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Pasteuria nishizawae sp. nov., a mycelial and endospore-forming bacterium parasitic on cyst nematodes of genera *Heterodera* and *Globodera*

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SUMMARY

This study describes *Pasteuria nishizawae* sp. nov., a fourth species of the genus *Pasteuria*. This mycelial and endospore-forming bacterium parasitizes the adult females of cyst-forming nematodes in the genera *Heterodera* and *Globodera*. The distinct ultrastructural features and unique host range found for this bacterium separate it from two closely related species, *Pasteuria penetrans*, which parasitizes several species of root-knot nematodes of the genus *Meloidogyne*, and *Pasteuria thornei*, which appears to parasitize only one species of the root-lesion nematode, *Pratylenchus brachyurus*. Because these obligate bacterial parasites of nematodes have not been cultured axenically, the taxonomic relationships described here for each species are based mainly on developmental morphology, fine structure of the respective sporangia and endospores, and their pathogenicity on nematode species.

Key-words: *Pasteuria*, *Pasteuria nishizawae*, Soil, Nematode, Biological control; Taxonomy, New species.

INTRODUCTION

The species currently assigned to the genus *Pasteuria* (Sayre and Starr, 1989) and many of the other closely related bacterial pathogens, which remain to be characterized, have not been cultured *in vitro*, *i.e.*, apart from their respective nematode hosts. Consequently, these bacteria are found primarily as a result of their antagonistic associations with nematodes that

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parasitize crop plants. The early morphological characterizations of endospores of *Pasteuria* have been documented in the taxonomic literature of nematodes (Cobb, 1906; Thorne, 1940, 1961; Williams, 1960). More recent investigations have described the true bacterial nature of these parasites of nematodes (Imbriani and Mankau, 1977; Mankau, 1975a,b; Mankau and Prasad, 1977). In pathogenic associations with nematodes, the bacterial species *Pasteuria penetrans, P. thornei,* and the cyst nematode *Pasteuria* (CNP), which is named in this study, convert the biomass of each parasitized host nematode into as many as 2×10^6 bacterial endospores per root-knot nematode carcass (figure 1). The endospores, which are liberated into the soil from the remnants of the parasitized nematode, then attach to the cuticular surface of susceptible migratory J2s (fig. 2). When these nematodes continue their development, the attached bacterial endospores germinate and the germ tubes penetrate through cuticle into pseudocoelom where they form mycelia that eventually differentiate to produce endospores, thereby completing the life cycle of the bacterium (Sayre and Starr, 1985; Sayre and Wergin, 1977). Recently, many studies have ascertained the potential benefits of the species *Pasteuria* as biocontrol agents of the economically destructive plant-parasitic nematodes (Brown *et al.*, 1985; Mankau, 1973, 1980; Mankau and Prasad, 1972; Stirling, 1984, 1988). Because *Pasteuria* spp. have not been cultivated *in vitro,* investigators have worked with crude preparations, *e.g.,* spores derived from a single "strain" of *P. penetrans* parasitizing a root-knot nematode of the genus *Meloidogyne* which, in turn, was parasitizing tomato plants (Stirling and Wachtel, 1980). To continue investigations of these bacteria, this method remains indispensable; it does not yield isolates of a "single" bacterial strain for transfer and long term stability in repositories. The probability that any one sample of several hundred diseased nematodes represents one strain is questioned, because a population of these bacterial parasites apparently consists of several taxa with limited host ranges (Sayre and Starr, 1985; Sayre *et al.*, 1988). Because the limits of each actual or potential species in the genus *Pasteuria* are not known, one caution must now be made: Wherever the name *P. penetrans* (or its earlier synonyms, *"Duboscqia penetrans"* or *"Bacillus penetrans"*) appears in the literature, it might justifiably be replaced by "member(s) of the *P. penetrans* group", "a *Pasteuria* spp.", or a similar reference.

Taxonomic studies are available on *P. ramosa* (Sayre *et al.*, 1983), *P. penetrans* (Sayre and Starr, 1985) and *P. thornei* (Sayre *et al.*, 1988). The recent investigations of Nishizawa (1984, 1986, 1987) and others (Sayre *et al.*, 1991) present additional information on another member of this bacterial group, the cyst nematode *Pasteuria* (CNP). CNP parasitizes nematodes of the genera *Heterodera* and *Globodera.* We will present a summary of the observed differences among species of *Pasteuria* occurring in the cladoceran, *Moina rectirostris* and those that parasitize the following nematodes: *Meloidogyne* species, *Pratylenchus brachyurus, Heterodera elachista, Heterodera glycines* and *Globodera rostochiensis.* The recently found bacterium that parasitizes cyst nematodes will be described and named in this study.

MATERIALS AND METHODS

Terminology

The fine structure of the sporangia of bacterial parasites of nematodes will be used to taxonomically delineate species of *Pasteuria.* We have applied the terminology, which was suggested by Sussman and Halvorson (1966) and recently reviewed by Ierson

CNP = cyst nematode *Pasteuria.*
J2 = infective second stage juvenile.
LM = light microscopy.
LVFE(SEM) = low voltage field emission (SEM).
RKN = root-knot nematode.
RKP = root-knot *Pasteuria.*
RLP = root-lesion *Pasteuria.*
SEM = scanning electron microscopy.
TEM = transmission electron microscopy.
WFP = water flea *Pasteuria.*

(1988), to our discussion of the structural details of the *Pasteuria* species. The following terminology is used in this study: (a) endospore: the single asexual spore that develops within a sporangium and may be enclosed by an exosporium; (b) plasma membrane: the innermost septum that surrounds the central protoplast; (c) cortex: the electron-translucent band that opposes the plasma membrane; (d) inner coat: a narrow multilaminar band that lies exterior to the cortex; (e) outer coat: the wide outer electron-dense band; (f) exosporium: the outermost membrane of a typical Gram-positive bacterial spore that surrounds the structures defined above. Within the exosporium, the *Pasteuria* spp. have two additional structures: (g) epicortical layer: a discontinuous, electron-dense band and varies in its appearance from species to species; and the (h) episporic layer: peripheral fibers that are an essential part of the endospore and allow for attachment to the nematode host.

Material examined

Sources of host nematodes

For laboratory bioassays, pure populations of the following nematodes were reared aseptically on root explants (Lauritis *et al.*, 1982, 1983a,b; Huettel and Rebois, 1985): *Meloidogyne incognita, P. brachyurus, Heterodera zeae,* and four races of *H. glycines.* Individual nematode populations were harvested from the culture plates using the modified Baermann funnel technique of Niblack and Huang (1985). In the greenhouse and field studies where large populations of nematodes were needed, the following species were maintained in greenhouse pot cultures: *M. incognita* on tomato cv. Marglobe or Tiny Tim; *M. hapla* on strawberry; *M. javanica* on tomato cv. Marglobe; *H. glycines* on soybean cv. Kent; *H. zeae* on corn cv. Iowa Chief; and *P. brachyurus* on peanut cv. Florarunner. The details for rearing *M. incognita* and *P. brachyurus* have been previously reported (Sayre and Wergin, 1977; Sayre *et al.*, 1988). *H. glycines* was maintained in the greenhouse on soybeans (cv. Kent). The nematodes were extracted from soil using the methodology of Ayoub (1980).

Sources of the Pasteuria *spp.*

The procedure of Stirling and Wachtel (1980) was used to maintain cultures of *P. penetrans* on *M. incognita. P. thornei* endospores were obtained from the parasitized juveniles and adults of *P. brachyurus* (Sayre *et al.*, 1988; Starr and Sayre, 1988). The infected nematodes obtained from the root of the peanut (cv. Florarunner) were extracted in a mist chamber by the procedure of Chapman (1957). Cysts of *H. glycines* parasitized by CNP were imported from Japan. CNP was introduced into a population of *H. glycines* and then maintained in greenhouse pot cultures (Sayre *et al.*, 1991).

Microscopy and measurements

Light microscopy

No new materials were prepared for *P. ramosa, P. penetrans* or *P. thornei.* Alternatively, the archival collection of photomicrographic materials of these species, some of which were presented in previous publications (Sayre, Gherna and Wergin, 1983; Sayre and Starr, 1985; Sayre *et al.*, 1988; Starr and Sayre, 1988; and Sayre *et al.*, 1991), were used to compare structural features of these species with those of the CNP preparations. The preparative methods for *P. ramosa, P. penetrans* and *P. thornei* were similar to those used for CNP. Briefly, cysts of *H. glycines* found parasitized by CNP were selected from soil samples, crushed and mounted on microscope slides in water using the temporary method of Southey (1986). Photomicrographs of sporangia and other life stages of CNP were taken on "Kodak Tri-X Pan" film processed in "Microdol-X" using an automatic exposure 35-mm camera attached to a compound microscope (Nikon Microphot F-X) fitted with an interference contrast system (figs. 1-3). Morphometric data were obtained by measuring images on the enlarged photomicrographs. Whenever possible, 20 separate measurements were taken of each bacterial life stage, and presented as the mean plus or minus the standard deviation. The morphometric data of previous studies indicated that measurements taken of the life stages from light microscopy were greater than those obtained for identical features recorded by electron microscopy. This difference results from shrinkage of specimens that occurs during chemical fixation, dehydration and critical point drying (Sayre *et al.*, 1991). As a consequence, the morphometric data in table I indicates the procedure used to obtain each value: light microscopy (LM), scanning electron microscopy (SEM), transmission electron microscopy (TEM).

Scanning electron microscopy

Nematode life stages that had been encumbered or parasitized by CNP were chemically fixed for SEM. The nematode specimens containing the endospores of CNP were placed in 3.0 % solution of glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) for 1.5 h and then dehydrated in an ethanol series. Thereafter, the specimens were critical-point-dried, mounted on aluminum stubs, coated with gold-

palladium, and examined with a "Hitachi" HHS-2R, S430, or S530 SEM operating at 15 or 20 kV.

A new instrument (Wergin and Sayre, 1988; Wergin *et al.*, 1988) known as a low voltage field emission SEM (LVFE-SEM) was also used in the examination of CNP.

Transmission electron microscopy

For TEM, single cysts of *H. glycines* parasitized by CNP were hand-picked and individually placed in the wells of a microtitration plate (Falcon 3034) containing a droplet of tap water. The wells were then filled with molten 3.0 % water-agar at 50°C. After solidification, agar cores containing the cysts were removed from the plate, placed in 3.0 % glutaraldehyde in 0.05 M phosphate buffer (pH 6.8) for 3 h, washed in several changes of buffer, post-fixed in 2.0 % osmium tetroxide for 2 h, and dehydrated in an acetone series. Finally, they were infiltrated and embedded in a low-viscosity resin (Spurr, 1969). Silver-grey sections of selected nematodes, which were cut on a "Sorvall" model MT2 ultramicrotome using a diamond knife, were mounted on uncoated copper grids (75 by 100 mesh). The sections were stained for 10 min with 2.0 % aqueous uranyl acetate, and then for 5 min with 3.0 % lead citrate. The stained thin sections were viewed with a "Hitachi" model H500-H transmission electron microscope operating at 75 kV with 30-μm apertures.

Host specificity

Host specificity to CNP was determined on the basis of attachment of bacterial endospores to the cuticles of the plant nematodes and incidence of diseased cyst on roots of host crop plants (table II). Usually, only the J2 (infective second stage juvenile) were available from root explant cultures. However, when other stages (*i.e.*, eggs, third and fourth vermiform larval stages, and adult males) were available, they were also examined for possible attachment by the bacterial endospores. The J2 taken from cultures were counted in a Hawksley chamber (Southey, 1986), adjusted to 300 J2 per ml, and added to the endospore suspension in a 50-mm diameter Petri dish. To prepare suspensions of the infective endospores of CNP, a few hundred diseased cysts of *H. glycines* that were infected with CNP were hand-picked from the roots of soybean. The cysts were crushed to liberate the mature sporangia. The heavy sporangial suspension that resulted was filtered through a 500-mesh wire sieve to remove the cuticular debris of the nematode. A haemocytometer was used to count the number of sporangia in the suspension, and adjust the concentration to 5×10^5/ml. In the attachment trials, 50 ml of the suspension was added to equal volumes of tapwater containing 1000 J2/ml of the target nematode.

Suspensions of J2 and endospores were poured into a shallow pan containing less than 1 cm of water and then placed on a rotary shaker (50 rpm) for 24 to 3 h. The suspension was aerated to maintain the juveniles capable of penetrating plant roots. Samples of J2 were taken and examined at 250× to determine the level of endospore attachment to the cuticular surfaces of J2. When three to ten endospores had attached to a majority of J2, the larvae were pipetted around the roots of host plants to allow the bacterium the opportunity to complete its life cycle on its nematode host. After 45 days, host plants of the nematodes were harvested and their roots were examined for the incidence of diseased nematodes.

RESULTS AND DISCUSSION

Light microscopy

Measurements of the CNP strain from cysts that were crushed on microscope slides in tapwater were compared with the three known spe-

Figs. 1 and 2. Photomicrographs of sporangia of *P. penetrans*.

Fig. 1. Sporangia from a crushed parasitized female RKN, *M. incognita*. Bar = 10 μm.
Fig. 2. Juvenile of *M. incognita* with endospores of *P. penetrans* attached to its cuticular surface. Bar = 10 μm.

Fig. 3. Photomicrograph of life stages of the *P. nishizawae* sp. nov. parasite of *H. glycines*.

Early vegetative stages, cauliflower stage, quartets of sporangia, single mature sporangium. Bar = 10 μm. Inset illustrates numerous mature sporangia of CNP. Bar = 10 μm.

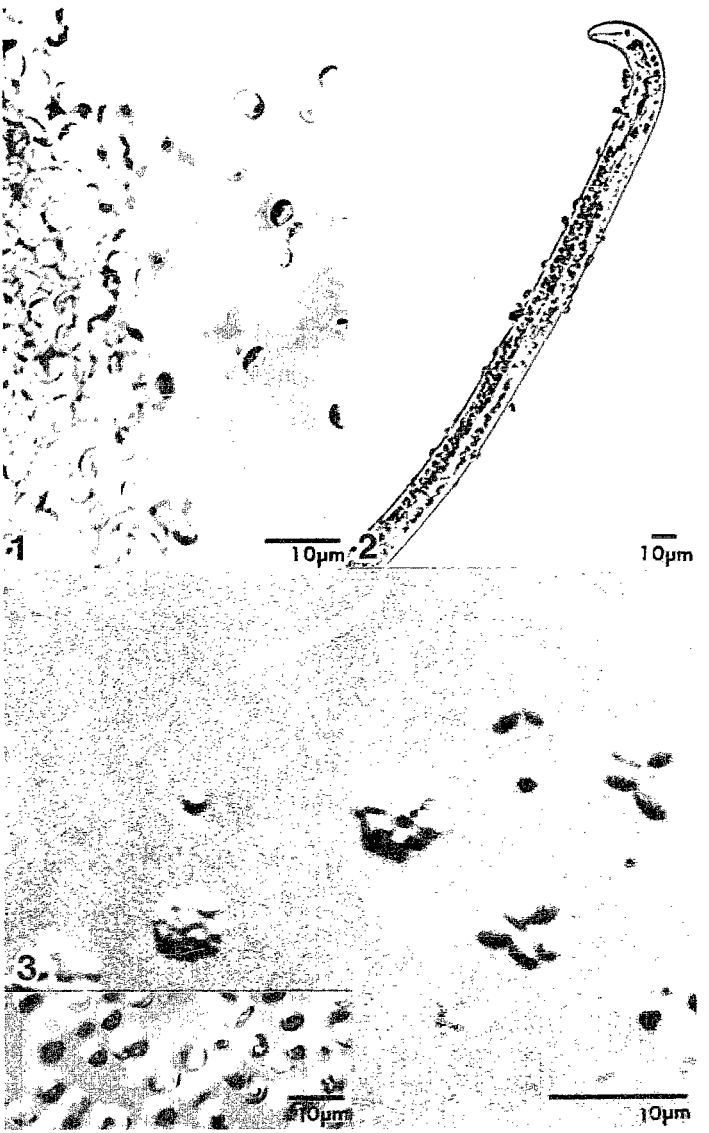

1
10μm
2
10μm
3
10μm
10μm

Table I. Comparison of *P. ramosa, P. penetrans, P. thornei,* and the *P.* sp. from cyst nematodes.

Trait	*P. ramosa* (WFP)	*P. penetrans* (RKP)	*P. thornei* (RLP)	*P. nishizawa* sp. nov. (CNP)
Colony shape	Like cauliflower floret	Spherical, to cluster of elongated grapes	Small, elongate clusters	Spherical, to cluster of elongated grapes
Sporangia:				
shape	Teardrop-shaped	Cup-shaped	Rhomboidal	Cup-shaped
diameter (μm)				
LM	3.3–4.1 (*)	4.5±0.3	3.5±0.2	5.3±0.3
TEM	2.12–2.77 (**)	3.4±0.2	2.4±0.2	4.4±0.3
height (μm)				
LM	4.8–5.7 (*)	3.6±0.3	3.1±0.2	4.3±0.3
TEM	3.4–4.35 (**)	2.5±0.2	2.2±0.2	3.1±0.3
sporangial wall's fate at maturity of endospore	Remains rigidly in place; external markings divide sporangium in three parts	Basal portion collapses inward on the developed endospore; no clear external markings	Remains rigid, sometimes collapsing at bases; no clear external markings	Basal portion collapses on the developed endospore; no clear external markings
exosporium	Not observed	Present, relatively smooth surface	Present, smooth	Present, velutinous to hairy surface
stem cell	Remains attached to most sporangia	Rarely seen; attachment of a second sporangium sometimes observed	Neither stem cell nor second sporangium seen	Occasional seen
Endospore:				
shape	Oblate spheroid, an ellipsoid, narrowly elliptic in section	Oblate spheroid, an ellipsoid broadly elliptic in section	Oblate spheroid, an ellipsoid sometimes almost spherical, narrowly elliptic in section	Oblate spheroid, an ellipsoid, narrowly elliptic in section
orientation of major axis to sporangium base	Vertical	Horizontal	Horizontal	Horizontal
diameter (μm)				
LM	2.1–2.4 (***)	2.1±0.2	1.6±0.1	2.1±0.2
TEM	1.2–1.5 (**)	1.4±0.1	1.3±0.2	1.6±0.2
height (μm)				
LM		1.7±0.2	1.5±0.1	1.7±0.1
TEM	1.37–1.61 (**)	1.1±0.1	1.1±0.1	1.3±0.1
partial epicortical wall	Not observed	Surrounds endospore laterally, not in basal or polar areas	Surrounds endospore somewhat sublaterally	Entirely surrounds endospore
Pore:				
presence	Absent	Present	Present	Present
characteristics		Basal annular opening formed from thickened outer wall	Basal cortical wall thins to expose inner endospore	Thickness of basal wall constant and is the depth of pore
diameter (μm)		0.3±0.1	0.1±0.0	0.2±0.0
Parasporal structures:				
fibers, origin and orientation	Long primary fibers arise laterally from cortical wall, bending sharply downward to yield numerous secondary fibers arrayed internally toward the granular matrix	Fibers arise directly from cortical wall, gradually arching downward to form an attachment layer of numerous shorter fibers	Long fibers arise directly from cortical wall, bending sharply downward to form an attachment layer of numerous shorter fibers	Same as *P. penetrans* but additional layer is formed on obverse surface of endospore

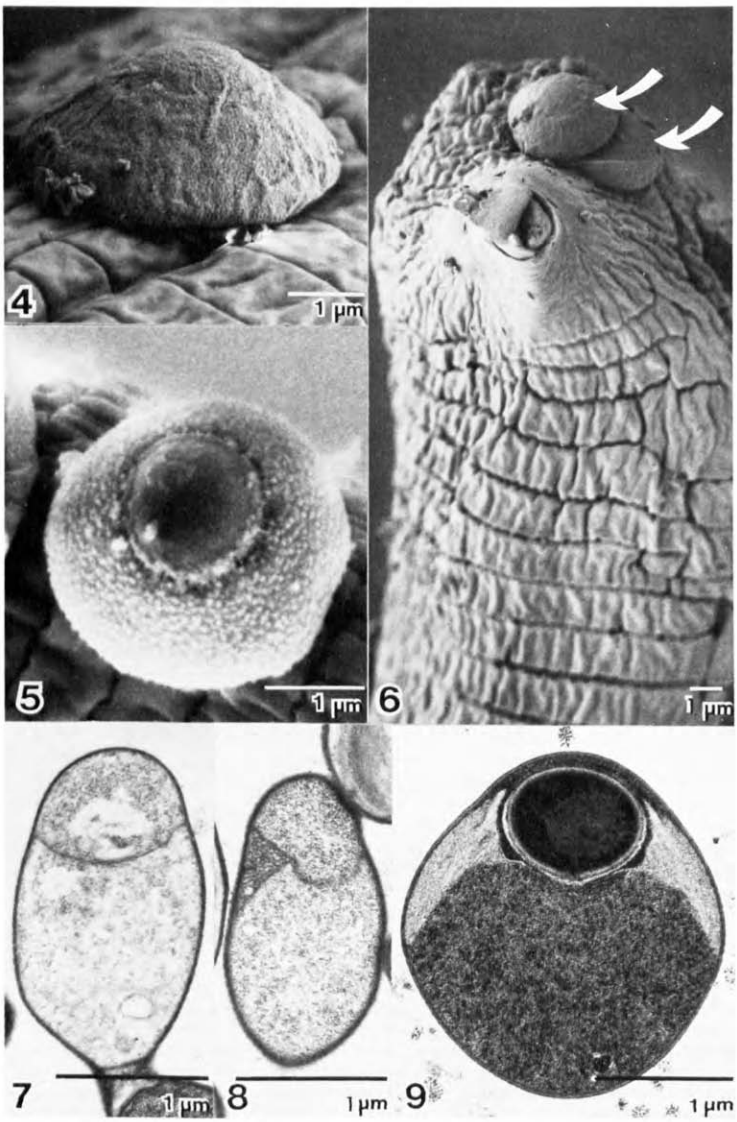
matrix, at maturity	Persists as fine granular material	Becomes coarsely granular; lysis occurs; sporangial wall collapses; base is vacuolate	Persists, but more granular; some strands are formed and partial collapse may occur	Persist, numerous strands are formed and partial collaspe may occur
Host	Cladocerans: *Daphnia; Moina*	RKN: *M. incognita*	Root-lesion nematodes: *P. brachyurus*	Cyst nematodes: *Heterodera* spp.
Completes life cycle in nematode juveniles		No, only in adult	Yes, in all larval stages and adult	No, only in adults
Location in host	Hemocoel and musculature; sometimes found attached to coelom walls	Pseudocoelom and musculature; no attachment to coelom walls seen	Pseudocoelom and musculature; no attachment to coelom walls seen	Pseudocoelom and musculature; no attachment to coelom walls seen
Attachment of spores on host	Spores not observed to attach or accumulate on surface of cladoceran	Spores accumulate in large numbers on cuticular surface	Spores accumulate in large numbers on cuticular surface	Spores accumulate on juveniles, rare on adult male
Mode of penetration of host	Not known; suspected to occur through gut wall	Direct penetration of nematode cuticle by hyphal strand	Direct penetration suspected but not seen	Direct penetration suspected but not seen
Source of host	Pond mud, freshwater	Soil, plants	Soil, plants	Soil, plants

(*) Sayre *et al.*, 1983. (**) Sayre and Starr, 1989. (***) Sayre and Starr, 1985.

Table II. Host specificity of CNP (the *Pasteuria* species from cyst nematodes) as scored by attachments of endospores to nematode juveniles.

Nematodes from which endospores originated on:	Attachment tried for these nematodes	Attached (+) or non-attached (−)
Heterodera glycines	Race 1 *H. glycines*	+
	Race 3 " "	+
	Race 4 " "	+
	Race 5 " "	+
	Heterodera trifolii	+
	Heterodera sp.	+
	Heterodera sp.	−
	Meloidogyne	−
Heterodera elachista (*)	*Globodera rostochienis*	+
	Helicotylenchus sp.	−
	Heterodera sp.	+
	Heterodera sp.	+
	Meloidogyne hapla	−
	Meloidogyne incognita	−
	Meloidodyne javanica	−
	Pratylenchus coffeae	−
	Pratylenchus penetrans	−
	Pratylenchus vulnus	−
	Various saprophagous	−

(*) Data prepared by Dr. T. Nishizawa.


4
1 µm
5
1 µm
6
1 µm
7
1 µm
8
1 µm
9
1 µm

cies of *Pasteuria* (table 1). The mature sporangia of CNP averaged 5.3 μm in diameter (fig. 3). The average height, from the margin of the dome of the central body to the base of the sporangium, was 4.3 μm. The central body had the shape of an oblate spheroid, with axes of 1.7 ± 0.1 by 2.1 ± 0.2 μm and was enveloped laterally by episporic fibers.

An individual sporangium in the immature quartet stage had a diameter of 1.8 μm and measured 3.2 μm in height from its point of attachment to the distal end (fig. 3). In the doublet configuration, the sporangium had a diameter of 4.1 μm and a height of 3.9 μm.

Light microscopy revealed significant size differences among the described species of *Pasteuria* (table I). These differences, in conjunction with the specificity of bacterial spore attachment to nematode species, served as a reliable indicator of strain differences and aided in the taxonomic characterization. Sporangia of CNP were larger than the other two bacterial isolates from nematodes; however, they were not the largest found in the genus. Another isolate of *Pasteuria,* which parasitizes *Hoplolaimus* spp., is much larger (*i.e.* 7 μm in diameter) and may be a different *Pasteuria* spp. (Sayre *et al.,* 1988). The numbers of sporangia in females of the root-knot nematodes (RKN) were nearly four-fold greater than those found for CNP in cyst nematodes. This difference was partly due to the larger volume of the RKN females as compared to smaller cysts of SCN and to the larger size of the CNP sporangia. In the veriform *Pratylenchus* spp., each stage of the nematode contained only a few hundred sporangia.

Scanning electron microscopy

Mature endospores of CNP with their episporal structures and associated perisporic remnants were found adhering to the surfaces of J2 and adult males of *H. glycines* (figs. 4 to 6). Two distinct forms of endospores occurred, which is similar to the situation for *P. penetrans.* In one form, the surface of the endospore was covered by the exosporium; in the other, the episporic wall structures were exposed. The surface of the first form appeared to be covered by a velutinous membrane, the exosporium, that encompassed the entire endospore (figure 4). In the other form, the exosporium had shed to expose an underlying central body, averaging 1.5 μm in diameter and 1.2 μm in height, that was surrounded by episporic fibers (figure 6).

The resolution and definition of the LVFE-SEM for viewing sporangia was far superior to that of LM. In particular, LVFE-SEM revealed remarkable details of the external morphology of sporangia and endospores of CNP that were

Figs. 4, 5 and 6. Scanning electron micrographs of CNP.

Fig. 4. An endospore of the *Pasteuria* sp. attached to the surface of a juvenile of *H. glycines.* The fine velutinous exosporium, which has been retained, hides the central body and its perisporic fibres. Bar = 1 μm.

Fig. 5. An endospore of CNP that has lost both of its sporangial coverings. The central exposed dome of the mature endospore is surrounded by downward-arching fibers that are covered with numerous hairlike projections.

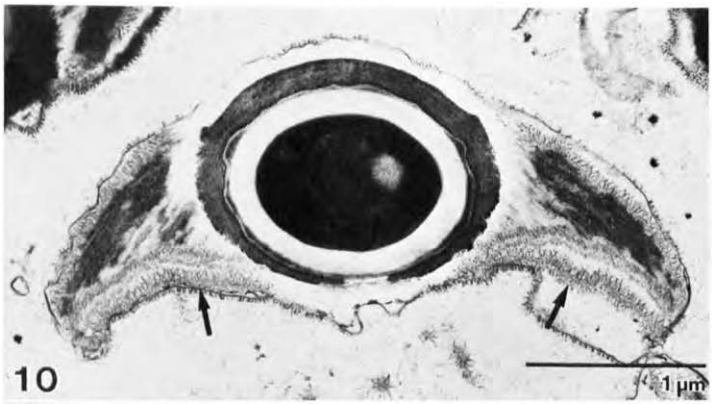
Fig. 6. The posterior of the male *H. glycines.* Two endospores (arrows) are attached near the slightly extended spicule. Bar = 1 μm.

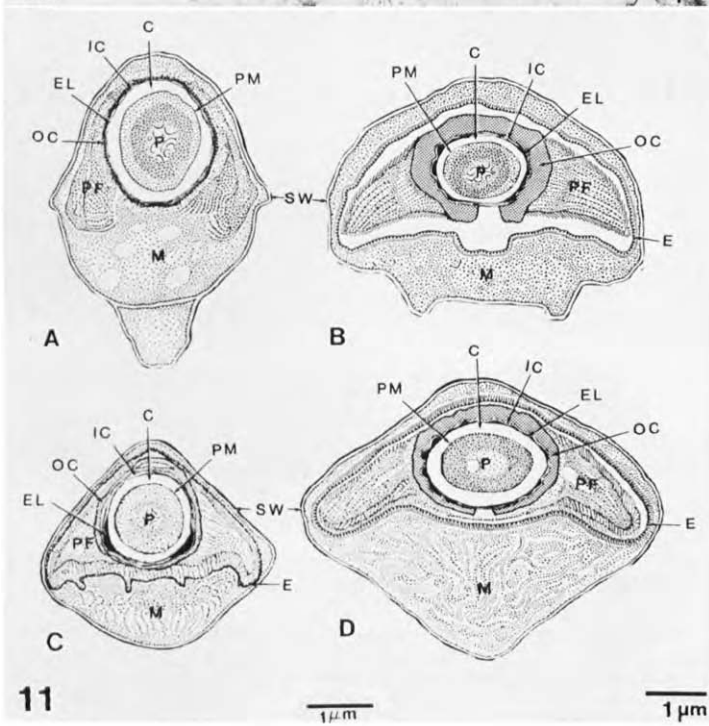
Figs. 7, 8 and 9. Transmission electron micrographs of immature sporangia of CNP.

Fig. 7. Ovate terminal cell of a microcolony with a septum that separates the forespore.

Fig. 8. A mesosome associated with the developing sporangium. Bar = 1 μm.

Fig. 9. A later developmental stage in a sporangium of CNP. Several thin spore coats have differentiated, but the peripheral fibers remain as ill defined white areas. Bar = 1 μm.


10
1 µm


C
IC
EL
PM
OC
P
PF
SW
M
A
B
E
D
11
1 µm
1 µm

not visible by LM. The textural appearance of the episporic wall coverings will be useful in delineating new taxons.

Transmission electron microscopy of CNP

Vegetative growth and sporogenesis

The developmental stages (*i.e.* growth, vegetative, mycelium and endosporogenesis) of CNP occurred in the distal or terminal cells of the dichotomously branching hyphal colonies. These cells were bounded by a compound wall, about 0.04 μm thick, composed of outer and inner membranes. The inner membrane constricts during the formation of septa that delineated successive cells, which enlarge and become ovate (fig. 7). The protoplasts of the ovate cells change from that having a granular matrix with numerous ribosomes to one with well-defined organelles. Mesosomes were often found associated with septa in thin sections of CNP (fig. 8). During the early development of the sporangia, transverse membranes formed within the cell and separated one-third of the distal end of the sporangium, the forespore, from the lower/basal parasporal portion. The protoplast in the upper portion of the cell condensed into an electron-dense central core that become encircled by a multilayered wall (fig. 9). After these changes occurred, the electron-translucent parasporal region developed laterally with respect to the endospore. Development of the parasporal region began with the appearance of lateral fibers, thickening of the multilayered endospore walls and the formation of the ellipsoidal endospore (fig. 9). Stages of endogenous spore formation of all *Pasteuria* sp. were typical of Gram-positive bacteria; but the formation of a fibrillar episporic wall distinguishes the genus *Pasteuria* from the genus *Bacillus*.

Mature sporangia

In a lateral view, sporangia of CNP retained a lenticular shape, which persisted until the outer sporangial wall degraded and exposed the velutinous exosporium. As a result, the endospore and its accompanying peripheral fibres become cup-shaped (figs. 10 and 11). The mature CNP endospore had a broadly elliptical central body whose axes measured 1.6 ± 0.2 by 1.3 ± 0.1 μm. The outer cortical wall was 0.2 μm thick at the mid-point of the endospore, but progressively tapered to 0.1 μm at the periphery of the pore. The partial hirsute layer, originating from the basal adhesion layer of the endospore, is a unique morphological feature of CNP (fig. 10), which was not found in other members of the *P. penetrans* group that we examined. Mature sporangia of water flea *Pasteuria* (WFP), root-knot *Pasteuria* (RKP) and root-lesion *Pasteuria* (RLP) appeared distinctly different from each other (fig. 11): sporangia of WFP were teardrop-shaped; those of RLP appeared rhomboidal; and RKP and CNP had a cup-shaped configuration. Additional differences that separated the latter two species included the physical arrangement (*i.e.* numbers and angle of fibre attachment) of the

Fig. 10. Transmission electron micrograph of a cross section of an endospore of the *Pasteuria* sp. found in cyst nematodes (CNP).

The additional partial layer (arrows), which is unique to this species of *Pasteuria*, can be observed on the obverse surface of the endospore. Bar = 1 μm.

Fig. 11. Line drawings taken from electron micrographs depicting sporangia of (11a) *P. ramosa*, (11b) *P. penetrans*, (11c) *P. thornei* and (11 d) *P. nishizawae* sp. nov.

The features used in the taxonomy of these species are labelled. Bar = 1 μm.
C = cortex; EL = epicortical layer; EX = exosporium; IC = inner coat; M = matrix; OC = outer coat; PF = peripheral fibers; PM = plasma membrane; PR = protoplast; and SW = sporangial wall.

peripheral fibres that occurred on the outer coats of their endospores. Qualitative differences that distinguished CNP from *P. penetrans* and *P. thornei* were associated with the spore coats. The epicortical layer of CNP completely surrounded the cortex, but was incomplete in the other two species. In addition, the depth of the basal pore of CNP reflected the thickness of the endospore wall. In the case of *P. penetrans*, the wall thickened and became doughnut-shaped around the pore. Alternatively, in *P. thornei* the wall thinned and resulted in an opening at the base of the endospore (fig. 11c).

The additional partial wall found in CPN was unique for a *Pasteuria* spp. It occurred on the observe face of the endospore and appeared to be formed from the attachment layer (fig. 10). Its function was not determined. The attachment of CNP endospores to males of *H. glycines* also helped distinguish it from the other two *Pasteuria* spp. that attach only to J2.

Life cycle of CNP

Similarities between the life cycles of CNP and *P. penetrans* are summarized in table 1. All life stages, except the mature sporangium of CNP, presented the same morphological appearance as those of *P. penetrans*. The developmental stages of CNP were in synchrony with those of its cyst nematode host; this was also the case for the *Pasteuria* found in RKN. This synchronous development was absent in *P. thornei* where the bacterial species apparently can completes its life cycle in any developmental stage of the nematode host.

A significant difference between CNP and *P. penetrans* was the ability of the endospores to attachment to the adult males of *H. glycines* (fig. 6). However, completion of the bacterium's life cycle within males has not been observed.

Host range studies of CNP

To establish the host ranges of CNP, RKP and RLP, we examined the ability of the endospores to attach to the respective J2 of the host; attachment was used as the criterion of susceptibility to the bacterial disease (fig. 2 and table II). The nematode hosts that we examined were restricted largely to the RKN and cyst nematodes. Endospores of CNP attached to the cyst nematodes but not to the RKN or the other plant-parasitic and free-living species that were tested. Conversely, endospores from RKN did not attach to J2 of cyst nematodes. The host range of CNP was unique; this was the first documented case of a *Pasteuria* species on cyst nematodes (Nishizawa, 1984), which included four species of *Heterodera* and the single species *G. rostochensis* (table II). However, over 60 other species of cyst nematodes exist which have not been screened for their susceptibility to CNP; therefore, additional hosts are likely and undescribed species of *Pasteuria* may be capable of parasitizing other cyst nematodes. In fact, another distinct isolate of CNP may have already been found by Bhattacharya *et al.* (1988) who recently reported that several *Heterodera* spp., were parasitized by a *P. penetrans* sp.; however, their bacterium parasitized *H. zeae* and *M. incognita* while ours does not.

The nematode hosts of *Pasteuria* spp. include about 175 species in nearly 70 genera from 10 orders (Starr and Sayre, 1988; Sturhan, 1985). Nematodes from each taxon have different feeding habits and life cycles that vary in duration. Therefore, each nematode species has a slightly different physiology. In order to survive, selection pressure favoured the bacterium whose physiology paralleled that of a particular nematode host. The one-to-one relationship that exists between these bacterial parasites and their nematode hosts suggests that the genus *Pasteuria* contains numerous unique bacterial species.

Morphological differences among the four bacteria investigated in this study lead us to conclude that the CPN isolate should be assigned a new epithet.

Taxonomic formalities

Description of *Pasteuria nishizawae* sp. nov. *Pasteuria nishizawae* sp. nov. (ni.shi.za'wae M. L. gen. n. nishizawae of Nishizawa; named for Tsutomu

Nishizawa, Japanese nematologist, who discovered and first investigated bacterial parasites of cyst-forming nematodes).

Gram-positive vegetative cell. Mycelium is septate, hyphal strands, 0.2-0.5 μm in diameter, branch dichotomously. The sporangia, which form by expansion of hyphal tips, are cup-shaped and measure about 4.3 μm in height and 5.3 μm in diameter. Each sporangium is divided into unequal parts. The smaller proximal body is not as refractile as the larger, rounded cup-shaped portion, which encloses an ellipsoidal central body. The central body, which is broadly elliptic in cross-section, having an axis of 1.7×2.1 μm, is surrounded laterally by perisporic fibers originating in the outer coat wall. The endospore, which is typical of the genus *Bacillus,* is resistant to heat and desiccation. Sporangia and vegetative cells are found in the pseudocoelom of the infected cyst-forming plant-parasitic nematodes. The epithet is now restricted to members of the genus *Pasteuria* with cup-shaped sporangia having central bodies that are broadly elliptic in cross section. They occur primarily as parasites of the adult female nematodes belonging to the genus *Heterodera* and *Globodera;* they may parasitize other plant-parasitic nematodes. Has not been cultivated axenically; the type descriptive material consists of the text and photographs herein. *P. nishizawae* differs from *P. penetrans, P. thornei* and other members of *Pasteuria* in host specificity, in size and shape of sporangia and endospores and in other morphological and developmental characteristics.

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Pasteuria nishizawae* sp. nov., bactérie à mycelium et formant une endospore parasite des nématodes à kystes du genre *Heterodera* et *Globodera

Dans cette étude est décrite une espèce nouvelle, *Pasteuria nishizawae,* la 4e espèce du genre *Pasteuria.* Cette bactérie à mycelium et formant une endospore, parasite les femelles adultes des nématodes du genre *Heterodera* et *Globodera.* Des caractères ultrastructuraux distincts et une unique gamme d'hôtes trouvés pour cette bactérie, la séparent de deux espèces étroitement apparentées, à savoir *P. penetrans* qui parasite plusieurs espèces de nématodes des nœuds raciniaires des plantes, du genre *Meloidogyne,* et de *P. thornei* qui semble parasiter seulement une seule espèce de nématode des lésions raciniaires, *Pratylenchus brachyurus.* Etant donné que ces parasites bactériens obligés des nématodes n'ont pas pu être cultivés de façon axénique, la description des liens de parenté taxonomique pour chaque espèce, est basée principalement sur la morphologie du développement, sur la structure fine des sporanges et endospores respectifs, et sur le pouvoir pathogène sur les diverses espèces de nématodes.

Mots-clés: Pasteuria, Pasteuria nishizawae, Sol, Nématode, Lutte biologique; Nouvelle espèce, Taxonomie.

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