Light and electron microscopical studies of the life cycle and developmental stages of a *Pasteuria* isolate parasitizing the pea cyst nematode, *Heterodera goettingiana*

Dieter STURHAN *, Rita WINKELHEIDE *, Richard M. SAYRE ** and William P. WERGIN ***

* *Biologische Bundesanstalt, Institut für Nematologie und Wirbeltierkunde, Toppheideweg 88, 48161 Münster, Germany,*
** *Nematology Laboratory, Plant Sciences Institute, 10300 Baltimore Avenue, Beltsville, MD 20705, USA, and*
*** *Electron Microscopy Laboratory, Natural Resources Institute, 10300 Baltimore Avenue, Beltsville, MD 20705, USA.*

Summary – In a *Pasteuria* isolate (HGP) found in the pea cyst nematode, *Heterodera goettingiana,* at Münster, Germany, the mode of infection, the developmental cycle and the morphology and ultrastructure of the developmental stages up to the immature sporangia are similar to those found in the three nematode-parasitic *Pasteuria* species previously described. The exclusive presence of all parasitic stages in the second-stage juveniles of *H. goettingiana* separates HGP from *P. penetrans* and *P. nishizawae*, which complete their life cycles in the females of *Meloidogyne* and *Heterodera*, respectively and do not infect migrating juveniles. HGP is also different from *P. thornei*, which parasitizes both juvenile and adult stages of *Pratylenchus*. The ultrastructural, morphological and morphometric features, which are different from those previously described for other species of *Pasteuria*, together with the distinction in host range and developmental stage specificity suggest that HGP should be assigned a new species within the genus *Pasteuria*.

Résumé – *Étude en microscopie optique et électronique du cycle biologique et des différents stades d'un isolat de* Pasteuria *parasitant le nématode à kystes du pois,* Heterodera goettingiana – L'étude d'un isolat de *Pasteuria* (HGP) observé sur le nématode à kystes des pois, *Heterodera goettingiana* à Münster, Allemagne, a montré que le mode d'infection, le cycle biologique, la morphologie et l'ultrastructure des différents stades jusqu'aux sporanges immatures y sont similaires à ceux des trois autres *Pasteuria* attaquant les nématodes parasites des plantes précédemment décrits. La présence de l'ensemble des stades parasites chez les juvéniles de deuxième stade de *H. goettingiana* sépare cet isolat HGP de *i)* P. penetrans et de *P. nishizawae* qui complètent leur cycle dans les femelles de *Meloidogyne* et d'*Heterodera*, respectivement, et *ii)* de *P. thornei* qui parasite les juvéniles et les adultes de *Pratylenchus*. Des caractères ultrastructuraux, morphologiques et morphométrique différents de ceux décrits chez les autres espèces de *Pasteuria*, de même que des différences dans la gamme d'hôtes et dans les stades de développement, suggèrent que l'isolat HGP représenterait une nouvelle espèce du genre *Pasteuria*.

Key-words : *Pasteuria*, nematode-parasitic bacterium, pea cyst nematode, *Heterodera goettingiana*, development, life cycle, morphology, ultrastructure, transmission electron microscopy.

Three species of *Pasteuria* bacteria parasitic on nematodes have so far been described : (1) *Pasteuria penetrans* (Thorne, 1940) Sayre & Starr, 1985 associated with *Meloidogyne incognita* (Kofoid & White) and probably parasitic on other species of root-knot nematodes; (2) *Pasteuria thornei* Starr & Sayre, 1988 described on *Pratylenchus brachyurus* (Godfrey), its type host, as well as on other species of *Pratylenchus* and (3) *Pasteuria nishizawae* Sayre, Wergin, Schmidt & Starr, 1991 which parasitizes the cyst nematodes *Heterodera glycines* Ichinohe, *H. elachista* Oshima, *H. trifolii* Goffart and *Globodera rostochiensis* (Wollenweber).

Several additional observations have reported *Pasteuria* parasites occurring on other cyst-forming nematodes. Esser (1980) described a *Pasteuria* infection of *Heterodera leuceilyma* Di Edwardo & Perry found in Florida. Sturhan (1985) listed *H. avenae* Wollenweber

and *H. goettingiana* Liebscher as hosts of *Pasteuria* in Germany as well as an unidentified *Heterodera* host in Nicaragua. In a more recent publication (Sturhan, 1989), *H. schachtii* Schmidt, was added as a nematode host in Germany. Page and Bridge (1986) reported *Pasteuria* infections in *Heterodera* sp. in India and in *Cactodera cacti* (Filipjev & Schuurmans Stekhoven) in Bolivia. Bhattacharya and Swarup (1988) listed *H. avenae*, *H. cajani* Koshy, *H. graminis* Stynes, *H. sorghi* Jain *et al.* and *H. zeae* Koshy *et al.* as additional bacterial hosts. Abrantes and Vovlas (1988) found a *Pasteuria* infection in *H. fici* Kirjanova from Italy. More recently, Davies *et al.* (1990) listed *H. avenae* as a host in England.

A *Pasteuria* population parasitizing the pea cyst nematode, *Heterodera goettingiana*, and apparently showing potential as a biological control agent, was found at Münster, Germany. Preliminary observations on its

biology and pathogenicity, morphology and fine structure have been published (Winkelheide, 1990; Sayre *et al.*, 1991 *c;* Sturhan & Winkelheide, 1991), along with light microscopical studies on its development and morphology (Winkelheide & Sturhan, 1993). This paper details the light and electron microscopical observations on the life cycle and the developmental stages of the *Pasteuria* isolate, which differs from the *Pasteuria* species previously described; therefore, this isolate appears to represent a new species.

Nomenclature, including detailed comparisons with previously described species of *Pasteuria,* will be treated separately. In the present study, the *Pasteuria* isolate that parasitizes second-stage juveniles (J2s) of *H. goettingiana* will be referred to as HGP.

Materials and methods

SOURCES OF NEMATODES

Pasteuria infected nematodes were obtained from a *H. goettingiana* culture originally isolated in Göttingen, Germany, the type locality of this cyst nematode species, and has been maintained on peas in a 1 m² microplot on the experimental field of the Institut für Nematologie und Wirbeltierkunde of the Biologische Bundesanstalt at Münster. In 1983 it had been observed for the first time that this population of *H. goettingiana* was heavily infested with *Pasteuria* bacteria. Some soil from the microplot was also moved to the greenhouse for propagation of the nematodes and their bacterial parasites. The nematodes were mostly isolated from soil samples using a modified centrifugation method with a MgSO₄ solution (Müller, 1980) that recovered also immobile stages and dead nematodes.

LIGHT AND ELECTRON MICROSCOPY

Light microscopy (LM) observations were made on nematodes fixed in TAF, processed to anhydrous glycerin through the slow evaporation method and then either mounted permanently on slides or prepared as temporary mounts consisting of parasitized fresh nematode specimens that were squashed or crushed in water to release their body contents. Measurements were taken of the bacterial stages by using an eyepiece micrometer on an Orthoplan® Leitz microscope equipped with an interference contrast system. Photomicrographs were made with a Zeiss® Photomicroscope III also equipped with a DIC system. An inverted microscope, Leitz Diavert®, was used to study spore attachment and for spore counting.

Preparation for transmission electron microscopy (TEM) was the same as previously described (Sayre *et al.*, 1991 *a*). A Hitachi model H500-H transmission electron microscope, operating at 75 kV with 30 μm apertures, was used for viewing the thin sections.

Morphometric data were obtained by measuring structures on enlarged TEM micrographs. The sources of measurements have been indicated by LM for values obtained by light microscopy or TEM for those from the transmission electron microscope.

TERMINOLOGY

Terms applied to describe the fine structure of the developmental stages of HGP agree with those suggested by Sussman and Halvorson (1966) and recently reviewed by Iterson (1988). The terminology used in this paper is as follows : (a) endospore is the single asexual spore that develops within a sporangium and is enclosed by an exosporium; (b) plasma membrane is the innermost layer that surrounds the central protoplast; (c) the cortex is the adjacent layer that appears as a wide electron-translucent zone and is followed by (d) the inner coat, a much narrower multilaminar band and finally (e) the outer coat, a wide electron-dense wall. All these structures are surrounded by (f) the exosporium, a delicate membrane that delineates the outermost layer of a typical Gram-positive bacterial spore. Within the exosporium, *Pasteuria* endospores have additional structures that include (g) the epicortical layer, which is a discontinuous, electron-dense band around the cortex that varies in appearance from species to species, and (h) the perisporium, largely consisting of peripheral fibers, which are an essential part of the *Pasteuria* endospore and probably allow its attachment to the nematode cuticle.

Our definitions for structures differentiated with the light microscope consist of the highly refractile " core " of the spore, which probably corresponds to the protoplast plus the plasma membrane and cortex, and the " coat ", a less refractile wall encircling the central core. The central part of the entire endospore, limited by the outer coat and distinctly differentiated from the translucent perisporium, will be referred to as the " central body ".

Results

Infection by HGP was exclusively observed in second-stage juveniles of *H. goettingiana* isolated from soil. On rare occasions a few spores were observed attached to the cuticle of males; however, in these instances no evidence of penetration or internal infection was found. Similarly, no infections of females and cyst contents were observed (Winkelheide, 1990; Winkelheide & Sturhan, 1993). Therefore, in this study all results on the developmental cycle, the morphology and the ultrastructure of HGP refer to observations made on the bacterial parasite infecting the J2s of *H. goettingiana*.

ATTACHMENT OF SPORES

Endospores normally attach to the cuticle of the nematodes along their " basal " side; occasionally spores appear to be loosely adhering to the cuticle along their polar side or their " rim ", which consists of the perisporal fibers. Spores may be present from the lip region to the hyaline tail terminus of the juveniles; no preferred

sites of attachment are evident, although the majority of spores is found along the anterior portion of the nematode. Frequently rod-like bacteria and debris are affixed to the surface of germinated endospores or are closely clustered around their bases.

In apical view, the endospores attached to the nematode cuticle are circular and have an evenly rounded central body with a distinctly offset wall (" coat "). In a germinated spore only a distinct wall is retained around the empty core (Fig. 1).
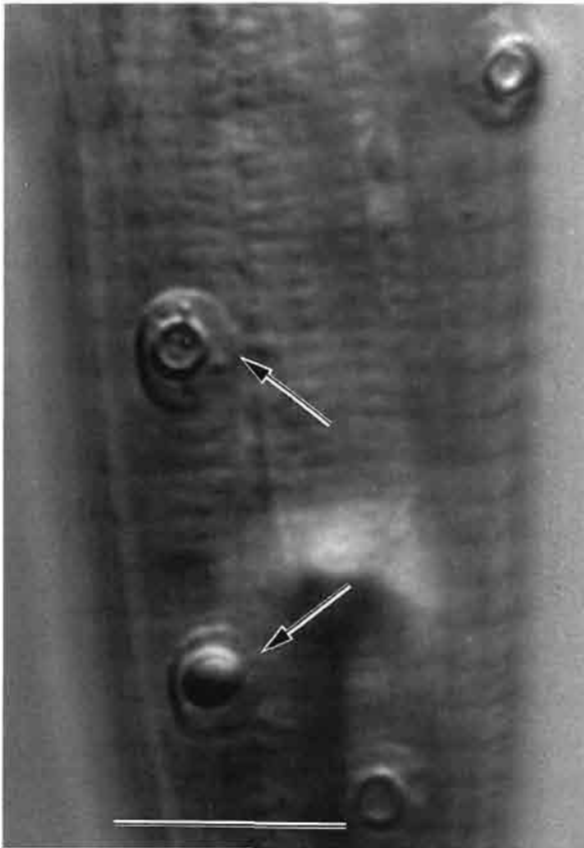
Fig. 1. *Endospores of* Pasteuria *sp. (HGP) attached to the cuticle of a* Heterodera goettingiana *second-stage juvenile (J2). The "full" spore indicated by the lower arrow shows a refractile bulging central body, while "empty" spores (upper arrow) show a circular ring whose center is devoid of protoplast. (Bar = 10 μm.)*

TEM observations indicate that in a germinated spore the exosporium appears to deteriorate; in Fig. 2 only remnants can be seen at the basal and apical sides of the spore. In ungerminated spores, the spore cortex appears smaller and less distinct (Fig. 10 C, D). The attachment layer seems to gradually detach from the cuticle of the nematode (Fig. 2 B, right side of spore). " Pores " are occasionally observed with the LM on the cuticle of second-stage juveniles. These openings mark the sites of

the penetration tubes and of developing microcolonies of *Pasteuria* that occur beneath the cuticle within the body of the nematode.

PENETRATION

On germination a germ tube emerges through a central pore at the basal side of the spore (Fig. 3 A, B). At the same time, the protoplast shrinks and separates from the spore walls, possibly due to breakdown of the cortex. The germ tube, which measures almost 0.5 μm in diameter (LM) and attains a length of around 2-3 μm, penetrates the cuticle and hypodermis of the nematode and forms a vesicle-like expansion at its end. At the sites of penetration, a " bulging " of the hypodermis is occasionally observed.

After evasion of the protoplast, the outer and inner coats and remnants of the cortex can still be distinguished in the TEM (Fig. 2 A, B; the germinal pore and the germ tube are not present in this section). " Empty " endospores, which attach to the surface of the nematode, are easily differentiated from ungerminated endospores with the light microscope (Fig. 1).

Generally, most of the endospores that adhere to the nematode juveniles are empty; only a few spores appear to penetrate the cuticle and give rise to multiple infections. More than 25 " empty " spores/nematode have been observed without any indication of cuticle penetration and/or infection initiation. In " empty " spores, the germinal pore of the endospore is generally visible at high magnification with the LM, but not in ungerminated " full " spores. There is evidence that germ tubes, which do not penetrate the cuticle, emerge between the " rim " of the perisporium and the cuticle of the nematode as was observed by Birchfield and Antonopoulos (1976).

VEGETATIVE GROWTH

The first sign of infection observed with the LM is a densely granulated area near the tip of the germ tube. This area increases in size and a rounded cauliflower-like mycelial colony develops, which later mostly becomes elongate or grape-like (Fig. 4). The maximum diameter of a microcolony was 10.4 μm, the greatest length 16.5 μm. The mycelial colonies are generally located in the pseudocoelom of the nematode, but are occasionally found in the hypodermal region or embedded in cavities in muscle tissue.

The well differentiated hyphae comprising the colonies are septate (Fig. 7); the diameter of a mycelial hypha is about 0.5 μm. The hyphae are bounded by a wall, 0.03 μm thick, consisting of an inner and an outer membrane. The inner membrane constricts to form the septations which delineate individual cells.

In advanced developmental stages, fragmentation of the thalli separates the mycelial masses, which are then transported in the fluid of the pseudocoelomic cavity through the body of the host nematodes. These frag-
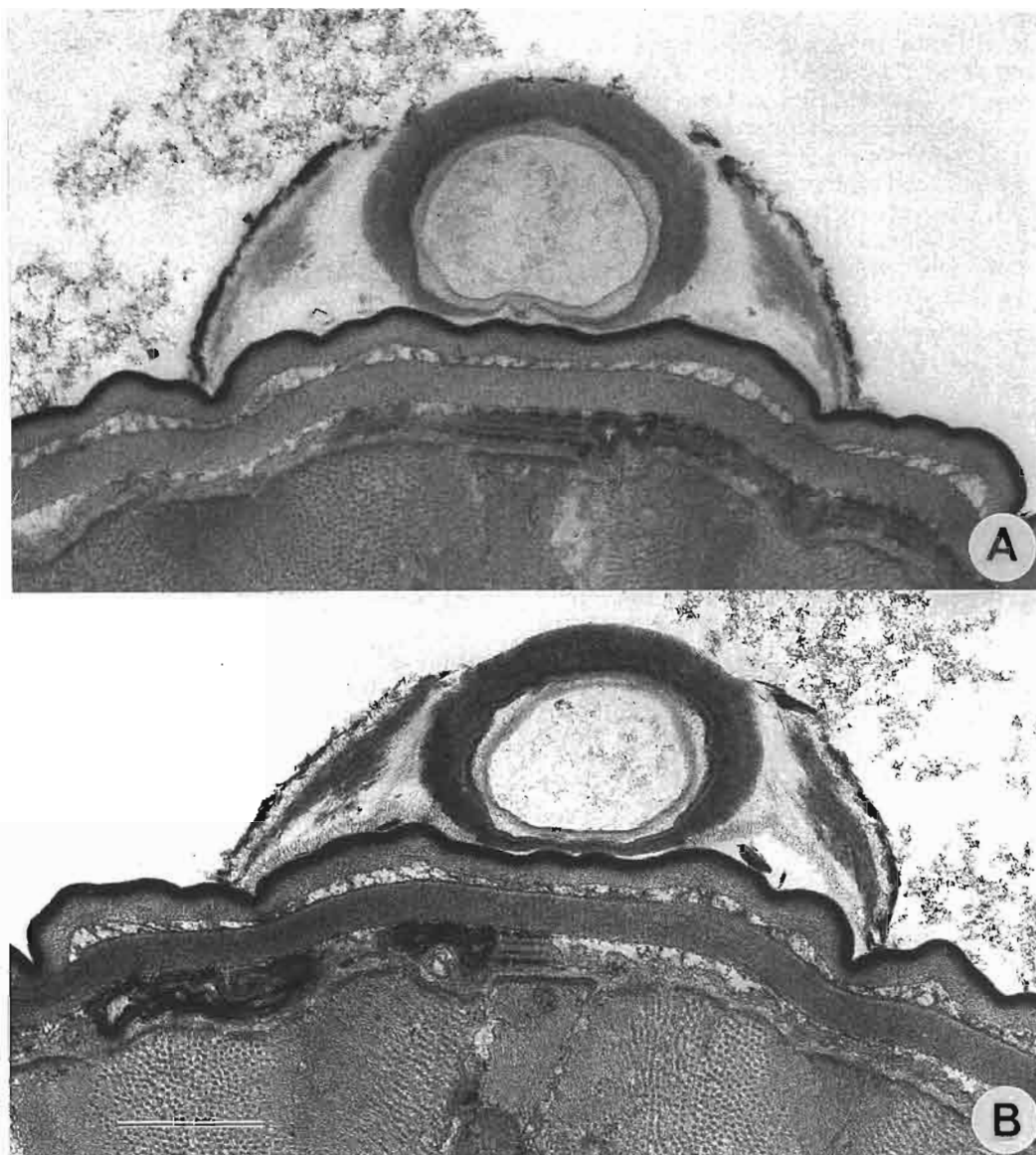
Fig. 2. *Transmission electron micrographs showing a cross section through an " empty " endospore of HGP on the cuticle of J2 of H. goettingiana. A : This endospore shows some degradation but has not yet begun to detach from the cuticle of the nematode; B : The cortex, inner and outer coats are present, exosporium is lacking above the central body and apparently dissolving above the perisporium; detachment from cuticle is beginning on the right side. (Bar = 1 μm.)*

ments are dichotomously branching thalli that give rise to microcolonies of four, eight or more terminal cells (Fig. 6 A, B). Later, the terminal cells increase in size and become oval. Fragments of mycelial colonies with either four or two ovally enlarged terminal cells are frequently seen (quartets and doublets in Fig. 6 C, D). The average size of a quartet is 5.0 μm by 3.8 μm; the terminal cells are around 2.4 μm long, around 1.4 μm wide and usually connected by three intercalary cells. In the doublet configuration the terminal cells measure 2.9-

3.5 μm by 1.4-1.6 μm. They are connected by a single cell that measures about 1 μm.

Cross sections of parasitized nematodes quite often reveal life stages of the bacterium ranging from filamentous vegetative stages to mature sporangia (Figs 5, 8). The vegetative growth phase of the bacterium and the immature sporogenesis stages are often found centrally located in the nematode, while the mature sporangia tend to be located at the periphery or beneath the cuticle (Fig. 7).
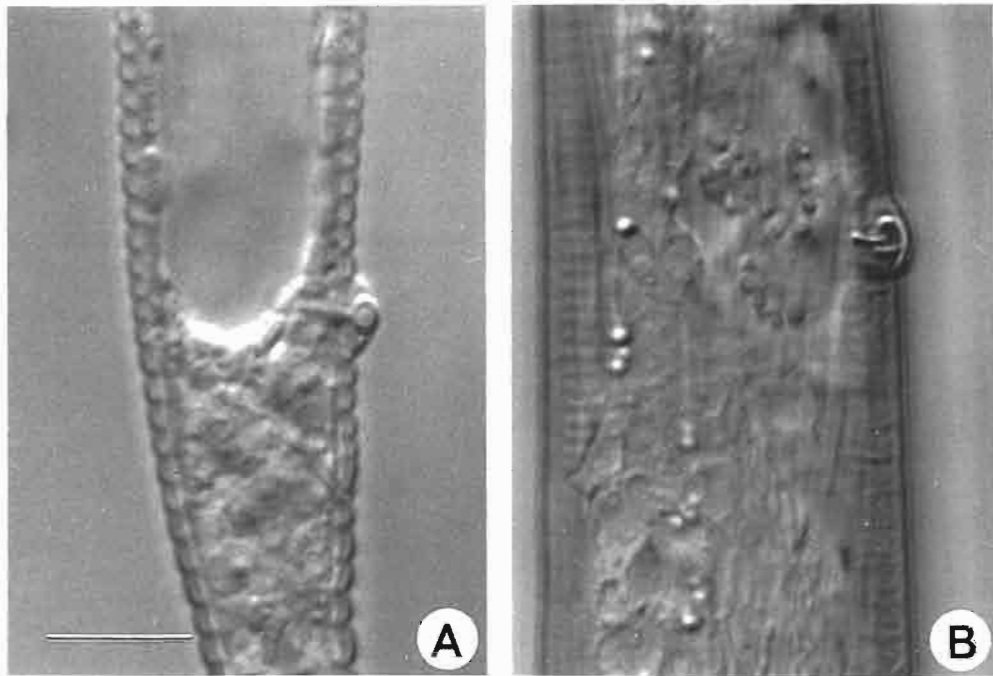
Fig. 3. *Photomicrographs showing penetration phenomenon. A : A long germ tube has formed under the saucer-shaped endospore that is attached to the cuticle of a* H. goettingiana *J2 anterior to the anus; B : A mycelial colony has developed under a germinated endospore.* (Bar = 10 μm.)
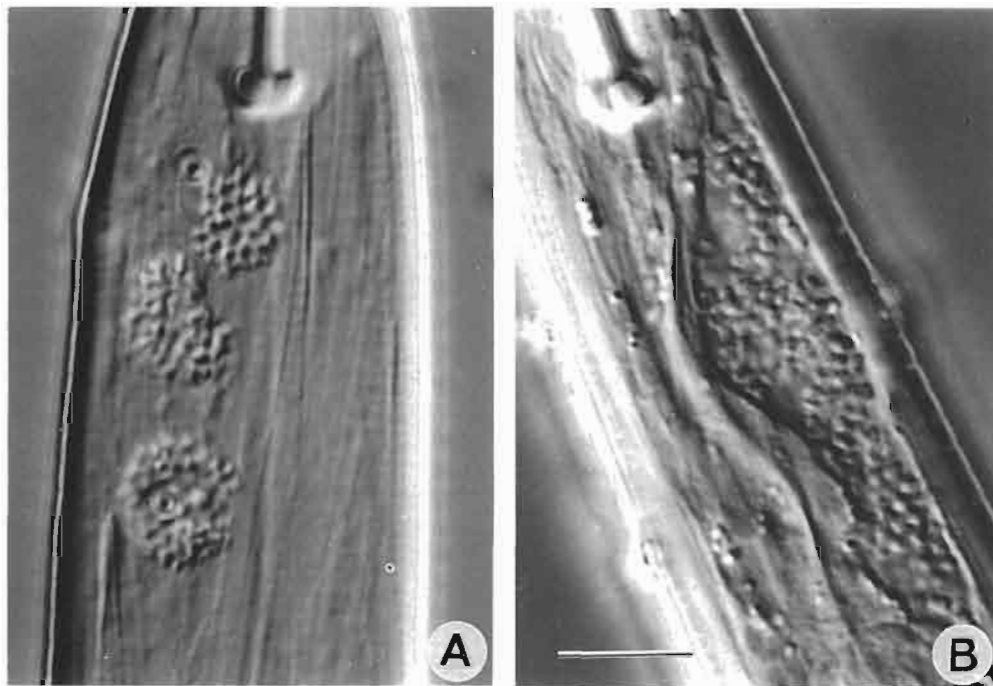
Fig. 4. *Photomicrographs showing rounded (A) and elongated (B) mycelial colonies of HGP that have formed in the nematode's oesophageal region below attached endospores.* (Bar = 10 μm.)
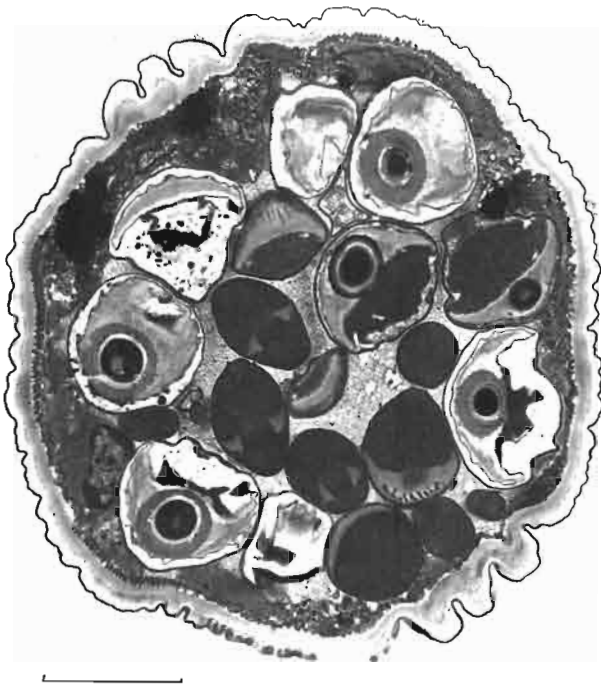
Fig. 5. *TEM cross section through mid-body region of a J2 of* H. goettingiana, *showing different stages of sporogenesis and mature sporangia with fully developed endospores of HGP.* (Bar = 5 µm.)

SPOROGENESIS

HGP exhibits the stages of endospore formation found in the endospore-forming Bacillaceae and as previously described for species of *Pasteuria.*

The earliest stage of the sporogenesis in HGP consists of expanded mycelial tips that are separated from parent colonies (Fig. 9 A). In sloughing off from the microcolony, the sporangium sometimes exhibits two small basal appendages (Fig. 9 A, C), which are remnants of the mycelial wall that broke from the microcolony. As a sporangium enlarges, a septum formed within the upper third of the cell separates the incipient forespore cytoplasm from the remainder of the spore mother cell. Unlike in previously described species of *Pasteuria,* mesosomes are apparently not associated with septations (Fig. 9 B). The septum growing around the forespore finally provides a double-layered membrane that encloses the condensed cytoplasm (Fig. 9 C, D). It characteristically forms an apex pointed towards the cytoplasm of the mother cell (Fig. 9 D). Meanwhile the sporangium has increased in size and attained an ovate to almost globular shape. The protoplast of the forespore exhibits a more electron-translucent central region, whereas the outer portion as well as the cytoplasm of the spore mother cell appear densely granular (Fig. 9 D). The electron-translucent area around the

forespore, which appears wing-like in lateral view and attains a fibrous appearance, gives rise to the perisporium which is characteristic of the mature endospores in all species of *Pasteuria* (Fig. 9 C, D). Variation in the condensation of these fibers may result in irregular striations.

At a more advanced sporangial stage, the cortex develops between the two membranes surrounding the forespore; it shows an inner electron-translucent zone and an opaque outer zone, around which an irregular granular epicortical layer eventually develops (Fig. 10 A). Subsequently, the outer spore coat is deposited on the surface of the outer membrane that finally becomes the laminar inner coat of the mature endospore. The outer coat gradually increases in thickness mainly at the upper side of the endospore and it attains a more or less homogenous fibrous appearance (Fig. 10 B).

Meanwhile the shape of the protoplast changes from rounded to slightly elliptical. The thin layer of electron dense mother cell cytoplasm around the apical part of the sporangium almost disappears and the granular material of the spore mother cell in the basal part gradually concentrates at the basal side of the endospore (Fig. 10 B). This spore mother cell material is finally engulfed by the delicate exosporium, which surrounds the entire endospore (Fig. 10 C).

SPORANGIA AND ENDOSPORES

Mature sporangia attain a thick lenticular to almost spheroidal shape; the apical part is rounded or slightly conoid and the basal part is irregularly shaped, i.e. rounded, flattened, conoid or dentate, due to partial collapse (Figs 10 C, D; 11; 12).

Sporangia released into water from infected fresh nematodes measured 5.2 (4.9-5.3) µm in diameter and 4.6 (4.2-5.2) µm in height (LM). With the TEM, measurements of sporangia within the nematodes were 3.92 (3.25-4.59) µm by 3.08 (2.64-3.56) µm. The ratio diameter/height is 1.11 and 1.27, for LM and TEM, respectively. A stem cell or attachment of a second sporangium is quite often observed (Fig. 11).

The mature endospores are saucer to bowl-shaped due to ventral bending of the distal area of the perisporium. The basal side is characterized by irregular lobes containing trapped matrix material. With the LM, a distinct spore wall (coat) can be differentiated which is surrounding the " core " of the endospore (Figs 1; 12 A). This wall has an even thickness of about 0.3 µm but decreases in thickness toward the center of the basal side of the spore. The refractile " central body ", consisting of the core plus coat, is easily differentiated from the more translucent circular perisporium. Endospores released from crushed nematodes are mostly still enclosed within the sporangia.
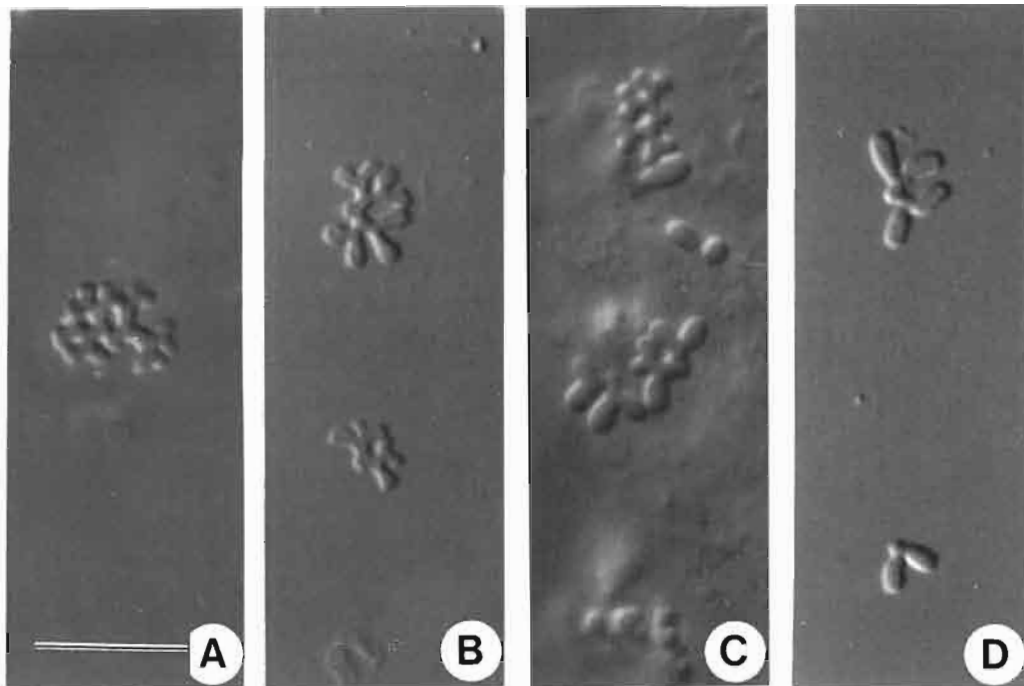
Fig. 6. *Photomicrographs showing fragmented mycelial colonies (A, B), quartets (C, D) and doublets (D).* (Bar = 10 μm.)

Free endospores that were released into water measured 5.0 (4.4-5.3) μm by 3.6 (3.2-4.1) μm (LM); the " central body " was 2.6 (2.4-2.9) μm by 2.0 (1.9-2.3) μm and the " core " was 1.9 (1.7-2.1) μm by 1.5 (1.3-1.9) μm. Spores, which were attached to the cuticle of *H. goettingiana* juveniles, had a diameter of 4.9 (4.5-5.3) μm. In apical view the diameter of the central body covers about half the width of the endospore and the diameter of the core about one quarter to one third.

In the TEM structural details of the sporangium and endospore can be observed (Fig. 11). The sporangium with a wall diameter of 0.05 μm encloses the endospore that is delimited by the exosporium. The electron-dense protoplast, which often shows a light central area, has an oblate spheroid shape and appears elliptical in lateral axial view with a horizontal orientation for the major axis. It is surrounded by the plasma membrane that appears to consist of several layers. This is followed by a wide electron-translucent cortex that often shows indistinct concentric striations.

Around the cortex layer is an irregular epicortical layer of granular particles, apparently originating from the mother cell cytoplasm that was entrapped in formation of the spore layers. This irregular epicortical layer is surrounded by the distinct " inner coat " consisting of around nine alternate dark and light longitudinal lines that form a laminar layer with nearly even thickness of about 0.03 μm. The number of lines and the diameter of the inner coat slightly decrease at the basal side of the endospore. The outer spore coat, which is fibrous in appearance, covers the laminar inner coat. It has its

greatest diameter at the polar side of the spore and gradually thins to the basal side, where a germinal pore of 0.3 μm diameter remains open. The surface of the fibrous outer coat is often irregular, not well delineated and may have a " fringed " appearance. Perisporic fibers are inserted here at a wide angle. They form a circular perisporium around the " central body " of the endospore and exhibit condensed regions mainly on their upper sides. The perisporium, which is mostly not distinctly offset from the outer coat, does not cover the apical and basal sides of the " central body ". The entire endospore is surrounded by the delicate membranous exosporium, which is irregularly folded over the apical region of the endospore. It generally encloses most of the mother cell cytoplasm at the basal part of the endospore and only rarely traps mother cell material along the apical side. The surface of the endospore enclosed within the exosporium is finely hirsute. The matrix of the spore mother cell around the endospore consists of coarsely granular to finely mottled material.

Measurements obtained from enlarged TEM images are as follows : 3.75 (3.11-4.37) μm by 2.34 (1.75-2.91) μm for the endospore; 1.94 (1.73-2.17) μm by 1.35 (1.26-1.45) μm for the central body and 1.03 (0.89-1.13) μm for the protoplast.

Discussion

The *Pasteuria* isolate found on the J2 of *H. goettingiana,* and the three *Pasteuria* species found on other nematodes are similar in their modes of infection, developmental cycles and in the gross appearance of the morphology and ultrastructure of the developmental stages.

Fig. 7. *Transmission electron micrograph showing a cross section through a J2 of* H. goettingiana *colonized by vegetative stages of microcolonies as well as various stages of sporogenesis.* (Bar = 1 µm.)

In all hitherto known species that parasitize nematodes, infection is through direct penetration of the cuticle by germinating spores that attach to the surface of the nematodes. The life cycle beginning with the vegetative mycelial colonies and extending to the mature sporangia is completed largely within the pseudocoelomic cavity but eventually extended to the entire body of the nematode. The sporangia are released into the soil when the nematode carcass degrades. When and where the endospores are released from the sporangia remains unknown.

The stages of the endospore formation and most of the structural details of the developmental stages are the same as those described for other endospore-forming bacteria. The mycelial cell walls of HGP are typical of the Gram-positive bacteria, but mesosomes which are often found with other bacteria, were not observed in HGP. No obvious differences to previously described *Pasteuria* species exist in the various stages of the life cycle beginning with the vegetative colonies and extending to the immature sporangia. However, in the later stages of sporogenesis and in the mature sporangia and endospores differences become evident. The differences involve the arrangement of perisporal structures, the development of the various spore coats, the presence of entrapped mother cell cytoplasm and certain dimensions.

The *H. goettingiana Pasteuria* is distinctly different from *P. thornei* in shapes and sizes of the sporangia and
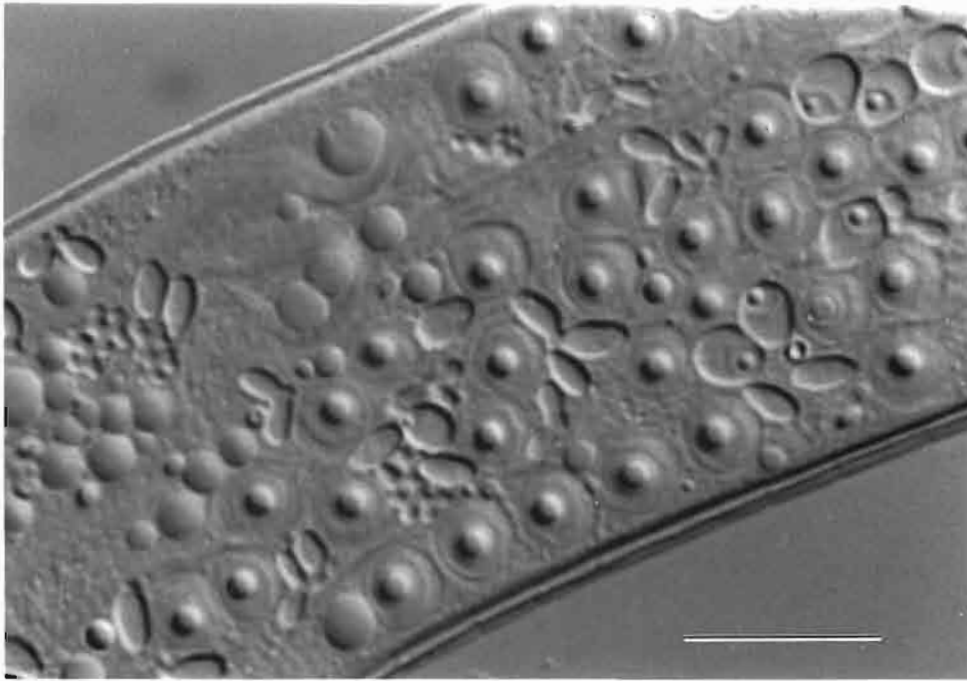
Fig. 8. *Photomicrograph of the mid-body region of a* Heterodera goettingiana *J2 showing HGP developmental stages, including mycelial fragments, doublets, early to late sporogenesis stages and nearly mature endospores.* (Bar = 10 μm.)

the endospores (Sayre *et al.,* 1988; Starr & Sayre, 1988). Differences between HGP and *P. penetrans* are less distinct; however, measurements of sporangia, the " central body " and the " core " of the endospore are smaller in *P. penetrans;* the outer coat of the endospore forms a distinct thickening around the basal germinal pore, and the appearance of the basal part of sporangium and endospore is different (Sayre & Wergin, 1977; Sayre & Starr, 1985). HGP most closely resembles *P. nishizawae* (Sayre *et al.,* 1991 *b, c*). It can be distinguished from this species by (1) the appearance of the basal portion of the endospore, which exhibits irregular extensions through enclosures of large parts of cytoplasm of the spore mother cell within the exosporium; (2) the presence of a simple basal adhesion layer in the endospore (double layer in *P. nishizawae*) and; (3) the gradual decrease of the outer endospore coat to form the basal pore (reduction to about one half the diameter at apical region in *P. nishizawae*).

A distinct character which differentiates HGP from *P. nishizawae* and from *P. penetrans* is that in the former the infection and completion of the life cycle only occurs and in second-stage juveniles. Different developmental stages of *Pasteuria* were present simultaneously within the same host specimen. In both other *Pasteuria* species, spores attach to the cuticle of migrating second-stage juveniles, but penetration of the nematode cuticle takes place only after the juveniles have invaded the roots of their host-plants; development is completed in the adult female stage. Infection of females or cyst contents by

HGP was never observed. Similarly, males of *H. goettingiana* are obviously no suitable hosts. Earlier observations of *Pasteuria* sporangia within cysts of *H. goettingiana* by Sayre *et al.* (1988, Table I) were erroneous. In *P. thornei,* infection and completion of the life cycle is possible in *Pratylenchus* juveniles as well as adults (Starr & Sayre, 1988).

The ability of HGP to develop and complete the life cycle only in the second-stage juveniles has significant consequences. The number of spores produced per host specimen must be low because the food reserve in the J2s is limited and feeding on host plant has not begun. Alternatively, in *P. penetrans* and *P. nishizawae* the spores germinate and develop when the nematode is feeding in the plant tissue. In these cases, the bacteria can rely on a continuous nutrient supply, the availability of the reproductive systems, and the voluminous bodies of the females in root-knot and cyst nematodes, which results in 4000 times *(P. penetrans)* resp. 900 times *(P. nishizawae)* more spores per host specimen than in HGP, for which an average number of 500 spores per host specimen was recorded (Mankau, 1975; Nishizawa, 1986; Winkelheide & Sturhan, 1993). In *P. thornei* a few hundred spores were found in the various vermiform stages of *Pratylenchus brachyurus,* which are comparable in size to second-stage juveniles of *Heterodera.* But unlike HGP, *P. thornei* can complete its entire life cycle in juvenile and adult stages which when feeding on host roots, supply continuously nutrients for the bacte-
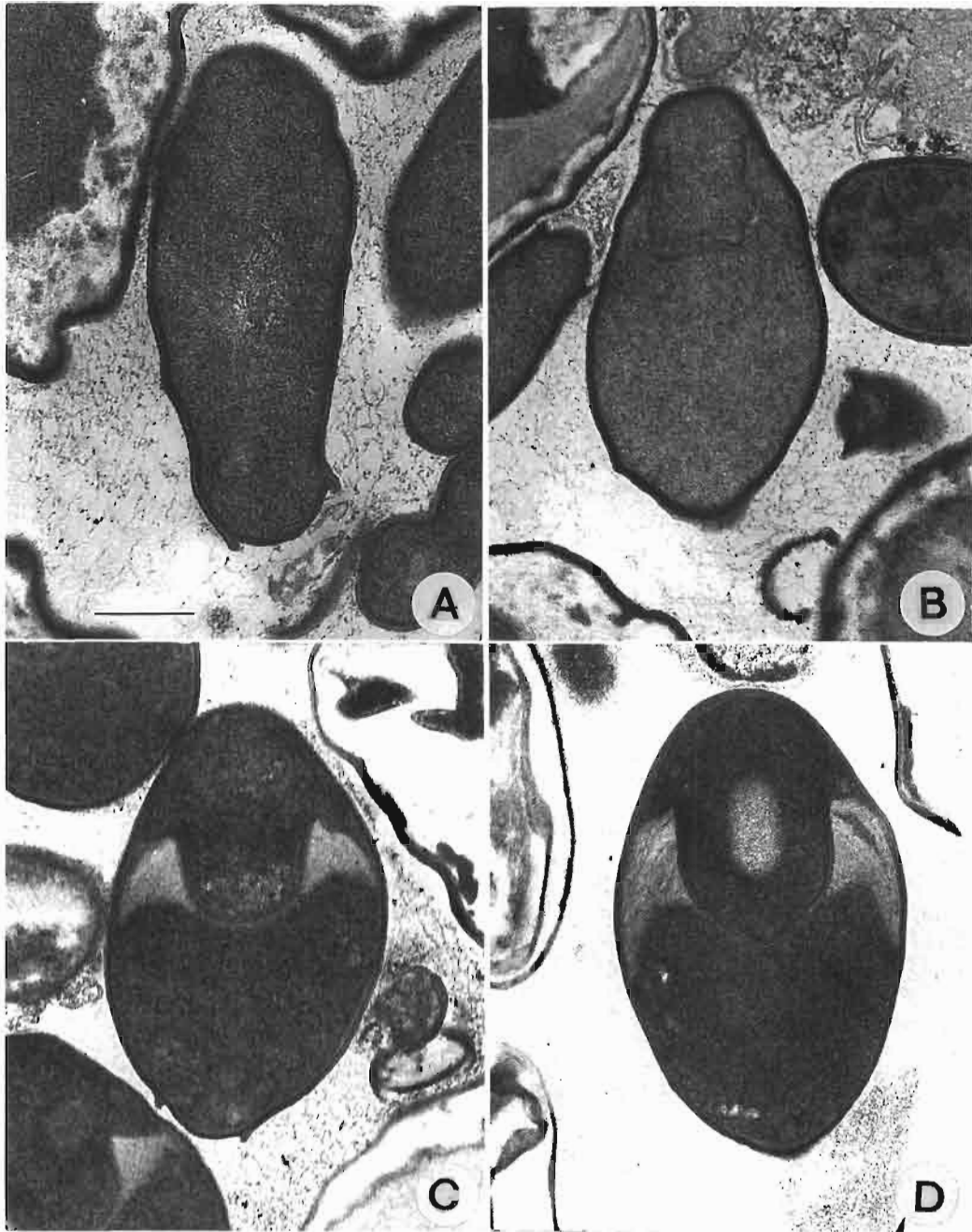
Fig. 9. *Median sections through early sporangial stages of HGP. A : Swollen distal branch of a microcolony that becomes a sporangium, containing dense granular material; B : Formation of an anterior septum that separates the forespore from the basal parasporal structures; C : A forespore has begun to condense in the anterior of the sporangium and is laterally surrounded by two small electron transparent areas that will become perisporal fibers; D : A discernible double layer wall has formed around the developing endospore in the anterior of the sporangium. (Bar = 1 μm.)*

rial parasites (Sayre *et al.*, 1988; Starr & Sayre, 1988). In this respect HGP is comparable to *Pasteuria* isolates found in the citrus nematode *(Tylenchulus semipenetrans)* and the oat cyst nematode *(Heterodera avenae),* where infection is obviously also restricted to second-

stage juveniles (Fattah *et al.*, 1989; Davies *et al.*, 1990). Therefore, although HGP appears to have great potential as biological control agent, the low rate of spores that is produced per host specimen will probably only slowly increase the density of spores in soil.
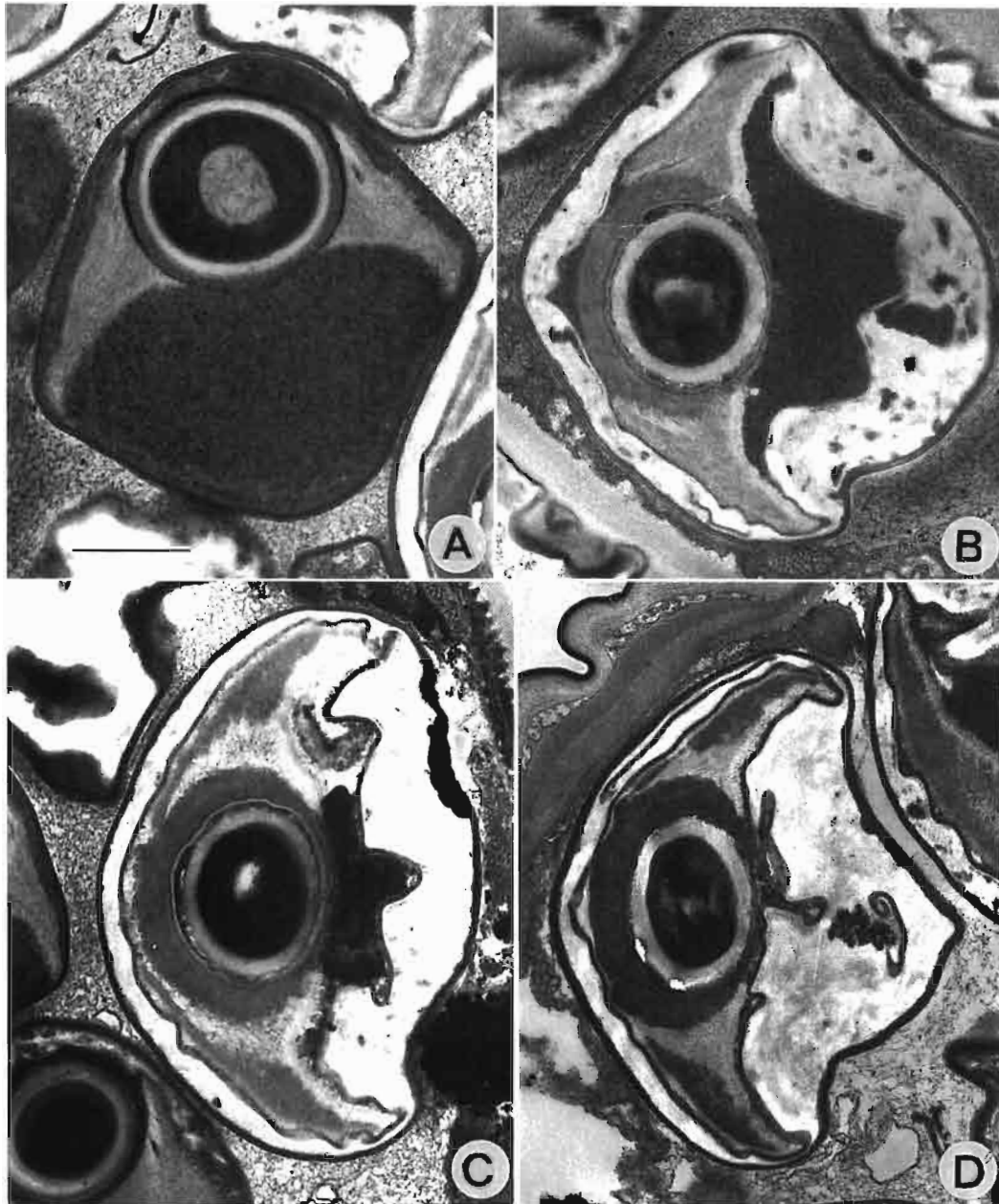
Fig. 10. *Median sections through nearly mature sporangia of HGP showing late stages of sporogenesis. A : An almost round protoplast is surrounded by a multilayered wall with clearly defined perisporal region. The basal portion of sporangium is filled with mother cell cytoplasm, while only a narrow band of mother cell material covers the developing endospore in the apex; B : Endospore with thick outer spore walls and fully extended perisporal fibers gives the typical cup-shape to the nearly mature sporangium. Remnants of the mother cell cytoplasm are scattered patches of electron dense material found largely inside the exosporium. The basal matrix is filled partially with remnants of the mother cell; C : The partially collapsed sporangial wall and thin inner exosporium surround a broadly elliptic endospore. The mother cell material is restricted to the basal area of the sporangium and is enclosed within the exosporium; D : The sporangium contains a fully mature endospore. The coats have their greatest thickness on the polar side of the spore and gradually thin down to the basal side.* (Bar = 1 μm.)

Fig. 11. *Median section through a sporangium of HGP containing a nearly mature endospore. Within the encircling exosporium is electron dense mass of entrapped mother cell cytoplasm. A small remnant of a cell of the microcolony is attached to the base of the mature sporangium.* (Bar = 1 μm.)
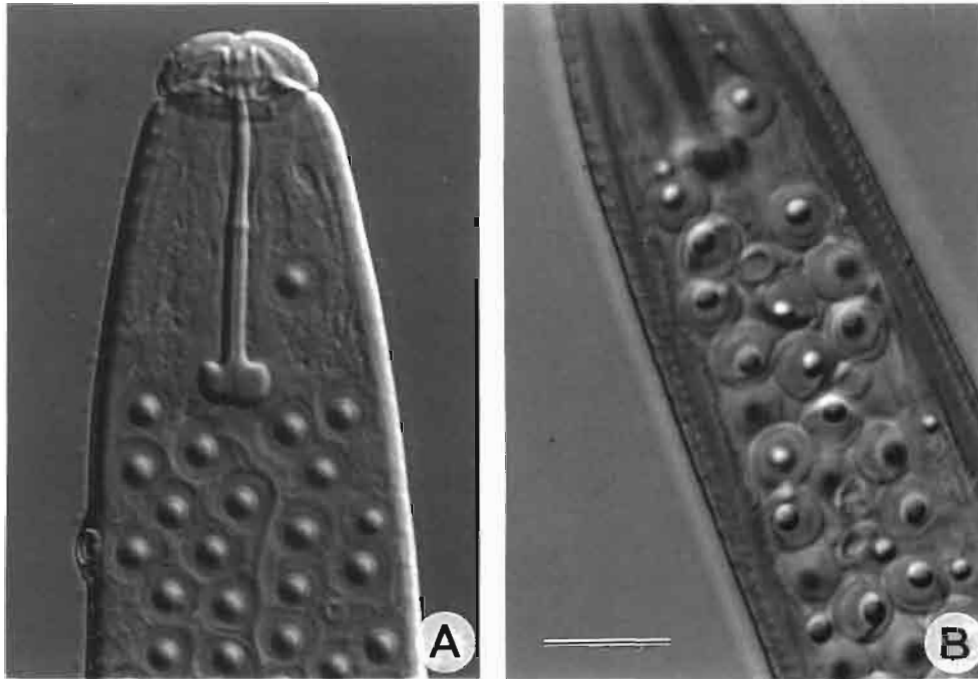
Fig. 12. *Photomicrographs showing mature endospores in the anterior end of* H. goettingiana *second-stage juveniles in apical view (A) and in various positions (B).* (Bar = 10 μm.)

In host specificity HGP differs, in particular, from *P. penetrans* and *P. thornei* which infect *Meloidogyne* and *Pratylenchus* species, respectively. In host range studies spores that were extracted from *H. goettingiana* juveniles adhered to the cuticle of some other members of the Heteroderidae family, including species of *Globodera* and *Meloidodera,* but not on *Meloidogyne* or *Pratylenchus* species (Winkelheide, 1990; Sturhan & Winkelheide, 1991).

In conclusion, differences in the morphology and ultrastructure, in the host-range and in the ability to develop only in J2s appear to justify assigning the *Pasteuria* isolate from the pea cyst nematode to a species that is separate from the three *Pasteuria* spp. previously described on nematodes. This taxonomical treatment will be published separately.

References

Abrantes, I. M. de O. & Vovlas, N. (1988). A note on parasitism of the phytonematodes *Meloidogyne* sp. and *Heterodera fici* by *Pasteuria penetrans. Canad. J. Zool.,* 66 : 2852-2854.

Bhattacharya, D. & Swarup, G. (1988). *Pasteuria penetrans* a pathogen of the genus *Heterodera,* its effect on nematode biology and control. *Indian J. Nematol.,* 18 : 61-70.

Birchfield, W. & Antonopoulos, A. A. (1976). Scanning electron microscopic observations of *Dubosqia penetrans* parasitizing root-knot larvae. *J. Nematol.,* 8 : 272-273.

Davies, K. G., Flynn, C. A., Laird, V. & Kerry, B. R. (1990). The life-cycle, population dynamics and host specificity of a parasite of *Heterodera avenae,* similar to *Pasteuria penetrans. Revue Nématol.,* 13 : 303-309.

Esser, R. P. (1980). A bacterial spore parasite on nematodes. *Florida Dept. Agric. & Consumer Serv., Div. Plant Industry, Nematol. Circ. No.* 63, 3 p.

Fattah, F. A., Saleh, H. M. & Aboud, H. M. (1989). Parasitism of the citrus nematode, *Tylenchulus semipenetrans,* by *Pasteuria penetrans* in Iraq. *J. Nematol.,* 21 : 431-433.

Mankau, R. (1975). *Bacillus penetrans* n. comb. causing a virulent disease of plant parasitic nematodes. *J. Invert. Pathol.,* 26 : 333-339.

Müller, J. (1980). Ein verbessertes Extraktionsverfahren für *Heterodera schachtii. NachrBl. dt. PflSchutzdienst,* 32 : 21-24.

Nishizawa, T. (1986). On a strain of *Pasteuria penetrans* parasitic to cyst nematodes. *Revue Nématol.,* 9 : 303-304.

Page, S. L. J. & Bridge, J. (1986). Observations on *Pasteuria penetrans* as a parasite of *Meloidogyne acronea. Nematologica,* 31 : 238-240.

Sayre, R. M. & Starr, M. P. (1985). *Pasteuria penetrans* (ex Thorne, 1940) nom. rev., comb. n., sp. n., a mycelial and endospore-forming bacterium parasitic in plant-parasitic nematodes. *Proc. helminth. Soc. Wash.,* 52 : 149-165.

Sayre, R. M., Starr, M. P., Golden, A. M., Wergin, W. P. & Endo, B. Y. (1988). Comparison of *Pasteuria penetrans* from *Meloidogyne incognita* with a related mycelial

and endospore-forming bacterial parasite from *Pratylenchus brachyurus. Proc. helminth. Soc. Wash.,* 55 : 28-49.

SAYRE, R. M. & WERGIN, W. P. (1977). Bacterial parasite of a plant nematode : Morphology and ultrastructure. *J. Bacteriol.,* 129 : 1091-1101.

SAYRE, R. M., WERGIN, W. P., NISHIZAWA, T. & STARR, M. P. (1991 *a*). Light and electron microscopical study of a bacterial parasite from the cyst nematode, *Heterodera glycines. J. helminth. Soc. Wash.,* 58 : 69-81.

SAYRE, R. M., WERGIN, W. P., SCHMIDT, J. M. & STARR, M. P. (1991 *b*). *Pasteuria nishizawae* sp. nov., a mycelial and endospore-forming bacterium parasitic on cyst nematodes of genera *Heterodera* and *Globodera. Res. Microbiol.,* 142 : 551-564.

SAYRE, R. M., WERGIN, W. P. & STURHAN, D. (1991 *c*). Comparison of the fine structure of *Pasteuria* sp. from *Heterodera glycines* with a related bacterium parasitizing *H. goettingiana. Nematologica,* 36 : 390.

STARR, M. P. & SAYRE, R. M. (1988). *Pasteuria thornei* sp. nov. and *Pasteuria penetrans* sensu stricto emend., mycelial and endospore-forming bacteria parasitic, respectively, on plant-parasitic nematodes of the genera *Pratylenchus* and *Meloidogyne. Annals Inst. Pasteur, Microbiol.,* 139 : 11-31.

STURHAN, D. (1985). Untersuchungen über Verbreitung und Wirte des Nematodenparasiten *Bacillus penetrans. Mitt. Biol. Bundesanst. Land-Forstwirtsch., Berlin-Dahlem,* 226 : 75-93.

STURHAN, D. (1989). New host and geographical records of nematode-parasitic bacteria of the *Pasteuria penetrans* group. *Nematologica,* 34 (1988) : 350-356.

STURHAN, D. & WINKELHEIDE, R. (1991). Studies on a *Pasteuria* isolate from *Heterodera goettingiana. Nematologica,* 36 : 395.

SUSSMAN, A. S. & HALVORSON, H. O., Eds (1966). *Spores their dormancy and germination.* New York, Harper & Row, 354 p.

VAN ITERSON, W., Ed. (1988). *Inner structures of bacteria.* New York, Van Nostrand Reinhold Co., 501 p.

WINKELHEIDE, R. (1990). *Untersuchungen über nematodenparasitäre Actinomyceten der Gattung* Pasteuria. *M. Sc. thesis,* Inst. Microbiol., Univ. Münster, 158 p.

WINKELHEIDE, R. & STURHAN, D. (1993). Lichtmikroskopische Untersuchungen zur Entwicklung und Morphologie eines *Heterodera goettingiana* parasitierenden Bakteriums der Gattung *Pasteuria. Zentralbl. Mikrobiol.,* 148 : 109-116.