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Differential Adhesion and Infection of Nematodes by the Endoparasitic Fungus *Meria coniospora* (*Deuteromycetes*)

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The conidia of the endoparasitic fungus *Meria coniospora* (*Deuteromycetes*) had different patterns of adhesion to the cuticles of the several nematode species tested; adhesion in some species was only to the head and tail regions, on others over the entire cuticle, whereas on others there was a complete lack of adhesion. After adhesion, the fungus usually infected the nematode. However, adhesion to third-stage larvae of five animal parasitic nematodes, all of which carry the cast cuticle from the previous molt, did not result in infection. *M. coniospora* infected animal parasitic nematodes when this protective sheath was removed. Seven preparations of sialic acid (*N*-acetylneuraminic acid) gave three types of response in adhesion-infection of nematodes: (i) a significant reduction in conidial adhesions; (ii) no interference with adhesion, but a 10-day delay in infection; and (iii) a delay in infection by 2 to 3 days. The current results support previous findings indicating involvement of sialic acids localized on nematode cuticles in recognition of prey by *M. coniospora*.

Most endoparasitic nematophagous fungi are obligate parasites and spend their entire vegetative lives within infected nematodes. Only the conidiophores, which penetrate through the nematode cuticle and emerge to the surrounding environment, and the conidia exist outside the nematode corpus. The conidia of *Meria coniospora*, like those of the majority of endoparasitic nematophagous fungi, adhere to the surface of the nematode cuticle. When the conidia mature they produce a bud at the distal end, and they stick to the cuticle by means of this bud. A mucus or adhesive covering the conidial buds has been visualized with transmission and scanning electron microscopy (10; H.-B. Jansson, A. van Hofsten, and C. van Mecklenburg, Antonie van Leeuwenhoek, J. Microbiol. Serol., in press). The first step of the infection process (conidial adhesion) was studied by Jansson and Nordbring-Hertz (3) with three bacteria-feeding and two plant-parasitic nematodes. For one of the bacteria feeders, *Panagrellus redivivus*, it was shown that *M. coniospora* conidia adhered only to the cephalic region of females, males, and larvae and also to the tails of the males. The attachment sites appeared to be on or close to the nematode chemosensory structures. The two plant parasites, *Aphelenchoides fragariae* and *Ditylenchus destructor*, were also infected in the mouth region, but conidia frequently attached over the whole cuticle.

In experiments designed to inhibit adhesion, the conidia of *M. coniospora* were treated with different sugar haptens. Of 21 carbohydrates tested, only sialic acid caused significant inhibition of conidial adhesion to *P. redivivus* (3). Furthermore, treatment of *P. redivivus* with the enzyme sialidase or the sialic acid-specific lectin limulin resulted in reduced conidial adhesion, indicating the importance of sialyl moieties for specific binding of *M. coniospora* to the nematode cuticle. Treatment of the conidia with trypsin also reduced adhesion, suggesting the involvement of a protein in conidial adhesion; a lectin binding specifically to sialic acid was proposed (4).

In the current study we present further information on *M. coniospora* adhesion and infection of different nematode species and also on the differential effects on infection of several sialic acid preparations.

MATERIALS AND METHODS

Organisms. *M. coniospora* Drechsler (CBS615.82) was cultivated on diluted corn meal agar plates (CMA 1:10; 1.5% agar; Difco Laboratories). Conidia were collected by flooding the plates with sterile water or 3-*N*-morpholinepropanesulfonic acid buffer (pH 7.2; Sigma Chemical Co.). The bacterial-feeding nematodes *P. redivivus* and *Caenorhabditis elegans* were cultivated axenically in a heme medium (5) or in a liver medium (15). Nematodes were harvested with a Baermann funnel and washed several times by alternate centrifugations (5,900 × *g*, 1 min) and buffer changes before use in the experiments. The animal parasitic nematodes *Ostertagia ostertagi*, *Ostertagia circumcincta*, *Nematospiroides dubius*, *Haemonchus contortus*, and *Trichostrongylus colubriformis* were kindly supplied by Gerald Coles, Department of Zoology, University of Massachusetts, Amherst. *Bursaphelenchus xylophilus* was a gift from Anne Dorrance of this department. Other nematode species (Table 1) were collected from soil or plant samples and extracted by the Baermann funnel technique.

The animal parasites were all tested as infective third-stage larvae, which are encased in the cast cuticle of the second molt. To remove the cast cuticle from third-stage *N. dubius*, the nematodes were treated in 0.2% sodium hypochlorite for 15 min (2), and the exsheathing was observed under dark-field illumination. The exsheathed larvae were then used in the infection experiments. First-stage larvae of *N. dubius* from newly hatched eggs were also examined in adhesion-infection studies.

Adhesion and infection. The techniques for studying infection of nematodes by *M. coniospora* were previously reported (3) and will only be briefly described here. To study adhesion of conidia or infection of the nematodes, ca. 10^7 conidia in water were spread on 5-cm plates of 1.5% water agar. After evaporation of the liquid, 10 to 500 nematodes in water or buffer were introduced to the plates. After 1 to 2 h, the nematodes were either hand picked or washed off the plates, and adhesion of conidia was observed under a light

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TABLE 1. Adhesion to and infection of 17 nematode species by *M. coniospora*

Adhesion group[a]	Infection[b]
Adhesion in cephalic region and tail (group 1)	
Panagrellus redivivus[c] (B)	+
Caenorhabditis elegans (B)	+
Anguina tritici (P)	+
Meloidogyne incognita (P)	+
Meloidogyne javanica (P)	ND
Aphelenchus avenae (P)	ND
Ostertagia ostertagi (A)	−
Ostertagia circumcincta (A)	−
Haemonchus contortus (A)	−
Trichostrongylus colubriformis (A)	−
Adhesion over entire cuticle (group 2)	
Pratylenchus penetrans (P)	ND
Ditylenchus destructor[c] (P)	ND
Aphenelchoides fragariae[c] (P)	ND
Criconemella xenoplax (P)	−
Nematospiroides dubius (A)	−
No adhesion (group 3)	
Xiphinema americanum (P)	−
Bursaphelenchus xylophilus (P)	−

[a] B, Bacteriophagous; P, plant parasitic; A, animal-parasitic nematodes (third-stage infective larvae).
[b] +, Infection; −, no infection; ND, not determined.
[c] Data from reference 3.

microscope at 100 to 200×. The sites of conidial attachment to the nematodes were recorded as follows: group 1, adhesion only to the mouth and tail; group 2, adhesion over the entire cuticle; group 3, no adhesion. A number of nematodes were left on the plates and observed daily for an interval of 10 days to establish whether infection of the nematode occurred.

Sialic acid specificity. The effect of sialic acid treatment of the conidia on adhesion to and infection of *P. redivivus* was investigated with the same techniques. The sialic acids, designated preparations A through G, were all *N*-acetylneuraminic acid from two companies and were of different origins as follows: A, E. Merck AG, Darmstadt, Federal Republic of Germany, product no. 24800 (>99% purity, synthetic); B, Sigma type II (60 to 80% purity, from egg); C, Sigma type III (90% purity, from egg); D, Sigma type IV (95% purity, synthetic); E, Sigma type VI (98% purity, from *Escherichia coli*); F, Sigma type VII (98% purity, from human urine); G, Sigma type VIII (98% purity, from sheep submaxillary gland). The conidia of *M. coniospora* were treated with the different *N*-acetylneuraminic acids at a concentration of 50 mM in 3-*N*-morpholinepropanesulfonic acid (pH 7.2, 5 mM) overnight. Conidia in buffer without sialic acid treatment served as controls. After incubation the conidia were added to water agar plates, and adhesion and infection of *P. redivivus* were studied as previously described.

C. elegans was also used to determine whether certain sugar haptens inhibited conidial adhesion. These studies with *M. coniospora* conidia paralleled those previously reported with *P. redivivus* (3) and were performed to determine whether response differences occurred between the two nematode species. The haptens tested were *N*-acetylneuraminic acid preparations A, B, and E, D-(+)-galactose, α-methyl-D-mannoside, D-(+)-glucosamine hydrochloride, L-(−)-sorbose, α-L fucose, *N*-acetyl-D-glucosamine, 3-O-methyl-α-D-glucopyranose, β-D-fructose, α-(+)-mannose, D-arabinose, *N*-acetyl-D-galactosamine, α-D-(+)-glucose, 2-deoxy-D-galactose, *N*-acetyl-D-mannosamine, and 2-deoxy-D-glucose.

Sialic acid displacement. To investigate whether the sialic acid bound to the conidia could be displaced, conidia were pretreated in three different ways before infection of *P. redivivus* as follows: (i) treated with sialic acid preparation B for 24 h and added directly to water agar plates followed by the nematode infection test; (ii) treated with sialic acid B for 24 h and washed five times with 5 mM 3-*N*-morpholinepropanesulfonic acid by repeated centrifugations (12,000 × *g*, 2 min) before the nematode infection experiments; (iii) treated with sialic acid B for 24 h, washed five times with 5 mM 3-*N*-morpholinepropanesulfonic acid and incubated in 5 mM 3-*N*-morpholinepropanesulfonic acid for 24 h before addition to the nematodes. Untreated conidia served as controls. The agar plates were checked daily, and the percentages of infected nematodes were recorded.

RESULTS

Adhesion and infection. The conidia of *M. coniospora* adhered with different distribution patterns to the cuticles of the several nematode species tested. These patterns were divided into three groups (Table 1). In group 1, adhesion was restricted to the cephalic region of larvae or adults of both sexes and to the tail region of the males of some of the species tested (i.e., *P. redivivus* and *C. elegans*). Adhesion was also to the tails of mature hermaphrodite *C. elegans*. Juveniles never had conidia attached to the tail. In group 2, conidia attached over the whole surface of the cuticle and were abundant also in the cephalic region. Group 3 nematodes consisted of only two species. These showed no conidial adhesion, signifying resistance to attack by the fungus.

Adhesion of conidia to the nematode surface did not always result in infection. Successful infection by the fungus generally resulted in the death of the nematode within 48 h. At 3 to 10 days after attachment of the conidia, the conidiophores emerged and grew along the length of the cadaver. Ten nematode species were observed for completion of the predation process; of these, the third-stage larvae of the five animal parasites and one plant parasite, *Criconemella xenoplax*, did not become infected. The third-stage larvae of *N. dubius*, where the cast cuticle had been removed, were infected by the fungus about 6 days after attachment. First-stage larvae of *N. dubius* were rapidly infected and died within 48 h of conidial adhesion.

Sialic acid specificity. Three different types of response to treatment of *P. redivivus* by the seven sialic acid preparations occurred (Table 2). The first type was confined to preparation A (the Merck product). This was the only treatment that significantly reduced adhesion of the conidia, with only a few nematodes showing attached spores. However, all of the nematodes to which conidia attached were infected and killed in 7 days. The second type of response, which resulted only from preparation B, showed no interference with adhesion, but a significant lag in infection up to 10 days after attachment. The third type of response, which ensued after treatment with the other five sialic acid preparations, gave normal attachment of conidia to nematodes, but a delay in infection for 2 to 3 days compared with untreated conidia. The same results were obtained when *C. elegans* was treated with sialic acid preparations A, B, and E (Table 2); furthermore, no inhibitory effect was observed when the other 15 sugar haptens were tested.

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TABLE 2. Effect on adhesion and infection of *P. redivivus* and *C. elegans* by *M. coniospora* after treatment of conidia with different sialic acid preparations[a]

Sialic acid	Adhesion	Infection	
		3 days	7 days
P. redivivus			
A	+	++	++++
B	++++	−	−
C	++++	++	++++
D	++++	++	++++
E	++++	++	++++
F	++++	++	++++
G	++++	++	++++
Untreated	++++	++++	++++
C. elegans			
A	++	++	++++
B	+++	−	−
E	++++	++	++++
Untreated	+++	++++	++++

[a] Adhesion (1 h after conidial treatment) and infection (3 and 7 days after adhesion): −, 0%; +, 1 to 25%; ++, 26 to 50%; +++, 51 to 75%; ++++, 76 to 100%.

Sialic acid displacement. Increased washing of conidia treated with sialic acid preparation B resulted in a gradual return to normal infection behavioral pattern of the fungus (Fig. 1). Specifically, the more the spores were washed, the shorter the lag time between attachment and infection. Spores washed and then soaked in buffer showed only 1 day of delay in infection.

DISCUSSION

The current experiments provide additional information on specific recognition and infection in the nematophagous fungus-nematode relationship. The sugar inhibition trials support previous evidence (3, 4) that sialic acid on the cuticle of *P. redivivus* and a sialic acid-specific lectin on *M. coniospora* conidia keys recognition between these organisms and extends these findings to *C. elegans*. Molecular differences of the cuticle surfaces among species are indicated by the generalized attachment of spores on some nematodes and the lack of attachment on others. We note elsewhere (14) that localized sugar residues near nematode chemosensilla probably derive from sensilla exudates. Therefore, since sensilla exudates are not an integral part of the cuticle makeup, the sugars contained in the exudates cannot be considered as indicators of differences in the chemical composition of the cuticle. There are, however, a number of reports in which differences in distribution and composition of cuticle surface carbohydrates have been demonstrated for free-living and plant-parasitic nematodes. Among these are *N*-acetylgalactosamine on *P. redivivus* (7) and on *Tylenchulus semipenetrans* and *Xiphinema index* (13), mannose-glucose and fucose on *P. silusiae* (9), and sialic acid residues on *T. semipenetrans* and *Meloidogyne javanica* (13). Other reports pointing to the differential makeup and importance of nematode surface sugars were the demonstration of quantitative stage-specific differences in *N*-acetylglucosamine residues for *C. elegans* (15) and observations on adhesion of *Corynebacterium* spp. to the cuticle of the nematode *Anguina* spp. (1). The accumulated evidence suggests differences in molecular recognition between nematodes that occupy similar environmental niches and other organisms.

The current results raise interesting questions relating to the mechanisms underlying cuticular penetration by *M. coniospora* conidia. As suggested previously (6), the initial recognition step represented by a lectin-carbohydrate interaction is the signal leading to penetration and digestion of the nematodes. As a working hypothesis, we speculate that penetration of the cuticle is preceded by collagenase induction in the conidia (or possibly the nematode) as would be required for breakdown of the primary cuticle structural protein, collagen. Collagenase has been reported from nematophagous fungi (12). Given enzyme induction as the step after recognition of the nematode by *M. coniospora*, the present results indicate that "living" cuticle is needed for inception of the penetration signal, for conidia attached to molted cuticles ensheathing living nematodes did not penetrate. The observed rapid infection of a stage of the same nematode species without the ensheathed cuticle, or from which the cast cuticle was removed, are consistent with this hypothesis. The report that the nematode-trapping fungus *Arthrobotrys oligospora* did not penetrate the cuticles of dead *P. redivivus* also agrees with these findings (8).

A clue to the stimulus required for the penetration signal derived from the observed delay of infection after treatment of conidia with sialic acid preparation B. A logical explanation for the results shown in Fig. 1 centers on the manner in which the several treatments would effect removal of sialic acid bound to recognition sites located on the adhesive buds. Sialic acid clearance from the recognition sites appears to have been achieved by washing. This is indicated by the nearly normal infection behavior of conidia subjected to thorough washing. On the other extreme, we speculate that a certain number of unwashed conidia treated with sialic acid were immature at the time of treatment. These conidia, upon maturity, would produce fresh adhesive buds with unblocked binding sites, and only after the time lag graphically illustrated in Fig. 1 would infection ensue.

The current results indicate that the seven sialic acids tested might differ in steric configurations. Furthermore, sialic acid consists of a nine-carbon basic configuration with substituents of five different sites (11). Among the substituents H, acetyl, glycolyl, methyl, phosphate, sulfate, and others have been recorded (11). *N*-Acetylneuraminic acid has one acetyl group and four H groups, and the substitution of one of these H groups, e.g., with another acetyl group

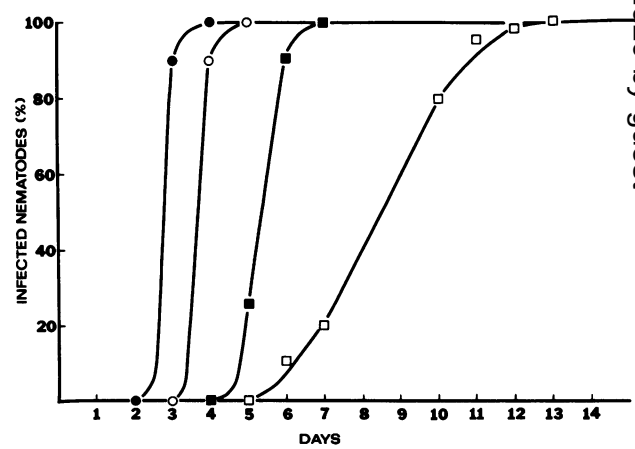

FIG. 1. Effect of pretreatment of *M. coniospora* conidia with Sigma type II sialic acid on infection of *P. redivivus*. Symbols: □, sialic acid alone (24 h); ■, sialic acid (24 h) followed by buffer washing; ○, sialic acid (24 h) plus incubation in buffer (24 h); ●, untreated conidia.

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would probably change the recognition sites between different sialic acids and the proposed conidial lectin. Whether one configuration of sialic acid is necessary for adhesion of the conidia and another is necessary for infection, or whether two sites of the same molecule are vital for both adhesion and infection, is unknown at present. Furthermore, as noted above, the several batches of sialic acids contained different amounts of undetermined types of impurities.

Our present hypothesis is that a lectin binding to sialic acid is located on the conidia of *M. coniospora*. We propose, as a possible pathway, that this lectin is involved in the adhesion of conidia to the nematodes when part of the sialic acid molecule has a certain configuration and another part of the molecule needs to be present to induce enzymatic penetration of the cuticle leading to successful infection of the nematodes. Such a two-stage model would explain the differing results both with the different sialic acids and the infection experiments with the different nematodes. At any rate, it appears logical that the penetration signal is keyed to the recognition of sialic acid residues on living cuticles. Studies based on the several aspects of the working hypotheses on pathways leading to nematode infection by *M. coniospora* conidia have been initiated.

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