viruses and bacteria (Sivan and Chet 1992; Herrera-Estrella and Chet 1998; Yedidia et al. 2003; Harman 2006).

Various mechanisms have been suggested for the biocontrol activity of *Trichoderma* against phytopathogenic fungi: direct interactions such as parasitism, enzymatic lysis, antibiosis and competition. Indirect interactions involve the stimulation of plant self-defence mechanisms, i.e., plant systemic induced resistance (Harman et al. 2004; Harman 2006; Viterbo et al. 2007a). Most of these processes are probably caused by multi-gene complexes (Harman 2000), and it can be assumed that biocontrol is a result of multi-mechanism action of the antagonist. Synergism between different forms of antagonism may occur (Elad and Freeman 2002; Howell 2003).

Most mechanisms, apart from competition, could potentially be involved in the biocontrol of nematodes. Enzymes such as chitinases, glucanases and proteases seem to be very important in the mycoparasitic process (Haran et al. 1996; Viterbo et al. 2002b). Chitinases and proteases of *Trichoderma* spp. are much similar to those of nematophagous fungi, and have the potential to attack nematodes (Morton et al. 2004). The processes of *Trichoderma* parasitism and the effects of fungal enzymes and metabolites on nematodes may occur in the soil, within roots and on the root surfaces, and induced systemic resistance mechanisms may also affect the nematodes. Microorganisms are affected by environmental conditions in the rhizosphere, and

since nematodes influence the quantity and quality of root exudates, they are likely to affect the physiology of such microorganisms in the rhizosphere (Kerry 2000).

Root-knot nematodes (RKNs), *Meloidogyne* spp. are sedentary, obligatory root endoparasites of great economic importance, and polyphagous species, such as *M. javanica* and *M. incognita* are among the major limiting factors in the production of field and plantation crops worldwide. RKNs are difficult to control because of their wide host range, short life-cycle, high reproductive rates and endoparasitic nature (Trudgill and Blok 2001; Manzanilla-Lopez et al. 2004). Therefore, these nematodes have been the main target for biocontrol by *Trichoderma*. The second-stage juveniles (J2s), which penetrate the roots and develop within them, induce a cascade of changes in the host plant, which lead to the formation of giant cells and galls. About 1 month after J2s penetration, the females lay out egg masses that contain nematode eggs enveloped in a gelatinous matrix (gm).

Several attempts have been made to use *Trichoderma* species to control plant-parasitic nematodes. Windham et al. (1989) reported reduced egg production in the root-knot nematode *M. arenaria*, following soil treatments with preparations of *T. harzianum* (T-12) and *T. koningii* (T-8). A combination of *T. harzianum* with neem cakes reduced the population of the citrus nematode *Tylenchulus semipenetrans* (Reddy et al. 1996). Among several other plant-based formulations of *T. harzianum* that were evaluated for the management of *M. incognita,* castor cake extracts showed the best biocontrol activity (Rao et al. 1998). Direct interactions between *T. harzianum* and the potato cyst nematode *Globodera rostochiensis* were demonstrated *in vitro* by Saifullah and Thomas (1996). The effect of *T. viride* metabolites on nematodes was demonstrated by implementing root-dip treatments with the fungal culture filtrate (Khan and Saxena 1997). *In vitro* assays with *T. virens* culture filtrates showed that low-molecular-weight, non-enzymatic factors inhibited egg hatching and impaired *M. incognita* second-stage juvenile mobility. The fungus, applied as seed treatment or root drenches, did not affect nematode *M. incognita* inoculation in greenhouse tests with tomato, but did achieve reductions in the nematode population on pepper roots (Meyer et al. 2000, 2001). *Trichoderma*-nematode interactions, has been studied by an Israeli group with main emphasis on RKNs, combining applied and fundamental research. Several *Trichoderma* species and isolates have been evaluated as biocontrol agents against *M. javanica* and *M. incognita* with various crops and experimental conditions. Significant results of nematode control and plants' growth improvements were achieved (Sharon et al. 2001; Spiegel et al. 2007).

Aiming to improve the biocontrol process, modes of action of the fungus against the root-knot nematodes have been investigated and are described in this chapter: Attachment and parasitic capabilities of *Trichoderma* on RKNs were demonstrated and the mechanisms were investigated (Sharon et al. 2007). Antibodies that bind to *M. javanica* surface served as a tool for further investigations of the fungal attachment to nematodes; antibodies were found to improve parasitism *in vitro* (Sharon et al. 2009b). Involvement of proteolytic and chitinolytic activities during parasitism has been investigated. *Trichoderma* metabolites affected the nematodes and differences were observed between the various isolates. Indirect effects of fungal root

colonization on the nematodes were demonstrated using split-root systems, suggesting induced systemic resistance mechanisms in the host plants (Sharon et al. 2009a). Understanding the fungus-nematode-plant interactions and the mechanisms involved in the biocontrol process for various *Trichoderma* species and isolates might contribute to the development of optimal implementation methods to improve biocontrol agents.

8.2 *Trichoderma* Biocontrol Activity Against Root-Knot Nematodes

Trichoderma asperellum-203 and *T. atroviride* IMI 206040 (both fungi were previously defined as strains of *T. harzianum*) exhibited biocontrol activity against *M. javanica* in soil (Sharon et al. 2001). Several other *Trichoderma* species and isolates (3 isolates of *T. asperellum*: 44, GH11 and 34; *T. harzianum* 248; *T. hamatum* 382) have been also evaluated as biocontrol agents against *M. javanica* and *M. incognita*. Those *Trichoderma* isolates had shown biocontrol activity against plant pathogenic fungi. Significant biocontrol activities against the RKNs were obtained with several vegetable crops, such as: tomatoes, cucumbers, egg plants and lettuce, as well as with ornamentals. Experiments were conducted with pots, up to 50 L containers, in growth-chambers and in microplots. Peat-wheat bran *Trichoderma* preparations were applied to different soils (or potting mixes) 1–2 weeks before planting and/or to the potting mix of the growing seedlings. *Trichoderma*-treated plants exhibited reduced galling indices and egg production, while weights of shoots, fruits and roots were higher and flowering was improved. Fungal application to both seedlings and pots improved the results (Sharon et al. 2001, 2007; Spiegel et al. 2007). *Trichoderma* species and isolates were tested for compatibility on agar plates. Some isolates belonging to same species (*T. asperellum*) showed compatibility - no distinct barrier was observed in their meeting line. In pot experiments of dual isolates combinations, improved biocontrol was achieved with some combinations, while others did not show better results and sometimes were even worse than each isolate alone (Spiegel, Sharon, Chet unpubl.).

8.3 Attachment and Parasitism

Parasitism is probably an important mode of action and attachment is one of the initial steps of it. *Trichoderma asperellum*-203 and *T. atroviride* showed the ability to parasitize nematode eggs and J2s (Sharon et al. 2001). Mechanisms involved in the attachment and parasitism processes were investigated, with special attention to the role of the gelatinous matrix (gm) in direct nematode-fungus interactions. It was

found that the gm enables fungal attachment and enhances parasitic abilities of most isolates, which could also utilize it as a nutrient source. Fungal conidia can attach to nematode egg masses and to eggs and J2s that had contact with the gm, whereas gm-free J2s and eggs are almost unattached by fungal conidia. However, differences were observed among the various *Trichoderma* species and isolates, in their attachment and parasitism capabilities, indicating the specificity of the processes (Sharon et al. 2007).

Conidia attachment and parasitism processes were microscopically monitored *in vitro*. A green fluorescent protein (GFP)-expressing *T. asperellum*-203 construct was used to observe the parasitism process (Fig. 8.1). Observations made by scanning electron microscopy (SEM) enabled a more detailed insight on this process, showing typical fungal parasitic behavior, including tight attachment of spores and hyphae, coiling of hypae around J2 and appressoria-like structures formation. Gelatinous matrix-free eggs and J2s were penetrated only by few fungal hyphae and colonized. The role of the gm in attachment was studied by using gm that had been separated from egg-masses. Conidia were agglutinated by a gm suspension (enhanced in presence of Ca^{2+}) and their germination was improved (Sharon et al. 2007). A biomimetic system based on nylon fibers, originally developed and used by Inbar and Chet (1994) for investigations of mycoparasitism, was modified and used with gm-coated fibers. It successfully expressed the specific triggering of fungal attachment and parasitic growth patterns by the gm, similar to the parasitism on the nematodes (Fig. 8.2) (Sharon et al. 2007). *Trichoderma* parasitic patterns on nematodes and nylon fibers, resembled mycoparasitc behavior (Viterbo et al. 2007a) and patterns induced by lectins derived from host fungi (Inbar and Chet 1994, 1997).

Hyphae of *T. atroviride*, which was the most effective parasite of the J2s, showed higher tendency to coil around the J2s than those of *T. asperellum*-203. Similar results with respect to the coiling process have been obtained in fungal-fungal biomimetic interactions using nylon fibers, especially after induction with a G-protein activator (Omero et al. 1999). The signal-transduction pathways downstream of the recognition event have recently been intensively investigated, with a focus on the role of G-protein α-subunit genes (Zeilinger et al. 2005). Further investigations may determine whether similar pathways are involved in gm induction of fungal parasitic behavior.

To understand *Trichoderma*–nematode direct interactions, the effects of *M. javanica* surface-binding antibodies on the parasitism was studied. The nematode's surface coat (SC) is considered to be important in recognition events involving plant hosts and microbial antagonists (Spiegel and McClure 1995; Kerry and Hominick 2001; Koltai et al. 2002; Morton et al. 2004). The nature of *Meloidogyne* species SCs has been studied (Spiegel et al. 1995, 1997; Lin and McClure 1996) and antibodies have been used to characterize surface antigens and the interactions with plant hosts (Gravato-Nobre and Evans 1998; Lopez de Mendoza et al. 1999) and microorganisms (Spiegel et al. 1996; Davies 2005).

Meloidogyne javanica (J2s or J2s and eggs) surface-binding monoclonal (MAb) and polyclonal (PAb) antibodies were tested for their effects on the

Fig. 8.1 Parasitism of
Trichoderma asperellum-203
(constitutively expressing
GFP construct) on
Meloidogyne javanica
on (**a**) eggs, bar=50 μm, and
on (**b**) second-stage juvenile,
bar=20 μm
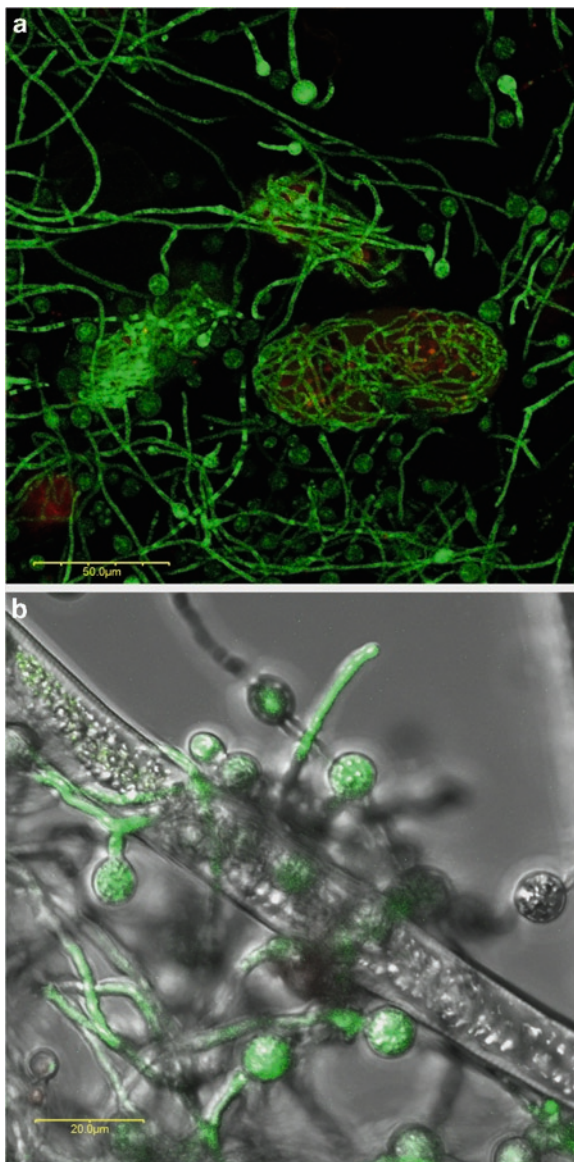
nematode-*Trichoderma* interactions. Those antibodies inhibited J2s movement and
therefore reduced root penetration (Sharon et al. 2002, 2009b). Parasitism of
T. asperellum-203 and *T. atroviride* on nematode egg masses, eggs and juveniles
was enhanced when antibodies were incorporated into *in-vitro* parasitism bioassays.
Parasitism on gm-free and J2s was also improved, compared to controls without
antibodies that almost did not attach fungal conidia. Improved parasitism could be
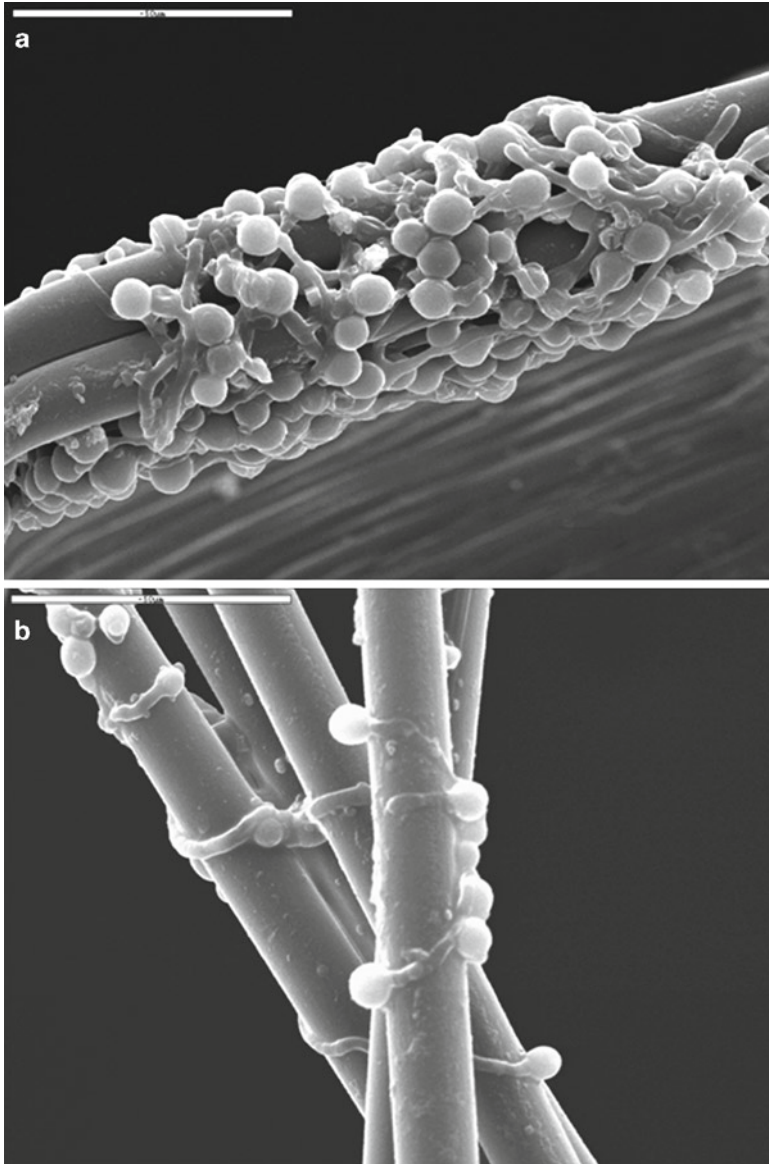
Fig. 8.2 Scanning electron micrographs of nylon fibers coated with gelatinous matrix (gm). (**a**) *Trichoderma asperellum*-203 conidium attachment, bar=50 μm. (**b**) Fungal parasitic-like behavior of *T. atroviride*: tight adhesion of hyphae and coiling, bar=50 μm

due to bilateral binding of the antibodies to the nematodes and conidia, enabling better conidial attachment to the nematodes. Enhanced germination of antibody-bound conidia further improved parasitism. Differences were observed among antibodies in their effects on fungal parasitism and their interaction with *Trichoderma* species.

Focus was made mainly on the egg- and juvenile-binding MAb MISC, which had been raised against *M. incognita* (race 3) and had exhibited specificity to fucosyl-bearing epitopes (Gravato-Nobre et al. 1999). Binding of MISC to *M. javanica* egg masses, eggs and J2s was inhibited by pretreatment of the MAb with fucose; therefore, the fucose-specific lectin, UEA-I, was used, and it also resulted in specific enhancement of conidial binding to nematodes and conidial agglutination, similar to the effect of the antibody. The labeling of gm and gm-originated eggs with UEA-I and its specific inhibition by the carbohydrate fucose indicate that the gm contains fucose residues.

A model for fungal conidia attachment to nematodes (Fig. 8.3) suggests that carbohydrate-lectin-like interactions might be involved in this process; such interactions are sometimes Ca^{2+}-dependent (Sharon et al. 2007, 2009b). This model addresses the roles of fucose and fucose-specific antibody and lectin; nevertheless, other carbohydrates/lectins interactions might be involved in these attachment processes. On the surface coat of gm-free J2s of *M. javanica* there are fucose-, mannose- and glucose-binding proteins (carbohydrate recognition domains CRDs) (Sharon and Spiegel 1996; Spiegel et al. 1995, 1997), and fucose residues. The gm contains fucose and fucose-binding domains (FBD) (Sharon and Spiegel 1993), molecules that also occur on *Trichoderma* conidia (Elad et al. 1983). Fucose inhibited conidia attachment to J2s, conidia agglutination by gm suspension and their attachment to nylon fibers; attachment was also inhibited after periodate treatment of nematodes. The model suggests that during J2's hatch from egg mass, gm, which
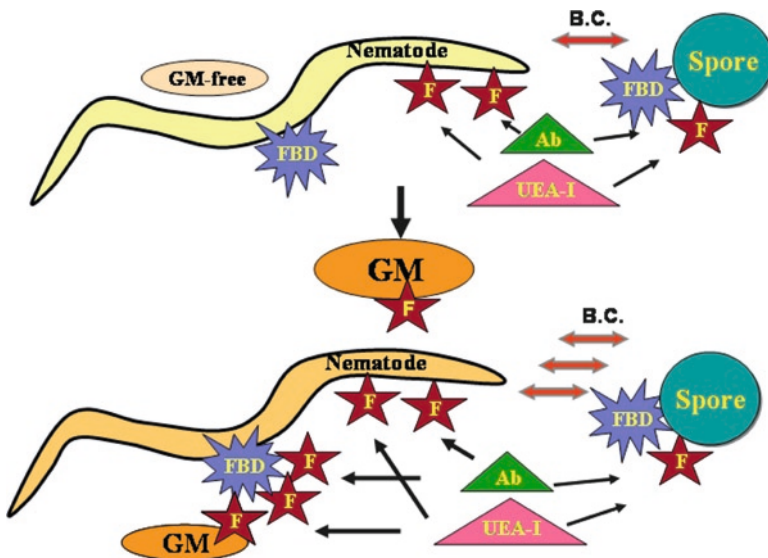

Fig. 8.3 Attachment model of *Trichoderma* spores to *Meloidogyne javanica* second-stage juveniles. *GM* gelatinous matrix, *F* fucose, *FDB* fucose-binding domain, *Ab* antibody, *Bc* biocontrol

contains carbohydrates such as fucose, binds to the J2s surface coat and this can alter their binding affinity to the fungal conidia that contain fucose-binding domains. As a result, gm-J2s are efficiently attached and parasitized by the fungus. Carbohydrate residues, such as fucose, on the surface of the nematode and fungal conidia can be involved in the antibody- and lectin-mediated improved attachment and parasitism.

One of the most interesting features of the nematode SC is its dynamic nature: there is a continuous turnover that involves shedding and replacement of the surface antigens (Lin and McClure 1996; Spiegel et al. 1997; Blaxter and Robertson 1998). However, surface-characterization studies of *Meloidogyne* species have been performed mainly on gm-free J2s and no attention has been paid to the role of the gm and its effect on interactions between nematodes and microorganisms. The MAb MISC has also been observed to label the gm of *M. incognita* (race 3) and the rectal glands, where the gm originates (Hu et al. 2000). Nevertheless, the fate of gm-originated components on the surface of *Meloidogyne* J2s and during the SC turnover process remains unclear.

The gm plays a key role in the process of *Trichoderma* conidia attachment to the nematode and in the ensuing parasitism. The gm is usually considered a defensive envelope that protects the eggs against microorganisms and enables the egg mass to survive in the soil (Sharon et al. 1993). Bacteria that were agglutinated by the gm could not reproduce in its presence, whereas others, which were not agglutinated, utilize the gm as a nutrition source and reproduce (Sharon et al. 1993). Thus, the ability of some *Trichoderma* species to be agglutinated by the gm and grow on it is unique, and partially accounts for their ability to attack RKNs.

Direct parasitism of *Trichoderma* on nematode life-stages on the roots might be important for a successful biocontrol process. The potential ability to parasitize nematode life-stages *in planta* was demonstrated with *T. asperellum*-203, which interacted with penetrating J2, and with females and egg masses on roots in soil, thereby interfering with the reproduction process (Sharon et al. 2007). The high affinity of this isolate as a root-surface colonizer (Yedidia et al. 1999) probably enhances these parasitic fungus-nematode interactions on the root surface. The ability of the fungus to colonize nematode penetration holes in the root might contribute to plant defense against secondary pathogens that usually exploit the penetration of the roots by the nematodes.

8.4   Lytic Enzymes and Metabolites

Following the attachment process, fungal lytic activities are induced in order to digest the host. For efficient parasitism, a biocontrol agent should overcome several barriers that protect the nematodes from the external environment. The eggshell forms an important barrier that is composed of three layers: an outer – vitelline (protein), middle – chitinous and an inner lipo-protein layer. The amino acid

composition of the eggshell indicates that it probably contains collagen-like proteins, which provide the tough, resilient properties associated with eggshells (Morton et al. 2004). The chitinous layer provides strength to the eggshell and is the thickest and most obvious layer; that of *M. javanica* is thicker than those of other plant-parasitic species. This layer protects the lipid layer, which determines the permeability and protects from harmful chemicals (Wharton 1980). A combination chitinolytic and proteolytic enzymes is required to disrupt the eggshell (Tikhonov et al. 2002; Khan et al. 2004), although chitinolytic capacity is probably the most important activity on the eggshells (Morton et al. 2004).

Another barrier is the cuticle that is composed mainly of collagens (Blaxter and Robertson 1998).

The role of *Trichoderma* lytic enzymes in plant defense and in fungal biocontrol processes was reviewed by Viterbo et al. (2002b), Markovich and Kononova (2003) and by Steyaert et al. (2003). Synergistic actions of different hydrolytic enzymes have been reported (Elad and Freeman 2002). Proteolytic activities of *Trichoderma* have not been investigated extensively as those of other lytic enzymes such as chitinases, but they have recently begun to be explored.

8.4.1   Proteases

One of the most studied *Trichoderma* proteases is a 31-kDa basic proteinase (Prb1), produced by *T. atroviride* strain IMI 206040, which was identified and characterized as a serine protease and belongs to the S8 family. The gene encoding this proteinase was cloned by Geremia et al. (1993). The gene expression was repressed by glucose and induced by fungal cell wall preparations of *R. solani* or chitin (Flores et al. 1997). This enzyme was subjected to nitrogen catabolite repression (Olmedo-Monfil et al. 2002). Transgenic fungal lines, carrying multiple copies of *prb1,* revealed improved biocontrol activity against *R. solani* in cotton plants (Flores et al. 1997). Those lines were used also to study the role of this proteinase in fungus-nematode interactions. Line P-2 exhibited improved nematode biocontrol capacity in soil and on all nematode life- stages that were tested *in vitro*, indicating that this proteinase is involved in the nematode biocontrol process (Sharon et al. 2001). Involvement of the *prb1* gene in nematode parasitism was supported by microscope observations, using a GFP inducible reporter construct (Provided by Prof. A. Herrera-Estrella, Mexico), which showed that expression of this gene was induced during fungal parasitism on the various life stages of the root-knot nematode, especially those that involve gm (Sharon et al. 2007).

Other protease activities were detected in *T. atroviride* during nematode parasitism process. Amino acid sequencing of peptides from these proteases revealed peptides with similarity to some acid proteases. The proteolytic profile of *T. asperellum* strains differed from that of *T. atroviride*; in *T. asperellum*-203, the Prb1 seems not to be involved in nematode parasitism. Nevertheless, some proteases presented alleviated activities during parasitism. In some *Trichoderma* isolates, protease activities were very low or not detected (Spiegel, Sharon, Chet unpubl.). Differences

in proteolytic capabilities of the various *Trichoderma* species and isolates might partially account for their different capabilities in parasitism on *Meloidogyne* life-stages.

PRA1, a serine-protease with trypsin-like activity was isolated from *T. harzianum* CECT 2413 (Suarez et al. 2004). This 28 kDa protease might be related to myco-parasitic interactions and exhibited nematicidal activity. PRA1 was found to be induced by conditions simulating antagonism, to be subject to nitrogen and carbon derepression, and to be affected by the pH of the culture medium, its optimal pH range being 7–8. Purified preparations of PRA1 reduced *M. incognita* egg hatch during *in vitro* assays and this nematicidal effect was enhanced by the use of fungal culture filtrates (CFs), suggesting that PRA1 has additive or synergistic interactions with other proteins produced during the antagonistic activity of the fungus (Suarez et al. 2004). Suarez et al. (2007) characterized the genes of six novel endopepti-dases from *T. harzianum* CECT 2413, belonging to different families. Gens within a family are differently regulated in response to different culture conditions, sug-gesting that they have diverse functional roles.

8.4.2   *Chitinases*

The chitinolytic system of *Trichoderma* and its role in mycoparasitism have been intensively investigated (Kubicek et al. 2001). Several chitinases and their related genes have been isolated from *Trichoderma* spp. growing in media containing chitin as a sole carbon source. Generally, carbon starvation, products of chitin degrada-tion, fungal cell-walls, and colloidal chitin are thought to induce chitinolytic enzyme expression, whereas glucose and other easily fermented carbon sources serve as repressors (Viterbo et al. 2002b).

N-acetylglucosaminidases. Two GlcNAcases, CHIT73 and CHIT102, were detected, isolated and identified in *Trichoderma asperellum*-203 growth medium (Ramot et al. 2004); the genes *exc1* and *exc2* encode for these enzymes, respectively. These enzymes were up-regulated by glucosamine and CHIT102 formed homodimers. CHIT102 was the first chitinase to appear upon contact with *S. rolfsii*, therefore, it has been speculated that it plays a unique role in triggering the expression of other chitinolytic enzymes (Haran et al. 1996; Viterbo et al. 2002b). The gene *nag1* in *T. atroviride* is a homologue of *exc1y* from *T. asperellum*. The Nag1 was extensively investigated by Brunner et al. (2003), who showed that it is essential for chitinase induction by chitin and, therefore, is of major relevance in biocontrol.

Exochitinases (Chitobiosidases). When grown on crab-shell chitin as the sole carbon source, a chitobiosidase of 40 kDa was secreted from *T. atroviride* P1 (Harman et al. 1993).

Endochitinases.   An endochitinase of 42 kDa has been isolated from several different strains of *Trichoderma*; it is believed also to be a key enzyme in the mycoparasitic interaction (Carsolio et al. 1999; Zeilinger et al. 1999). Two more endochitinases – of

37 and 33 kDa – were reported in *T. harzianum* (Viterbo et al. 2002b), and a new endochitinase termed CHIT36 (previously designated CHIT33 by Haran et al. (1996)), was isolated from *T. harzianum* isolate TM (Viterbo et al. 2001). The CHIT36 from *T. asperellum*- 203 is very similar (Viterbo et al. 2002a). A 37-kDa endochitinase has been isolated from *T. harzianum* 109 (De Marco et al. 2000).

Induction of chitinolytic activities during fungal parasitism on nematodes was demonstrated using GFP reporter constructs: the endochitinases CHIT36 and CHIT42 in *T. asperellum*-203 and *T. atroviride* P1, respectively and the *β-N*-acetyl-D-glucoseaminidases (Hexoaminidases) CHIT102 and Nag1 in those species, respectively. As in the case of proteinase Prb1, the presence of gm enhanced the production of chitinilytic enzymes (Sharon et al. 2009a).

Steyaert et al. (2003) suggested that there was co-regulation of the genes *prb1* and *chit42* in *T. hamatum*. The genes *prb1* and *chit42* were not induced by lectins in the fungal biomimetic system but were induced by diffusible factors from the host fungus (Cortes et al. 1998), a process that involves regulation pathways other than coiling and conidiation processes (Rocha-Ramirez et al. 2002).

8.4.3   Effects of Environmental Conditions on Enzymes

There is growing evidence for the effect of ambient pH on the expression and activity of fungal extracellular enzymes. In the regulation of many proteases, pH plays prominent role (St. Leger et al. 1998). In response to environmental signals, enzymes production during pathogenesis is probably regulated by the structural elements of the host, nutrient limitation, and the ambient pH. Fungi may be able to adjust the pH of micro-environments to facilitate optimal enzyme activity (Morton et al. 2004) and ecological niches may be actively improved and protected by the fungi during plant-*Trichoderma* interactions (Suarez et al. 2004).

Environmental different pH and nutritional conditions can be crucial for the production and activity of fungal enzymes and metabolites that affect the nematodes. Proteases that are apparently involved in nematode biocontrol required acidic pH conditions for activity, similar to the optimal conditions for chitinases activities (Schickler et al. 1998). Some of the parasitism-related proteolytic and chitinolytic enzymes presented nitrogen catabolite repression. Enzymes such as Prb1 are induced in presence of nitrate and repressed by ammonium and are capable of responding to different environmental conditions that may reflect stress conditions (Olmedo-Monfil et al. 2002). Micro-environments, in soil and rhizosphere, supporting preferable conditions for *Trichoderma* biocontrol activity may improve the process.

8.5   Antibiotics Production

Trichoderma species can produce a variety of secondary metabolites, including antibiotic compounds, which may contribute to the biocontrol processes (Howell 1998). The nature and roles of antibiotic peptides that belong to the peptaibol group

have been intensively studied (Szekeres et al. 2005). Peptaibols generally exhibit antimicrobial activity, which is thought to arise from their membrane activity and their ability to form pores in lipid membranes. A peptaibol synthetase gene has been cloned (Wiest et al. 2002) and further studies suggested that peptaibols are critical in the chemical communication between *Trichoderma* and plants as triggers of non-cultivar-specific defense responses (Viterbo et al. (2007b)). *Trichoderma virens* produces gliotoxin and gliovirin and also peptaibols. Parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotic action of *Trichoderma* against phytopathogenic fungi has been reported (Schirmböck et al. 1994). The antifungal action of enzymes reinforced by synergism with antibiotics was comprehensively reviewed by Kubicek et al. (2001).

Nematicidal activity against *M. javanica* J2s was detected mainly in *T. atroviride* culture filtrates (CFs); the active component/s showed low molecular weight (MW) and heat sensitivity. The nematicidal activity was specifically increased during parasitism on egg-masses (Sharon et al. 2007). Immature eggs exhibited reduced hatching rates in presence of CF, whereas hatching of mature eggs was enhanced. The effect of CF on eggs was contributed by both the enzymatic fraction, which contained proteases and chitinases, and by the low-MW component/s. (Spiegel, Sharon, Chet unpubl.). Appropriate candidates responsible for such nematicidal activity might be antibiotic peptides, such as peptaibols (Sharon et al. 2007). Such molecules have been identified and sequenced in *T. atroviride* by Oh et al. (2000).

8.6   Induced-Resistance

Trichoderma strains that are capable of root interaction induce metabolic changes in plants that increase resistance to a wide range of plant-pathogenic microorganisms and viruses. This response seems to be broadly effective for many plants, which indicates that there is little or no plant specificity (Harman et al. 2004). *Trichoderma* strains produce or release a variety of compounds that induce localized or systemic responses, and this explains their lack of pathogenicity to plants. These elicitors include peptides, proteins and low-molecular weight compounds (Yedidia et al. 2000; Harman et al. 2004; Viterbo et al. 2004). These root-microorganism associations cause substantial changes to the plant proteome and metabolism. Plants are protected from numerous classes of plant pathogens by responses that are similar to systemic acquired resistance (SAR) and rhizobacteria-induced systemic resistance (RISR). In the SAR pathways there is direct production of pathogenesis-related (PR) proteins by the plant, mediated by either salicylic acid or jasmonic acid as signaling molecules. However, in RISR the PR proteins and phytoalexins are not induced in the absence of attack by plant pathogens (Harman et al. 2004).

Analysis of signal molecules involved in defense mechanisms, and application of specific inhibitors, indicated the involvement of jasmonic acid and ethylene in the protective effect conferred by *Trichoderma* spp. against the leaf pathogen *Pseudomonas syringae* pv. *lachrymans*. Moreover, examination of local and systemic gene expression revealed that *T. asperellum*-203 modulated the expression of genes

involved in the jasmonate/ethylene signalling pathways of ISR in cucumber plants. Subsequent challenge of *Trichoderma*-preinoculated plants with the leaf pathogen resulted in higher systemic expression of the pathogenesis-related genes encoding for chitinase 1, β-1, 3-glucanase, and peroxidase relative to non-inoculated, challenged plants (Shoresh et al. 2005). The MAPK signal transduction pathways, both of the plant and *Trichoderma,* are important for the induction of systemic resistance (Viterbo et al. 2007a). Alfano et al. (2007) showed that *T. hamatum* 382 induced resistance response in tomato against bacterial spot of tomato and its pathogen *Xanthomonas euvesicatoria*. Fungal actively induced systemic changes in plant physiology and disease resistance through systemic modulation of the expression of stress and metabolism genes.

The indirect effects of fungal root colonization on *M. javanica* in pot experiments, using split-roots systems, was examined. Nematode infection was reduced, and inhibition of nematode development and egg production were recorded in root-halves that had not been exposed to the fungus, indicating potential involvement of systemic induced resistance mechanisms in the nematode biocontrol process (Sharon et al. 2009a). The effect on egg production might be due to the higher systemic expression of pathogenesis-related (PR) proteins, such as chitinases and peroxidases (Yedidia et al. 2000; Shoresh et al. 2005) that are produced in *Trichoderma*-preinoculated plants during the systemic response.

Jasmonic acid suppresses nematode infestation on tomato roots (Cooper et al. 2005). It is known to be transported from foliage to roots, where it can have a wide range of effects on plants development and metabolism. Jasmonates influence root growth and nutrient partitioning, which could potentially affect nematode parasitism (Cooper et al. 2005). Induced resistance seems to be an important indirect mechanism of the nematode biocontrol process. Further investigations are required to elucidate the pathways that mediate these systemic responses that affect the nematodes.

8.7   Interactions of *Trichoderma* with Other Nematodes and Microorganisms

The potential of *Trichoderma* to control other phytophagous nematodes is most promising, as was demonstrated *in vitro* with several nematode species. Culture filtrates (CFs) immobilized different plant-parasitic nematodes; nevertheless, non-target and beneficial nematode species were not harmed by direct parasitism with *T. asperellum*-203, nor by *T. atroviride* CFs (Spiegel, Sharon, Chet unpubl.).

The differing responses of nematode groups to fungal metabolites might be due to wide differences among the structures and compositions of their cuticles, which affect the permeability. The epicuticle is made up of lipids, and it appears to act as a hydrophobic barrier to diffusion. Lipids and glycolipids are presented on the surfaces of free-living nematodes and animal parasites, but there is very limited knowledge of their presence in plant-parasitic nematodes (Blaxter and Robertson 1998).

Interactions of different *Trichoderma* isolates with other organisms and micro-organisms in the ecological systems and the possible effects on nematode biocontrol processes have not been deeply investigated. Competition between *Trichoderma* and other microorganisms might interfere with root colonization and biocontrol processes. *Trichoderma* spp. in general have been found to be highly resistant to a variety of toxins and other compounds, including antibiotics produced by other microorganisms, plant antimicrobial compounds and chemical fungicides (Harman et al. 2004). Synergy between mycorrhizal fungi and *Trichoderma* has been shown, as well as synergy between *Trichoderma* enzymes and bacterial antibiotics (Harman et al. 2004). Mixtures of different root-colonizing biocontrol agents can provide better results than any one agent used on its own (Whipps 2001). However, the abilities of combinations of beneficial root-colonizing microorganisms to improve plant performance have been inadequately examined in either managed or natural ecosystems (Harman et al. 2004).

Kok et al. (2001) reported that egg masses of *Meloidogyne* species from soils contained a bacterial community significantly greater than that of the rhizosphere. They suggested that the egg masses microflora may be an important factor in determining the success of nematode biocontrol agents. Interestingly, a strain of *Trichoderma* that strongly reacted against the biocontrol agent *V. chlamydosporium* was found among *M. fallax* egg masses microflora.

8.8 Concluding Remarks and Future Prospects

Trichoderma isolates are very unique biocontrol agents as they present a wide range of activities and interactions in their ecosystems as free-living, plant symbionts or parasites of plant pathogens such as fungi and nematodes. Some isolates have the potential to serve as broad-spectrum plant protection agents and growth promoters.

Several modes of action are involved in the activity of *Trichoderma* against nematodes: direct parasitism, which involves attachment and enzymatic digestion by proteolytic and chitinolytic enzymes, production of nematicidal metabolites, as well as indirect effects of induced systemic resistance in the host plants. Different species and isolates can specialize in distinct modes, so that the combined application of some compatible isolates may result in a synergistic effect. Understanding the main mode of action of different isolates may improve their application methods, implementation sites (i.e. soil, roots) rates and timing. Formulations can be improved and designed to support and enhance production of enzymes and metabolites. Better understanding of the processes involved in biocontrol could lead to developments in selection of active biocontrol isolates.

Chen and Dickson (2004) described different groups of fungal antagonists of nematodes: predacious fungi, endoparasites of vermiform nematodes, parasites of sedentary females and eggs, fungi that produce antibiotic substances, and vesicular-arbuscular mycorrhizal fungi (VAM) or VAM-like fungi. There is no clear-cut

distinction between the categories, and some fungi can belong to more than one category. It is evident that in some *Trichoderma* species we can find all of these features.

The mechanisms potentially involved in the *Trichoderma* parasitism process on the nematode were studies mainly in the nematode-fungus interaction level. The presence of host plant triggers the *Trichoderma* to produce specific enzymes and metabolites (Harman et al. 2004; Viterbo et al. 2007b), that may also affect the nematodes. The tritrophic direct and indirect interactions should be further studied from ecology to molecular levels. Further applied and fundamental studies will enable the development of *Trichoderma* biocontrol potential also against other plant-parasitic nematodes.

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