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Adhesion of Conidia of *Drechmeria coniospora* to *Caenorhabditis elegans* Wild Type and Mutants

HANS-BÖRJE JANSSON[1]

Abstract: Adhesion of conidia of the endoparasitic fungus *Drechmeria coniospora* to the cuticles of the wild type and four different head defective mutants of *Caenorhabditis elegans*, and subsequent infection, was studied. The conidia adhered around the sensory structures in the head region, vulva, and occasionally to other parts of the cuticle in both mutant and wild type hosts. Infection took place after adhesion to the head region by penetration through the cuticle, and, following adhesion around the vulva, through the natural orifice. Infection was not observed after adhesion to other parts of the cuticle. Adhesion was reduced after treatment of the nematodes with Pronase E. Adhesion returned towards normal again within 2 hours, indicating that the proteinaceous material emanating from the sensory structures was rapidly replaced.

Key words: adhesion of conida, biological control, *Caenorhabditis elegans*, cuticle, *Drechmeria coniospora*, endoparasite, infection, mutant, nematode, nematophagous fungus, penetration, Pronase E, proteolytic enzyme, sensory structure.

The endoparasitic nematophagous fungus *Drechmeria coniospora* (Drechsler) W. Gams & Jansson infects a narrow range of nematodes (9,12). The conidia of the fungus adhere by means of an adhesive bud to the bacteriovorous nematode *Panagrellus redivivus* at the chemosensory structures in the head region, amphids and inner labial papillae of juveniles as well as adults, and to the caudal papillae of male specimens (13). After contact the conidia form an appressorium and a thin infection peg that penetrates the nematode cuticle close to the sensory pores. Trophic hyphae are then formed and digest the nematode (5, 10,13). The signals involved in adhesion and infection of nematodes are suggested to be mediated by proteins on the adhesive bud of the conidia and in the proteinaceous matrix materials emanating from the sensilla pores (9). The involvement of these exudates in nematode chemotaxis has been discussed by several authors (1,2, 7,18), although the exact mechanism is still unknown.

In the nematode *Caenorhabditis elegans*, several mutants with various defects in the amphids as well as other parts of the head are available (17). By studying adhesion and subsequent penetration of *C. elegans* in the wild type and in mutants, it might be possible to find variations in the infection patterns. In this paper I describe results obtained in such an investigation.

Materials and Methods

The fungus *Drechmeria coniospora*, designated isolate no. 5 by Jansson (9), was grown in 2% Malt Extract Broth (Oxoid) on a rotary shaker at 100 rpm and at ambient room temperature (ca. 22 C) for 5–8 weeks. In this way the conidia were suspended in the liquid medium and were easy to separate from the hyphal pellets. The conidia were harvested and washed in sterile distilled water before use.

The *Caenorhabditis elegans* wild type and mutants (see Table 1) were obtained from the *Caenorhabditis* Genetics Center, founded by the NIH National Center for Research Resources (NCRR). The nematodes were maintained on Nigon's agar with *Escherichia coli* OP-50 (17). Before use in the various experiments, the nematodes were washed several times in sterile water.

For adhesion experiments, a conidial suspension (ca. 10^9 conidia/ml) was spread onto the surface of a 1.5% water agar plate. After the surface of the agar dried, 100–200 nematodes were added to the plates. After 2 hours, nematodes were washed off the plates and adhesion of

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[1]Department of Microbial Ecology, Lund University, Ecology Building, S-223 62 Lund, Sweden.
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TABLE 1. Characterization of *Caenorhabditis elegans* mutants (17).

Strain	Gene	Description
CB3332	che-12	Weak FITC uptake by amphids and phasmids; amphid sheath cells fail to secrete matrix material; defective osmotic avoidance.
CB3687	che-14	Some FITC uptake by amphids but not by phasmids; abnormal uptake by CEP, ADE, PDE; abnormal amphid channel due to misjoining of sheath and socket cell.
CB1066	mec-1	Touch insensitive, lethargic; microtubule cells lack extracellular mantle, often displaced; some amphidial neurons also displaced.
CB648	vab-1	Notched head, variable dystrophy of ventral cephalic region, especially in L1.

conidia to the nematodes was observed. Nematodes with conidia adhering to the mouth region, vulva, and other parts of the nematode surface, as well as nematodes without conidia, were counted. The nematodes were separated into adults, 3rd- and 4th-stage juveniles, and 1st- and 2nd-stage juveniles. Approximately 100 nematodes of each category were counted, and the experiments were repeated three times. Alternatively, nematodes with adhering conidia were hand-picked, added to microscope slides, and observed and photographed using a Nikon Optiphot II microscope equipped with interference phase contrast optics.

Protease treatment of the nematodes was performed with 700 units/ml of Pronase E (Merck) in 10 mM Tris-HCl buffer, pH 7.5, for 1 hour at 30 C as described by Jansson (9). The numbers of conidia adhering to treated and untreated nematodes were compared. The reappearance of normal adhesion behavior was studied on nematodes kept in the Tris buffer for various lengths of time following treatment with Pronase E. Approximately 100 nematodes of each category were counted, and the experiments were repeated three times. Heat denatured (95 C, 15 minutes) enzyme had no effect.

RESULTS

Adhesion of conidia: The conidia of *D. coniospora* adhered to all mutants and wild type *C. elegans* (Table 2). About half of the nematodes had spores adhering to their surfaces, which is less than usually found with, for instance, *P. redivivus*. The conidia usually adhered at the head region (Table 2). There were no differences between conidial adhesion to juvenile stages and to adults.

All nematodes were infected near the mouth region (Fig. 1A), and hermaphrodites also were infected near the vulva (Fig. 1B). Infection occurring at the vulva appeared to proceed via the natural opening (Fig. 1B); in contrast, head infection always seemed to occur via the cuticle. When infection took place through the cuticle, appressorium and the infection peg were often observed (Fig. 1A).

The mutant CB3332, which lacks amphidal matrix production (Table 1), became infected in the head region like the other mutants tested. The adhesion and infection of CB3332 generally appeared to take place at the tip of the lips (Fig. 1A).

The mutant CB648 (mouth is slightly misplaced, Fig. 1C), became infected around the mouth region, as in the other mutants and in the wild type, suggesting that the sensilla structures are intact. Adhesion of conidia and infection in the vulva also occurred (Fig. 1C).

Large adult nematodes were capable of ingesting many conidia, filling the entire digestive system with spores (Fig. 1D). Apparently the conidia become ground in the basal bulb; the conidia cannot grow inside the nematode or after defecation.

Pronase E treatment: When the nematodes were treated with the proteolytic enzyme, the adhesion of conidia was reduced by 40–60% compared with untreated controls (Fig. 2). When the nematodes were washed and left in the buffer for various times after pronase treatment, adhesion

TABLE 2. Adhesion of *Drechmeria coniospora* conidia to wild type and mutant strains of *Caenorhabditis elegans*.†

C. elegans	Head	Body	Vulva	No spores
		Wild type		
Adults	33.3 ± 12.4	6.7 ± 2.8	10.8 ± 5.3	49.3 ± 11.3
3L-4L	51.4 ± 10.0	4.8 ± 1.5		47.2 ± 9.5
1L-2L	44.2 ± 8.9	2.9 ± 1.2		53.0 ± 9.4
		648		
Adults	27.4 ± 4.7	13.5 ± 5.7	18.9 ± 5.8	40.3 ± 9.9
3L-4L	27.1 ± 5.3	16.9 ± 5.3		56.0 ± 7.1
1L-2L	22.2 ± 5.2	1.8 ± 1.2		76.0 ± 5.6
		1066		
Adults	19.2 ± 5.9	13.9 ± 7.0	12.8 ± 10.5	54.1 ± 11.9
3L-4L	33.5 ± 6.5	13.1 ± 5.4		53.4 ± 5.3
1L-2L	31.1 ± 8.2	19.7 ± 5.3		49.3 ± 8.4
		3687		
Adults	34.5 ± 10.3	14.0 ± 12.8	2.0 ± 1.8	51.5 ± 12.5
3L-4L	26.8 ± 6.3	17.4 ± 12.7		55.8 ± 10.2
1L-2L	40.9 ± 9.6	12.2 ± 10.6		47.0 ± 9.1
		3332		
Adults	53.9 ± 9.3	43. ± 2.7	2.1 ± 0.9	39.6 ± 8.8
3L-4L	46.0 ± 8.0	10.6 ± 5.2		43.4 ± 8.7
1L-2L	42.3 ± 10.3	6.3 ± 2.5		51.4 ± 10.2

† Percentage of nematodes with conidia adhering (mean ± standard error).

behavior returned towards normal again within 2.5 hours (Fig. 2). Heat denatured enzyme had no effect on nematode behavior (data not shown). No differences were observed between the wild type *C. elegans* and the mutants.

DISCUSSION

The *C. elegans* wild type and the various mutants used in this study did not differ in their pattern of conidia adhesion and infection by *D. coniospora*. There was a lower attachment and infection level than usually found in *P. redivivus*, which is often 75–100% (12). A similar low degree of infection was also reported to occur with *D. coniospora* on the nematode *Acrobeloides buetschilii* (6). As in many other nematode species, the conidia adhering to the head region were capable of infecting the nematodes. In females of *P. redivivus* we have never observed infection to take place in the vulva region, as was the case with *C. elegans* hermaphrodites. In contrast to the cuticular penetration in the head region it seemed that infection in the vulva region occurred through the natural opening, which probably constitutes the easiest way to enter the host. The penetration of the head region of *P. redivivus* always proceeded through the cuticle (5).

The consumption of large amounts of conidia by adult *C. elegans* has also previously been shown to occur with large specimens of *P. redivivus* (14). Apparently the nematodes are capable of crushing the conidia (14), together with bacteria in the basal bulb, and might obtain nutrients in this way.

In the mutant CB3332, which lacks amphidial matrix production, adhesion of the conidia took place at the tip of the lips, which is the location of the inner labial papillae (16). These results indicate that the sensilla exudates from the inner labial papillae may be as important for adhesion of the conidia as are the amphidial exudates. The apparently normal adhesion and infection to the CB648 mutant indicates that the defects of this mutant are not important for adhesion of conidia and infection.

The treatment of *C. elegans* with Pronase E resulted in a reduction of the conidial adhesion indicating that the proteinaceous material surrounding the sensilla pores is digested. Similar results were also obtained

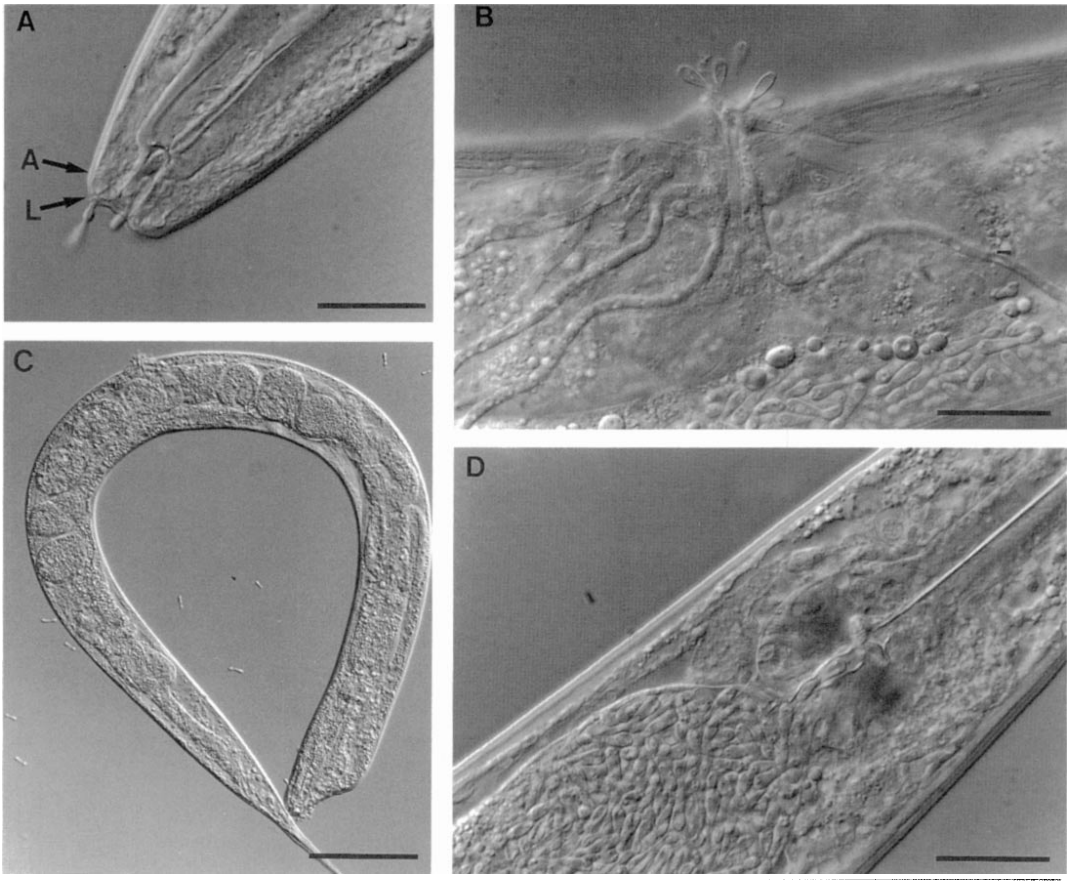

FIG. 1. Adhesion and infection of *Caenorhabditis elegans* wild type and mutants by conidia of *Drechmeria coniospora* after ca. 24 hours. A) Adhesion of conidia to mutant CB3332 at the head region, showing early infection. Note inner labial papilla (L) and amphid (A). B) Infection of wild type *C. elegans* at the vulva region. C) Infection of mutant CB648 with head defect. Conidial adhesion takes place around the mouth, which is slightly misplaced, and at the vulva. D) Mutant CB1066 adult with oesophagus filled with conidia of *D. coniospora*. Note conidium in the basal bulb of the pharynx. Scale bar is 10 μm in A, B, and D, and 50 μm in C.

with *P. redivivus* (9). When the nematodes were washed and left in buffer after the protease treatment the nematodes responded almost normally after less than 2.5 hours. These results show that the sensilla exudates, which are purportedly important for conidial adhesion, are renewed either by new production or by excretion of preformed matrix material. The function of these exudates is still a matter of speculation (3). In animal parasitic nematodes, changes in the nematode cuticle may take place between 10 minutes and 5 hours after entrance of their hosts, depending on species (15). The production, excretion, and importance of a surface coat, glycocalyx, or accessory layer is still a matter of controversy (4). Such a surface coat may be important in infection of many nematophagous fungi, but in *D. coniospora* it is probable that adhesion is mediated, not by the surface coat, but by sensilla exudates, which may adhere to the cuticle in a manner similar to the surface coat.

The involvement of glycoproteins in nematode chemotaxis was shown by Jansson et al. (11), where chemotaxis was inhibited after treatment of *C. elegans* and *P. redivivus* with trypsin and two carbohydrate-splitting enzymes. After trypsin treatment, the nematodes resumed normal

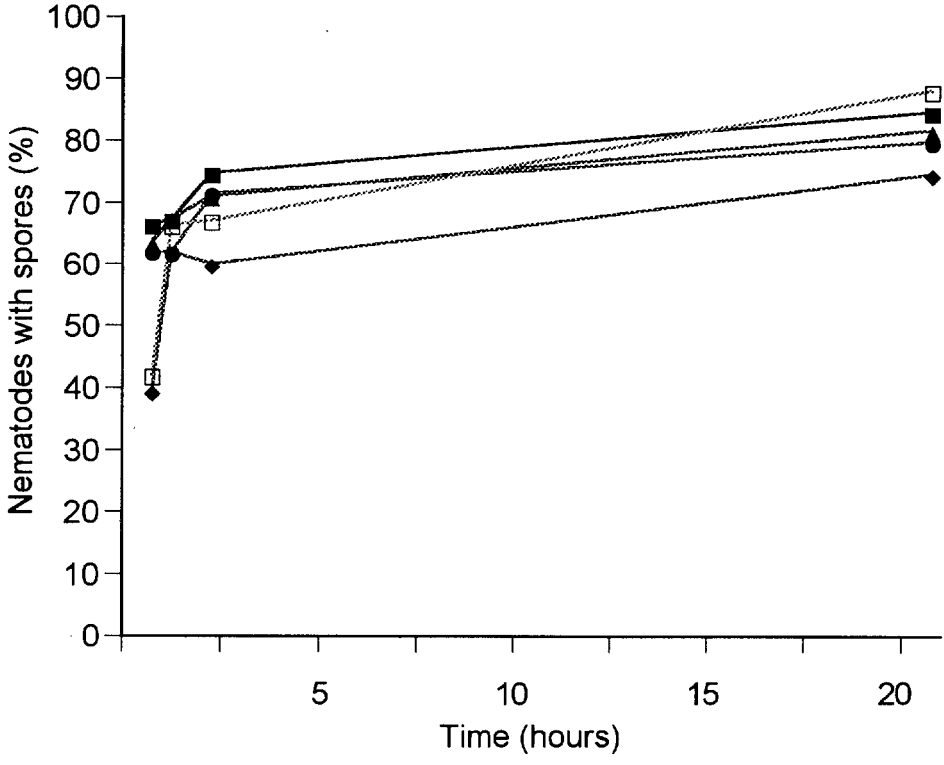

FIG. 2. Adhesion of conidia of *Drechmeria coniospora* to wild type and mutants of *Caenorhabditis elegans* after treatment of the nematodes with Pronase E. Values are relative to untreated controls (=100%) and give the percentage of nematodes with spores. Each point in the diagram is the mean of three replicates. For clarity, the standard errors are not included in the diagram. The standard errors vary from 0.7–14.5 at the various points. ■ = wild type, □ = CB3687, ♦ = CB1066, ▲ = CB3332, ● = CB648.

chemotactic behavior within 4–8 hours. In these chemotaxis experiments, it was suggested that proteolytic treatment interfered with the chemoreceptors and thus temporarily reduced chemotaxis (8,11). The similarities between the effects of the proteolytic enzymes indicate that the effects on chemotaxis may also have been due to hydrolysis of matrix material in the sensilla pores and in that way reduced the chemotactic ability of the nematodes. Investigations of the importance of the matrix material in the adhesion of conidia will continue by isolation and determination of this material as well as the proteinaceous material on the adhesive bud of the conidia. This work is now in progress.

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