Ultrastructural Investigation of the Mycoparasitic Interaction between *Stachybotrys elegans* and its host *Rhizoctonia solani*

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Stachybotrys elegans, a soil-borne fungus, has been described as a destructive mycoparasite of *Rhizoctonia solani* [1]. Detailed light and ultrastructural studies of *R. solani* hyphal and sclerotial cells parasitized with *S. elegans* revealed that parasitism of host cells was characterized by hyphal attachment and enhanced production of extracellular fibrillar material during contact, penetration of host cell walls and proliferation of trophic hyphae within the host cytoplasm [2].

S. elegans produces two exo- and one endo-acting chitinases when grown on chitin. We purified to homogeneity one of the exo-acting chitinases, β-*N*-acetylhexosaminidase, and partially characterized its physical and biochemical properties. The native enzyme has a molecular mass of 120 kDa when determined by gel filtration, and 68 kDa by SDS-PAGE which suggests that the protein may occur as a dimer in solution. The purified β-*N*-acetylhexosaminidase is most active at pH 5.0 and at 40° C; it hydrolyzed the ρNP-*N*-acetyl-β-D-glucosaminide with apparent K_m of 84.6 μM. Polyclonal antibodies raised against the 68 kDa β-*N*-acetylhexosaminidase (NAG-68) indicated that the antibody is highly specific and recognizes the protein in crude filtrate preparation (Fig. 1) [3].

Transmission electron microscopy observations of areas sampled from the interaction zone of both fungi in dual culture revealed that *S. elegans* penetrated *R. solani* thick hyphal cell walls and the cytoplasm of the host cells was in an advanced state of disintegration (Fig. 2). Interestingly, in sections sampled from areas where both fungi were in close vicinity but not in direct interaction, *R. solani* cytoplasm was highly altered (data not shown). This indicates that the mycoparasite can affect its host in advance to penetration. At penetration sites (Fig. 3), the extent of the extracellular fibrillar material surrounding S. elegans cells was often abundant. In order to demonstrate that an exo-acting chitinase of *S. elegans* is involved in the degradation process of *R. solani*, we carried out immunocytochemical investigation using the characterized antibody described here. These studies are complemented with other cytochemical tests for the localization of chitin.

References

[1] Benyagoub M. *et al*. 1994. Mycological Research 98:493-505.

[2] Benyagoub M. *et al*. 1996. Mycological Research 100:79-86.

[3] Taylor et al. 2002. Purification and characterization of an extracellular exochitinase, β-*N*-acetylhexosaminidase from the fungal mycoparasite *Stachybotrys elegans*. Can. J. Microbiol. (in press).

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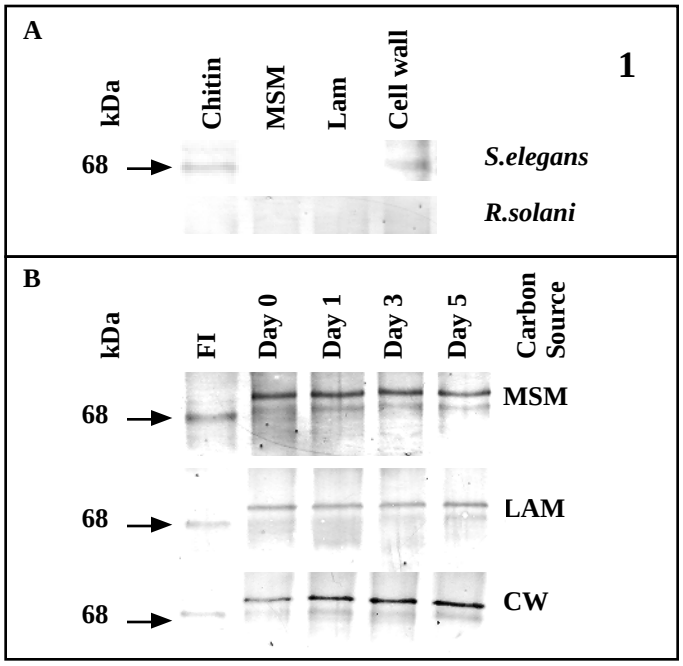

Figure 1: Western blot analysis of *S. elegans* and *R. solani* interactions using a polyclonal antibodies raised against the purified 68-kDa exochitinase. (A) Intercellular proteins (250 ng/lane) extracted from *S. elegans* and *R. solani* grown on MSM supplemented with different carbon sources ($0.5 g.L^{-1}$). Chitin (CHT), laminarin (LAM), *R. solani* cell wall fragments (CW), or no carbon source (MSM). (B) Intercellular proteins extracted from the interaction zone of *S. elegans* and *R. solani* in dual culture plates 0,24, 72 and 120 h after contact. NAG-68kDa in fraction F I (240 ng /lane)

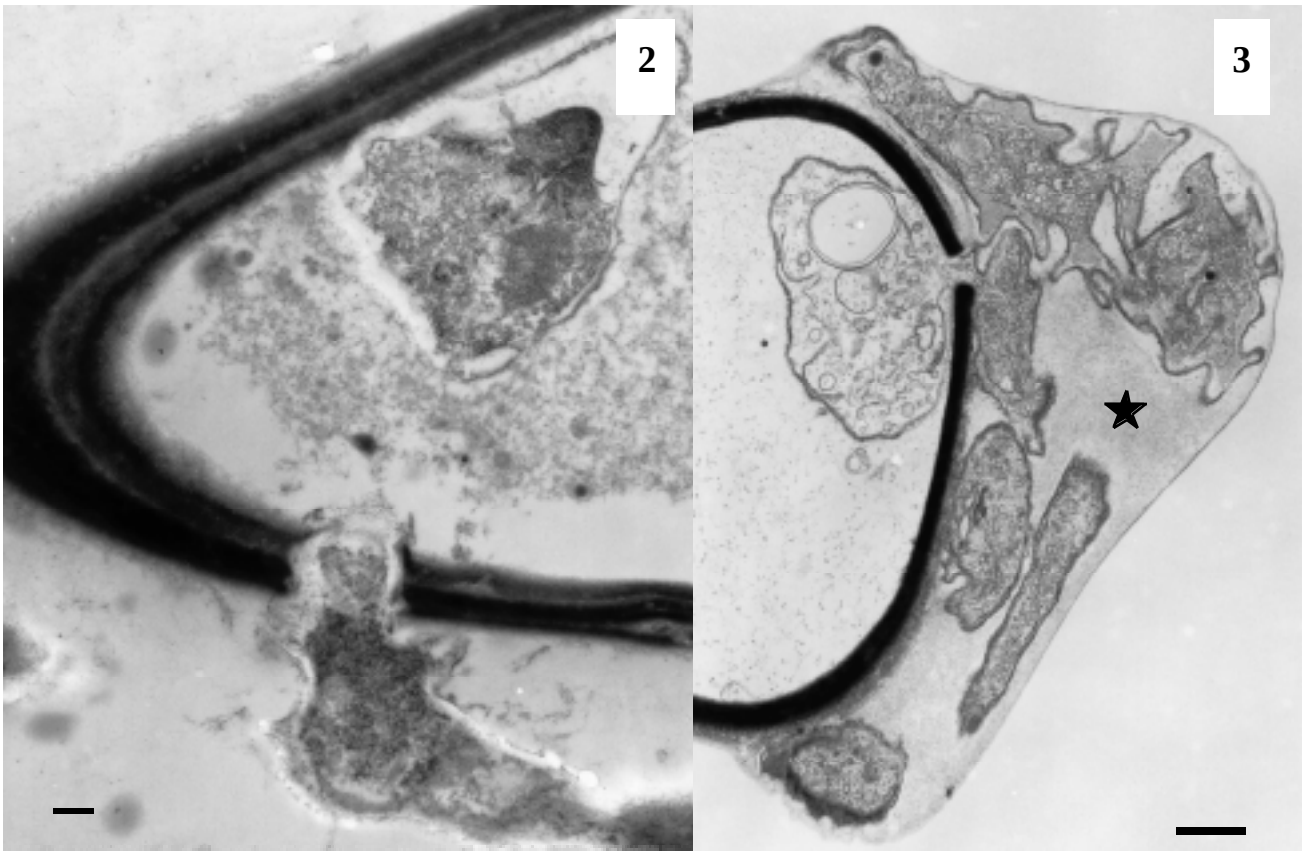

Figure 2: Penetration of *R. solani* hypha by *S. elegans*. The host cytoplasm is severely altered, no organelle is discernible. Scale bar = 500 nm. Figure 3: Moribund hypha or *R. solani* that has been penetrated by *S. elegans*. The cytoplasm of the host cell is desintegrated. The *S. elegans* hyphae of unusual form are included in a fibrillar matrix (∗) outside the hypha of *R. solani*. Scale bar = 1 μm.

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