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A Centrifugation Method for Attaching Endospores of *Pasteuria* spp. to Nematodes[1]

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Abstract: Attachment of relatively low numbers of endospores from two isolates of *Pasteuria* spp. to several species of nematodes was consistently achieved in 2–5 minutes with a centrifugation technique. The rate of attachment of *Pasteuria penetrans* at 10^4 endospores/0.1 ml/tube to second-stage juveniles (J2) of *Meloidogyne javanica, M. incognita* race 1, *M. incognita* race 3, and *M. arenaria* races 1 and 2 in two tests averaged 4.4, 5.2, 0.1, 0.3, and 0 endospores per J2, respectively. The rate of attachment *Pasteuria* sp. at 10^3 endospores/0.1 ml/tube to individuals of *Hoplolaimus galeatus, Belonolaimus longicaudatus, M. arenaria* race 1, *M. javanica,* and *M. incognita* race 1 in two tests averaged 0.8, 0.04, 0, 0, and 0 endospores per nematode, respectively. The rate of attachment of *P. penetrans* to *M. javanica* at 10^3, 10^4, or 10^5 endospores/0.1 ml/tube from two tests averaged 1.0, 5.7, and 28.3 endospores per J2, respectively. All of the J2 had endospores attached following centrifugation in tubes with 10^4 and 10^5 endospores/0.1 ml/tube.

Key words: bacterium, biological control, centrifugation, endospore, *Meloidogyne arenaria, M. incognita, M. javanica,* method, nematode, *Pasteuria penetrans, Pasteuria* sp.

Pasteuria spp., endospore-forming bacterial parasites of nematodes, have been demonstrated as effective biological control agents of plant-parasitic nematodes in greenhouse and microplot experiments (5–8,13) and from field observations (3,9, 11). Endospore–host attachment studies are the first step in establishing host ranges of *Pasteuria* spp. and determining their efficacy. Researchers conducting such studies have relied on nematode movement through soil (6), water (7), or agar (14), each laden with endospores, or agitation of nematode–endospore–water suspensions (2,4,8,12). The time needed for attachment with these techniques ranged from 1 hour to several days and usually required densities of 10^3 endospores per nematode or greater.

To date, most studies on *Pasteuria* spp. have been conducted with endospores obtained from female cadavers of the endoparasitic nematodes, *Meloidogyne* spp. Large numbers of endospores are relatively easy to collect because each cadaver may contain up to 2.5 million endospores. *Pasteuria* spp. that are pathogenic to ectoparasitic nematodes, however, are more difficult to study because collecting endospores from their cadavers is labor intensive. In this case, endospore-filled cadavers must be extracted from the soil and hand picked. They yield approximately 2,000 endospores per nematode (pers. obs.).

The purpose of this paper is to report on a fast and effective method of attaching relatively low numbers of endospores to nematodes by centrifugation.

Materials and Methods

Nematode populations: The nematodes used in this study originated from greenhouse isolates maintained at the University of Florida, Gainesville. *Meloidogyne* spp. were cultured on tomato (*Lycopersicon esculentum* cv. Rutgers). Eggs of *Meloidogyne* spp. were extracted from roots treated with 0.5% sodium hypochlorite (10) and caught on a 25-µm-pore sieve, rinsed, and placed on a Baermann funnel (1). Second-stage juveniles (J2) hatched from these eggs were no more than 3 days old when used in the experiment. *Belonolaimus longicaudatus* and *Hoplolaimus galeatus* were cultured on Bermudagrass (*Cynodon dactylon* cv. Tiftgreen) and extracted by Baermann funnel.

Bacterial cultures: Pasteuria penetrans P-100 was isolated from a *Meloidogyne* spp. population collected in Pasco County. The isolate was cultured in the greenhouse on

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Meloidogyne javanica growing on tomato roots. To obtain endospores, air-dried galls were crushed with a mortar and pestle in 10 ml tap water. Endospores were separated from root debris by passing them through a 25-μm-pore sieve.

Pasteuria sp. H-1 originated from a population of *H. galeatus* on Bermudagrass in Alachua County. Endospores of H-1 were extracted from hand-picked, endospore-filled cadavers of *H. galeatus* collected from Bermudagrass. These cadavers were crushed in 2 ml water with a tissue grinder previously treated with a water repellant (Repel-Silane, LKB, Bromma, Sweden). For both isolates, the endospore concentrations were determined with a hemacytometer counting chamber.

Host-range studies: Host range of *P. penetrans* P-100 was determined using 0.1 ml of a 10^5/ml endospore–water suspension, and 0.1 ml of a 2,000 J2/ml water suspension of *Meloidogyne arenaria, M. incognita,* and *M. javanica.* These were placed in 0.25-ml previously silanized microfuge tubes and centrifuged at 9,500*g* for 2 minutes using a Beckman microfuge (Beckman, Palo Alto, CA). Nematodes were removed from the tubes with a pipette and placed on glass slides for observation. Twenty individuals per combination were observed. Numbers of nematodes with endospores attached and the number of endospores attached per nematode were counted. Except for *M. arenaria* race 2, this test was conducted simultaneously on *M. javanica, M. arenaria* race 1, or *M. incognita* races 1 and 3.

Host ranges of *Pasteuria* sp. H-1 were determined as described above, but with suspensions of 10^4 endospores/ml, 1,000 mixed-life stages of *B. longicaudatus* or *H. galeatus,* and 2,000 J2/ml of *Meloidogyne arenaria, M. incognita,* or *M. javanica.*

Spore density and attachment studies: The rate of attachment of P-100 to *M. javanica* was tested with treatments of 10^3, 10^4, or 10^5 endospores/0.1 ml/tube with the technique described.

All above mentioned treatments were replicated five times, and experiments were repeated.

Results and Discussion

Meloidogyne javanica and *M. incognita* race 1 had the highest percentage of J2 with P-100 endospores attached, averaging 95 and 82% for *M. javanica,* and 100 and 96% with *M. incognita* race 1 in Tests 1 and 2, respectively (Table 1). These nematodes also had the highest number of endospores attached per J2. Attachment to *M. incognita* race 3 and *M. arenaria* race 1 was relatively low. Endospores of P-100 did not attach to *M. arenaria* race 2 in either test.

Endospore attachment of isolate H-1 was greatest on *H. galeatus.* Thirty percent and 39% of the nematodes in Tests 1 and 2, respectively, had endospores attached (Table 2). The rate of attachment was poor on *B. longicaudatus.* Only 2% and 6% of the nematodes in Tests 1 and 2, respectively, had endospores attached. No attachment occurred on the three *Meloidogyne* spp. tested.

The rate of attachment of endospores to nematodes increased approximately five to six times for each 10-fold increase in numbers of endospores used (Table 3). An average of 55 and 67% of the J2 had endospores attached in Tests 1 and 2, respectively, when endospore densities were 10^3. All J2 had endospores attached at densities of 10^4 and 10^5.

The most common technique used for evaluating the attachment of endospores

Table 1. Average number of endospores of *Pasteuria penetrans* P-100 attached to 20 second-stage juveniles (J2) of five *Meloidogyne* spp., and average percentage of J2 with endospores attached, after centrifugation with 200 J2 and 10^4 endospores/0.1 ml/tube.

	Test 1		Test 2	
Nematode	Number	%	Number	%
M. javanica	6.1 ± 2.2	95	2.6 ± 0.76	82
M. incognita race 1	7.0 ± 1.2	100	3.4 ± 0.5	96
M. incognita race 3	0.1 ± 0.1	4	0.1 ± 0.3	3
M. arenaria race 1	0.1 ± 0.1	8	0.4 ± 0.3	8
M. arenaria race 2	0	0	0	0

Data are means ± SD of five replicates.

TABLE 2. Average number of endospores of *Pasteuria* spp. H-1 attached to 20 specimens each of five nematode species and average percentage with endospores attached, following centrifugation with 10^3 endospores/0.1 ml/tube and 100 *Hoplolaimus galeatus* or *Belonolaimus longicaudatus* (mixed life stages) or 200 second-stage juveniles of *Meloidogyne* spp. per centrifuge tube.

Nematode	Test 1		Test 2	
	Number	%	Number	%
H. galeatus	0.6 ± 0.4	30	1.0 ± 0.2	39
B. longicaudatus	0.02 ± 0.03	2	0.06 ± 0.04	6
M. arenaria race 1	0	0	0	0
M. javanica	0	0	0	0
M. incognita race 1	0	0	0	0

Data are means ± SD of five replicates.

on nematodes has been by agitating endospore–nematode–water suspensions. This procedure may take ⩾1 hour, and 100% attachment is difficult to achieve. Our centrifugation method requires only 2 minutes for completion, and the rate of endospore attachment to nematodes can be controlled by adjusting the density of endospores in the suspension. Attachment of endospores to large numbers of nematodes is possible by using larger centrifuge tubes. We have obtained 100% attachment on 3×10^4 J2 of *M. javanica* with 5×10^5 endospores of isolate P-100 in a 15-ml conical centrifuge tube (5,550*g*, 5 minutes). Other tube sizes or centrifugation speeds (*g*) have not been evaluated. This technique is an improved method of attaching endospores to nematodes, thereby providing a more reliable means of studying the host ranges of *Pasteuria* spp. We routinely use *Meloidogyne* J2 that each have 5–15 endospores attached following the centrifugation technique as inoculum for the buildup of *Pasteuria* sp. endospores. This technique also allows for studies of *Pasteuria* spp. collected from nematode species that yield relatively few endospores per cadaver.

TABLE 3. Average number of endospores of *Pasteuria penetrans* attached to 20 second-stage juveniles (J2) of *Meloidogyne javanica* and average percentage of J2 with endospores attached following centrifugation with 200 J2 and 10^3, 10^4, or 10^5 endospores/0.1 ml/tube.

Endospore number	Test 1		Test 2	
	Number	%	Number	%
10^3	0.8 ± 0.3	55	1.1 ± 0.4	67
10^4	4.8 ± 0.4	100	6.6 ± 1.1	100
10^5	22.8 ± 1.0	100	33.8 ± 3.4	100

Data are means ± SD of five replicates.

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