Biomimics of Fungal Cell-Cell Recognition by Use of Lectin-Coated Nylon Fibers

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When the mycoparasitic, biocontrol fungus Trichoderma harzianum was allowed to grow on nylon fibers treated with concanavalin A or Sclerotium rolfsii lectin, it coiled around the nylon fibers and produced hooks in a pattern similar to that observed with the real host hyphae. The incidence of interaction between T. harzianum and S. rolfsii lectin-treated fibers was significantly higher than that of the controls (untreated or blocked activated fibers). These findings provide direct evidence for the role of lectins in mycoparasitism.

Several species of fungi from the genus Trichoderma are very potent biocontrol agents of some soil-borne plant-pathogenic fungi (4). Direct parasitism of Trichoderma spp. on live mycelium of other fungi has been reported. Trichoderma organisms coil around the host hyphae, produce hooks and appresorium-like bodies, and penetrate the host cell wall (5-8).

Twenty years ago, Dennis and Webster (6), using plastic threads of a diameter similar to that of Pythium ultimum hyphae, concluded that the coiling of Trichoderma organisms was not merely a contact stimulus. The Trichoderma hyphae never coiled around the threads but rather grew over or followed them in a straight course.

Recently, LSR, a lectin produced by the soil-borne plant pathogen Sclerotium rolfsii, was purified and partially characterized (1a, 3). The ability of different strains of Trichoderma to attack S. rolfsii was correlated with the agglutination rate of their conidia, which is caused by this lectin. This suggests that lectins play a role in the interaction and recognition between Trichoderma spp. and soil-borne plant-pathogenic fungi (3). However, clear evidence is required.

We hereby report a system, based on covalent binding of lectins onto a surface of nylon fibers, which mimics the host fungus hyphae and enables the examination of the role of lectins in mycoparasitism.

Concanavalin A (Con A) is a plant lectin obtained from Concanavalia ensiformis (jack bean). It binds to d-glucose and d-mannose and is similar to LSR in both carbohydrate binding and antigenicity (1). We therefore used Con A type III (Sigma) to establish the system. Fibers of nylon 66 (13-μm approximate diameter; kindly supplied by Nilit, Migdal-Haemek, Israel) were extended on polyester grids (7 by 7 mm, 420-μm pore size) and their edges were fastened with cyanoacrylate glue (Loctite). Covalent binding of lectins to the nylon fibers was carried out, under sterile conditions, by the two-step activation method (9) with a few modifications. Fibers were treated by refluxing in 0.5 N sulfuric acid in methanol for 2.5 h at 64°C. Fibers were then washed in distilled water and in 1% NaHCO3 for 90 min and then placed in glutaraldehyde (12.5%) for 1 h. The lectin (2 mg of protein per ml of a 0.1 M sodium borate solution [pH 8.5] as a coupling buffer) was then allowed to react with the activated fibers for 4 h at 25°C under continuous stirring.

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FIG. 1. Microscopic appearance of nylon fibers treated with FITC-Con A. Fibers were washed with cold phosphate-buffered saline and observed with a standard RA Zeiss fluorescence microscope, using filter set 18. (a) Untreated fibers; (b) FITC-Con A-treated fibers.
After the fibers were washed in distilled water and in 1% KCl for 1 h (to eliminate possible adsorbed lectin), reduction was carried out with NaBH₄ (0.5 mg/ml of phosphate-buffered saline [pH 7.4]) for 3 h at 25°C in order to stabilize the lectin-nylon complex and block free active sites on the nylon (11). Fluorescein isothiocyanate-conjugated Con A (FITC-Con A; Bio-Makor, Rehovot, Israel) was applied to ascertain binding of the lectin onto the fibers (Fig. 1).

*Trichoderma harzianum* was grown aseptically on the nylon fibers in a petri dish containing water agar supplemented with glucose (0.5%, wt/vol). After 24 h in the dark (to prevent conidiation), fibers were removed and examined under a scanning electron microscope. *T. harzianum* apparently recognized the Con A-treated fibers as the host hyphae. It attached to the fibers and, in response, coiled and formed hooks around them (Fig. 2). Incubation with methyl-α-D-mannopyranoside inhibited the interaction of *Trichoderma* hyphae with the Con A-treated fibers (data not shown). These results strongly support the idea that LSR may be involved in the recognition between *Trichoderma* spp. and *S. rolfsii*. Moreover, the observation suggests that LSR on the cell surface of *S. rolfsii* probably induces the formation of fungal structures involved in mycoparasitism, namely, hyphal coils and hooks. To test this hypothesis, LSR was separated and lyophilized (1a) from the culture filtrate of the fungus after 5 days of incubation in synthetic medium (12) (SM). This preparation, consisting mainly of LSR (about 85%, and a few other minor proteins with no agglutination activity, as previously reported by Barak and Chet (1a)), was used for further studies.

An experiment was carried out by incubating *T. harzianum* with LSR-treated fibers, blocked activated fibers (blocking was carried out by reduction with NaBH₄, as previously described), or untreated fibers, as described above. Scanning electron micrographs show clear differences between the interaction of *T. harzianum* with LSR-treated fibers and the control (Fig. 3 and 4). In the control treatments, *T. harzianum* could grow uninterruptedly across and along the fibers (Fig. 3a and 4a), supporting the observations of Dennis and Webster (6). On the other hand, wherever the *Trichoderma* hyphae attached to the LSR-treated fibers, it coiled around the fibers, produced hooks, and altered its growth direction (Fig. 3b and 4b).

The incidence of interaction, determined as the number of coils produced by the *Trichoderma* organisms per millimeter of nylon fiber, was significantly higher with the LSR-treated fibers than with the blocked activated fibers or the untreated control (Fig. 5).

Many discussions and speculations were reported on the possible role of lectins in microbial interactions (10) and in mycoparasitism (1a, 3, 7). Our results reveal that treatment of nylon fibers with lectins can imitate living hyphae and change the behavior and morphogenesis of filamentous fungi. This phenomenon may clarify the role of lectins in cell-cell surface recognition during mycoparasitism.

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FIG. 3. Scanning electron micrograph of *T. harzianum* mycelium grown on nylon fibers. (a) Untreated fibers; (b) LSR-treated fibers. Coils formed by *T. harzianum* can be observed in many places (arrows) when the fibers are treated with the lectin, whereas no specific interaction is seen with the untreated fibers. Bars, 100 µm.
FIG. 4. Scanning electron micrograph of *T. harzianum* mycelium grown on nylon fibers. (a) Untreated fibers; (b) LSR-treated fibers. In the untreated control, the fungus hyphae grew across or along the fiber without interaction. Coating the fibers with LSR stimulated *Trichoderma* coil formation. Bars, 10 μm.
FIG. 5. Incidence of interaction between T. harzianum and the various nylon fibers. Evaluation was carried out by counting the number of coils produced by T. harzianum after each treatment in 20 different fields (200 by 150 μm) under the scanning electron microscope. The number of the coils was then correlated to the total length of fibers present at each field. Treatments: untreated fibers (control); blocked activated fibers (BAF); LSR-treated fibers (LTF). Bars represent standard errors.

REFERENCES

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