

PASTEURIA PENETRANS INFECTING MELOIDOGYNE SPP. IN EGGPLANT IN EGYPT

R.A. Bakr¹, M.E. Mahdy and E.M. Mousa

Agricultural Botany Department, Faculty of Agriculture, Minufiya University, Shibin El-Kom, Egypt

Summary. A survey was undertaken in three different governorates of the newly reclaimed lands in Egypt, cultivated with different vegetable crops, to detect the presence of the endoparasitic bacterium *Pasteuria penetrans* on root-knot nematodes, *Meloidogyne* spp. Root and soil samples were collected in infected fields from the Shibin El-Kom and El-Sadat (El-Minufiya), El-Tahrir and Kom Hamada (El-Behira), and El-Salhiya (El-Sharkiya) regions. *Pasteuria penetrans* was detected on second-stage juveniles and females of *Meloidogyne javanica*, from eggplant, only in Kom Hamada, El-Behira governorate. Studies with the transmission electron microscope revealed that diameter and height of the endospores were 3.35 μm and 1.42 μm , respectively.

Key words: Biocontrol agent, endoparasitic bacteria, *Meloidogyne javanica*, root-knot nematodes.

In Egypt, root-knot nematodes, *Meloidogyne* spp., cause economic damage to many crop plants and are considered as a limiting factor for crop production, especially in the newly reclaimed lands. These lands have been reclaimed from the Egyptian deserts in the last decades, are cultivated with fruit trees and vegetables and are irrigated with river Nile water or under ground water. *Pasteuria penetrans* (ex Thorne, 1940) Sayre *et* Starr, 1985 is a gram-positive, mycelial, endospore-forming bacterium with septate mycelium, and is an obligate parasite bacterium on plant parasitic nematodes (Mankau and Imbriani, 1975). It is one of the more promising biocontrol agents for suppressing field populations of several economic plant parasitic nematodes throughout the world, especially *Meloidogyne* spp., on different host plants (Chen and Dickson, 1998; Mahdy, 2002; Mousa and Mahdy, 2006; Mousa *et al.*, 2008; Chaudhary and Kaul, 2010). The host preference of *P. penetrans* appears to be related to nematode populations rather than to nematode species (Stirling, 1985). *Pasteuria penetrans* is known to attach differentially to second-stage juveniles (J_2) from the same field (Davies *et al.*, 1991). It is widely distributed in agricultural soil and has been found attached to plant parasitic nematodes in many countries (Sayre and Starr, 1988; Sturhan, 1988; Chen and Dickson, 1998). In Egypt, the only report on this endoparasite is that of El-Saedy and Mokbel (2007) who obtained two isolates of *P. penetrans* (*Pp*), parasitizing J_2 of *Meloidogyne* spp., from infected banana roots at El-Behira governorate and grapevine roots at Kafer EL-Sheikh governorate.

Therefore, a survey was undertaken to ascertain the presence of the endoparasitic bacterium *P. penetrans* as-

sociated with root-knot nematodes in three different governorates of the newly reclaimed lands in Egypt.

The survey was conducted in field crops infested with root-knot nematodes in Shibin El-Kom and El-Sadat (El-Minufiya), El-Tahrir and Kom Hamada (El-Behira), and El-Salhiya (El-Sharkiya) regions. Root and soil samples were collected from tomato (*Solanum lycopersicum* L.), cucumber (*Cucumis sativus* L.), eggplant (*Solanum melongena* L.), pepper (*Capsicum annuum* L.) and watermelon (*Citrullus vulgaris* Schrad) infected by *Meloidogyne* spp. The predominant species in these fields were *M. incognita* (Kofoid *et* White) Chtw. and *M. javanica* (Treub) Chitw., and the females most infected by *P. penetrans* were of *M. javanica* as identified by observation of perineal patterns according to Hartman and Sasser (1985).

Soil samples. Fifty samples, each composed of five sub-samples, were collected from the rhizosphere of the plants to a depth of 15-30 cm, using a garden trowel. The five sub-samples were then carefully mixed, kept in a polyethylene labelled bag, sealed and brought to the laboratory for nematode extraction. Two hundred and fifty grams of each soil sample were used to extract nematodes with the sieving and modified Baermann tray technique according to Southey (1986). After 72 hours, the extracted J_2 of *Meloidogyne* spp. were examined for the presence of *P. penetrans* spores adhering to the nematode cuticle under a microscope at 400 \times magnification.

Root samples. The infected root systems were removed carefully from the soil with a trowel, collected in plastic bags and labeled. A part of each root sample was examined directly and 20 adult females of *Meloidogyne* spp. were dissected from the galls. They were observed for the presence of *P. penetrans*. Females of *Meloidogyne* spp. infected with *P. penetrans* were distinguished by their opaque dull creamy white to amber colour com-

¹ Corresponding author: ramadanbaker82@yahoo.com

pared to glistening white healthy females, as described by Mankau and Imbriani (1975). The other part of the infected roots was soaked in tap water in glass beakers for 3-4 days or until the roots became softened (Ratnasoma and Gowen, 1996). Adult females of *Meloidogyne* spp. were then separated from the softened roots, by washing under a vigorous jet of tap water onto 500 μm and 250 μm sieves, collected in distilled water in a small flask and then examined for their infection by *P. penetrans*. Mature females were crushed in a little drop of water on a glass slide, covered with a cover slip and then examined for the presence of *P. penetrans* endospores or other stages of the life cycle under a microscope at 400 \times magnification.

Image analysis and studies with transmission electron microscope at 15000 \times , 20000 \times and 30000 \times magnification of mature *P. penetrans* spores from infected adult females of *M. javanica* were also done at the Regional Center for Mycology and Biotechnology, Al-Azhar University, Nasser city, Cairo, Egypt.

Pasteuria penetrans was detected in J₂ and mature females of the root-knot nematodes, in soil or root sam-

ples, respectively, only in the Kom Hamada region, El-Behira governorate. *Pasteuria penetrans* was found in females and adhering to the cuticle of J₂ of *M. javanica* on infected eggplant (Figs 1A, B).

The studies with the transmission electron microscope showed that diameter and height of the endospores were 3.35 μm and 1.42 μm , respectively (average of 19 spores) (Figs 2 and 3). Spore shape and size of this isolate of *P. penetrans* are similar to those reported by Sayre and Starr (1985) infecting root-knot nematodes. The low occurrence of *P. penetrans* in the selected area, on vegetable crops, may be due to the use of pesticides and herbicides, which are used frequently in these fields and may be the cause of low natural infection in these locations (Verdejo-Lucas *et al.*, 1997). The sandy soil texture of the examined location may also have contributed to the migration of the bacterium spores into deeper soil layers following irrigation (Shahid *et al.*, 2010). The attachment rates may be variable due to the specificity of *P. penetrans* to different *Meloidogyne* populations of the same species, within a country or even within the same area (Tzortzakakis, 2008).

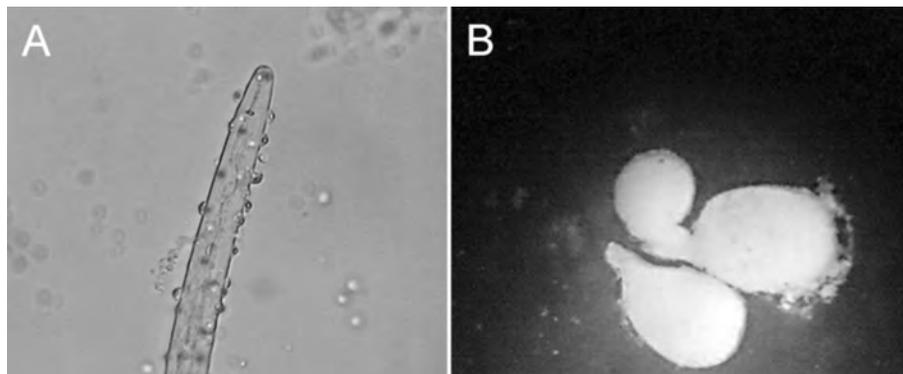


Fig. 1. A: Spores of the detected Egyptian isolate of *Pasteuria penetrans* attached to a second-stage juvenile of *Meloidogyne* sp.; B: Females of *Meloidogyne* sp. infected with the Egyptian isolate of *P. penetrans*.

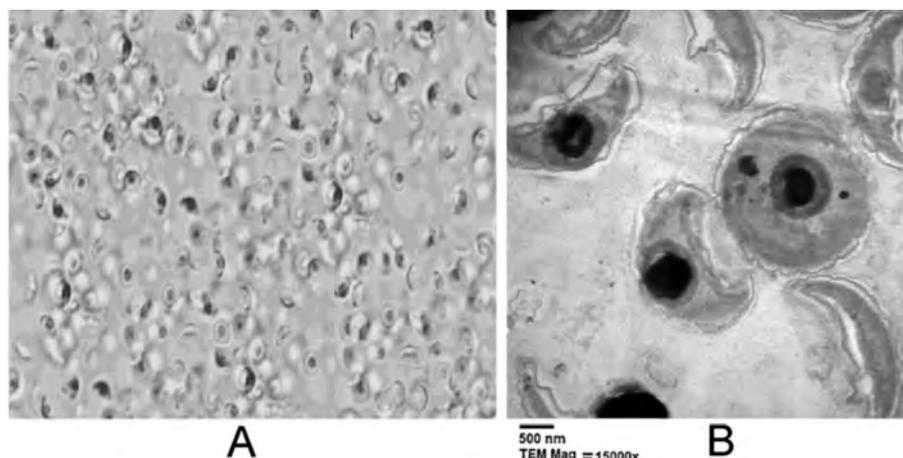


Fig. 2. Spores of the Egyptian isolate of *P. penetrans*. A: Under light microscope at 400 \times with Image Analysis System; B: Different shape of the spores at 15000 \times under Transmission Electron Microscope (Scale bar = 500 nm).

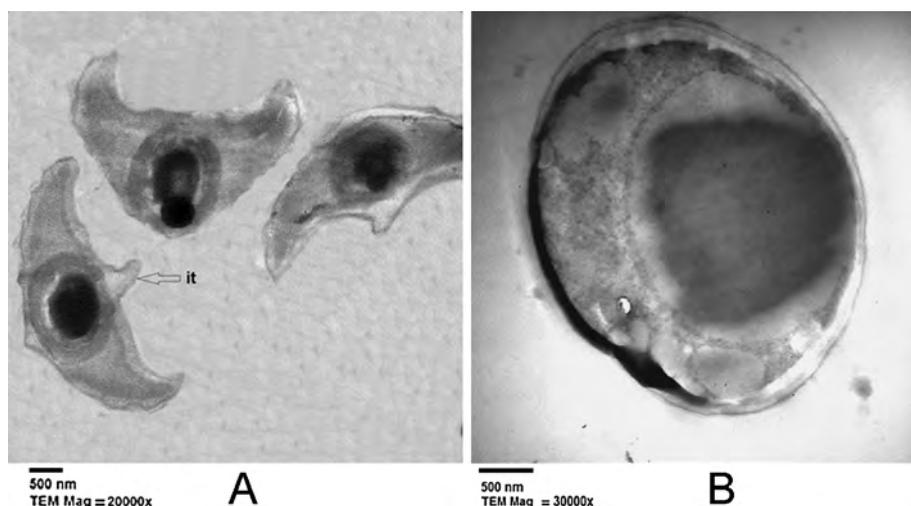


Fig. 3. Spores of the detected Egyptian isolate of *P. penetrans* (Pp EGY) under Transmission Electron Microscope. A: Showing the infection tube (it) at 20000 \times ; B: One spore at the magnification 30000 \times . Scale bars = 500 nm.

Our survey broadens information on the presence of the endoparasitic bacterium *P. penetrans* in Egypt, previously reported by El-Saedey and Mokbel (2007). Further investigations should be undertaken to explore the feasibility of using this local isolate of the bacterium for nematode management under field conditions.

LITERATURE CITED

- Chaudhary K.K. and Kaul R.K., 2010. Effect of the time of application on the biocontrol efficacy of *Pasteuria penetrans* against root-knot nematode *Meloidogyne incognita*. *Journal of Phytopathology*, 2/7: 70-74.
- Chen Z.X. and Dickson D.W., 1998. Review of *Pasteuria penetrans*: biology, ecology and biological control potential. *Journal of Nematology*, 31: 313-340.
- Davies K.G., De Leij F. and Kerry B.R., 1991. Microbial agents for the biological control of plant parasitic nematodes in tropical agriculture. *Tropical Pest Management*, 37: 303-320.
- El-Saedey M.A.M. and Mokbel Asmaa A., 2007. Control of the root-knot nematode, *Meloidogyne incognita* using two Egyptian isolates of *Pasteuria penetrans*. *Egyptian Journal of Phytopathology*, 35(2): 125-139.
- Hartman K.M. and Sasser J.N., 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. Pp. 69-77. In: *An Advanced Treatise on Meloidogyne - Vol. II Methodology* (Barker K.R. and Carter C.C. eds). North Carolina State University Graphics, Raleigh, USA.
- Mahdy M.E., 2002. Biological control of plant parasitic nematodes with antagonistic bacteria on different host plants. Ph.D. dissertation, Bonn University, Bonn, Germany, 182 pp.
- Mankau R. and Imbriani J.L., 1975. The life cycle of an endoparasite in some tylenchid nematodes. *Nematologica*, 21: 89-94.
- Mousa E.M. and Mahdy M.E., 2006. Differential rate in spore attachment of seven isolates of *Pasteuria penetrans* on the root-knot nematode *Meloidogyne javanica*. *Journal of Nematology*, 38: 284 (Abstract).
- Mousa E.M., Mahdy M.E. and Bakr R.A., 2008. *Pasteuria penetrans* as a biological control agent of root-knot nematodes in Egypt. 5th International Congress of Nematology, 13-18 July 2008, Brisbane, Queensland, Australia, p. 78 (Abstract).
- Ratnasoma H.A. and Gowen S.R., 1996. Spore attachment of *Pasteuria penetrans* on juveniles of *Meloidogyne incognita* as affected by pH and organic matter. *Nematologia Mediterranea*, 24: 283-285.
- Sayre R.M. and 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. *Proceedings of the Helminthological Society of Washington*, 52: 149-165.
- Sayre R.M. and Starr M.P., 1988. Bacterial diseases and antagonism of nematodes. Pp. 69-101. In: *Diseases of Nematodes* (Poinar G.O. and Jansson H.B., eds). CRC Press, Boca Raton, Florida, USA.
- Shahid M., Rehman A.U. and Javed N., 2010. Occurrence of *Pasteuria penetrans* in the fields of vegetable crops in Punjab, Pakistan. *Pakistan Journal Nematology*, 28: 329-333.
- Southey J.F., 1986. *Laboratory methods for work with plant and soil nematodes*. Ministry of Agriculture, Fisheries and Food, HMSO, London, UK, 202 pp.
- Stirling G.R., 1985. Host specificity of *Pasteuria penetrans* within the genus *Meloidogyne*. *Nematologica*, 31: 203-209.
- Sturhan D., 1988. New host and geographic records of nematode parasitic bacteria of the *Pasteuria penetrans* group. *Nematologica*, 34: 350-356.
- Tzortzakakis E.A., 2008. Attachment of *Pasteuria penetrans* spores on populations of *Meloidogyne javanica* and *M. incognita* virulent and avirulent on the Mi gene of resistant tomato. *Helminthologia*, 45: 54-56.

