Partial characterization of a Pasteuria sp. attacking the citrus nematode, Tylenchulus semipenetrans, in Florida (1)

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Summary – Juveniles and males of the citrus nematode, Tylenchulus semipenetrans, infected with a Pasteuria sp. were detected in soil samples collected from a citrus grove in Central Florida. In laboratory studies, three to sixteen endospores attached to cuticles of individual nematodes. Mature endospores were observed within the nematode body 18 days after endospore attachment. Parasite development was asynchronous within individual nematodes. In males, the region of the body filled with spermatocytes was not colonized. Infected second stage juveniles and males each contained 320–400 endospores 18 days after endospore attachment at 25 °C. Adult females infected by Pasteuria sp. were not detected nor were endospores observed attached to the cuticles of adult females. Endospores of the Pasteuria sp. did not attach to the cuticles of Meloidogyne incognita, M. javanica, Radopholus cirophilus, or R. similis.


Key-words: bacteria, biological control, Citrus, parasitism, Pasteuria, Tylenchulus, nematodes.

Pasteuria penetrans (Thorne) Sayre & Starr have been associated with Tylenchulus semipenetrans Cobb in France (Corsica), Florida, Iraq, and Samoa (Sturhan, 1985, 1989; Fattah et al., 1989; Walter & Kaplan, 1990). To date, the only published detailed report of a Pasteuria sp. attacking T. semipenetrans characterized the endospores within the nematode's pseudocoelom and illustrated endospore attachment to the nematode body wall of second-stage juveniles in Iraq (Fattah et al., 1989). A Pasteuria sp. was among a diverse group of predators, parasites, and antagonists of the citrus nematode identified in a survey of Florida citrus groves (Walter & Kaplan, 1990). The purpose of this paper is to further characterize the Pasteuria sp. detected in that survey.

Materials and methods

Life cycle and development

Second-stage citrus nematode juveniles (J2) and adult males were retrieved from 50 cm³ soil samples collected from a citrus grove using modified Baermann funnels. Approximately 250 females were collected by either mechanically removing them from untreated roots or by enzymatically treating roots to facilitate release of intact females prior to dissection of females from the roots (Kaplan & Davis, 1990). The grove was located in Ona, Florida, where a Pasteuria penetrans-like organism attacking citrus nematodes was previously detected (Walter & Kaplan, 1990).

Citrus nematodes were examined using an inverted light-microscope (200×) and those with Pasteuria sp. endospores attached to their cuticles were transferred to individual drops of sterile distilled water on microscope slides and observed at 400× with a light microscope. Three J2 or males containing Pasteuria sp. endospores were individually transferred to watch glasses (BPI) containing 200 μl of sterile distilled water. A dissecting needle was used to break open and crush the parasitized nematodes in order to release the Pasteuria sp. endospores.

After endospore release, 200 μl of water containing 100 J2 and males were added to each of the BPI watch glasses containing Pasteuria sp. endospores. These were

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subsequently placed on a shaker in moist chambers in the dark at 25°C. The citrus nematodes added to the endospore suspension were hatched from eggs isolated from a citrus grove located in Ocoee, Florida, where Pasteuria sp. had not been detected. To attain adequate numbers of freshly hatched citrus nematodes, infested fibrous roots of sour orange (Citrus aurantium L.) were cut into small pieces (ca 5 mm) and 250 ml of water was added to create a slurry of root pieces. This was vigorously shaken for 5 min and then passed through a series of sieves (2000-, 354-, 90-, 63-, and 30-μm opening) to separate the eggs from root pieces, soil particles, and debris. Nematode eggs and fine soil debris were collected on a 20-μm opening nylon-mesh sieve. Citrus nematode eggs were then isolated by adding 10.0 ml of 1.103 specific gravity MgSO₄⋅7H₂O beneath 20 ml of the egg-debris suspension and centrifuged for 10 min (2000 g). Eggs were collected again on the 20-μm opening sieve. The eggs were then rinsed with distilled water. The egg suspension was placed in a vial fitted with a 20-μm nylon mesh over the open end. The vial was inverted for 20 min into a watch glass filled with water to allow males and J2 that had previously hatched to migrate through the 20-μm nylon mesh as before. These nematodes were added to the citrus grove in Central Florida (Fig. 1A). Three to six-endospores were collected from the soil samples collected from a citrus grove in Central Florida (Fig. 1A). Three to sixteen endospores were attached to the cuticles of J2 and males after the Pasteuria sp.-free citrus nematodes were added to the Pasteuria sp.-endospore suspension. The diameter of endospores attached to the nematode cuticles were 2.7 ± 0.3 μm and their central cores were 1.4 ± 0.2 μm. The morphology of the Pasteuria sp. detected in the Florida citrus grove was not significantly different from that reported from Iraq (Fattah et al., 1989) where the endospore diameter was 2.6 μm. From published photographs, the central core diameter of that isolate was estimated to be 1.6 μm (± 0.1).

Three watch glasses were removed from the moist chamber at 3-day intervals over a 30-day period and the nematodes fixed in TAF (Courtney et al., 1955). Nematodes in each of the watch glasses were then examined using an inverted light-microscope (200 x). Nematodes examined for the presence of endospores or other Pasteuria sp. life cycle stages on the nematodes at 1000-1600 x with a Zeiss Photomicroscope II using Normal-sky optics fitted with a DAGE MTI CCD72 video camera and viewed on a DAGE MTI-HR 1000 high resolution monitor. Developmental stages of the parasite, morphometrics (diameter of endospore and central core) of endospores attached to the nematode cuticle and within the nematode host, the location of endospores within the nematode body, and the number of endospores within each of 20 J2 and males were determined with a Hamamatsu Argus-10 Image processor and recorded with a Panasonic Time Lapse Video Cassette Recorder AG-6750A. Images recorded for publication were either photographed off the screen or produced with a Sony Color Video Printer UP 3000.

**Host specificity**

An experiment was conducted to determine if the Pasteuria sp. would attach and/or parasitize Meloidogyne incognita, M. javanica and Tylenchulus semipenetrans collected from Pasteuria sp.-free greenhouse pot cultures. The burrowing nematodes, Radopholus similis, retrieved from carrot disk cultures (Kaplan & Davis, 1990) were also evaluated. Then, 200 cm³ soil samples from the Ocoee site were placed in incubators. A 5.0-ml aqueous suspension containing 1000 nematodes was added to each soil sample. The samples were incubated at 25°C for 5 days and then placed on Baermann funnels. Nematodes retrieved from each of five samples per nematode species were examined using an inverted light-microscope (200 x) for the presence of attached endospores. Individuals with endospores attached to their bodies were transferred to BPI watch glasses containing distilled water and incubated in a dark moist chamber at 25°C. Nematodes were examined for the presence of endospore development 20 days after placement in incubators.

**Results and discussion**

Males and J2 of T. semipenetrans infected with Pasteuria sp. were detected in soil samples collected from a citrus grove in Central Florida (Fig. 1A). Three to sixteen endospores were attached to the cuticles of J2 and males six days after the Pasteuria sp.-free citrus nematodes were added to the Pasteuria sp.-endospore suspension. The diameter of endospores attached to the nematode cuticles were 2.7 ± 0.3 μm and their central cores were 1.4 ± 0.2 μm. The morphology of the Pasteuria sp. detected in the Florida citrus grove was not significantly different from that reported from Iraq (Fattah et al., 1989) where the endospore diameter was 2.6 μm. From published photographs, the central core diameter of that isolate was estimated to be 1.6 μm (± 0.1).

Mature endospores ("fried-egg" shaped) appeared within the bodies of T. semipenetrans 18 days after endospore attachment (Fig. 1B-C). In contrast to P. penetrans which developed synchronously in Meloidogyne (Sayre & Starr, 1985), development of Pasteuria sp. was asynchronous within a single citrus nematode (Fig. 1D). In contrast to the Pasteuria sp. isolated from Iraq (Fattah et al., 1989), the Florida Pasteuria sp. isolate produced sporangia and endospores within males (Fig. 1E). The endospores probably attached to males after they had reached the adult stage (Fig. 1F). Infected second-stage juveniles and males contained 320-400 endospores 18 days after attachment.

Adult female specimens of many sedentary and several migratory species of plant parasitic nematodes have also been found filled with endospores of members of the P. penetrans group (Davies et al., 1990; Giblin-Davis, 1990). However, endospores were not detected on the cuticles or within mature citrus nematode females.
Fig. 1. Second-stage juveniles (J2) and males of Tylenchulus semipenetrans parasitized by Pasteuria sp. A: J2 with mature endospore (ME) attached to tail and containing mature endospores and sporangia; B: Pasteuria sp.-filled J2 broken to release mature endospores (ME); C: Detail of mature endospores released from broken J2; D: Asynchronous development of Pasteuria sp. within the J2 as evidenced by sporangia (S) and mature endospores (ME); E: Mature endospore (ME) attached to head region of male T. semipenetrans with germ tube (GT); F: Mature endospore (ME) attached to male cuticle in vicinity of spermatocytes.

Examination of citrus nematode females is complicated by the parasitic habit of the citrus nematode; intracellular invasion of the root accompanied by the enlargement of the anterior half of the female makes it difficult to attain entire females without breaking them at the point where they emerged from roots (semi-endoparasite). That is, the anterior end of the nematode generally remains in the root when the posterior portion and body contents are lost. However, it is likely that if present, endospores would have been observed when females
were broken away from the root surface. In an attempt to circumvent this, tissue-softening enzymes were used to release females from root tissue, but none of the females retrieved had endospores attached to their cuticles or within their bodies.

Citrus nematode J2 and males remain in soils for extended periods of time. This, in conjunction with the relatively short life cycle of Pasteuria sp. (18 days), would be conducive to the development of mature endospores in the migratory stage of the nematode. Isolates of other Pasteuria sp. that complete their life cycles in vermiform stages of some other sedentary plant parasitic nematodes have also been noted to have a rapid life cycle (Sayre & Starr, 1988; Giblin-Davis, 1990).

Pasteuria sp. did not attach to cuticles of Meloidogyne incognita, M. javanica, Radopholus similis, or R. similis. However, Pasteuria sp. did become attached to T. semipenetrans J2 and males that migrated through the soil. After 20 days incubation, endospore development was observed in several T. semipenetrans J2 and males. This suggests that Pasteuria sp. is specific for the citrus nematode.

Pot cultures of the Florida isolate of Pasteuria sp. have been established using T. semipenetrans on citrus trees growing in Pasteuria sp.-infested soil and by direct infestation with Pasteuria sp.-infected J2 and males. However, it has been difficult to recover enough Pasteuria sp.-colonized nematodes to determine the effect of Pasteuria sp. on citrus nematode population dynamics. Failure to detect females filled with endospores suggests that previously described techniques for P. penetrans culture (Stirling & Wachtel, 1980) are not useful for this citrus nematode parasite since Pasteuria sp.-infected stages occur in the soil. Although the objective of this study was not directed toward development of a culture method for Pasteuria sp. on citrus nematode, the ability of Pasteuria sp. to attach, penetrate, and produce endospores under laboratory conditions suggests that this may provide a means of Pasteuria sp. population increase. We are exploring this possibility.

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References


