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Occurrence and hosts of nematode-parasitic bacteria of the genus *Pasteuria* in Vietnam

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Summary. Nematodes in 74 of a total of 132 soil samples from forests and some agricultural and coastal sites in various parts of Vietnam were found to be attacked by *Pasteuria*. Among 123 host records, the majority were members of the order Tylenchida (with members of Heteroderidae, *Helicotylenchus* spp. and *Meloidogyne* spp. predominating), followed by Dorylaimida. Many of the nematode taxa are reported as hosts for *Pasteuria* for the first time. In almost half of the samples with *Pasteuria* several different nematode taxa were simultaneously attacked by the bacterial parasites. Differences in dimensions and other morphological characteristics of the sporangia and endospores observed in various host nematodes indicate that several *Pasteuria* species are present in Vietnam. Their role as antagonists of plant-parasitic and other soil-inhabiting nematodes is still unknown.

Key words: agriculture, bacteria, coast, forest, hosts, nematodes, parasites, *Pasteuria*, soil, Vietnam.

Endospore-forming and mycelial bacteria of the genus *Pasteuria* have gained increasing interest over the last 20 years because of their potential use for biocontrol of plant-parasitic nematodes. In addition to one *Pasteuria* species known from cladocerans (*P. ramosa*), four species have been described from plant-parasitic nematodes so far, *P. penetrans* from *Meloidogyne*, *P. thornei* from *Pratylenchus*, *P. nishizawae* from *Heterodera* and *P. usgae* from *Belonolaimus*. Most of the recent studies on *Pasteuria* have concentrated on *P. penetrans* from root-knot nematodes. As well as plant-parasitic nematodes, many other soil-inhabiting nematodes have been reported as hosts of *Pasteuria*. In the most recent compilation of *Pasteuria* hosts more than 300 nematode taxa are listed, the majority being members of the orders Tylenchida and Dorylaimida (Chen & Dickson, 1998). Most, if not all, *Pasteuria* species or isolates appear to be highly host specific with host ranges generally restricted to closely related nematode taxa. It is assumed that a high number of different *Pasteuria* species exists, many of which can be distinguished in morphological details and in dimensions of the sporangia and endospores.

Pasteuria is known to occur worldwide. It has been recorded from a large number of countries in all continents, often from individual nematode taxa, most commonly from root-knot nematodes and other plant-parasitic nematodes, but in general only rarely from other soil-inhabiting or freshwater nematodes. There are only a very few publications so far dealing with studies on frequency of occurrence in a country or region and on the range of host nematodes observed in particular samples, sites or areas.

In this paper, data on frequency of *Pasteuria* presence in Vietnam, based mainly on studies of nematode samples from natural forests and a few from agricultural and coastal sites, and a list of soil-inhabiting nematodes taxa found attacked by these bacteria are presented. There are no previous records of *Pasteuria* (formerly under the generic names *Duboscqia* and *Bacillus*) for Vietnam.

MATERIAL AND METHODS

Soil samples were collected in natural forests and at a few agricultural and coastal sites in different parts of Vietnam. Each sample consisted mainly of 3-4 cores taken from the upper 0-35 cm soil layer. Nematodes were extracted from soil samples by a sieving-decanting procedure combined with a filtering technique (Nguyen & Ngu-

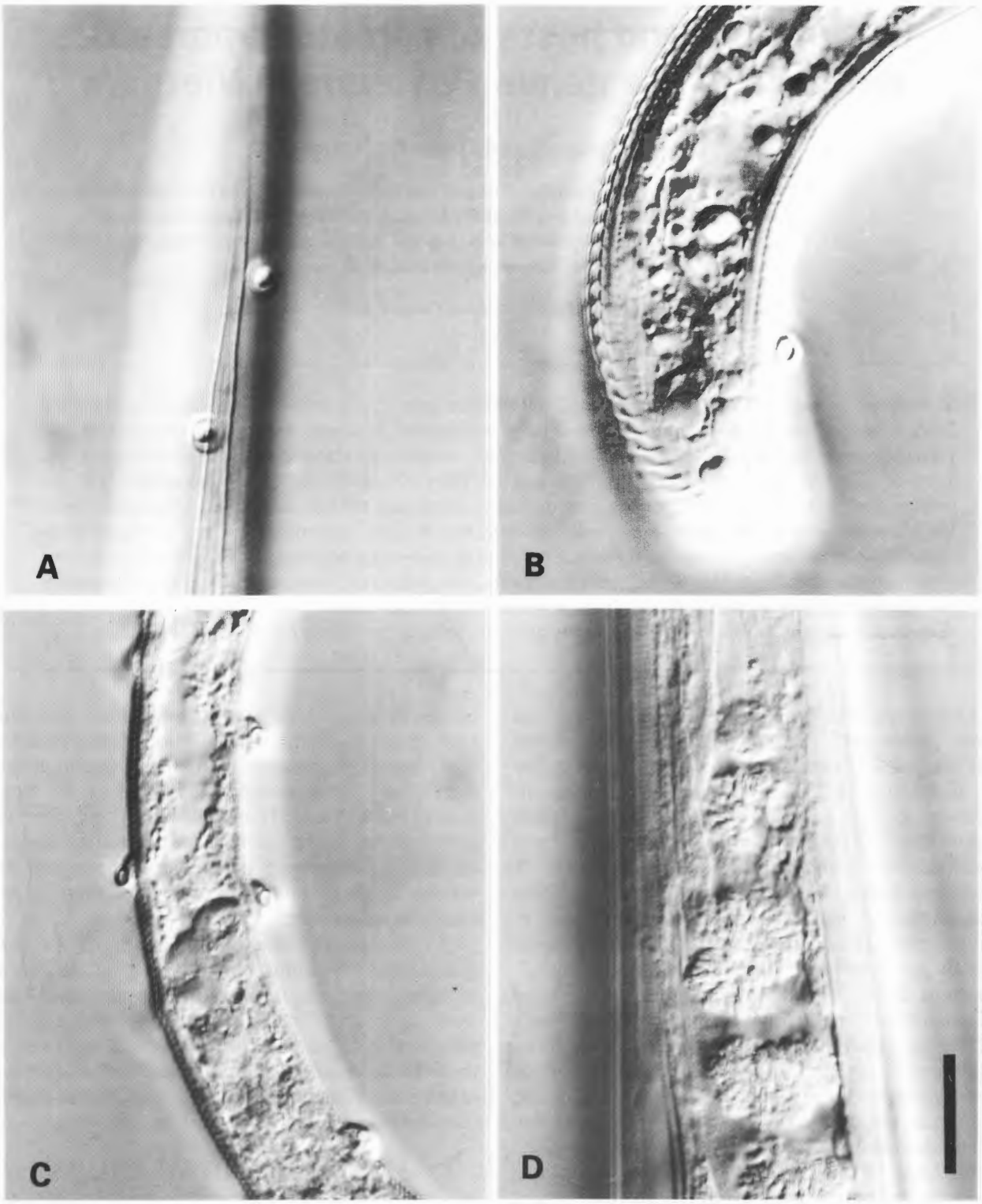

Fig. 1. A: Two *Pasteuria* endospores on anterior end of a *Meloidogyne* second-stage juvenile; B: Germinated endospore on posterior end of *Scutellonema brachyurum*; C: Germinated endospores on cuticle and mycelial cells in pseudocoelom of a *Helicotylenchus* juvenile; D: Mycelial colonies in the oesophagus region of *Carcharolaimus* sp. (Scale bar = 15 µm).

Table 1. Nematode hosts of *Pasteuria* in Vietnam.

Nematode	Number of samples	Nematode stage*	Presence of *Pasteuria***	Biotope
Order Tylenchida				
Basiria sp.	1	F, M	C, P	forest
Cephalenchus sp.	1	F, M	C, P	forest
Cryphodera spp.	14	J	C	forest
Cryphodera sp.	1	M, J	C	forest
Discocriconemella sp.	1	F	P	forest
Filenchus spp.	6	F, M, J	C, P	forest
Helicotylenchus dihystera	2	F	C	agriculture
Helicotylenchus erythrinae	1	F, M	P	forest
Helicotylenchus spp.	7	F, M, J	C, P	forest
Helicotylenchus spp.	8	F, M, J	C	forest
Heterodera sp.	3	J	C	forest
Heteroderidae gen. indet.	3	J	C	forest
Hirschmanniella mucronata	2	F, M, J	C	agriculture
Hirschmanniella oryzae	1	F	C, P	agriculture
Hirschmanniella shamini	1	F, M, J	C	agriculture
Meloidogyne spp.	5	J	C	agriculture
Meloidogyne spp.	12	J	C	forest
Meloidogyne spp.	1	J	C	coast
Pratylenchus dianthus	2	F	C	forest
Pratylenchus coffeae	2	F, M	P	forest
Pratylenchus sp.	2	F, J	C, P	forest
Pratylenchus sp.	1	F, M	C	forest
Rotylenchus sp.	1	J	C	forest
Scutellonema brachyurum	1	F	C, P	forest
Scutellonema paramonovi	1	F	C	forest
Sphaeronema sp.	1	J	C, P	forest
Trophonema sp.	1	J	C, P	forest
Tylenchorhynchus dalatensis	1	F, M	C, P	forest
Tylenchorhynchus leviterminalis	2	F, M, J	C	forest
Tylenchorhynchus sp.	2	F	C	forest
Tylenchulus sp.	3	J	C	forest
Order Dorylaimida				
Axonchium sp.	2	J	P	forest
Axonchium sp.	1	J	P	agriculture
Basirotyleptus sp.	2	F	P	forest
Belondira sp.	2	J	P	forest
Carcharolaimus sp.	1	J	P	forest
Dorella sp.	1	F	P	forest
Laimydorus sp.	1	F	P	forest
Leptonchidae gen. indet.	1	F	P	forest
Longidorella sp.	1	F	P	forest
Oriverutus sp.	1	J	C, P, I	agriculture
Xiphinema ensiculiferum	1	F	P	forest
Xiphinema insigne	1	F, J	P	forest
Xiphinema longicaudatum	1	F, J	P	forest
Xiphinema sp.	1	J	P	forest

Table 1. (continued). Nematode hosts of *Pasteuria* in Vietnam.

Order Enoplida				
Alaimus sp.	2	F, J	P	forest
Amphidelus sp.	1	F	P	forest
Amphidelus sp.	1	F, J	C, P	forest
Etamphidelus sp.	1	J	P	forest
Order Triplonchida				
Paratrichodorus paramirzai	1	J	P, I	forest
Tobrilus sp.	1	J	P, I	forest
Tripyla sp.	1	F	P, I	agriculture
Order Araeolaimida				
Aphanolaimus brasiliensis	1	F, J	P	forest
Aphanolaimus brasiliensis	1	F	C	forest
Aphanolaimus seshadrii	2	F, J	C, P	forest
Aphanolaimus sp.	4	F, J	P	forest

* F = female, M = male, J = juvenile. ** C = cuticle, P = pseudocoelom, I = intestine.

yen, 1993). Sieving was first through a screen with 0.5 mm openings to remove coarse particles, followed by filtering through a 200 mesh (75 µm openings) sieve to retain the sediment containing nematodes. After gentle washing, the sediment was transferred to special 75 mm diameter and 20 mm high sieves with 75 µm openings. Each of these sieves was placed in a Petri dish (90 mm diameter) and left for 48 h at room temperature. The nematodes migrating through the sieves were subsequently killed by gentle heat and fixed with TAF. From the nematode suspensions obtained, a total of 132 (119 from forests, 11 from agricultural sites and 2 from coastal sites) were microscopically checked for the presence of *Pasteuria*, with bacterial spores attached to the nematodes cuticle or with an internal infection. Permanent microscope slides were prepared from many of the attacked nematode specimens for subsequent detailed studies.

RESULTS

Pasteuria was found on or in nematodes at all sampling sites, in 74 of the 132 nematode suspensions checked (= 56% of all samples were positive for *Pasteuria*). The presence of spores attached to the nematode cuticle and/or internally was found in a wide range of nematode taxa (Table 1). Figures 1 and 2 show various stages of development in different nematodes: ungerminated endospores on the cuticle (Fig. 1A), germinated endospores (Fig. 1 B, C, Fig. 2C), germinal tube penetrating the nematodes cuticle and early mycelial cells in the pseudocoelom (Fig. 1C), large mycelial colonies (Fig. 1D), advanced sporogenesis stages and fully developed sporangia (Fig. 2A), and mature sporangia in the pseudocoelom or almost filling the body cavity of the nematodes (Fig. 2B-D). Species identification was possible only for rather few of the hosts, because of too little material available for identification, only juveniles present in the samples or presence of obviously still undescribed nematode species. The majority of *Pasteuria* records are for members of the order Tylenchida, followed by Dorylaimida and with only low numbers in other orders (Table 2). Among the Tylenchida, *Pasteuria* was most frequently seen in second-stage juveniles of heteroderids (*Cryphodera*, *Heterodera* and an unidentified genus), where more than one-third of all populations showed *Pasteuria* attack. In root-knot nematodes (*Meloidogyne* spp.) and in spiral-nematodes *Helicotylenchus* spp.), 30% of all samples of these nematodes were positive for *Pasteuria*. Attack by *Pasteuria* was not observed in members of, e.g., the order Rhabditida, which were generally rare in the nematode suspensions.

Pasteuria endopores were commonly found on the cuticle of females, males and juveniles in members of the order Tylenchida but only exceptionally seen attached to the cuticle of any dorylaim nematode, where generally various developmental stages of *Pasteuria* were observed in the pseudocoelom. It is remarkable that in the plant-parasitic nematode *Paratrichodorus paramirzai* fully developed *Pasteuria* endospores were seen also in the intestinal lumen (Table 2). *Pasteuria* endospores were also found in the intestine of the predacious nematode *Stenonchulus* sp. (not included into Table 1), which may have

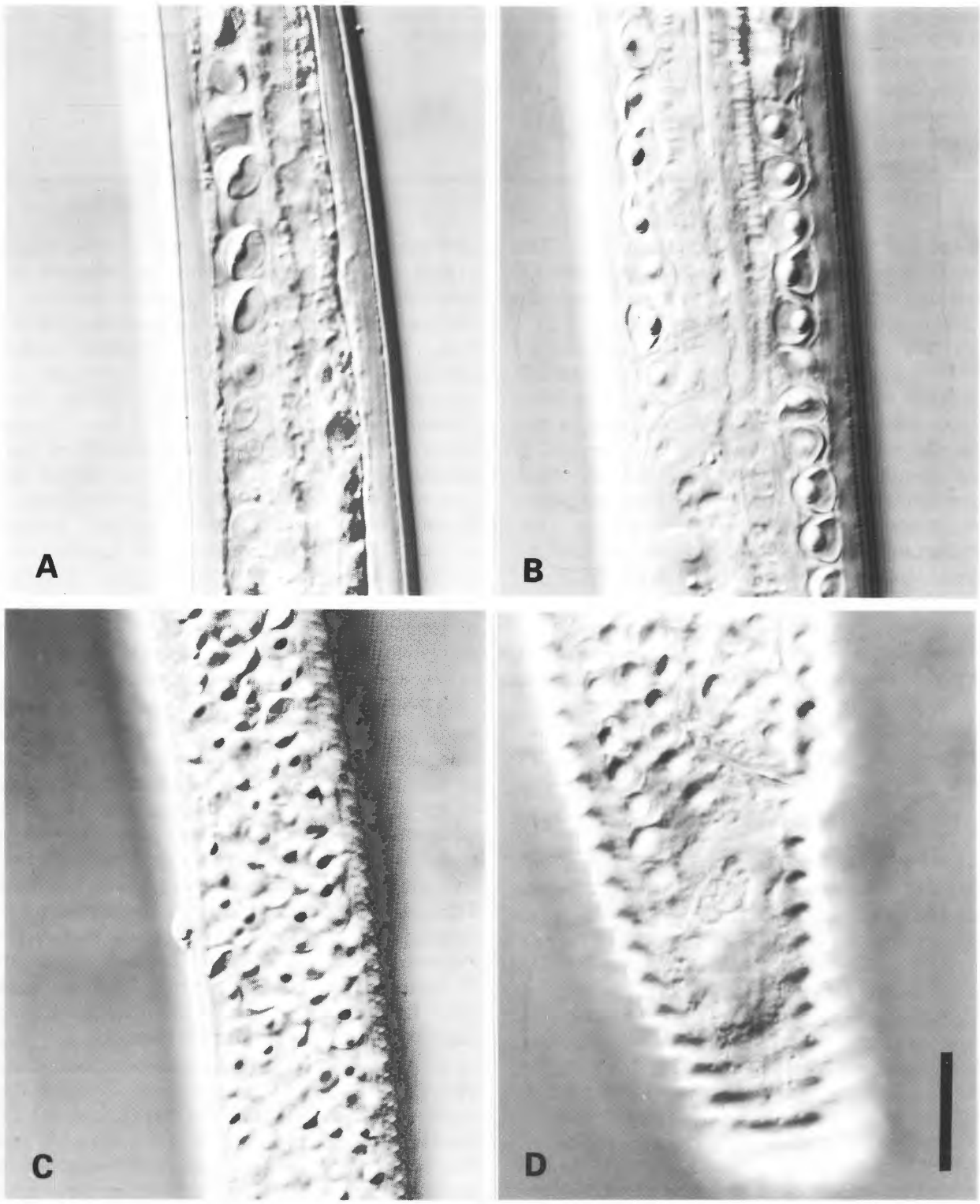

Fig. 2. A: Developing and mature *Pasteuria* sporangia in the oesophagus region of a *Xiphinema* juvenile; B: Mature sporangia in the oesophago-intestinal region of an *Axonchium* juvenile; C: Germinated endospore and mature sporangia in a *Hirschmanniella oryzae* female; D: Mature sporangia in posterior part of a *Discocriconemella* female. (Scale bar = 15 μm).

Table 2. Summarized records of *Pasteuria* hosts in Vietnam (n=123).

Nematode order	Number of records	Percentage of records
Tylenchida	90	73.1 %
Dorylaimida	17	13.8 %
Enoplida	5	2.2 %
Triplonchida	3	2.4 %
Araeolaimida	8	6.5 %

ingested the spores with the nematode prey. This may be true also for *Tobrilus*, *Tripyla* and the dorylaim *Oriverutus*, but these nematodes had also an infection of the pseudocoelom (Table 1).

In 45% of all nematode samples 'positive' for *Pasteuria*, the bacterial parasites were observed in more than one nematode species. In 15 of these samples *Pasteuria* occurred simultaneously in two nematode taxa, in nine samples in three and in three samples in four taxa. In the nematode suspensions with four taxa the following nematodes were attacked by *Pasteuria*: *Cryphodera* sp., *Helicotylenchus* sp., *Rotylenchus* sp. and *Axonchium* sp. in one sample, *Filenchus* sp., *Basirotyleptus* sp., *Paratrichodorus paramirzai* and *Amphidelus* sp. in the second sample, and *Tylenchorhynchus leviterminalis*, *Helicotylenchus* sp., *Heterodera* sp. and *Tylenchulus* sp. in the third sample. *Pasteuria* in the different hosts were, in most cases, distinguishable in dimensions and in other morphological details of the sporangia and the endospores.

DISCUSSION

The studies have shown that *Pasteuria* commonly occurs in Vietnam and that these nematode-parasitic bacteria are widely distributed in the country. Their actual incidence may even be higher than 56% of all samples or sites studied, because in many of the samples only low numbers of nematodes were present and so the chance of finding specimens attacked by *Pasteuria* is limited. Moreover, nematodes damaged or killed by the parasites cannot be isolated by the method used, in which the nematodes have to move through sieves.

There are only very few reports worldwide, where all nematode taxa isolated from soil samples have been checked for the presence of *Pasteuria* or studies on the presence of these parasites in particular countries or regions (Sturhan, 1985, 1988a; Subbotin *et al.*, 1994; Ciancio *et al.*, 1994; Barooti, 1989). Most studies were confined to plant-parasitic nematodes (Elekcioglu, 1995; Verdejo-Lucas *et al.*, 1997; and others) or even to certain genera such as *Meloidogyne* (Spaull, 1981; and others). Based on his own studies, Sturhan (1985) assumed an occurrence of *Pasteuria* at at least every tenth site in Germany and in the Azores, and at least in 5% of all soil samples from Iran. Subsequently, Sturhan (1988b) reported almost every fourth site studied in Germany to be positive for *Pasteuria*. Barooti (1989) found *Pasteuria* in 1.5% of all samples in Iran. Verdejo-Lucas *et al.* (1997) observed *Pasteuria* on plant-parasitic nematodes in 19 (=12%) of 160 soil samples from various crops in northeastern Spain. In South Africa, Spaull (1981) observed *Pasteuria* attack in 34% of the *Meloidogyne* populations in sugarcane fields. In Vietnam the rate of samples with *Pasteuria* in root-knot nematodes is expected to be higher than the observed 30%, because in many of the samples with *Meloidogyne* spp. only very few second-stage juveniles were present.

Among the nematode taxa on Table 1 several are reported as *Pasteuria* hosts for the first time (compared with the most recent host list compiled by Chen & Dickson, 1998). However, attachment of the endospores at the cuticle, does not necessarily mean that the spores germinate, cause an internal infection and develop to sporangia with endospores in the pseudocoelom of the nematode. Thus, nematode taxa with spores only seen on the cuticle may not all be considered as hosts of *Pasteuria*. *Pasteuria penetrans* in *Meloidogyne* and *P. nishizawae* in *Heterodera* spp., however, penetrate the nematodes cuticle only after the second-stage juveniles have invaded the host roots.

Our observations confirm previous observations that *Pasteuria* from different hosts mostly appear to be highly host-specific, with hosts confined to a species, a genus or closely related nematode taxa. The wide range of sporangia and endospore dimensions observed among *Pasteuria* on various nematode taxa and differences in certain morphological structures indicate that many different *Pasteuria* species are present in Vietnam. In particular, *Pasteuria* from Enoplida and Triplonchida hosts differed from the most common appearance of *Pasteuria penetrans* and the other species described from nematodes so far.

It is not known, which role *Pasteuria* spp. play in the nematode communities and the ecosystems in Vietnam, but it may be assumed that these parasitic bacteria are of importance as antagonists of nematodes. In particular, studies on *P. penetrans* have shown that the presence of these antagonists in soil and their application as biological control agents have considerable influence on populations of root-knot nematodes (for details see, e.g., Chen & Dickson, 1998).

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REFERENCES

Barooti, S. 1989. Distribution of *Pasteuria penetrans*, a parasite of nematodes in Iran. *Iranian Journal of Plant Pathology* 25: 9-10, 23-28.

Chen, Z.X. & Dickson, D.W. 1998. Review of *Pasteuria penetrans*: Biology, eology, and biological control potential. *Journal of Nematology* 30: 313-340.

Ciancio, A., Bonsignore, R., Vovlas, N. & Lamberti, F. 1994. Host records and spore morphometrics of *Pasteuria penetrans* group parasites of nematodes. *Journal of Invertebrate Pathology* 63: 260-267.

Elekcioglu, I.H. 1995. Occurrence of *Pasteuria* bacteria as parasites of plant-parasitic nematodes in the east Mediterranean region of Turkey. *Nematologia mediterranea* 23: 213-215.

Ko, M.P., Bernard, E.C., Schmitt, D.P. & Sipes, B.S. 1995. Occurrence of *Pasteuria*-like organisms on selected plant-parasitic nematodes in the Hawaiian Islands. *Journal of Nematology* 27: 395-408.

Nguyen, N.C. & Nguyen, V.T. 1993. Modified method for extraction of nematodes from soils and plant tissues. *Achievements of Science and Technology Applied into Practice*. National Center for Science and Technology 1: 41-45 (in Vietnamese).

Spaull, V.W. 1981. *Bacillus penetrans* in South African plant-parasitic nematodes. *Nematologica* 27: 244-245.

Sturhan, D. 1985. Untersuchungen über Verbreitung und Wirte des Nematodenparasiten *Bacillus penetrans*. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 226: 75-93.

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

Sturhan, D. 1988b. Bakterien der Gattung *Pasteuria* als Nematodenparasiten. *Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem* 245: 447-448.

Subbotin, S.A., Sturhan, D. & Ryss, A.Yu. 1994. Occurrence of nematode-parasitic bacteria of the genus *Pasteuria* in the former USSR. *Russian Journal of Nematology* 2: 61-64.

Verdejo-Lucas, S., Espanol, M., Ornat, C. & Sorribas, F.J. 1997. Occurrence of *Pasteuria* spp. in northeastern Spain. *Nematologia mediterranea* 25: 109-112.

Sturhan D., Nguyen N.C. Паразитирующие в нематодах бактерии рода *Pasteuria* во Вьетнаме: распространение и хозяева.

Резюме. Поражение бактериями рода *Pasteuria* отмечено для нематод в 74 пробах из 132 образцов почвы, собранных в лесах, а также в некоторых сельскохозяйственных угодьях и прибрежных участках Вьетнама. Среди 123 выявленных нематод-хозяев, большую часть составляют нематоды отряда Tylenchida (с доминированием различных Heteroderidae, а также *Helicotylenchus* spp. и *Meloidogyne* spp.). Второе место по числу поражений *Pasteuria* занимают Dorylaimida. Для многих таксонов нематод поражение *Pasteuria* отмечено впервые. Почти в половине проб, содержащих зараженных нематод, поражение *Pasteuria* отмечено одновременно у нематод нескольких различных таксонов. Различия в размерных и морфологических особенностях спорангиев и эндоспор, выявленных у изученных нематод, указывает на присутствие нескольких видов *Pasteuria* во Вьетнаме. Значение этих бактерий, как антагонистов фитопаразитических и почвенных нематод все еще не определена.