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Effects of Monoclonal Antibodies, Cationized Ferritin, and Other Organic Molecules on Adhesion of *Pasteuria penetrans* Endospores to *Meloidogyne incognita*

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Abstract: The incidence of adhesion of *Pasteuria penetrans* endospores to *Meloidogyne incognita* second-stage juveniles (J2) was studied after pretreatment of the latter with monoclonal antibodies (MAb), cationized ferritin, and other organic molecules in replicated trials. Monoclonal antibodies developed to a cuticular epitope of *M. incognita* second-stage juveniles gave significant reductions in attachment of *P. penetrans* endospores to treated nematodes. MAb bound to the entire length of J2 except for the area of the lateral field, where binding was restricted to the incisures. Since reductions in attachment with MAb treatment were modest, it is uncertain if these results implicated a specific surface protein as a factor that interacted in binding of the endospore to the nematode cuticle. Endospore attachment was decreased following treatment of the nematode with the detergents sodium dodecyl sulfate (SDS) and cetyltrimethylammonium bromide (CTAB). Endospore attachment to live nematodes was significantly greater than attachment to dead nematodes. Attachment rates of three *P. penetrans* isolates to *M. incognita* race 3 varied between isolates. The effects of neuraminidase, pronase, pepsin, trypsin, lipase, and Na periodate on endospore attachment were inconsistent. The cationic dye alcian blue, which binds sulfate and carboxyl groups on acidic glycans, had no consistent effect on endospore attachment. The incidence of endospore attachment was significantly lower but modest, at best, for nematodes that were treated with cationized ferritin alone or cationized ferritin following monoclonal antibody. The lack of consistency or extreme reduction in most experiments suggests that attachment of *P. penetrans* spores to *M. incognita* is not specified by only one physico-chemical factor, but may involve a combination of at least two physico-chemical factors (including surface charge and movement of the J2). This points to a need for analysis of combined or factorial treatment effects.

Key words: adhesion, bacterium, binding site, biological control, cationized ferritin, cuticle, *Meloidogyne incognita,* monoclonal antibody, nematode, *Pasteuria penetrans,* root-knot nematode, surface charge, surface coat.

The specificity of attachment of endospores of *Pasteuria penetrans* to the cuticle of *Meloidogyne* spp. second-stage juveniles (J2) has been investigated with different probes (Bird et al., 1989; Davies and Danks, 1992, 1993; Davies et al., 1994; Davis et al., 1988; Spiegel et al., 1996) but is still poorly understood. Bird et al. (1989) and Persidis et al. (1991) suggested that carbohydrate residues on the endospore and nematode surfaces were involved in attachment. Persidis et al. (1991), using polyclonal antiserum raised to endospore antigens, suggested that attachment involves proteins that are highly glycosylated with N-acetylglucosamine. Davies and Danks (1992) and Davies et al. (1992) reported that proteins on the surfaces of the nematode and endospore may play a role in attachment. More recent results from pretreatment of *M. javanica* J2 implicated carbohydrates, carbohydrate-recognition domains, and a 250-kDa surface coat antigen in attachment (Spiegel et al., 1996). Polyclonal antibodies developed to the 250-kDa antigen did not possess carbohydrate-recognition domains and bound to most of the J2 surface except the head region. New results support the involvement of hydrophobic interactions and fibronectin-like residues on *P. penetrans* endospores (Afolabi et al., 1995; Davies et al., 1996).

The perplexity of the mechanism of attachment is further exemplified by the observation that endospores of one isolate of *P. penetrans* had greater affinity to J2 from

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one female than to those from another, even when the females were obtained from the same site in a field (Davies et al., 1991). The highly heterogeneous nature of *P. penetrans* populations has been demonstrated using monoclonal antibodies raised to epitopes on endospores (Davies et al., 1994). The amount and nature of proteins on the surface of *P. penetrans* endospores may account for host specificity (Davies et al., 1992; Persidis et al., 1991). Differences in surface characteristics of J2 also have been demonstrated (Davies and Danks, 1992). Other factors that affect endospore attachment to the cuticle, such as increased attachment following sonication (Davies et al., 1988; Stirling, 1985) and optimum temperature for attachment (Hatz and Dickson 1992; Stirling et al., 1990), have been reported.

The presence of negatively charged groups on the surface of the nematode cuticle has been shown previously (Himmelhoch et al., 1977). Afolabi et al. (1995) suggested that surface charge density may play a role in *P. penetrans* adhesion to nematodes. The importance of negatively charged groups has been shown in the attachment of fungal spores to the nematode cuticle, specifically in the attachment of *Meria coniospora* to the nematodes *Panagrellus redivivus* and *Caenorhabditis elegans* (Jansson et al., 1985). The reduction in fungal spore attachment observed after treatment with neuroaminidase (Jansson et al., 1985) provided a rationale for inclusion of that enzyme in this study with *P. penetrans*. Previous reports have shown that cationized ferritin binding to dead (glutaraldehyde-fixed) and live *Caenorhabditis elegans* gives the same surface charge pattern (Himmelhoch et al., 1977; Zuckerman et al., 1979).

The current study was an attempt to advance our understanding of the molecular interaction of *P. penetrans* and its nematode host. It reports on the effects of a monoclonal antibody to a cuticular epitope of *M. incognita* J2 on *P. penetrans* attachment, and also the effects of pretreatment of J2 with cationized ferritin, detergents, enzymes, and other organic reagents on endospore attachment observed over at least five trials or replications. The results show that none of the treatments dramatically reduced endospore attachment.

Materials and Methods

Organisms: Meloidogyne incognita (Kofoid and White) Chitwood race 3 (obtained from M. A. McClure, University of Arizona, Tucson, AZ) was cultured either on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) or eggplant (*Solanum melongena* L. cv. Black Beauty) in a greenhouse. An isolate of *P. penetrans* Sayre and Starr (Starr and Sayre, 1988) was obtained from Tifton, Georgia, by R. M. Sayre, Beltsville, Maryland, and used in all assays. A California isolate obtained from R. Mankau, University of California, Riverside, and a Florida isolate obtained by the third author also were used in one comparative study. In all trials, *P. penetrans* endospores were obtained from air-dried roots containing endospore-filled females (Stirling and Wachtel, 1980).

Preparation of monoclonal antibodies and attachment studies: Meloidogyne incognita race 3 J2 were collected from hatched eggs (McClure and Stynes, 1988). Approximately 25,000 J2 were mixed with Freund's complete adjuvant and the mixture injected into the peritoneum of Balb/c mice. Four injections of 25,000 J2/injection were given at 7-day intervals and, 34 days after the fourth injection, a final dose of the same preparation was administered. Three days after the final injection, the mice were sacrificed and their splenocytes fused with murine myeloma cells (Oi and Herzenberg, 1980; Sharon et al., 1980). Hybridoma cells were cultured in D-MEM medium (Life Technologies, Grand Island, NY) containing 7% fetal calf serum.

Hybridoma supernatants were screened for antibodies to the nematode's surface by incubating live J2 in the hybridoma culture supernatant, followed by treatment with goat-antimouse-FITC secondary antibody. Treated nematodes were observed with a fluorescence compound microscope, and cell lines selected on the basis of intensity of antibody binding to cuticle surface were

cloned. An antibody isotyping kit (Boehringer Mannheim, Indianapolis, IN) was used to determine that the antibody produced by the cloned cell line used here was type IgG_1,κ. Binding of the monoclonal antibody to the J2 surface also was assayed by treatment of the labeled nematode with goat-antimouse secondary antibody conjugated to colloidal gold followed by intensification with silver (BB International, Cardiff, UK). The culture medium (D-MEM) containing the monoclonal antibody produced by the cloned cells was lyophilized and used for endospore attachment assays without further purification.

For attachment studies, lyophilized antibody-hybridoma supernatant was dissolved in sterile, distilled water to a concentration of 1.7 mg antibody/supernatant/ml. Lyophilized culture medium supernatant (diluted in water to 1.7 mg/ml) was used as the control. Three milliliters of rehydrated antibody or control solution were placed in a sterile scintillation counting vial, and 6,000 J2 were added. After 1 hour of incubation, excess antibody or control solution was removed, J2 were washed with sterile distilled water, and 6,000 *P. penetrans* endospores/ml in 100 µl of water were added. Air from an aquarium pump, delivered through a 1-cm^3 tuberculin syringe with a 26G needle, was bubbled into the solution overnight at 30 °C. The solution was then poured into a sieve with 10-µm-pore openings. The sieve allowed the unattached endospores to pass through but retained the J2. The J2 were washed from the sieve and mounted live on a hemocytometer counting slide for observation. Counts were made of the number of endospores attached to each of 116 J2 in each treatment. Treatments were antibody + hybridoma medium, hybridoma medium alone, and water. Three categories were established to record the rate of attachment: 0 to 2 endospores/J2, 3 to 15 endospores/J2, and more than 15 endospores/J2. The experiment was replicated 12 times. Data were subjected to analysis of variance, and means were separated with Duncan's multiple range test.

Parallel, but different, experiments were run in Florida using the centrifugal method for attachment of bacterial endospores (Hewlett and Dickson, 1993). Two J2 of *M. incognita* race 3 were placed in a 0.25-ml microfuge tube in 100 µl of water, and an equal volume of rehydrated antibody (1.7 mg/ml) or culture medium was added. After mixing, the preparation was incubated for either 4 or 8 hours. The antibody or culture medium was then removed by centrifugation-rinsing two times, and a 100-µl suspension of 10,000 *P. penetrans* endospores was added. The tubes were centrifuged at 9,500*g* for 2 minutes and the nematodes removed for counting of attached endospores. Each treatment was replicated five times and each experiment was repeated.

Differential attachment of three P. penetrans isolates: Attachment of three *P. penetrans* isolates ('Georgia', 'California', and 'Florida') to *M. incognita* race 3 (maintained at the University of Massachusetts) was studied. The procedures were the same as for the first study described herein with the exception that the endospore-nematode mixture was not aerated during incubation. Endospores attached to 20 nematodes were counted for each of three replicates.

Effects of chemicals on attachment: Because *P. penetrans* and *Meloidogyne* spp. can vary geographically, the effects of several previously tested chemicals as well as some untested chemicals on attachment were evaluated. Enzymes used to pretreat the nematodes under aeration were neuraminidase Type V (Sigma N2876, Sigma, St. Louis, Missouri), 2 Units/ml in buffer (Na acetate 10 mM, $CaCl_2$ 4 mM; pH 5.5), 3 hours at 37 °C; pronase (Sigma P5147), 7 to 15 Units/mg as 50 Units/ml in PBS (pH 7.3), 3 hours at 37 °C; trypsin (Sigma T8462), 10 mg/ml, PBS, pH 7.2, 3 hours at 37 °C; lipase (Sigma L3001) (11 Units/mg) at 2 Units/ml; and pepsin, 5 mg/ml (Bird and Zuckerman, 1989). Sodium acetate buffer was used at pH 4, 3 hours at 37 °C, for both lipase and pepsin. Each enzyme test was replicated eight times, except pepsin, which was replicated six times. Endospore attachment in the corresponding buffer alone served as the control.

Since alcian blue (a cationic dye) can

block acidic groups, which exist in *C. elegans* cuticle (Himmelhoch and Zuckerman, 1983), nematodes were treated with alcian blue (5% in sodium acetate buffer, pH 5.5). Buffer alone served as the control, and there were three replicates. Another chemical, sodium metaperiodate, was prepared as a 10-mM solution in 2 mM Na acetate, pH 5.5, and held on ice in the dark for 1 hour. These chemicals were tested by the scintillation vial assay using endospore suspensions of 4,000 endospores/ml.

Detergents: Experimental conditions for detergents were 1% CTAB (Sigma MTA-5) or 2% sodium dodecyl sulfate (SDS) in PBS. SDS and CTAB were held at room temperature for 1 hour. Newly hatched J2 were incubated in detergent in watch glasses in a laminar-flow hood. After 1 hour, J2 were concentrated by centrifugation in a 1.5-ml sterile Eppendorf tube and washed three times with sterile tap water, followed by addition of an endospore suspension (4,000 endospores/ml). The preparation was incubated overnight at 30 °C with aeration, after which nematodes were collected on a 10-µm-pore screen and mounted on a hemocytometer slide for observation of attachment. Controls were J2 similarly exposed to endospores but incubated for 1 hour in appropriate buffer.

Attachment to live or dead nematodes: Comparisons were made between endospore attachment to live and dead nematodes to determine whether J2 movement affected binding of endospores to the cuticle. Juveniles were heat-killed at 60 °C for 20 minutes. Previous studies showed that the surface charge pattern on dead (glutaraldehyde-fixed) nematodes was similar to that on live nematodes (Himmelhoch et al., 1977; Zuckerman et al., 1979). Conditions for endospore attachment were the same as for the monoclonal antibody studies, including aeration. This study was replicated six times, and observations on attachment were made on 240 nematodes.

Effect of cationized ferritin: The potential involvement in attachment of negatively charged groups on the cuticle surface was investigated. *Meloidogyne incognita* J2 (8,000) were exposed to cationized ferritin to block negative charges. In the first experiment, J2 were first exposed to monoclonal antibody for 1 hour as described herein. Cationized ferritin (1 mg/ml in 0.15 mM NaCl) was then added in sterile, distilled water for 1 hour. The solution containing nematodes was concentrated by centrifugation, washed once with sterile distilled water, and brought up to 3 ml in sterile, distilled water held in a scintillation counting vial. *Pasteuria penetrans* endospores (8,000/ml) were added and incubated overnight under vigorous aeration at ambient room temperature. Endospore attachment was then recorded. The second experiment with cationized ferritin proceeded in the same way, but without pretreatment of the nematodes with the monoclonal antibody.

Results and Discussion

Effect of monoclonal antibody on attachment: Monoclonal antibody bound to the entire length of the *M. incognita* J2 (Fig. 1A) except for the area of the lateral field, where binding was restricted to the incisures (Fig. 1B). There was a significant reduction in the numbers of *P. penetrans* endospores attached to J2 when the J2 were pretreated and incubated with monoclonal antibody plus spores compared to water and medium-only controls ($P \leq 0.05$) (Table 1). Medium alone did not reduce attachment (Table 1). Bovine serum albumin in culture medium has been shown to bind to *P. penetrans* endospores (Afolabi et al., 1995), but in the current study it did not prevent discrimination of the antibody effect in most cases. The probability of the antibody binding to *P. penetrans* endospores would tend to be much less for a monoclonal compared to a polyclonal antibody treatment. If MAb bound to the endospores and/or J2, it would decrease binding of the endospores to the J2. For the highest endospore attachment category (>15 spores/J2), it was possible to discriminate some inhibition of attachment by MAb (Fig. 2). Results of these and parallel tests conducted in Florida with different methods showed that, in most trials, attachment of

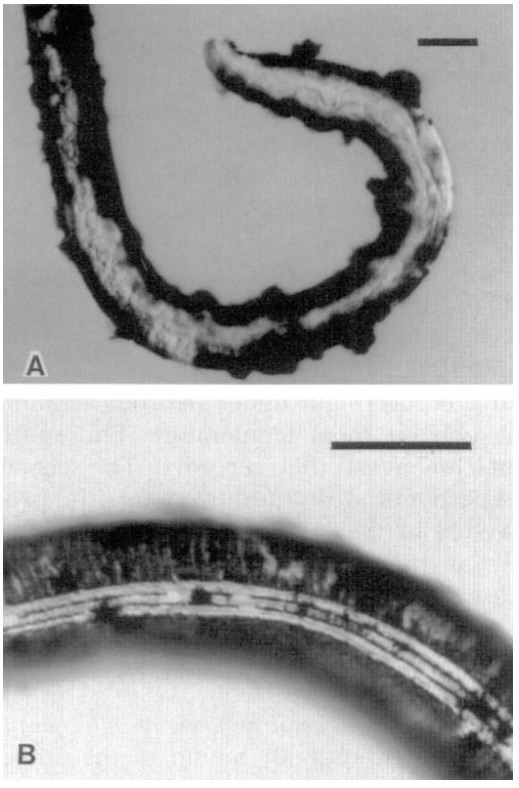

FIG. 1. Second-stage juveniles of *Meloidogyne incognita* race 3 treated with monoclonal antibody to their surfaces. Labeling was visualized (black deposits) by treatment with gold-conjugated, goat-antimouse secondary antibody followed by silver intensification of the conjugate. A) Anterior end of second-stage juvenile. B) Detail of labeling at midbody where binding was restricted to the lateral field incisures. Bars = 10 µm.

endospores in water or medium was greater than with antibody-treated nematodes (Tables 1,2; Fig. 2). The MAb effect on attachment was not dramatic and was not consistent over all endospore categories.

Attachment of endospores from three isolates of Pasteuria penetrans to M. incognita race 3: In each of the three trials, attachment to *M. incognita* J2 was higher for the Georgia isolate than for the Florida or California isolates ($P \leq 0.05$) (Fig. 3). Stirling (1985) also noted differences in attachment of different isolates of *P. penetrans* to *M. incognita* and other root-knot nematode species. *Pasteuria penetrans* is known to attach differentially to J2 sampled from the same field (Davies et al., 1991).

Alcian blue and enzymes: Alcian blue and

TABLE 1. Attachment of *Pasteuria penetrans* Florida isolate endospores to *Meloidogyne incognita* race 3 following treatment of second-stage juveniles (J2) with monoclonal antibody or hybridoma culture medium.

Treatment	Incubation time (hours)	Endospores per J2
Water	4	32.6 a
Water	8	35.6 a
Antibody + medium	4	14.6 b
Antibody + medium	8	15.5 b
Medium	4	44.2 a
Medium	8	24.4 a

Means followed by the same letter did not differ significantly ($P > 0.05$) according to Duncan's multiple range test. Treatments were replicated five times, and each mean is the average of two runs.

the enzymes tested gave inconsistent results with increases and decreases in attachment of *P. penetrans* endospores (data not shown). Certain groups (such as OSO_3- and certain COO- groups on acidic glycans or proteins) may not have contributed directly to the attachment of *P. penetrans* endospores or more probably were not affected by these treatments.

Detergents: CTAB consistently reduced the attachment of *P. penetrans* endospores ($P \leq 0.05$) (Table 3). SDS also reduced the rate of endospore attachment in 8 of 9 trials (*P*

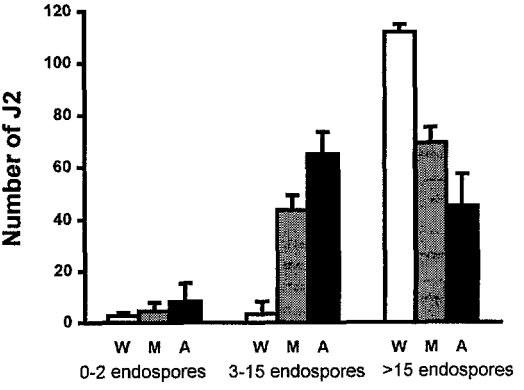

FIG. 2. Effect of treatment with monoclonal antibody on attachment of *Pasteuria penetrans* endospores to *Meloidogyne incognita* race 3. Incidence of attachment is based on the averages of 12 replicates, where 116 nematodes were scored for each treatment. Three categories were established for attachment (0 to 2, 3 to 15, and >15 endospores). Bars represent means ± standard error. W = Water, M = Medium alone, A = Medium + antibody.

TABLE 2. Effects of a monoclonal antibody combined with treatment by cationized ferritin on attachment of *Pasteuria penetrans* endospores (8,000 endospores/ml) to *Meloidogyne incognita* second-stage juveniles (J2) (8,000 J2/ml).

Treatment	Number of J2 with: 0–2 endospores per nematode	3–15 endospores per nematode	>15 endospores per nematode
Antibody + medium	2.0 ± 0.0 b	31.5 ± 2.1 c	82.5 ± 2.1 b
Medium	5.0 ± 1.4 a	12.0 ± 9.9 d	99.0 ± 8.5 a
Antibody + medium-ferritin	0.5 ± 0.7 d	52.5 ± 7.8 a	63.0 ± 7.1 d
Medium-ferritin	1.0 ± 0.0 c	38.0 ± 2.8 b	77.0 ± 2.8 c

Means in a column followed by a common letter are not significantly different according to Duncan's multiple range test ($P = 0.05$). Numbers are the means ± standard error of five replications, and each mean is the average of two runs.

≤ 0.05). The reductions recorded for the CTAB and SDS trials were of different magnitudes (Table 3). Spiegel et al. (1996) also observed a reduction of attachment following pretreatment of J2 with SDS. Sodium periodate gave inconsistent results, with decreases or increases in attachment, or no effect on attachment (data not shown). Persidis et al. (1991) reported that pretreatment of *M. incognita* race 2 J2 with SDS or SDS extracts of endospore fragments did not inhibit attachment of *P. penetrans* endospores to the nematode. Davies and Danks (1993) reported no effect of Na periodate on endospore attachment when nematodes only were pretreated; however, pretreatment of the endospores with sodium periodate reduced attachment to nematodes. 1,2-dihydroxy groups may be more exposed on the endospore than on the nematode surface. Davies and Danks (1993) also observed that SDS at room temperature significantly increased endospore attachment but significantly decreased endospore attachment at elevated temperatures. The significant decrease in attachment following SDS treatment at room temperature in the present study differed from that reported by Davies and Danks (1993), but the isolate of *P. penetrans* and the race of *M. incognita* in the current study were different. Also, 2% SDS was applied for 1 hour, whereas Davies and Danks (1993) tested 1% SDS for 2 hours.

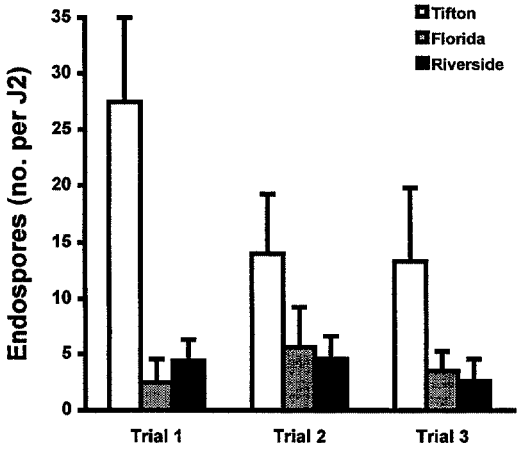

FIG. 3. Attachment of endospores from three isolates of *Pasteuria penetrans* (Tifton, Florida, Riverside) to *Meloidogyne incognita* race 3 second-stage juveniles (J2). The ratio of endospores per J2 was 6,000/20. The experiment was replicated three times. Bars represent means (of the three replicates) ± standard error.

TABLE 3. Numbers of *Pasteuria penetrans* endospores attaching to *M. incognita* second-stage juveniles following pretreatment of juveniles with detergents.

Trial number	Control (PBS)	Detergents[a] CTAB	SDS
1	43 ± 5	23 ± 10 (23%)[b]	14 ± 4 (67%)
2	76 ± 20	35 ± 6 (54%)	33 ± 6 (57%)
3	88 ± 12	45 ± 6 (49%)	53 ± 10 (40%)
4	63 ± 40	29 ± 2 (54%)	—
5	33 ± 2	20 ± 2 (39%)	9 ± 1 (73%)
6	16 ± 1	13 ± 1 (19%)	16 ± 2 (0%) NS
7	44 ± 8	9 ± 1 (80%)	4 ± 1 (91%)
8	86 ± 16	32 ± 10 (63%)	11 ± 5 (87%)
9	48 ± 12	36 ± 17 (25%)	35 ± 5 (27%)
10	37 ± 3	28 ± 3 (24%)	24 ± 4 (35%)

[a] Numbers are means ± standard error. Trials 4 to 7 and 10 are based on 25 replicates, trials 1 and 8 are based on 14 replicates, and trials 2 and 9 are based on 15 replicates. Dash (—) = No observation. Attachment of endospores in detergent trials was significantly higher or lower than attachment in control, except for SDS in trial 6. NS = not significantly different.

[b] Data in parentheses represent percent reduction in attachment from the control.

CTAB gave consistent reduction in endospore attachment (Table 3), whereas no reduction was observed in another study (Davies and Danks, 1992), in which CTAB was tested. Since detergent-pretreated nematodes were incubated overnight with *P. penetrans* endospores and a consistent decrease in attachment was observed, any significant recovery of the J2 surface probably occurred in 16 to 20 hours. There is no consistent observation for these detergent treatments among the various research results.

Attachment to live or dead nematodes: The incidence of endospore attachment to dead (heat-killed) nematodes was 2- to 12-fold less than to live nematodes in all six trials ($P < 0.05$) (Fig. 4). However, endospores still attached to ca. 12% of dead J2. These results differ from those of Stirling et al. (1986), who reported equal attachment to live nematodes and nematodes heat-killed at 60 °C and 100 °C for 5 and 10 minutes, respectively.

Effect of cationized ferritin on endospore attachment: The blocking of negative charges on the nematode cuticle surface with ferritin conjugated to the monoclonal antibody caused an additive reduction in endospore attachment when compared to treatment with the medium containing antibody only, when nematodes with up to 2 or >15 endospores attached were counted ($P \leq 0.05$) (Table 2). There was only a small reduction in the 0-to-2 endospore category and increased attachment in the 3-to-15 endospore category. Conjugation of ferritin and antibodies has been shown to be a useful tool that gives decisive results in the study of nematode surface characteristics (Himmelhoch, 1982).

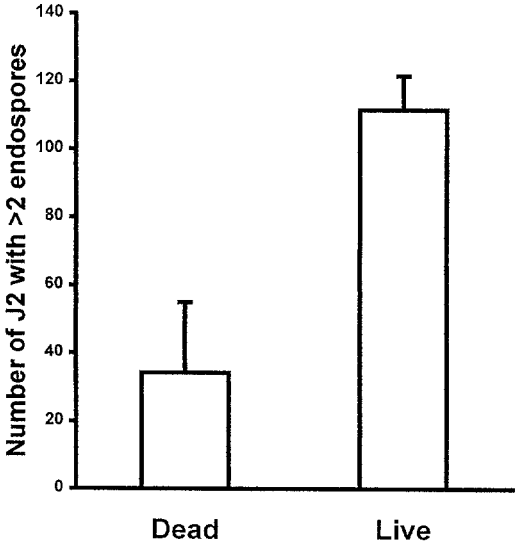

FIG. 4. Attachment of *Pasteuria penetrans* endospores to live or dead *Meloidogyne incognita* race 3 second-stage juveniles (J2). Bars represent means of six replicates (240 nematodes per treatment) ± standard error.

Treatment with cationized ferritin alone gave a reduction of attachment but only when nematodes with the highest numbers of endospore attached were counted. This suggests that negative charges (or groups proximal to negative charges) on cuticle-surface molecules may not play the main role in attachment of *P. penetrans* endospores (Table 4). Where cationized ferritin increased attachment (in the lower endospore categories), the mechanism of attachment to possible net negative electric dipoles or charges could be non-group-specific.

Davies and Danks (1993) and Afolabi et al. (1995) suggested the attachment of *P. penetrans* to *M. incognita* J2 probably was governed by several different ligands. The results of the current study supported this view but further suggested that an interplay of at least two physico-chemical factors may be involved in the attachment of the endospore to the nematode surface. The monoclonal antibody against a cuticular epitope of unknown structure bound to cuticular sites and appeared to block adhesion of *P. penetrans* endospores. The indications were that cationized ferritin following monoclonal antibody or ferritin alone blocked attachment by occupying negatively charged sites or dipoles on the surface of the nematode cuticle. This suggested that alteration of the surface charge of cuticular epitopes (by modifying pH or ionic strength of the surrounding medium, for example) would influence attachment. Davies et al. (1994) noted differences in endospore attachment at pHs 4, 7, and 9. The electronegative potential at the endospore surface is known to

TABLE 4. Effects of cationized ferritin treatment on attachment of *Pasteuria penetrans* endospores to *Meloidogyne incognita* race 3 second-stage juveniles (J2).

Treatment	Number of J2 with:		
	0–2 endospores per nematode	3–15 endospores per nematode	>15 endospores per nematode
0.15 M NaCl	0.5 ± 0.7 c	15.0 ± 1.4 b	100.5 ± 0.7 b
Cationized ferritin in 0.15 m NaCl	2.0 ± 0.7 b	38.0 ± 5.7 a	75.5 ± 6.4 c
Distilled water	5.0 ± 0.7 a	5.0 ± 2.8 c	110.5 ± 3.5 a

Means ± standard error in a column followed by a common letter are not significantly different according to Duncan's multiple range test ($P = 0.05$). Numbers are the means of 12 replications (116 nematodes per replicate).

change with the type and concentration of electrolytes and pH (Alfolabi et al., 1995).

In aqueous solution the net negative charge on the surface of the nematode (Himmelhoch et al., 1977) and bacterium (Afolabi et al., 1995) would tend to prevent binding through electrostatic repulsive forces alone. The monoclonal antibody and cationized ferritin probably block (prevent) important strongly and weakly polarizing electric dipole interactions between variously charged groups on either surface. Alteration of the ionic strengths of the suspension medium and vigorous aeration and agitation probably reduce the influence of the aqueous environment on the dynamic coulombic repulsion between the endospore and nematode surfaces. A polyclonal antiserum raised to *P. penetrans* endospores has been shown to affect endospore surface charge density (Afolabi et al., 1995). The role of attractive and repulsive forces in other host-microbe interactions has been studied and supports the involvement of surface charge in attachment (Chang and Hsu, 1995; Jones and O'Shea, 1994; Romantschuk, 1992).

The differential attachment of various *P. penetrans* isolates to a single race of *M. incognita* indicates the existence of intraspecific variation in the molecular composition of the bacterial endospore surface. Although many endospore surface proteins are conserved (Davies et al., 1994), there may be differences in the quantity and nature of certain proteins that correspond to host specificity and resultant charge densities. Endospore surfaces and nematode cuticles of subpopulations of both *P. penetrans* and *M. incognita* have been shown to be highly heterogeneous serologically (Davies et al., 1994). The chemicals used in this study had different effects. The inconsistency in the results of some trials and the varying degrees of attachment observed in this study suggest that early (docking) phases in the binding of endospores to the cuticle of moving J2 may possibly be non-specific and reversible. These initial phases could involve changes in Donnan potentials at the interface of the nematode and(or) bacterial surfaces that are probably followed by irreversible attachment stages (as evidenced by endospores that successfully attached to the nematodes in this study). The mechanism of attachment of the base of *P. penetrans* endospores to J2 is still not understood. Future studies should investigate the effects of two or more treatments and their interactions on adhesion.

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