

OCCURRENCE OF *PASTEURIA* FORMS FROM A BIOSPHERE RESERVE IN MEXICO

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ABSTRACT

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Three localities and five land uses with different degrees of disturbance were selected inside the buffer zone of the Los Tuxtlas Biosphere Reserve in Veracruz, Mexico, and eight sampling points were located in each one. From each sampling point, one hundred nematodes were randomly collected and mounted for identification and detection of associated *Pasteuria* forms. *Pasteuria* endospores infecting nematodes were observed in two of three localities, and in four different land uses (secondary forest, maize fields, white lily and pasture fields). A total of 1200 nematodes belonging to six genera were found as infected by or with adhering endospores of *Pasteuria* spp., including *Helicotylenchus exallus* (1116), *Pratylenchus* (44), *Mesocriconema* (20), *Tylenchus* (10), *Plectus* (6) and *Aporcelaimium* (4). These findings represent the first report of *Pasteuria* species from Mexico.

Key words: biological control, endoparasitic bacteria, *Helicotylenchus*, Los Tuxtlas, *Pratylenchus*.

RESUMEN

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Dentro de la zona de amortiguamiento de la Reserva de la Biósfera Los Tuxtlas en Veracruz, México, se seleccionaron tres localidades y cinco usos de suelo con diferentes grados de disturbio; en cada sitio se ubicaron ocho puntos de muestreo. De cada punto de muestreo se tomaron y montaron al azar, 100 nematodos para la identificación y detección de formas de *Pasteuria* asociadas. Las endosporas de *Pasteuria*, infectando o adheridas al cuerpo de los nematodos, se observaron en dos de las tres localidades y en cuatro usos de suelo diferentes (bosque secundario, maizales, azucena y pastizales). Se encontraron endosporas de *Pasteuria* spp., ya sea infectando o adheridas al cuerpo de un total de 1200 nematodos, pertenecientes a seis géneros y una especie, incluyendo *Helicotylenchus exallus* (1116), *Pratylenchus* (44), *Mesocriconema* (20), *Tylenchus* (10), *Plectus* (6) y *Aporcelaimium* (4). Estos hallazgos representan el primer reporte de especies de *Pasteuria* presentes en México.

Palabras clave: bacterias endoparásitas, control biológico, *Helicotylenchus*, Los Tuxtlas, *Pratylenchus*.

Pasteuria spp. (Bacillaceae), are gram-positive, endospore-forming bacteria including several species parasitic in nematodes. They are obligate parasites distributed worldwide and have been isolated from several soil environments and/or found as adhering to or inside a wide range of nematode species (Chen and Dickson, 1998; Chen *et al.*, 2000; Ciancio *et al.*, 1998;

Sayre and Starr, 1988). These include more than 300 hosts belonging to about 117 genera of free-living, predatory and plant parasitic nematodes (Atibalentja *et al.*, 2004; Chen and Dickson, 1998; Sayre and Star, 1988). *Pasteuria* spp. have been used as biological control agents because of parasitism on nematodes of economic importance (i.e., *Meloidogyne* and *Het-*

erodera) (Anderson *et al.*, 1999; Brito *et al.*, 2003; Cetintas and Dickson, 2004; Chen *et al.*, 1996; Chen *et al.*, 1997; Chen and Dickson, 1997a and b; Hewlett *et al.*, 1994; Jonathan *et al.*, 2000; Rojas-Miranda and Marbán-Mendoza, 1999; Sayre and Starr, 1988; Serracin *et al.*, 1997). *Pasteuria* spp. endospores represent both the survival and infective stage of the parasite, and can persist in soil for long periods of time, apparently without loss of viability (Chen *et al.*, 2000). Upon contact, the endospores attach selectively to the cuticle of their corresponding nematode hosts, and their adhesion provides the first step in the life cycle of the bacterium, since it is essential for reproduction within the infected host tissues (Freitas *et al.*, 1997).

Traditional taxonomic arrangement of these endoparasitic bacteria has been based on endospore morphology and nematode host preference. In fact, both criteria have been the basis for the distinction of the recognized species of *Pasteuria*: *P. penetrans* (the first species described from nematodes and parasitic in *Meloidogyne* spp.), *P. nishizawae* (parasitic in *Heterodera* and *Globodera* spp.), *P. thornei* (parasitic in *Pratylenchus* spp.) and *Candidatus Pasteuria usgae* (parasitic in *Belonolaimus longicaudatus*) (Atibalentja *et al.*, 2004; Chen *et al.*, 1997; Giblin-Davis *et al.*, 2001; Giblin-Davis *et al.*, 2003; Sayre and Starr, 1988). However, according to Ciancio *et al.* (1998), morphometric and host-related criteria are not consistent enough when applied to the identification of *Pasteuria* forms. The examination of a large number of host and *Pasteuria* associations showed the occurrence of forms with different morphometrics but parasitic in nematodes within the same genera or species, or forms with similar morphometrics but parasitic in remarkably distant hosts. Actually, several molecular studies have been performed in order to classify and establish the phylogenetic posi-

tion of *Pasteuria* species relative to new forms found around the world and other endospore-forming bacteria (Anderson *et al.*, 1999; Bekal *et al.*, 2001; Sturhan *et al.*, 2005; Wang *et al.*, 2007). In this context, any new report about occurrence of *Pasteuria* spp. or forms will contribute to our knowledge about the taxonomy, distribution and parasitism ecology of this group of nematode parasitic bacteria.

During a study on the diversity of nematodes carried out under different land use intensities in the Los Tuxtlas Biosphere Reserve, in Veracruz, Mexico, nematodes belonging to different genera were observed to be infected by or with adhering endospores of *Pasteuria*. *Pasteuria* has not been reported previously in Mexico. This is the first evidence that the bacteria are present in Mexican localities. Morphology of isolates is illustrated and data on morphometry and nematode hosts are also provided.

Study Area and Sampling

Soil samples were collected inside the Los Tuxtlas Biosphere Reserve (18°10'-18°45' N, 94°42'-95°27' W), located in Veracruz, Mexico. Forty sampling points were selected in three different localities (Lopez Mateos, Venustiano Carranza and San Fernando) covering five land uses (natural forest, secondary forest, white lily, pasture fields and maize fields) from each one. In each sampling point, eight subsamples (100 g) were taken from depth of 10 to 30 cm, mixed and carried in plastic bags to the laboratory. Forty soil samples were taken per locality and 120 in total from the Reserve.

Processing and Mounting Nematodes

Nematodes were extracted from 300 cm³ of soil of each composed sample by sieving and then by the sugar flotation

method (Hooper, 1986). Once extracted, the nematodes were gently killed by heat at 60°C and fixed with cold formalin 4% (Franco *et al.*, 2000); mass nematode suspension of each sample was dehydrated to glycerol by a mass and slow method, and nematodes were mounted in glycerol using wax ring as sealer (Franco *et al.*, 2000). One hundred nematodes from each sampling site were randomly picked and mounted to be observed using a light microscope. Nematodes infected by or with adhering endospores of *Pasteuria* forms were detected and bacterial endospores were measured with at 1000×.

Different *Pasteuria* spp. endospores were observed infecting or adhering to nematodes from samples corresponding to four different land uses in two localities: San Fernando (five sampling points) and Venustiano Carranza (four sampling points) (Table 1). Land uses where *Pasteuria* forms were found are: secondary forest (two positive sampling points), maize fields (two points), white lily (two points) and pasture fields (three points). A total of 1200 nematodes belonging to six genera were found infected or in association with *Pasteuria* spp.: *Helicotylenchus exallus* (1116 specimens), *Pratylenchus* (44), *Mesocriconema* (20), *Tylenchus* (10), *Plectus* (6) and *Aporcelaimium* (4) (Table 1).

Pasteuria spp. mature endospores observed inside the vermiform stages of their nematode hosts or adhering to their cuticle appeared structurally similar to those reported in other studies (Kaplan, 1994; Sorribas *et al.*, 2000). The endospores were found adhering to the cuticle and/or filling the body of specimens belonging to different genera or widely separate taxa (Figs. 1 and 2). The endospore and central core diameters of propagules adhering to the nematodes ranged from 4.55 to 4.83 µm, and 1.61 to 1.96 µm. The endospore height ranged

from 1.47 to 1.86 µm. The number of endospores per nematode was variable, with some specimens showing only one endospore adhering to the cuticle (Fig. 2C and D), others with 10-30 adhering propagules, whereas 10% appeared totally filled. Differing from other host genera, most of the *Mesocriconema* specimens were filled with endospores (Fig. 1A and B).

The frequency and occurrence of nematodes parasitized by *Pasteuria* spp. in the study area could be underestimated since the survey's primary objective was to study the nematode diversity in soils under different land use intensities. Endospore-filled specimens may have been lost during extraction or mounting. On the other hand, it is important to acknowledge that the presence of *Pasteuria* endospores adhering to a nematode cuticle, as noted in many cases of this study, does not necessarily mean that *Pasteuria* can parasitize that nematode, because the simple endospore attachment does not warrant eventual germination, host penetration and subsequent infection. Regardless, nematodes with *Pasteuria* endospores were found in two localities, San Fernando and Venustiano Carranza, with levels of disturbance higher than those observed in Lopez Mateos (in which *Pasteuria* was not found). Moreover, *Pasteuria* forms were more frequent in intensive production systems than in those less disturbed, i.e. disturbed forest. Only two sampling points of the secondary forest, one in each locality, contained *Pasteuria* forms, while the remaining localities with *Pasteuria* forms corresponded to systems with human intervention (pasture and maize fields and white lily).

Continued observations on the occurrence of *Pasteuria* forms in this area as well as in other regions of Mexico will help to enhance our knowledge about *Pasteuria* distribution and associated nematode hosts. Moreover, additional assays will be

Table 1. Localities, host nematodes, land uses and associated *Pasteuria* forms measurements, from the Los Tuxtlas Biosphere Reserve, Veracruz, Mexico.

Sampling point	Host nematode	Locality/land use	Dimensions (μm)		
			Endospores	Central core diameter	Endospore height
SF7P	<i>Aporcelaimium</i> (4)* <i>Helicotylenchus exallus</i> (685)	San Fernando/pasture fields 18°19'19" N, 94°52'83" W Altitude: 1119 masl	4.69 \pm 0.13	1.96 \pm 0.03	1.77 \pm 0.06
SF4P	<i>Helicotylenchus exallus</i> (408)	San Fernando/pasture fields 18°19'16" N, 94°52'87" W Altitude: 1117 masl	4.69 \pm 0.22	1.80 \pm 0.11	1.76 \pm 0.04
SF19A	<i>Plectus</i> (6)	San Fernando/secondary forest 18°18'58" N, 94°53'27" W Altitude: 1029 masl	4.83 \pm 0.72	1.87 \pm 0.14	1.50
SF4M	<i>Pratylenchus</i> (6)	San Fernando/maize fields 18°16'87" N, 94°53'29" W Altitude: 1029 masl	4.79 \pm 0.25	1.76 \pm 0.14	1.80 \pm 0.11
SF34M	<i>Pratylenchus</i> (6)	San Fernando/maize fields 18°16'89" N, 94°53'09" W Altitude: 754 masl	4.79 \pm 0.25	1.61 \pm 0.16	1.47 \pm 0.17
VC16	<i>Tylenchus</i> (10)	Venustiano Carranza/white lily 18°20'74" N, 94°45'73" W Altitude: 231 masl	4.75 \pm 0.29	1.80 \pm 0.09	1.53 \pm 0.14
VC17	<i>Mesocriconema</i> (11)	Venustiano Carranza/white lily 18°20'83" N, 94°45'96" W Altitude: 243 masl	4.60 \pm 0.16	1.95 \pm 0.04	1.86 \pm 0.05
VC32	<i>Helicotylenchus exallus</i> (23)	Venustiano Carranza/secondary forest 18°20'74" N, 94°45'20" W Altitude: 193 masl	4.55 \pm 0.20	1.94 \pm 0.07	1.78 \pm 0.04
VC9	<i>Pratylenchus</i> (32) <i>Mesocriconema</i> (9)	Venustiano Carranza/pasture fields 18°20'21" N, 94°46'27" W Altitude: 257 masl	4.66 \pm 0.09	1.81 \pm 0.06	1.80 \pm 0.05

*Numbers in parenthesis show the total number of specimens infected by or with adhering *Pasteuria* spp. endospores.

masl = meters above sea level.

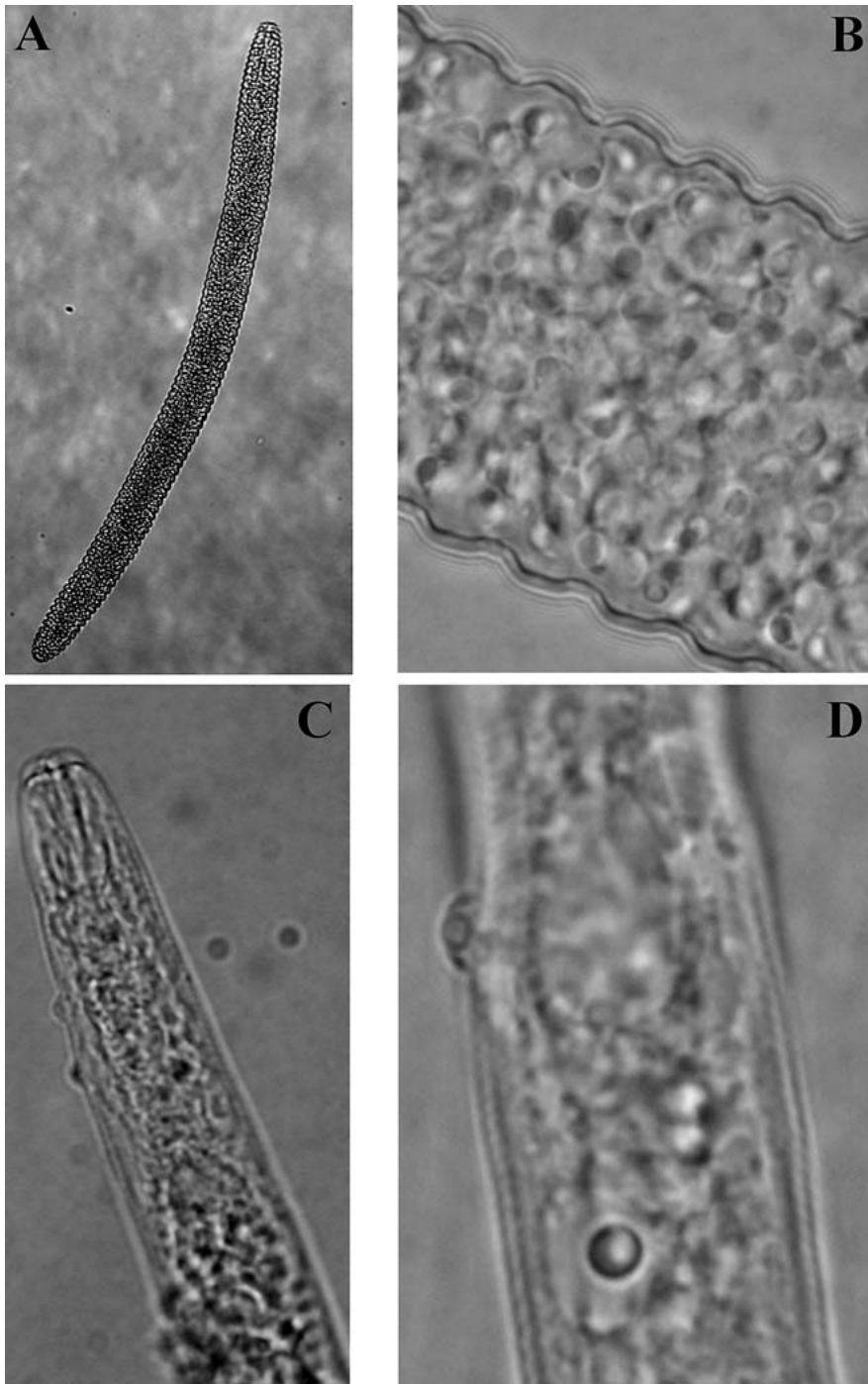


Fig. 1. *Pasteuria* sp. endospores infecting specimens of *Mesocriconema* sp. (A and B) and adhering to *Pratylenchus* sp. (C and D) from the Los Tuxtlas Biosphere Reserve, Mexico.

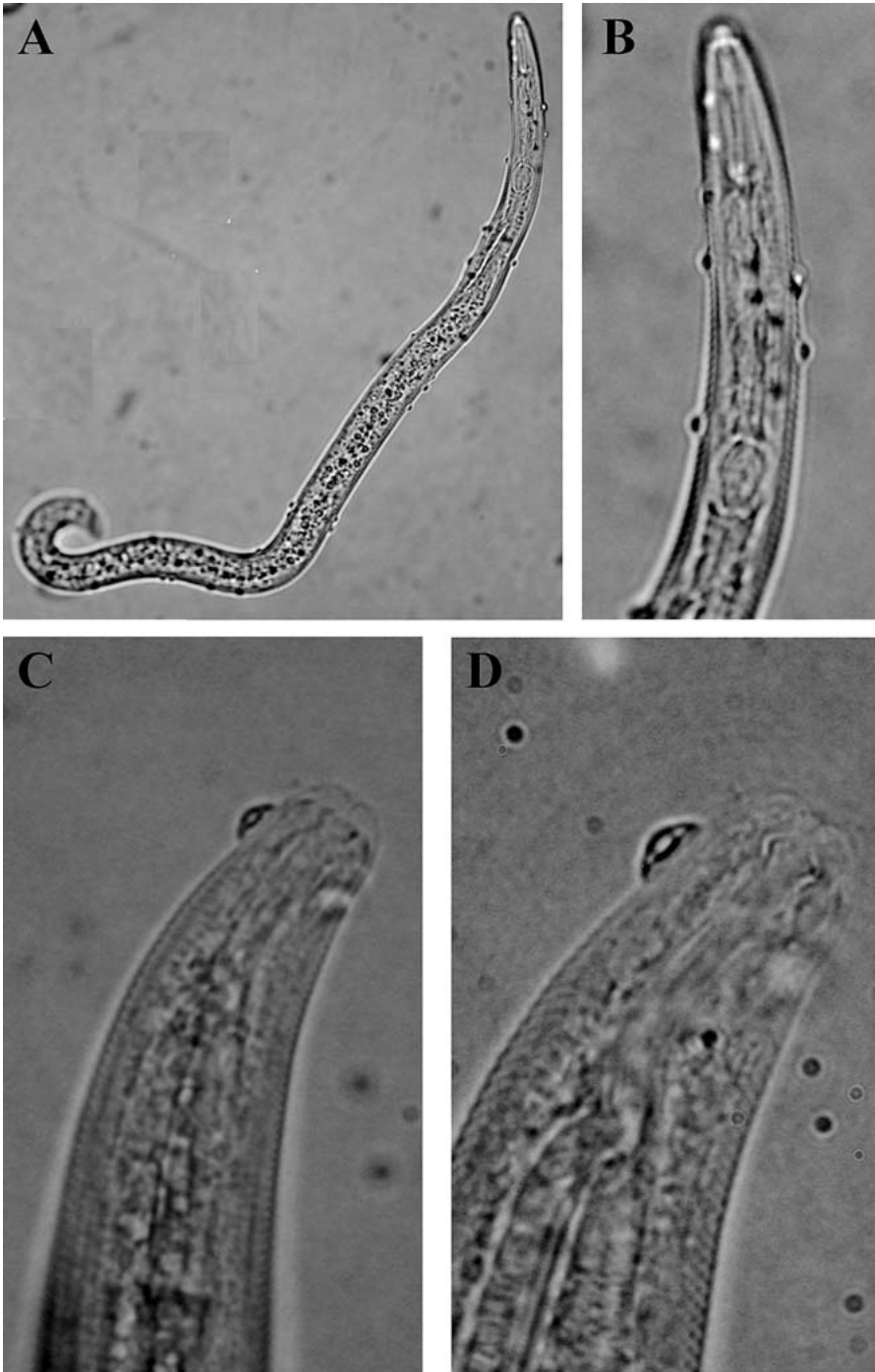


Fig. 2. *Pasteuria* endospores adhering to specimens of *Helicotylenchus exallus* (A and B) and *Plectus* sp. (C and D) from the Los Tuxtlas Biosphere Reserve, Mexico.

needed to elucidate the biology, biological control potential and ecological role of these *Pasteuria* forms from the Los Tuxtlas Biosphere Reserve.

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