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The role of recognition in the induction of specific chitinases during mycoparasitism by *Trichoderma harzianum*

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The induction of chitinolytic enzymes in the biocontrol agent *Trichoderma harzianum* during parasitism on *Sclerotium rolfsii* and the role of fungal–fungal recognition in this process were studied. A change in the chitinolytic enzyme profile was detected during the interaction between the fungi, grown in dual culture on synthetic medium. Before coming into contact with each other, both fungi contained a protein with constitutive 1,4-β-*N*-acetylglucosaminidase activity. As early as 12 h after contact, the chitinolytic activity in *S. rolfsii* disappeared, while that of *T. harzianum* (a protein with a molecular mass of 102 kDa, CHIT 102) greatly increased. After 24 h of interaction, the activity of CHIT 102 diminished concomitantly with the appearance of a 73 kDa 1,4-β-*N*-acetylglucosaminidase, which became clear and strong at 48 h. This phenomenon did not occur if the *S. rolfsii* mycelium was autoclaved prior to incubation with *T. harzianum*, suggesting its dependence on vital elements from the host. Cycloheximide inhibited this phenomenon, indicating that *de novo* synthesis of enzymes is taking place in *Trichoderma* during these stages of the parasitism. A biomimetic system based on the binding of a purified surface lectin from the host *S. rolfsii* to nylon fibres was used to dissect the effect of recognition. An increase in CHIT 102 activity was detected, suggesting that the induction of chitinolytic enzymes in *Trichoderma* is an early event which is elicited by the recognition signal (i.e. lectin–carbohydrate interactions). It is postulated that recognition is the first step in a cascade of antagonistic events which triggers the parasitic response in *Trichoderma*.

Keywords: *Trichoderma*, mycoparasitism, recognition, chitinases, lectins

INTRODUCTION

Parasitism is one of the mechanisms by which fungi from the genus *Trichoderma* antagonize other fungi (including plant pathogenic fungi), thereby serving as biocontrol agents of plant diseases (Baker, 1987; Chet, 1987, 1990). Parasitism by *Trichoderma* spp. is considered to be necrotrophic (destructive), whereby the relationship results in the destruction and death of the host fungus (Barnett & Binder, 1973). *Trichoderma* spp. attach to the host hyphae by coiling, hooks or appressorium-like bodies and penetrate the host cell walls by secreting lytic enzymes such as 1,3-β-glucanase and chitinase (Elad *et al.*, 1983).

Abbreviations: GlcNAc, *N*-acetylglucosamine; 4-MU-(GlcNAc), 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide; 4-MU-$(GlcNAc)_2$, 4-methylumbelliferyl β-D-*N*,*N*′-diacetylchitobioside; 4-MU-$(GlcNAc)_3$, 4-methylumbelliferyl β-D-*N*,*N*′,*N*″-triacetylchitotriose.

The activity of these enzymes during the parasitism of *Trichoderma harzianum* on the soilborne plant pathogenic fungus *Sclerotium rolfsii* has been shown using fluorescein isothiocyanate (FITC)-conjugated lectins or Calcofluor White M2R. Localized cell-wall lysis was indicated by the appearance of fluorescence at points of interaction between the antagonist and its host. Enzymic activity and antagonism were diminished in the presence of cycloheximide (Elad *et al.*, 1983).

A considerable amount of research has been carried out in recent years on the chitinolytic system of *Trichoderma* (De La Cruz *et al.*, 1992; Haran *et al.*, 1995; Harman *et al.*, 1993; Lorito *et al.*, 1993; Ulhoa & Peberdy, 1991a, b, 1992, 1993). It has been found to be a complex system consisting of six distinct enzymes. Using three different fluorescent analogue substrates, the system was found to be composed of two 1,4-β-*N*-acetylglucosaminidases

(CHIT 102 and CHIT 73) and four endochitinases (CHIT 52, CHIT 42, CHIT 33 and CHIT 31) (Haran *et al.*, 1995). All these enzymes were induced and excreted by *T. harzianum* (strain TM) during growth in liquid medium with chitin as sole carbon source. CHIT 102 could be detected intracellularly at a low constitutive level, even when *Trichoderma* was grown on glucose (Haran *et al.*, 1995).

In all of these studies, the expression and regulation of chitinases in *Trichoderma* were studied in liquid cultures supplemented with different carbon sources, e.g. chitin, purified fungal cell walls, glucose or *N*-acetylglucosamine (GlcNAc) (De La Cruz *et al.*, 1993; Haran *et al.*, 1995; Ulhoa & Peberdy, 1991a, 1993). Clearly, cell-wall-degrading enzymes, as well as other changes, e.g. morphological (coiling and appressorium formation) and cytological, were induced in both *Trichoderma* and the host fungi during the parasitic interaction (Benhamou & Chet, 1993; Cherif & Benhamou, 1990; Elad *et al.*, 1983; Goldman *et al.*, 1992, 1994; Lora *et al.*, 1994; Schirmbock *et al.*, 1994). However, which of the above-described *Trichoderma* chitinases is expressed during mycoparasitic interactions and what triggers their expression is still unknown. Recently, we were able to show that a lectin present on the cell wall of *S. rolfsii* serves as a recognition signal which induces coils and appressorium-like structure formation in *T. harzianum* (Inbar & Chet, 1992, 1994). When a purified lectin from *S. rolfsii* was bound to nylon fibres in a biomimetic system that simulated the host hyphae, it specifically induced the formation of mycoparasite-related structures in *Trichoderma* (e.g. coils, hooks, appressorium-like bodies and hyphal loops). Secretion of adhesive material aiding in the establishment of the interaction of *Trichoderma* with the lectin-treated fibres was also observed (Inbar & Chet, 1994).

In the present study, the induction of specific chitinases in *T. harzianum* during its parasitic interaction with *S. rolfsii* was studied by detection of chitinase activity using PAGE. The biomimetic system (Inbar & Chet, 1992) was used to investigate the involvement of lectins in triggering chitinase activity in *Trichoderma*.

METHODS

Culture of organisms. *Sclerotium rolfsii* Sacc. type A, ATCC 26325, was maintained on a synthetic medium (SM; Okon *et al.*, 1973). For lectin production and purification, *S. rolfsii* was grown in liquid SM supplemented with 5×10^{-6} M polyoxin D (Barak & Chet, 1990). After 5 d, the culture filtrate (50 ml) was collected and dialysed for 24 h at 4 °C against 20 mM Tris/HCl buffer (pH 7·0; 2 × 5 l) (crude agglutinin). *Trichoderma harzianum* Rifai, T-Y, was grown on potato dextrose agar (PDA; Difco). Dual cultures were carried out by growing the fungi on a cellophane membrane which covered a 90 mm Petri dish containing SM supplemented with 0·2% (w/v) glucose. Plates were inoculated with PDA strips (3 × 15 mm) carrying mycelium of the fungi, placed parallel, 6 cm apart, and incubated at 28 °C in the dark.

Enzyme production and identification of activity. At the indicated time, mycelium from the interaction zone, where *Trichoderma* parasitized *S. rolfsii*, was collected aseptically and washed with sterile distilled water. Washed mycelium, in sterile distilled water, was then homogenized by Ultra-Turrax (TP 18/10, IKA-WERK) for 3 min at 4 °C and sonicated for 3 min at 4 °C with 50% pulses using a small microtip (Sonicator model W-375, Heat Systems-Ultrasonics). Finally, mycelium was homogenized for 3 min at 4 °C with a Heidolph R2R-50 homogenizer and centrifuged for 20 min at 20000 ***g*** at 4 °C. The supernatant was collected and dialysed against distilled water (4 × 5 l) for 24 h at 4 °C. Proteins were then freeze-dried and kept at −70 °C until use. Protein concentration was determined according to Bradford (1976) using the Bio-Rad protein assay dye reagent and BSA as a protein standard.

The effect of cycloheximide on the appearance of chitinolytic enzymes during the interaction between *T. harzianum* and *S. rolfsii* was tested by growing the *Trichoderma* alone in 90 mm Petri dishes containing SM, on a cellophane membrane as above, for 48 h at 28 °C. Membranes were then aseptically transferred to new dishes centrally divided into two sections, one containing SM and the other SM supplemented with cycloheximide (20 μg ml^{-1}). Plates were then inoculated with *S. rolfsii* (on the unsupplemented SM) and further incubated under the same conditions. Proteins were obtained from the interaction zone at the indicated times, as described above.

For the identification of enzymic activity, proteins were prepared in Laemmli buffer (Laemmli, 1970) without 2-mercaptoethanol. Proteins were separated by SDS-PAGE in 1·5 mm gels with 10 or 12% (w/v) acrylamide in a Mini-Protean II cell (Bio-Rad) according to the manufacturer's instructions.

Chitinolytic enzymes were detected *in situ* using a set of three highly sensitive substrates that produce fluorescent products following enzymic hydrolysis: 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide [4-MU-(GlcNAc)]; 4-methylumbelliferyl β-D-*N*,*N*′-diacetylchitobioside [4-MU-$(GlcNAc)_2$]; 4-methylumbelliferyl β-D-*N*,*N*′,*N*″-triacetylchitotriose [4-MU-$(GlcNAc)_3$] (all from Sigma). These compounds function as dimeric, trimeric and tetrameric substrates, respectively (Haran *et al.*, 1995). Enzymes were reactivated by removing SDS, as per the casein/EDTA procedure described by McGraw & Green (1990). Gels were washed twice for 15 min in 200 ml 100 mM sodium acetate buffer, pH 4·8, prior to the detection procedure. Enzyme activity was detected on gels as described by Tronsmo & Harman (1993).

Immunological analysis. Proteins were separated by 12% SDS-PAGE with 2-mercaptoethanol and electroblotted onto nitrocellulose membranes. Western blot analysis was performed as described by Ausubel *et al.* (1990) using rabbit polyclonal antibody raised against purified 42 kDa endochitinase of *T. harzianum* strain P1 (kindly provided by Professor G. Harman, Cornell University, USA) or against CHIT 73 (Haran *et al.*, 1995) (prepared in our laboratory by Mrs S. Haran). Detection was performed by enhanced chemiluminescence according to the manufacturer's instructions (Amersham).

Biomimetic system. Lectin from *S. rolfsii* was purified and bound to nylon fibres as previously described by Inbar & Chet (1994). This biomimetic system was used to isolate the effect of recognition and to examine the role of the purified *S. rolfsii* lectin in the induction of *T. harzianum* chitinases during the parasitic interaction. *T. harzianum* was grown on a cellophane membrane covering SM supplemented with 0·2% (w/v) glucose as described above, in the presence of lectin-treated nylon fibres, nylon fibres without lectin or no fibres at all. After 12 h incubation, mycelium was collected and protein production and chitinolytic enzymes were detected as above.

All experiments were carried out at least three times. Representative results are shown.

RESULTS

Chitinolytic enzyme profiles in a dual culture of *T. harzianum* and *S. rolfsii*

Proteins from both *T. harzianum* and *S. rolfsii* were obtained during their interaction in dual culture at 0 h (just before contact) and at 12 h, 24 h and 48 h after contact. The proteins were separated and their chitinolytic activity detected as described in Methods.

Before contact, both fungi exhibited a single protein with constitutive chitinolytic activity (Fig. 1). The *S. rolfsii* chitinolytic protein (molecular mass of approximately 116 kDa) exhibited a high activity with all substrates tested (Fig. 1, lane 1). In *T. harzianum*, low constitutive chitinolytic activity could be detected before *Trichoderma* contacted *S. rolfsii*, from a protein of approximately 102 kDa (Fig. 1, lane 2). This protein hydrolysed all the substrates tested, albeit at reduced rates, from 4-MU-(GlcNAc) to 4-MU-$(GlcNAc)_2$ and 4-MU-$(GlcNAc)_3$ (not shown). For this reason, although it could be clearly seen in the original gel, it is not visible in Fig. 1. These data are in agreement with a previous study (Haran *et al.*, 1995) where this enzyme was determined to be 1,4-β-*N*-acetylglucosaminidase (CHIT 102). Twelve hours after contact between the *T. harzianum* and *S. rolfsii* colonies, the chitinolytic activity in *S. rolfsii* vanished, whereas that of CHIT 102 in *Trichoderma* dramatically increased (Fig. 1, lane 3). This activity began to decrease 12 h later (24 h after contact), concomitant to the appearance of two new proteins exhibiting chitinolytic activity: a 1,4-β-*N*-acetylglucosaminidase with a molecular mass of 73 kDa, and an endochitinase of approximately 50 kDa (Fig. 1, lanes 4 and 5). The latter enzyme exhibited very low activity with the trimeric and tetrameric substrates, and none with the dimeric one. These two chitinolytic enzymes remained active until *Trichoderma* totally covered the *S. rolfsii* colony (after 120 h) (not shown). When *T. harzianum* was grown for 48 h on a solid SM supplemented with chitin (0·2%, w/v) as sole carbon source, CHIT 102 was strongly active together with weak activity of the 50 kDa endochitinase (Fig. 1, lane 6). The above experiment was repeated, but this time the *S. rolfsii* mycelium was autoclaved prior to inoculating the plates with the antagonist and different results were obtained. For this experiment *S. rolfsii* was grown on the membrane until it reached the middle of the plate. Then, the membrane carrying the *S. rolfsii* mycelium was autoclaved, transferred to a new plate with SM and inoculated with *T. harzianum*. All other procedures were carried out as before. No chitinolytic activity was detected in *S. rolfsii* after autoclaving (Fig. 2, lane 1). Twelve hours after *Trichoderma* came into contact with the autoclaved mycelium of *S. rolfsii*, CHIT 102 was induced (Fig. 2, lane 2). However, in contrast to the results obtained in the living dual culture, in the interaction with the autoclaved *S. rolfsii* this enzyme remained active and became even more active with time, until 48 h post-contact, when the 50 kDa endochitinase appeared weakly (Fig. 2, lane 4). These results are similar to those obtained when *T.*

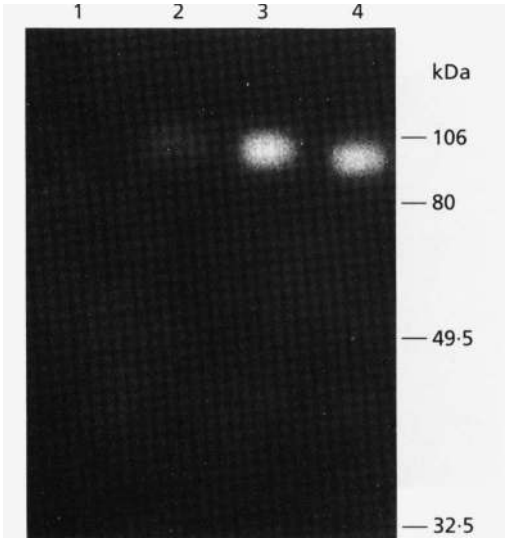

Fig. 2. **Profile of chitinolytic enzymes (50 µg per lane) obtained from a dual culture of *T. harzianum* and autoclaved mycelium of *S. rolfsii*. Chitinolytic activity was detected, following SDS-PAGE (10% acrylamide), using 4-MU-$(GlcNAc)_2$ as the substrate. Lanes: 1, proteins obtained from the autoclaved mycelium of *S. rolfsii*; 2, 3 and 4, proteins obtained from the interaction zone of *T. harzianum* with the autoclaved mycelium of *S. rolfsii* at 12 h, 24 h and 48 h post-contact, respectively.**

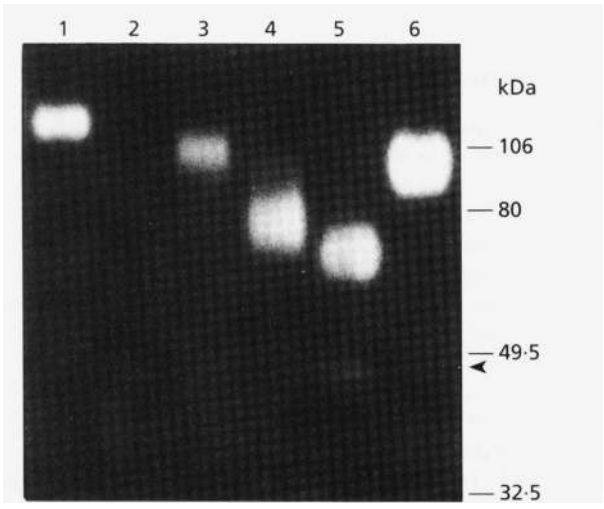

Fig. 1. **Profile of chitinolytic enzymes (50 µg per lane) obtained from a dual culture of *T. harzianum* and *S. rolfsii* grown on synthetic medium (SM) supplemented with glucose (0·2%, w/v). Chitinolytic activity was detected following SDS-PAGE (10% acrylamide) using 4-MU-$(GlcNAc)_2$ as the substrate. Lanes: 1, proteins produced by *S. rolfsii* before coming into contact with *T. harzianum*; 2, proteins produced by *T. harzianum* before contacting *S. rolfsii*; 3, 4 and 5, proteins obtained from the interaction zone at 12 h, 24 h and 48 h post-contact, respectively; 6, proteins produced by *T. harzianum* after 48 h incubation on solid SM with chitin as sole carbon source. The arrowhead indicates the position of the approximately 50 kDa endochitinase which was more clearly seen in the original gel.**

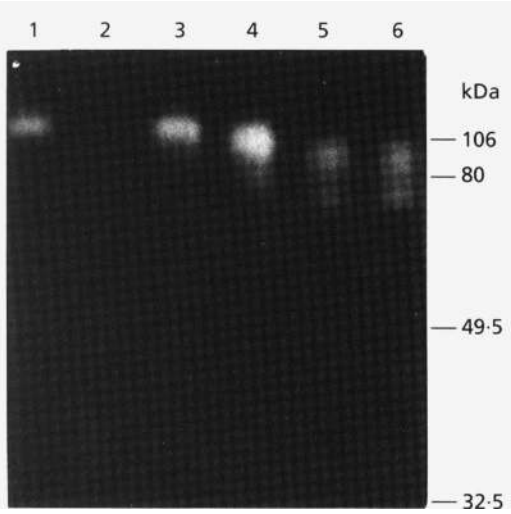

Fig. 3. Profile of chitinolytic enzymes (50 μg per lane) obtained from a dual culture of *T. harzianum* (grown on SM + cycloheximide, 20 μg ml^{-1}) and *S. rolfsii* (grown on SM) in centrally divided dishes. Chitinolytic activity was detected following SDS-PAGE (12% acrylamide) using 4-MU-$(GlcNAc)_2$ as the substrate. Lanes: 1, proteins obtained from the mycelium of *S. rolfsii* before contact with *T. harzianum*; 2, proteins produced by *T. harzianum* before contact with *S. rolfsii*; 3, 4, 5 and 6, proteins obtained from the interaction zone of *T. harzianum* with *S. rolfsii* at 6 h, 12 h, 24 h and 48 h post-contact, respectively.

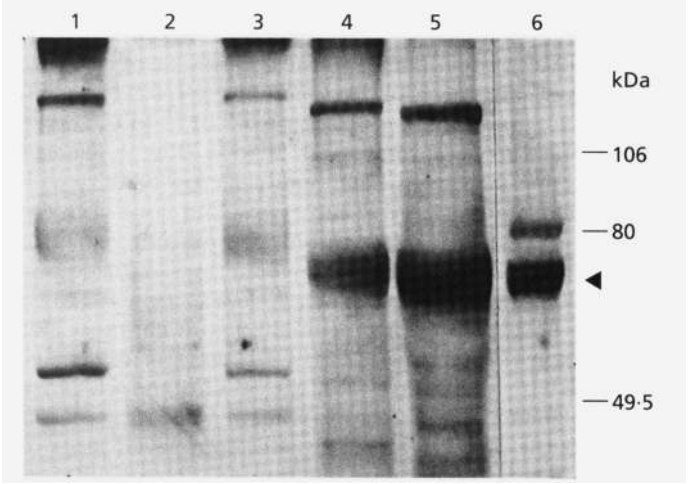

Fig. 4. Western blot analysis of SDS-PAGE (12% acrylamide) using rabbit polyclonal antibodies raised against CHIT 73 (a 73 kDa 1,4-β-*N*-acetylglucosaminidase from *T. harzianum*) (Haran *et al.*, 1995). Lanes containing 100 μg protein are as in Fig. 1. The control lane (6) contains 20 μg extracellular chitinolytic enzymes obtained from *T. harzianum* which was grown in liquid SM with chitin as sole carbon source. The arrowhead indicates the position of CHIT 73.

harzianum was grown on SM containing chitin (Fig. 1, lane 6), indicating the involvement of vital elements from the host *S. rolfsii* in this phenomenon, possibly proteases.

When *Trichoderma* was grown in dual culture in the presence of cycloheximide (20 μg ml^{-1}), the appearance of its chitinolytic enzymes was inhibited (Fig. 3). Even after 48 h interaction, CHIT 102 was still present, while CHIT 73 was only faintly apparent. This suggests that enzymes in *Trichoderma* are synthesized *de novo* during these stages of the parasitism.

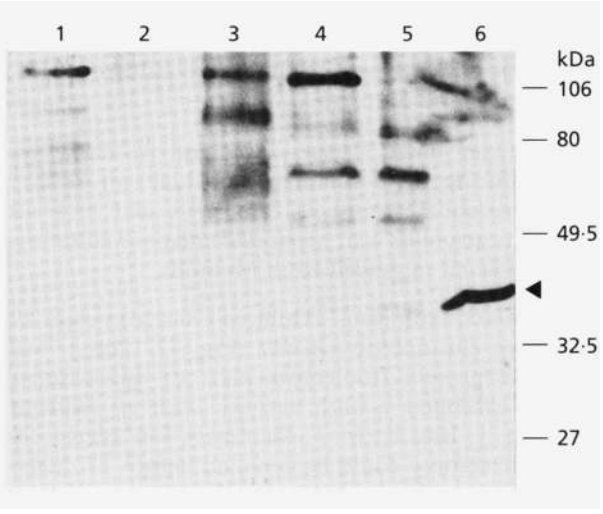

Fig. 5. Western blot analysis of SDS-PAGE (12% acrylamide) using rabbit polyclonal antibodies raised against CHIT 42, a purified endochitinase from *T. harzianum* strain P1. Lanes containing 100 μg protein are as in Fig. 1. The control lane (6) contains 20 μg extracellular chitinolytic enzymes obtained from *T. harzianum* which was grown in liquid SM with chitin as sole carbon source. The arrowhead indicates the position of CHIT 42.

Western blot analysis

Proteins from dual cultures of *T. harzianum* and *S. rolfsii* were analysed at various times (as in Fig. 1) by Western blot analysis using polyclonal antibodies raised against CHIT 73 (Haran *et al.*, 1995) (Fig. 4) and against a purified 42 kDa endochitinase from *T. harzianum* (Fig. 5). A positive reaction to the anti-CHIT 73 antibodies was detected as early as 24 h after contact had been made between the fungi (Fig. 4, lane 4). The reaction became stronger and more defined at 48 h (Fig. 4, lane 5). No reaction could be detected in *Trichoderma* before contact with its host (Fig. 4, lane 2). These results suggest that the 73 kDa chitinase present in this system is identical to the previously reported CHIT 73 against which the antibody was raised (Fig. 4, lane 6) (Haran *et al.*, 1995). During the parasitic interaction between *T. harzianum* and *S. rolfsii* in dual culture, the 42 kDa endochitinase was apparently either not expressed, or only expressed in small amounts. No reaction, or only a faint reaction, could be detected, even after 30 min exposure of the membrane (Fig. 5), confirming the results obtained from the chitinase activity gels.

Nonspecific reactions with both antibodies may result from the fact that proteins (100 μg per lane) were obtained from mycelium of both fungi grown in dual culture. Control lanes, containing 20 μg *T. harzianum* extracellular

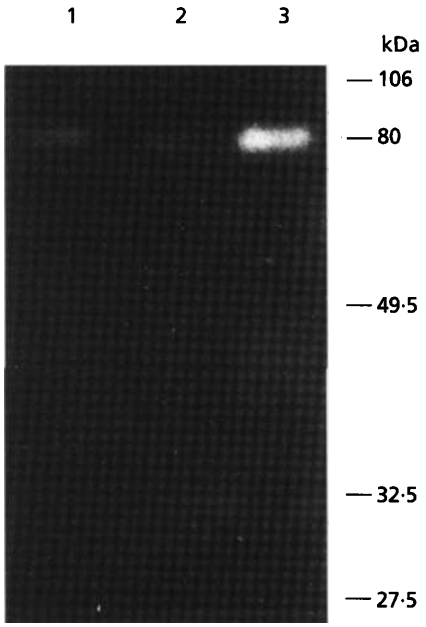

Fig. 6. Detection of chitinolytic activity in proteins (25 μg per lane) produced by *T. harzianum* grown in the biomimetic system in the presence or absence of variously treated nylon fibres. Lanes: 1, proteins obtained from *T. harzianum* grown on SM without nylon fibres; 2, proteins obtained from *T. harzianum* grown on SM, in the presence of untreated nylon fibres; 3, proteins obtained from *T. harzianum* grown in the presence of nylon fibres coated with a purified lectin from *S. rolfsii*. Detection was carried out using 4-MU-$(GlcNAc)_2$ as the substrate.

proteins, exhibited only a minor nonspecific reaction or none at all (Figs 4 and 5, lane 6).

Induction of chitinases in *T. harzianum* using the biomimetic system

To check whether the purified *S. rolfsii* lectin which serves as a recognition signal for *Trichoderma* during its interaction with *S. rolfsii* (Inbar & Chet, 1994) is also involved in the induction of chitinolytic enzymes in *Trichoderma*, *T. harzianum* was grown in Petri dishes with SM in the presence of the various nylon fibres as described in Methods. Proteins were produced and chitinolytic activity detected as before.

After 12 h incubation, a clear induction and increase in the activity of CHIT 102 was detected (Fig. 6, lane 3), as compared with the low constitutive activity in the control without nylon fibres (Fig. 6, lane 1). Growing *Trichoderma* in the presence of nylon fibres without lectin (Fig. 6, lane 2) resulted in no change in the activity of this enzyme as compared to the control. No induction of CHIT 102 could be detected when the purified *S. rolsfii* lectin, not bound to nylon fibres, was applied to colonies of *T. harzianum* via a Whatman paper saturated with the purified lectin (not shown). This observation suggests that the lectin serves as a signal only in association with the surface of the host's cell wall.

DISCUSSION

The involvement of fungal cell-wall-degrading enzymes (i.e. chitinases and 1,3-β-glucanases) in the mycoparasitic action of *Trichoderma* spp. towards phytopathogenic fungi has been demonstrated previously (Benhamou & Chet, 1993; Cherif & Benhamou, 1990; Elad *et al.*, 1982, 1983). Recently, Haran *et al.* (1995) reported the secretion of six different chitinolytic enzymes when *T. harzianum* (strain TM) was grown on liquid synthetic medium with chitin as sole carbon source. The present study was designed to determine which of the above described enzymes were active and how they were induced during mycoparasitism by *Trichoderma*. Chitinolytic enzymes were obtained from dual cultures of *T. harzianum* and *S. rolfsii* and detected in activity gels, as previously described by Haran *et al.* (1995). Our results revealed that as early as 12 h after contact between *T. harzianum* and *S. rolfsii*, activity of the constitutive 1,4-β-*N*-acetylglucosaminidase (CHIT 102; Haran *et al.*, 1995) is greatly increased. The presence of low constitutive activity of hydrolytic enzymes which are induced to increase their activity upon contact with the target has already been reported in several cases of host–parasite interactions (Benhamou *et al.*, 1990; Kolattukudy, 1985; Metraux & Boller, 1986). Kolattukudy (1985) suggested a model explaining the induction of the plant-cuticle-hydrolysing enzyme cutinase in a plant pathogenic fungal spore by monomers generated from the polymer (cutin). On the other hand, the expression of chitinase, which is present at basal levels in healthy plants, increases dramatically during pathogen attack (Benhamou *et al.*, 1990; Joosten & De Wit, 1988; Metraux & Boller, 1986). Twenty-four hours after contact between *T. harzianum* and *S. rolfsii*, CHIT 102 began to disappear and, concomitantly, CHIT 73 was activated. From then on, until the end of the incubation period, CHIT 73 was the major chitinolytic enzyme. This phenomenon was avoided by autoclaving *S. rolfsii* mycelium prior to its incubation with *T. harzianum*. In this case, as in the case of *T. harzianum* growing on SM with chitin as sole carbon source, CHIT 102 remained active up to 48 h after contact between *T. harzianum* and the sterile *S. rolfsii* mycelium. These results suggest that vital elements in the live mycelium of the host are responsible, at least in part, for this phenomenon. These might be proteases which are either already present or induced and released by *S. rolfsii* during the interaction with its antagonist *T. harzianum*. These putative proteases might digest CHIT 102 and at the same time activate CHIT 73. It was evident from the Western blot analysis that the 73 kDa chitinase appearing at 48 h is identical to the CHIT 73 described by Haran *et al.* (1995). Activation of chitinases (microsomal and cytosolic) by both endogenous and exogenous proteases has been reported for a number of fungal species (Adams *et al.*, 1993; Balasubramanian & Manocha, 1992; Yanai *et al.*, 1992). The apparent mechanism for chitinase regulation by proteolysis has been demonstrated by Yanai *et al.* (1992). Whether this is the case with the *Trichoderma–S. rolfsii* interaction and whether CHIT 102 is broken down to yield CHIT 73 are questions which warrant further research. Nevertheless, cycloheximide inhibited the

above-described phenomenon, suggesting that *de novo* synthesis of enzymes (chitinases and/or proteases) is required for its occurrence.

Recognition, attachment and coiling of *Trichoderma* around its host hyphae are early events, preceding host hyphal damage (Benhamou & Chet, 1993; Elad *et al.*, 1983; Inbar & Chet, 1994). Subsequently, a series of degradative events in the host begins (Benhamou & Chet, 1993; Elad *et al.*, 1983). Chitin oligomers released from the outer wall layers of the host were suggested to act as elicitors for synthesis of enzymes required for *Trichoderma* to penetrate the host cell wall (Benhamou & Chet, 1993; Elad *et al.*, 1982). Our results revealed that the induction of chitinolytic enzymes in *Trichoderma* during mycoparasitism is a very early event which is triggered by the recognition signal. Increased activity of CHIT 102 was detected when *T. harzianum* was grown on nylon fibres coated with the purified lectin of *S. rolfsii*. No chitin was present in this biomimetic system. Moreover, applying lectin which was not bound to fibres to colonies of *T. harzianum* revealed no increase in the activity of CHIT 102 as compared to controls. It is apparent from these and previous results (Inbar & Chet, 1994) that the recognition signal triggers differentiation processes in *Trichoderma* leading to the formation of infection structures concomitant with the induction of a chitinolytic enzyme which is needed for penetration of the host cell wall. Recently, the expression of *ech-42* (a gene encoding one of the endochitinases produced by *T. harzianum*) was found to be strongly enhanced during interaction with *Rhizoctonia solani*. Chitin as sole carbon source, as well as light, also induced high levels of translation of this gene, suggesting its developmental regulation (Carsolio *et al.*, 1994). Tunlid *et al.* (1992) suggested that in host–fungal-parasite interactions, following recognition, differentiation processes which lead to the formation of various infection structures are accompanied by nuclear division and cytoskeletal rearrangement. These processes take place simultaneously with the secretion of extracellular hydrolytic enzymes and adhesins (Tunlid *et al.*, 1992). This model was partially confirmed in our previous work (Inbar & Chet, 1994). A possible relationship between the cytoskeleton and chitin synthase regulation during cell-wall synthesis at the hyphal apex has been suggested by Wessels (1986). Adams *et al.* (1993) discussed the coordinated regulation of chitin synthase and chitinase and raised the question of whether cytoskeletal elements are also involved in the delivery and regulation of chitinases.

Schirmbock *et al.* (1994) demonstrated the parallel formation and synergism of hydrolytic enzymes and peptaibol antibiotics which are elicited in *T. harzianum* by cell walls of *Botrytis cinerea*. They suggested that the cascade of antagonistic events might be regulated by a common mechanism. Lora *et al.* (1994) investigated changes in gene expression patterns in *Trichoderma* elicited by pathogenic fungi. They speculated that oligosaccharides containing GlcNAc, which are generated by the partial degradation of fungal cell walls, act as elicitors which might trigger a general antifungal response in *Trichoderma*. It is evident from the present work that different chitinases are activated at different times during mycoparasitism by *Trichoderma*. Based on our results, we suggest that an earlier event, namely the recognition event which is mediated by lectin–carbohydrate interactions, serves as a signal that triggers a general parasitic response in *Trichoderma*. This response consists of morphological changes (i.e. coiling, appressorium formation, etc.) which involve cytoskeletal rearrangements and nuclear division. At the same time, induction of cell-wall-hydrolysing enzymes (e.g. chitinases) and changes in other related proteins, e.g. induction of cell-wall proteins (Lora *et al.*, 1994), or suppression of others (Goldman *et al.*, 1992), take place. Chitin oligomers or monomers may regulate further expression of the system as the process progresses.

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