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Life Cycle, Ultrastructure, and Host Specificity of the North American Isolate of *Pasteuria* that Parasitizes the Soybean Cyst Nematode, *Heterodera glycines*[1]

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Abstract: Light and transmission electron microscopy were used to investigate the life cycle and ultrastructure of an undescribed isolate of *Pasteuria* that parasitizes the soybean cyst nematode, *Heterodera glycines*. Studies also were conducted to determine the host specificity of *Pasteuria*. The endospores that attached to the cuticle of second-stage juveniles (J2) of *H. glycines* in soil did not germinate until the encumbered nematodes invaded soybean roots. Thereafter, the bacterium developed and completed its life cycle only in females. The stages of endosporogenesis were typical of *Pasteuria* spp. The mature endospore, like that of *P. nishizawae,* the only other *Pasteuria* known to infect *H. glycines,* produces an epicortical layer that completely surrounds the cortex, an outer spore coat that tapers progressively from the top to the base of the central body, and a double basal adhesion layer. However, subtle differences exist between the *Pasteuria* from North America and *P. nishizawae* with regard to size of the central body, nature and function of the mesosomes observed in the earlier stages of endosporogenesis, and appearance of the fibers lining the basal adhesion layer and the exosporium of the mature endospore. Endospores of the North American *Pasteuria* attached to J2 of *H. schachtii, H. trifolii,* and *H. lespedezae* but not to *Meloidogyne arenaria* race 1, *Tylenchorhynchus nudus,* and *Labronema* sp. Results from this study indicate that the North American *Pasteuria* is more similar to *P. nishizawae* than to any other known member of the genus. Additional evidence from comparative analysis of 16S rDNA sequences is needed to clarify whether these two *Pasteuria* belong to the same species.

Key words: Heterodera glycines, host specificity, life cycle, *Pasteuria* spp., soybean cyst nematode, taxonomy, ultrastructure.

The gram-positive, mycelial, and endospore-forming bacteria of the genus *Pasteuria* are obligate parasites that are associated only with invertebrate hosts (Sayre, 1993; Sayre and Starr, 1989). Apart from *P. ramosa,* the type species that occurs on cladoceran water fleas of the genera *Daphnia* (Ebert et al., 1996; Metchnikoff, 1888) and *Moina* (Sayre et al., 1979, 1983), the other species of *Pasteuria* that have been described are parasites of plant-parasitic nematodes. *Pasteuria* spp. have potential as biological control agents of nematodes (Atibalentja et al., 1998; Brown et al., 1985; Chen et al., 1996, 1997b; Duponnois and Ba, 1998; Giblin-Davis, 1990; Gowen et al., 1998; Nishizawa, 1987; Weibelzahl-Fulton et al., 1996). The three nematode-infecting *Pasteuria* species with nomenclatural standing include *P. penetrans* parasitic on root-knot nematodes, *Meloidogyne* spp. (Sayre and Starr, 1985; Starr and Sayre, 1988); *P. thornei* on root-lesion nematodes, *Pratylenchus* spp. (Starr and Sayre, 1988); and *P. nishizawae* on cyst nematodes of the genera *Heterodera* and *Globodera* (Sayre et al., 1991a, 1991b). A fourth nematode-infecting *Pasteuria* with provisional species designation (Murray and Schleifer, 1994; Murray and Stackebrandt, 1995; Stackebrandt et al., 2002), *Candidatus* Pasteuria usgae ex *Belonolaimus longicaudatus,* was described recently (Giblin-Davis et al., 2003). In addition to the above validly and provisionally described species, *Pasteuria* spp. have been reported worldwide from hundreds of nematode species distributed over more than 100 genera including plant-parasitic, entomopathogenic, predatory, and free-living nematodes (Chen and Dickson, 1998; Ciancio et al., 1994; Sayre and Starr, 1988; Sturhan, 1988).

For many years, *Pasteuria* spp. have eluded attempts for axenic cultivation (Bishop and Ellar, 1991; Williams et al., 1989). A breakthrough toward in vitro cultivation of *P. penetrans* was announced recently (Hewlett et al., 2002). However, the details of the technique remain proprietary information that might not be available to the public in the foreseeable future. In the absence of pure cultures prescribed by standard biochemical tests in bacterial systematics (Goodfellow and O'Donnell, 1993), members of the genus *Pasteuria* have been described mainly in terms of morphological, developmental, and pathological characteristics such as the shape and size of the sporangium and endospore, ultrastructure, life cycle, and host specificity (Davies et al., 1990; Giblin-Davis et al., 1990; Metchnikoff, 1888; Noel and Stanger, 1994; Sayre and Starr, 1989; Sayre et al., 1991a, 1991b; Starr and Sayre, 1988; Sturhan et al., 1994). In a few instances, morphological, ultrastructural, and host specificity data have been supplemented by 16S rDNA sequence analysis (Anderson et al., 1999; Atibalentja et al., 2000; Bekal et al., 2001; Ebert et al., 1996). The objectives of the present study were to elucidate the life cycle, ultrastructure, and host specificity of an undescribed isolate of *Pasteuria* that was reported as a parasite of the soybean cyst nematode *H. glycines* Ichinohe (Noel and Stanger, 1994) and to compare this *Pasteuria* with other members of the genus, especially *P. nishiza-*

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wae, the only *Pasteuria* previously known to attach to and infect *H. glycines* (Lee et al., 1998; Sayre et al., 1991a, 1991b).

Materials and Methods

Light microscopy (LM): Root systems were harvested at 2-day intervals beginning 8 days after germination of *H. glycines*-susceptible Williams 82 soybean planted in soil naturally infested with both *H. glycines* and *Pasteuria* in a greenhouse. Roots were macerated in 10% (v/v) Pectinex Ultra SP-L (Novo Nordisk BioChem North America, Franklin, NC) for 24 hours on a platform shaker (156 rpm) at room temperature (24 °C). The slurry was homogenized in a blender at high speed with three 20-s pulses, and the materials were washed through a series of 850-, 150-, and 37-µm-pore sieves. Juvenile stages of *H. glycines* were selected from the 150- and 37-µm fractions with the help of a stereomicroscope. The selected nematodes were mounted on temporary slides in 2.5% formalin, pH 7.0, and examined with interference contrast microscope. Measurements were made with an eyepiece micrometer, and photomicrographs recorded various stages of infection and the bacterium life cycle. Using the centrifugal flotation method (Jenkins, 1964), females and cysts of *H. glycines* were extracted from the rhizosphere of the same soybean plants and collected in a 180-µm-pore sieve. Individual females and cysts were selected, mounted, and examined under similar conditions as juveniles. Males were collected from infected roots placed in a mist chamber and examined for infection by *Pasteuria.*

Transmission electron microscopy (TEM): Pasteuria-infected females and cysts, recognizable by their opaque appearance, were selected from the nematodes that were extracted by the centrifugal flotation method (Jenkins, 1964) from the rhizosphere of 3-month-old soybean plants grown in infested soil in a greenhouse. The selected nematodes were fixed for 8 hours at room temperature in a 1:1 mixture of 4% (v/v) glutaraldehyde and 4% (v/v) paraformaldehyde in 0.05 M phosphate-buffer, pH 7.2. The samples were washed in the same buffer for 3 hours, with changes every 15 minutes, and allowed to incubate in the last buffer change at 4 °C overnight. Thereafter, the specimens were postfixed in a 0.05 M phosphate-buffered (pH 7.2) solution of 1% osmium tetroxide for 2 hours at room temperature, dehydrated in an ascending acetone series, and infiltrated for 16 hours at 40 °C. The samples were polymerized for 72 hours at 60 °C in an epoxy mixture that consisted of glycerol polyglycidyl ether (eponate 12 resin), dodecenyl succinic anhydride (DDSA), nadic methyl anhydride (NMA), and 2,4,6-tris(dimethylaminomethyl) phenol (DMP-30) (TED PELLA, Redding, CA). Survey sections (0.5-2 µm) were cut with a Reichert Ultracut E ultramicrotome (Leica, Wien, Austria) and examined at 1,000× for the presence of *Pasteuria.* Positive specimens were overlaid with LX112 epoxy resin and polymerized further for 18 hours at 65 °C. Ultrathin sections (90-100 nm) were cut and mounted on formvar-carbon-coated grids. Sections were stained with aqueous saturated uranyl acetate for 40 minutes and with the modified Sato's triple lead stain (Hanaichi et al., 1986) for 1 minute. Stained sections were viewed with a Hitachi H-600 transmission electron microscope (Hitachi, Tokyo, Japan) operated at 100 kV with 300-µm and 100-µm condenser and objective apertures, respectively. Measurements were obtained from enlarged micrographs. The terminology suggested by Sussman and Halvorson (1966) and reviewed by van Iterson (1984) was used to describe the ultrastructure of *Pasteuria.*

Endospore attachment tests: Pasteuria-infected females and cysts of *H. glycines* were selected and transferred individually into 1.6-ml microfuge tubes containing 0.1 ml tap water, in which the nematodes were crushed with a tissue grinder. A 10-µl aliquot was examined microscopically for the presence of mature endospores, and positive fractions were pooled in a 1.6-ml microfuge tube to constitute the stock suspension, the concentration of which was determined with a Levy-Hausser counting chamber (Arthur H. Thomas, Philadelphia, PA). The centrifugation method (Hewlett and Dickson, 1993) was used for attachment tests. Specifically, 0.1-ml suspensions containing 100,000 endospores/ml were added to equal volumes of tap water in separate 1.6-ml microfuge tubes that contained 100 2-day-old second-stage juveniles (J2) for *Heterodera* and *Meloidogyne* species, or 100 mixed stages for *Tylenchorhynchus nudus* and *Labronema* sp. (see Table 1). The mixtures were centrifuged for 2 minutes at 10,500g in an Eppendorf microcentrifuge and transferred into 60 × 15-mm culture dishes where the number of endospores attached to each of 20 nematodes was determined with the aid of an inverted microscope. The test was repeated at least twice for each of the nematode species and races investigated, and data were subjected to the analysis of variance (PROC GLM, SAS Institute, Cary, NC).

Results

Microscopic examinations (LM) showed no evidence of germination from the *Pasteuria* endospores that adhered to the cuticle of J2 collected from soil. In contrast, some of the J2 excised from the soybean roots contained endospores from which a germ tube had differentiated and penetrated into the body of the nematode (Fig. 1A). In apical view, germination was evidenced by the sunken and less refractile appearance of the central body, compared to an evenly rounded, bulging, and highly refractile central body in ungerminated

TABLE 1. Attachment of endospores of the North American isolate of *Pasteuria* that parasitizes *Heterodera glycines* to selected species of nematodes.[a]

Nematode species tested	Endospores per nematode[b]	Encumbered nematodes (%)
H. glycines race 1	2.3 C[c]	70 AB[c]
-″- race 2	2.1 C	62 AB
-″- race 3	1.5 D	49 B
-″- race 4	1.2 D	49 B
-″- race 5	1.2 D	52 B
-″- race 14	2.1 C	69 AB
H. schachtii	3.7 A	86 A
H. trifolii	2.1 C	73 AB
H. lespedezae	3.4 B	69 AB
Meloidogyne arenaria race 1	0.0 E	0 C
Tylenchorhynchus nudus	0.0 E	0 C
Labronema sp.	0.0 E	0 C

[a] Attachment tests involved 0.2-ml suspensions containing 10,000 endospores and 100 2-day-old second-stage juveniles (J2) for *Heterodera* spp. and *M. arenaria* race 1, or 100 mixed stages for *T. nudus* and *Labronema* sp.

[b] Figures are means of 2 to 10 replications, with at least 20 nematodes examined per replication. Numbers of endospores per nematode were transformed to $\ln(x + 1)$ prior to analysis of variance.

[c] Means followed by the same letter are not significantly different ($P > 0.05$) according to Fisher Least Significant Difference test.

endospores. Occasionally, remnants of both germinated and ungerminated endospores were found attached to a sloughed J2 cuticle. Following germination, cauliflower-like primary microcolonies were observed either in late J2 (Fig. 1B) or in early third-stage juveniles (J3) (Fig. 1C). Whereas the sex of J3 nematodes was not obvious, the vermiform fourth-stage male juvenile still folded in the old J3 cuticle clearly was distinct from the swollen flask-shaped fourth-stage female juvenile with developing ovaries. The distinction facilitated the monitoring of the fate of *Pasteuria* inside nematodes of either sex. It was observed that *Pasteuria* did not develop inside the fourth-stage male juveniles and adult males, despite the fact that endospores may occasionally adhere to the cuticle of the latter. In contrast, numerous secondary microcolonies originating from the fragmentation and proliferation of the primary microcolonies were found inside the body cavity of infected fourth-stage female juveniles and immature females (Fig. 1D). Grape-like clusters of early sporangia also were apparent in some specimens, indicating that sporulation occurred in both the fourth-stage female juveniles and immature females. As a result of such an asynchronous sporulation, infected females and cysts commonly contained mixtures of developmental stages of *Pasteuria* including grape-like clusters of early sporangia, octets, quartets, triplets, doublets, and individual sporangia (Fig. 1E, F). Ultimately, however, parasitized females and cysts were filled mainly with mature sporangia and endospores (Fig. 1G, H), the number of which varied with the size of the female or cyst, from 30,000 to 820,000, with mean and standard deviation of 314,000 and 234,000, respectively. The mature cup-shaped sporangia measured (mean ± standard deviation) 4.7 ± 0.3 × 3.7 ± 0.5 μm, and their highly refractile central body was 2.1 ± 0.3 × 1.7 ± 0.3 μm. In comparison, the developing sporangia in the quartet stage measured 2.4 ± 0.0 μm in diam., with a height of 4.0 ± 0.5 μm from the attachment point to the distal end. In the triplet configuration, sporangia were 2.5 ± 0.6 × 3.4 ± 0.3 μm compared to 2.9 ± 0.9 × 3.3 ± 0.4 μm in the doublet stage.

Six of the seven stages of bacterial endosporogenesis (Bechtel and Bulla, 1976; Chen et al., 1997a; Decker and Maier, 1975; Ellar and Lundgren, 1966; Holt et al., 1975; Ryter, 1965), from the forespore septum formation (stage II) to the maturation of the endospore (stage VII), were observed in the TEM study. Stage I, during which the nucleoid material condenses to form an axial filament, was not observed. The first recorded evidence of endosporogenesis was the formation of a bi-layered septum that divided the protoplast of an enlarged terminal cell of a dichotomously branching vegetative microcolony into a smaller distal region, the forespore, and a larger basal region, the mother cell or sporangium (Fig. 2A). Membranous, mesosome-like bodies were present in either of the two regions, but the association of those structures with the plasma membrane and their involvement with the forespore septum formation were not observed. During forespore engulfment (stage III), the newly formed septum bulged into the center of the sporangium, even while the developing endospores were still bundled together into grape-like clusters (Fig. 2B). At this point, the nascent perisporium appeared as two sub-lateral electron-translucent regions near the base of the forespore. At the peak of the engulfment process, the forespore protoplast condensed into a central body completely encircled by the two unit-membranes of the septum, hereafter termed the inner (plasma) forespore membrane and the outer forespore membrane, the latter overlaid with dense staining materials (Fig. 3A). The center of the central body was occupied by an electron-translucent region containing the DNA, while the nascent perisporium had expanded laterally to form the peripheral fibers. Cortex formation (stage IV) occurred gradually, as layers of electron-dense materials filled up the space between the primordial cell wall surrounding the inner forespore membrane and the outer forespore membrane (Fig. 3B). A thin layer of electron-transparent materials separates the primordial cell wall from the innermost cortex layer. As cortex expansion continued, the electron-transparent peripheral fibers became interspersed with strands of electron-opaque materials (Fig. 4A). During the synthesis of spore coats (stage V), the accumulation of electron-dense materials around the outer forespore membrane intensified markedly, resulting in the formation of a multilayered outer spore coat (Fig. 4A). Laterally and sub-laterally,

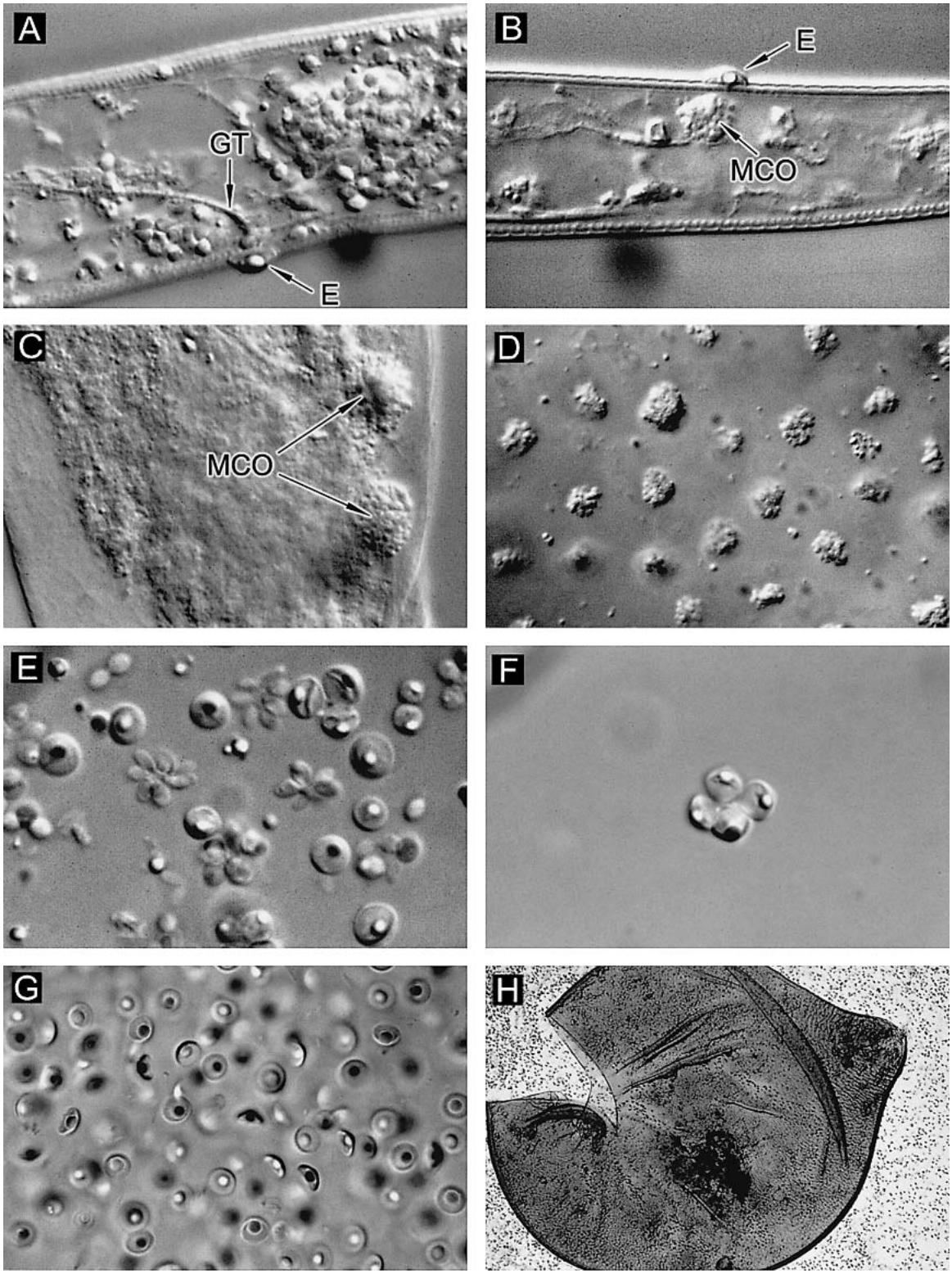

FIG. 1. Photomicrographs describing the life cycle of the North American isolate of *Pasteuria* that parasitizes *Heterodera glycines*. A) The *Pasteuria* endospore (E) that attaches to the cuticle of a second-stage juvenile (J2) in soil germinates soon after the J2 invades the soybean root by differentiating a germ tube (GT) that penetrates into the body of the nematode. B) Following germination, a primary vegetative microcolony (MCO) forms underneath the endospore inside the body of the J2. C) Formation of primary microcolonies also may take place inside the body of early third-stage juveniles. D) Subsequent fragmentation of primary microcolonies results in the formation of numerous secondary microcolonies that proliferate throughout the body cavity of fourth-stage female juveniles and immature females. E) Since *Pasteuria* sporulation is asynchronous, infected females and cysts commonly contain mixtures of developmental stages of the bacterium including octets, quartets, triplets, doublets, and individual sporangia. F) A quartet of sporangia from an infected female. G) Ultimately, parasitized females and cysts are filled mainly with mature sporangia and endospores. H) A parasitized female that was broken open to show her content of mature sporangia and endospores.

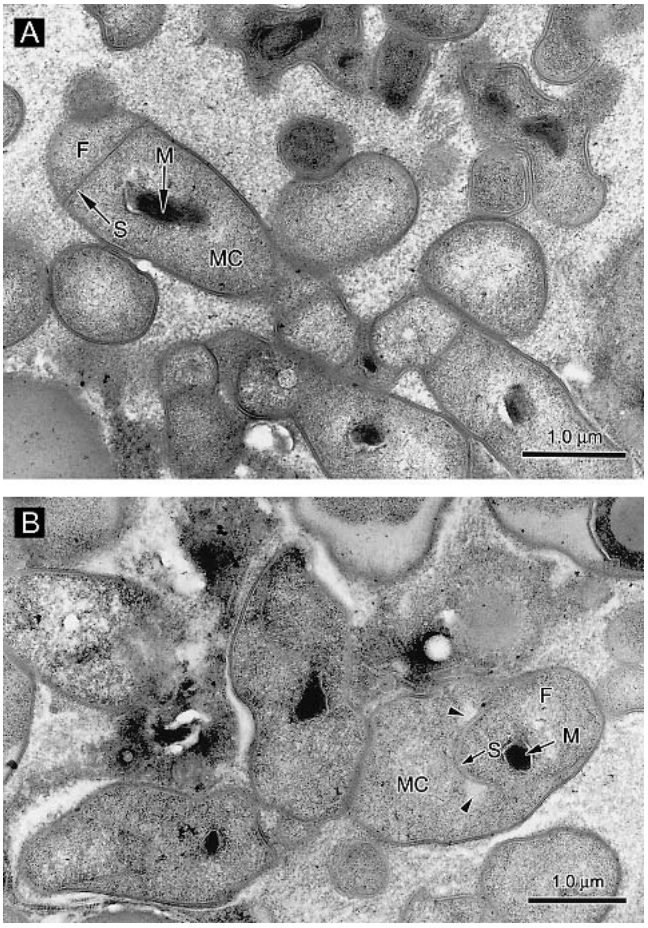

FIG. 2. TEM micrographs showing the early stages of endosporogenesis in the North American isolate of *Pasteuria* that parasitizes *H. glycines*. A) This section through a vegetative microcolony shows the stage II of endosporogenesis, when a septum (S) divides an enlarged terminal cell of a microcolony into a smaller distal region, the forespore (F), and a larger proximal region, the mother cell (MC) or sporangium. Mesosome-like bodies (M) may be present in either of the two regions. B) A cluster of developing endospores at the beginning of the engulfment process (stage III), when the forespore septum (S) bulges into the center of the mother cell (MC). The nascent perisporium appears as two sub-lateral electron-translucent regions (arrow heads) near the base of the forespore (F). Mesosome-like bodies (M) are also evident.

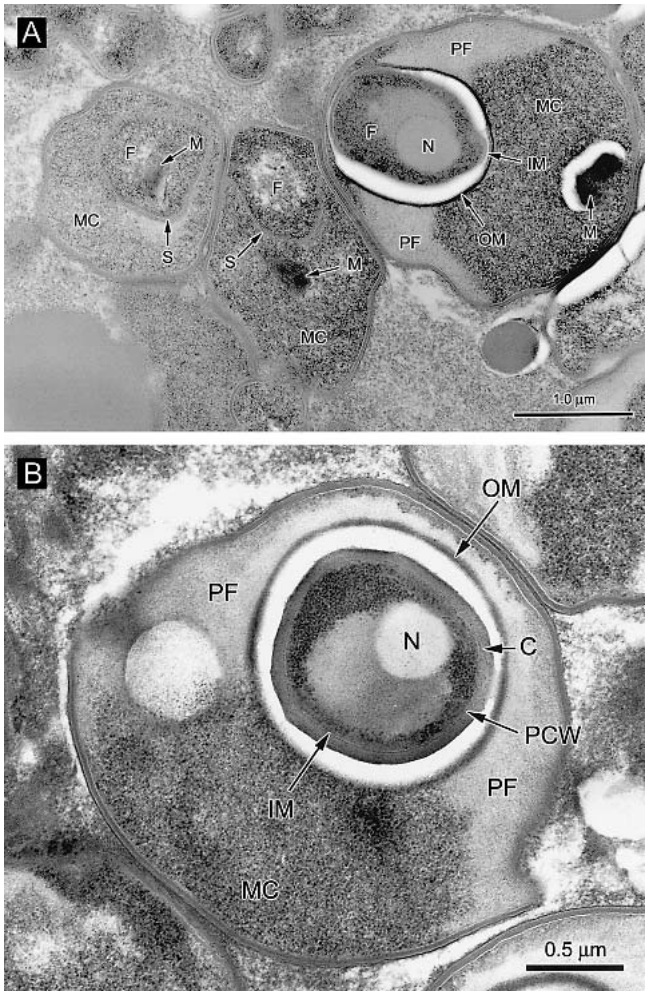

FIG. 3. TEM micrographs showing more advanced stages of endosporogenesis in the North American isolate of *Pasteuria* that parasitizes *H. glycines*. A) Section through a developing endospore at the end of the engulfment stage (right), when the forespore (F) protoplast condenses into a central body completely encircled by the two membranes of the septum: the inner forespore membrane (IM) and the outer forespore membrane (OM), which is overlaid by an electron-dense substance. The center of the central body is occupied by the nucleoid (N), and the nascent perisporium has expanded laterally to form the peripheral fibers (PF). Also evident in this micrograph are mesosome-like bodies (M), the mother cell matrix (MC), and the septum (S). B) A developing endospore at the beginning of stage IV, when concentric layers of cortex (C) gradually fill up the region between the primordial cell wall (PCW) and the outer forespore membrane (OM). The inner forespore membrane (IM), the mother cell matrix (MC), the nucleoid (N), and the peripheral fibers (PF) are also indicated.

the outermost layer of the outer spore coat exhibits densely packed fibrous micro-projections that stand perpendicular to the inner concentric layers (Fig. 4B). The outer spore coat (including the micro-projections) is thickest at the top of the central body, then tapers progressively to 0.2 µm at the endospore equator and to 0.1 µm or less around a 0.3 ± 0.1 µm-wide basal pore. A laminar inner spore coat with alternating layers of light and dense materials is present between the cortex and the outer forespore membrane. The major event in stage VI was the formation of a velutinous exosporium that encloses both the central body and the perisporium (Fig. 4B). Maturation of the endospore (stage VII) continued while it was still contained in the sporangium. At this stage, the bacterium (endospore + sporangium) corresponds to the mature sporangium viewed in LM and measures 4.4 ± 0.3 µm in diam. against 2.9 ± 0.3 µm in height. The endospore maturation culminated with the disintegration of the sporangium, at which time the free endospore appeared saucer-shaped, measuring 4.2 ± 0.3 µm in diam. and 1.6 ± 0.2 µm in height, with an elliptical 1.9 ± 0.3-µm × 1.5 ± 0.2-µm central body (Fig. 5A). In the mature endospore, the cortex appears less electron-dense than it was in stage IV, and it is surrounded by a diffuse, granular, and electron-dense epicortical layer. In some micro-

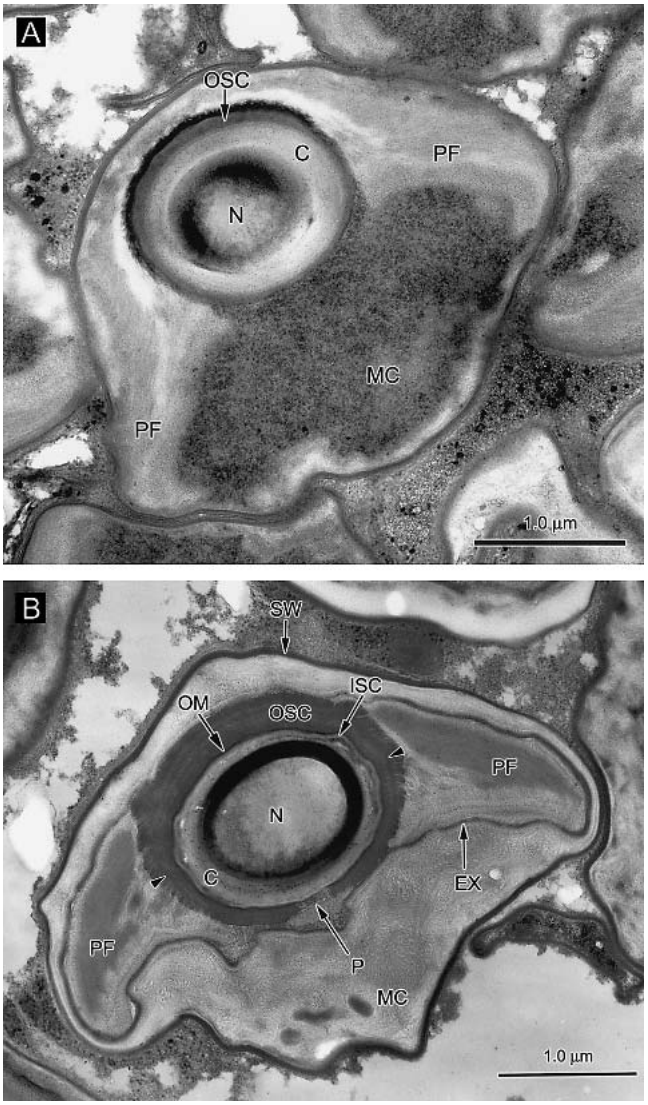

FIG. 4. TEM micrographs showing the final stages of endosporogenesis in the North American isolate of *Pasteuria* that parasitizes *H. glycines*. A) A developing endospore at stage V, when successive accumulation of electron-dense materials around the outer forespore membrane results in the formation of a thick outer spore coat (OSC). The electron-transparent peripheral fibers (PF) at this stage are interspersed with strands of dense staining materials. Also apparent in the micrograph are the cortex (C), the mother cell matrix (MC), and the nucleoid (N). B) Stage VI of endosporogenesis, when a velutinous exosporium (EX) forms around the endospore, which is still enclosed by the mother cell matrix (MC) surrounded by the sporangial wall (SW). Arrow heads indicate the boundaries between the micro-projections and the other layers of the outer spore coat (OSC). The cortex (C), the laminar inner spore coat (ISC), the nucleoid (N), the outer forespore membrane (OM), the basal pore (P), and the peripheral fibers (PF) are also shown.

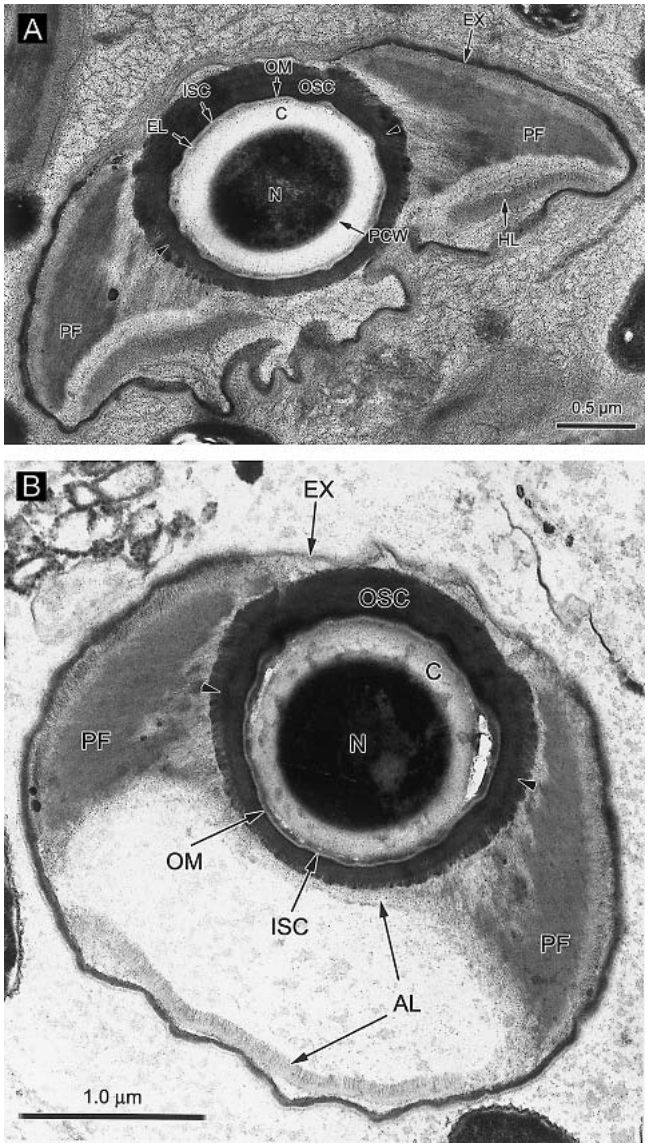

FIG. 5. TEM micrographs showing mature endospores of the North American isolate of *Pasteuria* that parasitizes *H. glycines*. A) A mature endospore in which the basal adhesion layer is fully retracted, giving the impression of the presence of an additional partial hirsute layer (HL). Arrow heads indicate the boundaries between the micro-projections and the other layers of the outer spore coat (OSC). The cortex (C), the epicortical layer (EL), the exosporium (EX), the laminar inner spore coat (ISC), the nucleoid (N), the outer forespore membrane (OM), the primordial cell wall (PCW), and the peripheral fibers (PF) are indicated. B) A mature endospore in which the basal adhesion layer (AL) is full extended and, therefore, no partial hirsute layer is visible. Arrow heads indicate the boundaries between the micro-projections and the other layers of the outer spore coat (OSC). The cortex (C), the exosporium (EX), the laminar inner spore coat (ISC), the nucleoid (N), the outer forespore membrane (OM), and the peripheral fibers (PF) are apparent.

graphs, the endospore exhibits a partial hirsute layer that probably originated from an invagination of the basal adhesion layer (Figs. 5A, B; 6).

In attachment tests, endospores from parasitized females and cysts of *H. glycines* race 4 attached only to J2 of the *Heterodera* species tested (Table 1). The efficiency of attachment varied, however, with the species and race of *Heterodera*. A higher number of endospores attached to J2 of *H. schachtii* than to those of other *Heterodera* spp. tested ($P < 0.05$). Likewise, a higher percentage (86%) of *H. schachtii* J2 were encumbered with at least one endospore, compared to 50% only for J2 of *H. glycines* races 3 to 5. The endospores of the North American *Pasteuria* did not attach to *Meloidogyne are-*

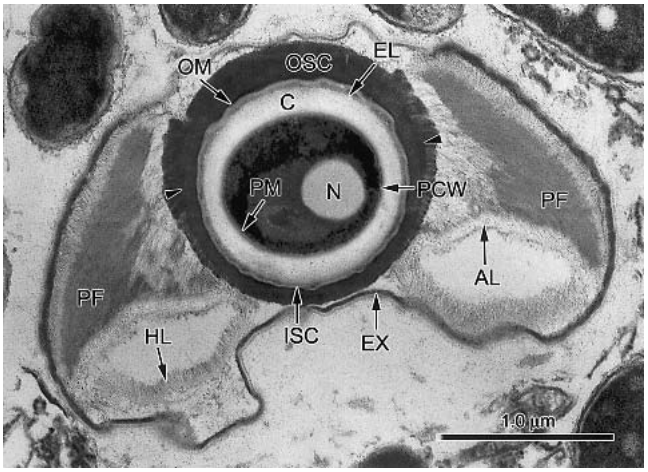

FIG. 6. A TEM micrograph showing a mature endospore of the North American isolate of *Pasteuria* that parasitizes *H. glycines*. The basal adhesion layer (AL) is fully retraced toward the base of the central body, but not on the lateral sides. This view leaves the impression of the presence of an additional partial hirsute layer (HL) on the obverse face of the endospore. Arrow heads indicate the boundaries between the micro-projections and the other layers of the outer spore coat (OSC). Also shown in the micrograph are the cortex (C), epicortical layer (EL), exosporium (EX), laminar inner spore coat (ISC), nucleoid (N), outer forespore membrane (OM), primordial cell wall (PCW), plasma membrane (PM), and peripheral fibers (PF).

naria race 1, *Tylenchorhynchus nudus,* and the Dorylaim *Labronema* sp. Results were similar with endospores collected from *H. glycines* race 3.

DISCUSSION

This study describes the life cycle of the North American isolate of *Pasteuria* that parasitizes *H. glycines,* from the germination of the bacterium on the invading J2 to the production of the next generation of endospores in adult females and cysts. Descriptions were based on microscopic examination of successive juvenile stages of *H. glycines* excised from soybean roots unlike those of *P. nishizawae,* which were based solely on examination of diseased cysts (Sayre et al. 1991a, 1991b). Although diseased cysts of *H. glycines* generally contain a mixture of cells in various stages of the *Pasteuria* life cycle, the bacterium development, especially endospore germination and germ tube penetration inside the nematode, must begin soon after the encumbered J2 invades the soybean root. Otherwise, the ungerminated endospore would be shed with the cuticle of the J2 when it molts into the J3, and there would be no *Pasteuria* infection. For this reason, observations based solely on diseased cysts provide an incomplete account of the life cycle of *Pasteuria,* which might explain why germination of *P. nishizawae* was not observed (Sayre et al., 1991a, 1991b). The finding that the *Pasteuria* endospores that attach to the cuticle of J2 in soil do not germinate until the encumbered J2 invades the soybean root raises at least two intriguing questions. First, what prevents those endospores from germinating before root invasion? Second, what triggers the endospore to germinate after the nematode has invaded the root? It is also interesting that *Pasteuria* develops only in females of *H. glycines,* since such a behavior is likely to have serious implications on the population dynamics of both the parasite and its host nematode and, therefore, on the prospects of using *Pasteuria* as a biological control agent of *H. glycines*. From the population dynamics viewpoint, the significance of *Pasteuria* developing only in females of *H. glycines* can easily be appreciated once it is understood that the effect of the number of endospores that fail to develop in males is to inflate the parasite death rate parameter in predator-prey models used to describe the *Pasteuria-H. glycines* interaction in soil (Atibalentja et al., 1998).

The life cycle of the North American isolate of *Pasteuria* is similar to that of *P. nishizawae* (Sayre et al., 1991a, 1991b) and *P. penetrans* (Starr and Sayre, 1988) in that all three *Pasteuria* spp. develop only in females of their respective nematode hosts. Development of *P. penetrans* in males of *M. arenaria* has been reported, but only under empirical conditions (Hatz and Dickson, 1992). The life cycle of the North American *Pasteuria* differs from the one exhibited by the *Pasteuria* isolates that develop exclusively in J2 of the oat cyst nematode *H. avenae* (Davies et al., 1990) and the pea cyst nematode *H. goettingiana* (Sturhan et al., 1994). A third form of life cycle exhibited by nematode-infecting *Pasteuria* is found in *P. thornei* and *Candidatus* Pasteuria usgae, which develop in both juveniles and adults of *Pratylenchus* spp. and *B. longicaudatus,* respectively (Giblin-Davis et al., 2003; Sayre et al., 1988; Starr and Sayre, 1988). A variant of this type of life cycle occurs on the *Pasteuria* isolate that parasitizes juveniles and males, but not females, of the citrus nematode *Tylenchulus semipenetrans* (Fattah et al., 1989; Sorribas et al., 2000).

The data from this study indicate that the sporangia and endospores of the North American *Pasteuria* are the most similar in shape, morphometrics, and ultrastructure to those of *P. nishizawae*. Together, these two *Pasteuria* form a group that stands out from all other described species and isolates in at least one respect. For instance, the *Pasteuria* isolate that parasitizes *H. goettingiana* (Sturhan et al., 1994) shares with the *H. glycines*-infecting *Pasteuria* the unique ability of the endospore to produce an epicortical layer that completely surrounds the cortex, and an outer spore coat that tapers progressively from the top to the base of the central body. However, unlike those of the *H. glycines*-infecting *Pasteuria,* mature endospores of the *H. goettingiana*-infecting *Pasteuria* contain portions of sporangium entrapped within the exosporium (Fig. 11 in Sturhan et al., 1994). Furthermore, endospores of the *H. goettingiana*-infecting *Pasteuria* lack the double basal adhesion layer observed in the *H. glycines*-infecting *Pas-*

teuria (Figs. 5,6 in this paper; Figs. 16,10 in Sayre et al., 1991a, 1991b, respectively).

In spite of their similarity, subtle differences exist between the North American *Pasteuria* and *P. nishizawae*. The central body of the mature endospore is slightly larger in the North American *Pasteuria* (1.9 ± 0.3 µm × 1.5 ± 0.2 µm) than in *P. nishizawae* (1.6 ± 0.2 µm × 1.3 ± 0.1 µm) (Sayre et al., 1991a, 1991b). The North American *Pasteuria* also differs from *P. nishizawae* with respect to the nature and putative function of the mesosomes present in the earlier stages of endosporogenesis. In the North American *Pasteuria,* mesosomes are laminar and have no apparent relationship with the plasma membrane nor do they seem to play any role in the forespore septum formation. The vesicular mesosomes of *P. nishizawae* were associated with forespore septum formation (Sayre et al., 1991a, 1991b). Mesosomes have also been observed in *P. ramosa* (Sayre et al., 1979, 1983), *P. penetrans* (Imbriani and Mankau, 1977; Mankau, 1975; Sayre, 1993; Sayre and Starr, 1985), and in the *Pasteuria* isolate S-1 that parasitizes *B. longicaudatus* (Giblin-Davis et al., 2001). In contrast, mesosomes were absent in *P. thornei* (Sayre et al., 1988; Starr and Sayre, 1988) and other isolates of *Pasteuria* (Chen et al., 1997a; Sturhan et al., 1994). Whether mesosomes are real prokaryotic structures or mere artifacts of conventional preparation techniques is still a matter of controversy (Aldrich et al., 1987; Dubochet et al., 1983; Ebersold et al., 1981; Fooke-Achterrath et al., 1974; Ghosh and Ghosh, 1977; Higgins et al., 1976; Holt and Leadbetter, 1969; Nanninga, 1968, 1971; Remsen, 1968; Silva et al., 1976; Strohl, 1979; van Iterson, 1984). The latest contribution to the debate dates back to 1987 when Aldrich and coworkers observed that mesosomes were always present when cells were fixed under suboptimal conditions, whereas they were absent in cells that were either freeze-fractured, freeze-substituted, or fixed under optimal conditions. However, the authors, who had previously observed mesosomes in freeze-substituted cells, refrained from dismissing all internal membranous structures in prokaryotes as artifacts. Other investigators have also reported mesosomes in freeze-fractured bacterial cells that had not been subjected to chemical fixation (Holt and Leadbetter, 1969; Nanninga, 1968; Remsen, 1968; Strohl, 1979). Interestingly, specimen preparation techniques for TEM were similar in all but one (Chen et al., 1997a) of the above-mentioned studies on *Pasteuria,* and yet mesosomes were observed only in some but not all of the *Pasteuria* investigated. Observations of this type support the hypothesis that the presence or absence of mesosomes may actually reflect differences in the organisms themselves rather than fixation artifacts.

In addition to differences in the size of the central body and in the appearance and function of mesosomes, the fibers lining the basal adhesion layer and the exosporium look more erected and abundant in the North American *Pasteuria* (Figs. 5, 6) than in *P. nishizawae* (Figs. 16,10 in Sayre et al., 1991a, 1991b, respectively). Inasmuch as surface features of *Pasteuria* endospores mediate their attachment to nematode cuticles and, hence, their host specificity (Afolabi et al., 1995; Bird et al., 1989; Davies et al., 1992, 1994; Persidis et al., 1991; Spiegel et al., 1996), variations in the amount and orientation of fibers are likely to have some consequences, at least on the efficiency of endospore attachment to host nematodes. It is unfortunate that the host range of *P. nishizawae* was reported only on a plus or minus basis (Sayre et al., 1991a, 1991b), which did not allow for comparison of attachment efficiency as was done in this study.

Pasteuria endospores have been reported to attach more readily to nematodes of their original host population than to nematodes from other populations (Davies et al., 1994; Oostendorp et al., 1990). In this study, endospores of the North American *Pasteuria* attached more to J2 of *H. schachtii* than to those of *H. glycines,* which suggests that *H. schachtii* may be the original host of this *Pasteuria.* This conjecture is the more likely as the microplots where *Pasteuria* was discovered were established in a field that had no history of either soybean cultivation or *H. glycines* infestation (Noel and Stanger, 1994). On the contrary, the field had history of sugarbeet production 20 to 30 years before the microplots were established (Noel, unpubl.). Furthermore, the soil used to infest the microplots came from an area that also produced sugarbeets many years ago. The soil was determined to be free of cysts before it was used in microplots.

In conclusion, the morphological, developmental, and pathological evidence presented in this study show that the North American *Pasteuria* is, in spite of minor differences, more similar to *P. nishizawae* than to any other *Pasteuria* previously described. However, it is not clear whether this similarity is sufficient enough to warrant merging the two *Pasteuria* into a single species. Since the 16S rDNA sequence of the North American *Pasteuria* is already available (Atibalentja et al., 2000), it would be desirable to obtain the homologous sequence from *P. nishizawae* and to conduct sequence similarity analyses that would supplement the current data and help resolve the taxonomic relationships between the two *H. glycines*-infecting *Pasteuria.*

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