Journal of Nematology 24(1):36–39. 1992.

Pasteuria sp. Parasitizing *Trophonema okamotoi* in Florida[1]

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Abstract: Two populations of *Trophonema okamotoi* parasitized by *Pasteuria* sp. were found on *Liquidambar styraciflua* (sweetgum) and on an unidentified tropical grass in north-central Florida. Endospores of this *Pasteuria* sp. attached to motile vermiform second-stage juveniles (J2) and males of *T. okamotoi,* but not to other developmental stages. Sporangia and new endospores were produced only inside the bodies of swollen and sedentary third- and fourth-stage juveniles and females that developed in the host roots. No egg masses were produced by infected *T. okamotoi* females. The endospore diameter from the tropical grass population was 4.93 μm and the central core diameter was 1.97 μm; measurements of endospores from the sweetgum populations were similar. Endospores that were collected from *T. okamotoi* and added to uninfected *T. okamotoi* and other plant-parasitic nematodes attached to J2 of *T. okamotoi* but did not attach to juveniles and adults of *Helicotylenchus pseudorobustus, Pratylenchus brachyurus,* or to J2 of either *Meloidogyne arenaria* race 1, *M. incognita* race 1, *M. javanica,* or *Tylenchulus semipenetrans. Pasteuria* sp. from *T. okamotoi* differed from the described *Pasteuria* species in endospore size, host preference, and rate of attachment.

Key words: bacterium, biological control, endospore, host range, nematode, *Pasteuria nishizawae, P. penetrans, P. thornei,* spore attachment, *Trophonema okamotoi.*

Three species of *Pasteuria* have been described: *Pasteuria nishizawae* Sayre, Wergin, Schmidt, & Starr from *Heterodera glycines, P. penetrans* Sayre & Starr from *Meloidogyne incognita,* and *P. thornei* Starr & Sayre from *Pratylenchus brachyurus* (7,9,12). Other *Pasteuria* species may exist because *Pasteuria* isolates varying in morphology, host range, and nematode life stage required for development have been found in different geographical regions (1,2,5). Reports of *Pasteuria* sp. parasitizing Tylenchulinae species are scarce and deal mainly with the occurrence of spore attachment to the vermiform second-stage juveniles (J2; 11,13,14). A *Pasteuria* sp. that attached and sporulated inside the body of *Tylenchulus semipenetrans* J2 was reported from Iraq (4).

In Florida, Tylenchulinae species occur often in uncultivated lands. *Trophonema okamotoi* is a tylenchulid commonly associated with *Tylenchulus palustris* in waterlogged soils. We found two isolates of *Pasteuria* sp. parasitizing *T. okamotoi* in northern Florida. Our objectives were to study the development, morphology, and specificity of these isolates of *Pasteuria* sp.

Materials and Methods

Forty 500-g samples of soil and roots were collected from sweetgum (*Liquidambar styraciflua*) in Taylor County, Florida, and from an unidentified tropical grass in Alachua County, Florida. Nematodes were extracted from soil by sugar flotation centrifugation (6). Swollen nematodes were dislodged from roots by spraying the roots with water and collecting the nematodes on a 45-μm-pore sieve. Nematodes were separated from debris with centrifugal flotation and were transferred to water agar (3) for observation and identification of developmental stages infected with *Pasteuria* sp.

Endospore and central core diameter for both isolates of *Pasteuria* sp. were measured with an eye-piece micrometer with a light microscope. For the Alachua County isolate, 15 endospores of *Pasteuria* from each of six diseased females were measured in water. Because of the scarcity of the Taylor County isolate, only 15 endospores were measured in glycerin. Endospores from each location were examined

Received for publication 5 April 1991.
[1] Contribution No. 429, Bureau of Nematology. Research supported in part by USDA Grant 89-34135-4574.
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with a Hitachi S4000 SEM. Parasitized nematodes were fixed in formalin and processed to anhydrous glycerin (10). They were placed on SEM stubs, smashed to release their endospores, and sputter-coated with gold. Spores were observed at acceleration voltages of 5 to 15 kV.

To determine the specificity of endospore attachment, spore-filled specimens of the Alachua County population of *T. okamotoi* were handpicked and their cuticles were ruptured in demineralized water to release endospores. These endospore suspensions (20,000/ml) were mixed with an equal volume (0.1 to 0.5 ml) of water containing 100 J2 of either *M. incognita* race 1, *M. arenaria* race 1, or *M. javanica;* or 40 J2 of *Tylenchulus semipenetrans;* or 40 juveniles and adults of *Helicotylenchus pseudorobustus, Pratylenchus brachyurus,* or *T. okamotoi.* The nematodes were maintained at room temperature and were observed for spore attachment daily for 10 days.

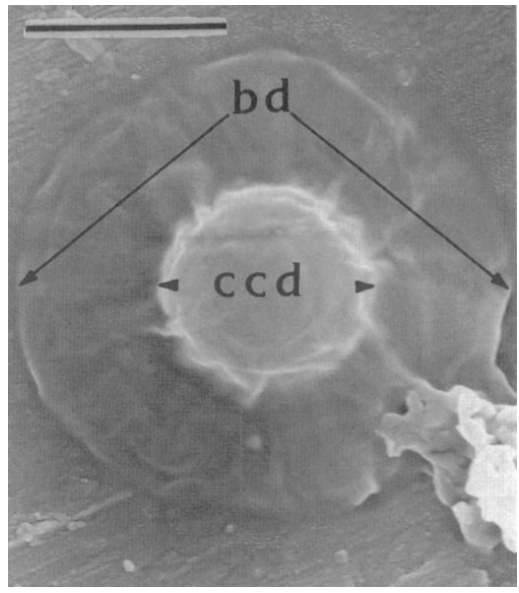

FIG. 1. SEM photograph (dorsal view) of a hat-shaped endospore of *Pasteuria* sp. collected from a swollen female of *Trophonema okamotoi.* bd = endospore body diameter, ccd = central core diameter. Scale bar = 2 μm.

RESULTS AND DISCUSSION

Morphology: Based on observations with light microscopy, the endospore diameter for the Alachua County isolate was 4.7–5.1 μm (mean 4.93 μm, SD 0.07) and the central core diameter was 1.8–2.2 μm (mean 1.97 μm, SD 0.07). The endospore diameter for the Taylor County isolate was 4.8–5.1 μm (mean 4.95 μm, SD 0.1) and the central core diameter was 1.8–2.1 μm (mean 1.99 μm, SD 0.09). *Pasteuria* sp. endospores from *T. okamotoi* are hat-shaped in dorsal view (Fig. 1). The diameter of these endospores is slightly larger than for *P. penetrans* (4.5 μm), considerably larger than for *P. thornei* (3.5 μm; 8), and somewhat smaller than for *P. nishizawae* (5.3 μm; 8). The diameters are within the range given for an isolate from *Tylenchorhynchus annulatus* from Florida but differ from those of five other isolates also from Florida (5).

The central core diameter of our *Pasteuria* sp. overlaps that of *P. penetrans* and *P. nishizawae* but is slightly larger than that of *P. thornei* (1.6 μm; 8). *Pasteuria* sp. endospores from *T. semipenetrans* (4) are also smaller (2.6 μm) than those of *Pasteuria* sp. from T. okamotoi.

Development: Eighteen percent and 0.1% of the *T. okamotoi* were infected with *Pasteuria* sp. in the Alachua and Taylor County populations, respectively. Endospore attachment was observed along the entire body of J2 (Fig. 2A, B) and males (Fig. 2C), with no preference to any region. Endospores were never observed inside J2, but spore-filled J3, J4, and females (Fig. 3A, B) were found. Infected, swollen females remained attached to the roots, but no eggs were observed in the gelatinous matrix that covered their bodies. Infected females did not contain visible reproductive organs; instead they were tightly packed with endospores and sporangia (Fig. 4).

The development of this isolate differed from that of *P. penetrans* on *Meloidogyne* spp. Endospores of *Pasteuria penetrans* usually attach only to J2 of *Meloidogyne* spp., and newly formed endospores are found only in females, although spore-filled J2 of

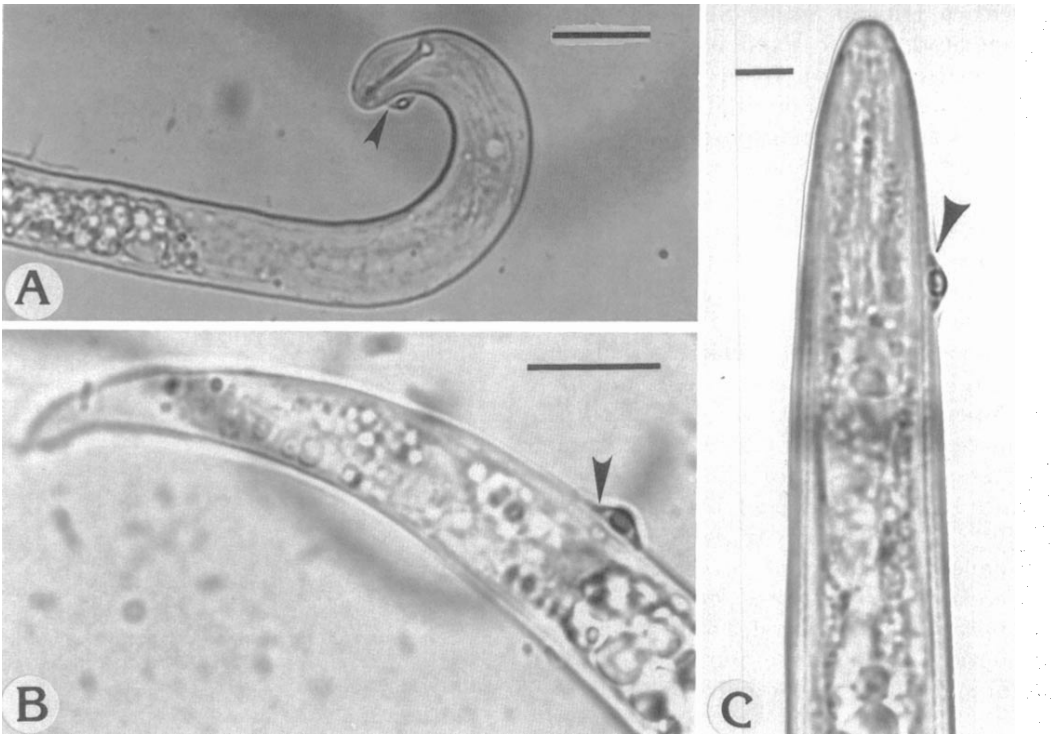

FIG. 2. Endospores of *Pasteuria* sp. adhering to vermiform stages of *Trophonema okamotoi*. Scale bar = 15 μm in A, 10 μm in B, and 4.2 μm in C. A, B. Endospores of *Pasteuria* sp. attached to the cuticle near the head and tail of a second-stage juvenile. C. Endospore of *Pasteuria* sp. attached to the cuticle near the head of a male.

this genus and other genera have been reported (1,2,5). Also, recently we observed spore-filled males of *Meloidogyne arenaria* that had apparently undergone sex reversal following treatment at 35 C (unpubl.). *Pasteuria nishizawae* also forms endospores

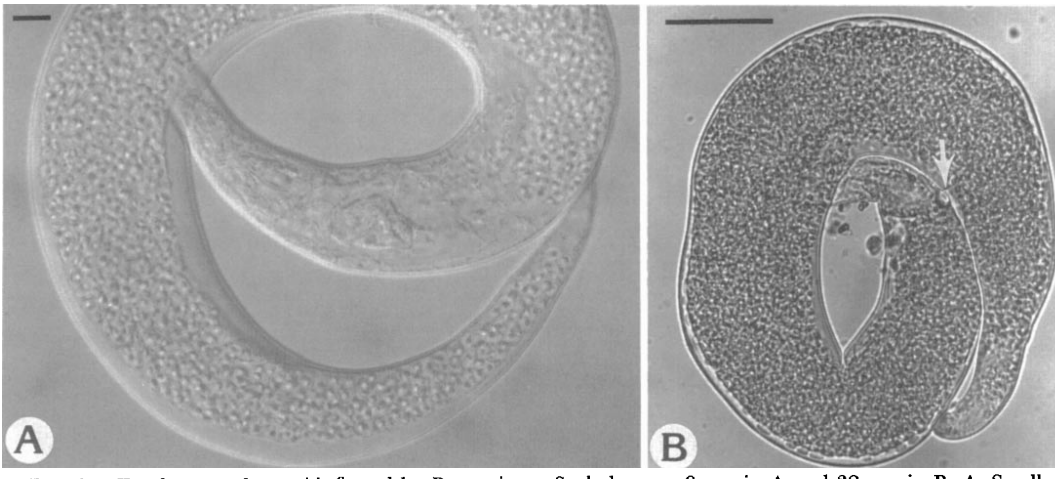

FIG. 3. *Trophonema okamotoi* infected by *Pasteuria* sp. Scale bars = 9 μm in A and 32 μm in B. A. Swollen fourth-stage juvenile with about two-thirds of the body filled with sporangia and endospores. B. Swollen female with body completely packed with sporangia and endospores that have obliterated all nematode organs. Arrow indicates vulva.

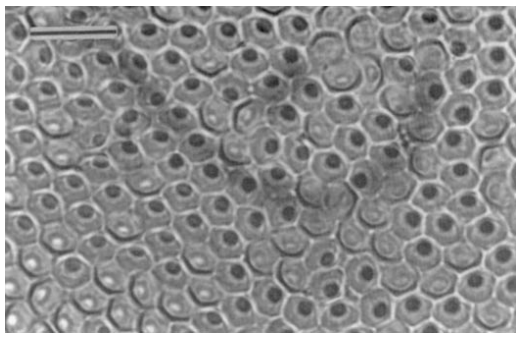

FIG. 4. Tightly packed endospores and sporangia of *Pasteuria* sp. after their release from an infected, swollen female of *Trophonema okamotoi*. Scale bar = 10 μm.

only in the females (8). In contrast, *Pasteuria thornei* spore attachment and endospore formation occur in all life stages of the host nematode *Pratylenchus brachyurus* (12), whereas a population of *Pasteuria* sp. parasitizing *T. semipenetrans* reproduces only in J2 (4).

Host range: Endospores of *Pasteuria* sp. from *T. okamotoi* females attached to J2 but not to other stages of *T. okamotoi* in laboratory experiments. The endospores did not attach to J2 of *T. semipenetrans, M. incognita, M. arenaria,* or *M. javanica,* or to any developmental stage of *H. pseudorobustus* or *P. brachyurus*. The endospore attachment rate to *T. okamotoi* was much lower than the attachment rates for *P. penetrans* to *Meloidogyne* spp. under similar experimental conditions (pers. obs.). We observed approximately five endospores attached per J2 of *T. okamotoi* after 5 days incubation compared with a similar attachment rate after only 24 hours for *P. penetrans* and *Meloidogyne* spp. when tested at the same endospore concentrations.

We conclude that the *Pasteuria* sp. reported herein differs from *P. nishizawae, P. penetrans,* and *P. thornei* in diameter of endospores and central core, host preference, and endospore attachment rate. Our observations strengthen the view that *Pasteuria* sp. "almost certainly constitutes an assemblage of numerous pathotypes and morphotypes; it probably also comprises a multiplicity of taxa, each of currently unknown breadth and categorical level" (7).

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