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Pasteuria penetrans and *P. nishizawae* attachment to *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla*

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Summary – This is the first report of endospores of *Pasteuria penetrans* and *P. nishizawae* isolates binding to juveniles of *Meloidogyne chitwoodi* and *M. fallax*. The patterns of endospore attachment to *M. chitwoodi* and *M. fallax* compared with the related *M. hapla* suggest that there are differences amongst these three temperate root-knot nematode species. Intraspecific variability in attachment of *P. penetrans* to juveniles of *M. chitwoodi* was demonstrated.

Keywords – bacterial parasite, biocontrol, spore attachment.

Meloidogyne chitwoodi and the closely related *M. fallax* are major pests of potato and are quarantine organisms in Europe. These nematodes and a third *Meloidogyne* species, *M. hapla*, are thought to be responsible for increased damage to a wide range of crops following decreased use of soil pesticides (Molendijk & Mulder, 1996).

Pasteuria species are mycelial, endospore-forming bacteria which parasitise phytoparasitic nematodes. Non-motile spores in the soil become attached to the cuticle of susceptible second-stage (J2) nematodes and, although in excess of 100 spores may become attached, one spore is sufficient to infect a nematode (Chen & Dickson, 1998). Three species of *Pasteuria* that parasitise plant-parasitic nematodes have been described: *P. penetrans*, on *Meloidogyne* species, *P. thornei*, on *Pratylenchus* spp., and *P. nishizawae* on *Heterodera* spp. and *Globodera* spp. (Sayre & Starr, 1989). Endospores of *P. penetrans* have not been found adhering to animal-parasitic or free-living nematodes (Mendoza de Gives *et al.*, 1999). However, variability in the specificity of attachment is clear as certain *Pasteuria* isolates can adhere to nematodes from a wide range of different genera (Sharma & Davies, 1996) while others are more host-specific (Davies & Danks, 1992; Kaplan, 1994). It is not known if development of the bacterium is successful in all cases (Hewlett & Dickson, 1994). These differences in attachment and penetration indicate complex interactions between the dynamic cuticle of the nematode and the surface of the bacterial parasite, and observations of patterns of *Pasteuria* attachment provide evidence for nematode cuticle diversity.

Pasteuria isolates have been reported on *M. hapla* in China, Japan, USA and Spain but there have been no reports of *Pasteuria* on either *M. chitwoodi* or *M. fallax* (Chen & Dickson, 1998). In this study, the attachment of *Pasteuria* spores from isolates from different geographical origins was assessed on a range of populations of *M. chitwoodi* and *M. fallax*. We also compared percentage attachment of spores between *M. chitwoodi*, *M. fallax*, *M. hapla* and *M. incognita.*

Materials and methods

The *Pasteuria* isolates were maintained as described by Stirling and Wachtel (1980) and those used are listed in Table 1. The nematode cultures (Table 2) had been maintained on susceptible tomato plants (cv. Money Maker) in glasshouse cultures. Juveniles were collected as described by Wishart *et al.* (2002). The species homogeneity of all nematode populations was tested using PCR (Wishart *et al.*, 2002) and mixed populations were eliminated from the study.

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Table 1. Pasteuria *isolates used in the attachment assays.*

Pasteuria isolate	Nematode host	Geographical origin	Source
P.p.1	*Meloidogyne javanica*	California, USA	S.R. Gowen[a]
P.p.A	*M. arenaria*	Beltsville, USA	R.M. Sayre[b]
P.p. Tusell	*M. hapla*	Spain	S. Verdejo-Lucas[c]
P.p. Senegal	*M. javanica*	Senegal	T. Mateille[d]
P. nishizawae	*Heterodera glycines*	Japan	T. Nishizawa[e]

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[b] USDA, Barc-west Bldg 011A, 10300 Baltimore Ave., Beltsville, MD 20705, USA.
[c] IRTA, Departamento de Protección Vegetal, Crta de Cabrils s/n, 08348 Cabrils, Barcelona, Spain.
[d] UMR 022 – CBGP (Centre de Biologie et de Gestion des Populations), Campus, International de Baillarguet CS 30 016,34988 Montferrier-sur-Lez Cedex, France.
[e] National Institute Agro-Environmental Sciences, Nematology Laboratory, Tsukuba, Ibaraki 305, Japan.

Table 2. *Populations of nematodes used in the attachment assays.*

Meloidogyne species	[a]Line/ Population	Country of origin
M. chitwoodi (race unknown)	Y1	from potato imported into the UK
	Y2	from potato imported into the UK
M. chitwoodi race 1	Ca	The Netherlands
	Carg	Argentina
	Ck	The Netherlands
	Cl	The Netherlands
	Cy	The Netherlands
M. chitwoodi race 2	Cbd	Washington, USA
	Cbf	Oregon, USA
M. chitwoodi race 3	Cbh	California, USA
M. fallax	Fa	The Netherlands
	Paimpol	France
M. hapla	Cyprus	Cyprus
M. incognita	Race 2	USA

[a] *M. chitwoodi* populations Y1 and Y2 were obtained from Dr Sue Hockland, CSL, York, UK from intercepted potato; *M. fallax* population Paimpol from Dr Didier Mugniéry, INRA, BP 35327, 35653 Le Rheu Cedex, France; the Cyprus population from Dr Emmanuel Tzortzakakis, National Agricultural Research Foundation, P.O. Box 2228, 71003, Heraklion, Crete, Greece.
All other *M. chitwoodi, M. fallax* isolates were provided by Dr Carolein Zijlstra, Leo Poleij and Dr Hans van der Beek, Plant Research International, 6700 AA Wageningen, The Netherlands: *M. incognita* from the SCRI collection.

Pasteuria assays

Spores of *Pasteuria* in 200 μl at 10^6 spores/ml were added to a silane-coated 1.5 ml tube and 200 μl of nematodes in suspension (1000 J2/ml) added and the mixture sedimented at 10 000 *g* for 3 min. The supernatant was removed and the pellet re-suspended in 100 μl sterile distilled water, placed on a glass slide, covered with a coverslip and observed with a microscope at $\times$400 magnification (Hewlett & Dickson, 1994). An average of 22 nematodes per treatment combination (ranging from ten to 45) were observed and the number of J2 with spores attached to the cuticle was counted. Spores of five isolates of *Pasteuria* (Table 1) were incubated with nematodes from ten populations of *M. chitwoodi*, two populations of *M. fallax*, one of *M. hapla* and one of *M. incognita* (Table 2).

Statistical analysis

Where *Pasteuria* spores showed attachment to nematodes in most *Pasteuria* $\times$ nematode populations combinations, the number of spores observed attaching was <10 with only three observations where it was >10. Consequently, all spore attachments were incorporated into one category of >0 and a value for percentage of nematodes with spores attached was obtained (Davies & Danks, 1992). A Generalised Linear Model (GLM) was used to perform an analysis of deviance using Genstat 6.1 (Payne *et al.*, 2002). As populations Y2 and Cbd had no attachment by any isolate of *Pasteuria* they were not included in the analysis.

Results

Spores of each *Pasteuria* isolate tested attached to J2 belonging to at least one *M. chitwoodi* population. The

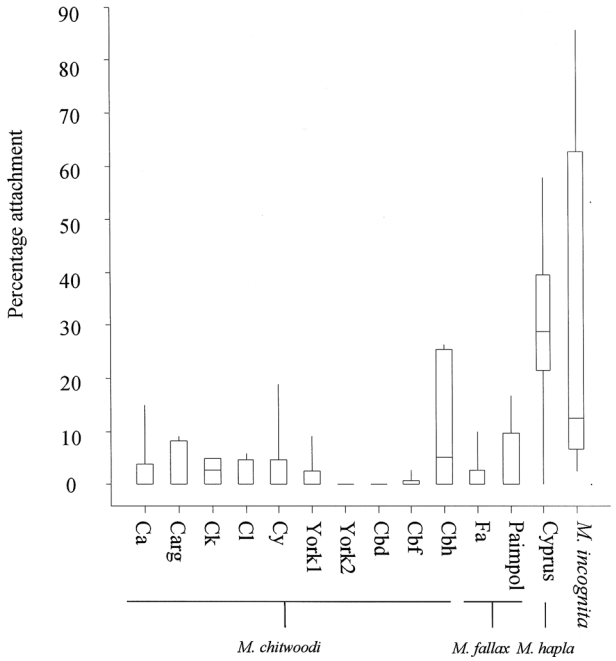

Fig. 1. *A box plot of the percentage attachment of spores of* Pasteuria *isolates to populations of* Meloidogyne *species. The box spans the interquartile range of the values, so that the middle 50% of the data lie within the box, with a horizontal line indicating the median. Vertical lines indicate the minimum and maximum values.*

percentage of J2 with spore attachment is shown in Figure 1. Of all the isolates, *P. nishizawae* (isolated from cyst nematodes), showed the closest compatibility with *M. chitwoodi* and *M. fallax* attaching to the J2 of half the populations of *M. chitwoodi* and both *M. fallax* populations. One isolate of *P. penetrans* (Tussell) showed attachment (60.7%) to the J2 of the *M. hapla* population (Cyprus) but <7% attachment to the J2 of four *M. chitwoodi* populations, one *M. fallax* and the *M. incognita* population. The *Pasteuria* isolate from Senegal had the lowest compatibility of all the isolates. All five *Pasteuria* isolates showed attachment to J2 of *M. incognita* (2.4-86%). Three interactions involved >10 spores attached per J2; *M. hapla* to P.p. Tussell (7.69%), *M. incognita* to P.p.A (50%), *M. chitwoodi* (Carg) to P.p.1 (4.5%).

The analysis of deviance showed that the major source of variation was the degree of attachment to the J2 in the different populations of *Meloidogyne*. There were also significant differences between the *Pasteuria* isolates and a small but significant interaction between *Pasteuria* isolate and nematode population. The higher levels and greater range of attachment found on the J2 of the *M. hapla* and *M. incognita* populations compared to most of the *M. chitwoodi* and *M. fallax* populations are illustrated in Figure 1.

The proportion of nematodes with attachment of *Pasteuria* spores to most of the *M. chitwoodi* and *M. fallax* populations was relatively small. With the exception of *P. nishizawae* spores binding to J2 of both *M. fallax* populations tested (10 and 17% attachment) and *M. chitwoodi* (Ca and Cbh) and P.p.1 spores to juveniles of Cy (19%) and Cbh (25%) *M. chitwoodi* populations, the proportion of attachment was less than 10%. No attachment was observed in 36/50 of the *Pasteuria*/nematode combinations.

Discussion

Previous studies have shown attachment of *Pasteuria* spores to juveniles of *M. hapla* and tropical *Meloidogyne* species but this is the first published report of any *Pasteuria* spores binding to *M. chitwoodi* or *M. fallax* (Chen & Dickson, 1998).

Spores of *P. penetrans* Tusell have been reported to attach specifically to *M. hapla* J2 (Espanol *et al.*, 1997) and not at all to juveniles of *M. incognita*, *M. arenaria* or *M. javanica*. In this study, *P. penetrans* Tusell spores showed the greatest levels of attachment to *M. hapla* confirming the species specificity found in previous studies (Kaplan, 1994). The 85.7% attachment of P.p.A spores to *M. incognita* and 12.5% attachment of P.p.1 to *M. incognita* juveniles were comparable the data obtained by Espanol *et al.* (1997), suggesting that this type of attachment test is reproducible. However, Mendoza de Gives *et al.* (1999) reported no attachment of *P. nishizawae* spores to *M. incognita* or *M. hapla* J2. This was in contrast to the present study, in which *P. nishizawae* spores attached to *M. hapla* (28.6%) and *M. incognita* (55%). Attachment of spores isolated from root-knot nematodes has previously been reported to be restricted to root-knot nematodes, whereas those isolated from cyst nematodes were observed to have a broader range of hosts (Mendoza de Gives *et al.*, 1999). Similar observations were made in this study where *P. nishizawae* (isolated from *H. glycines*) spores attached to J2 from more of the populations than other *Pasteuria* isolates (Fig. 1).

Meloidogyne chitwoodi, *M. fallax* and *M. hapla* (apart from *M. hapla* race B which was not tested in this study) reproduce by meiotic parthenogenesis and, therefore, may be expected to generate greater variability of surface coat than tropical, mitotic parthenogenetic *Meloidogyne* species (Davies *et al.*, 2001). The results of the attachment

assays revealed statistically significant differences among *M. chitwoodi* populations although only one *M. chitwoodi* population, Cbh, showed levels of attachment greater than 25%.

Also, *P. penetrans* isolates varied in the percentage attachment of spores to J2 within different populations. For example, spores of the isolate P.p.A had the greatest degree of attachment to *M. incognita* J2 but showed little or no attachment to J2 of the *M. chitwoodi* or *M. fallax* populations. Davies *et al.* (2001) found only two spore populations out of 25 attaching in the same way to the species tested (*M. incognita*, *M. javanica*, *M. arenaria*, *M. mayaguensis*, *M. hispanica* and *M. hapla*).

Pasteuria penetrans has potential as a biological control agent (Davies & Danks, 1992). This may be particularly so if isolates could be found that attach to *M. chitwoodi* populations such as Cbh and Cbd which have virulence to the major resistance gene(s) found in the wild potato *Solanum bulbocastanum* (Mojtahedi & Santo, 1994; Van der Beek *et al.*, 1999).

Although *Pasteuria* has not yet been obtained from field isolates of *M. chitwoodi* or *M. fallax* (Chen *et al.*, 1998) this study indicates that spores of *Pasteuria* do bind to their cuticles and the varying percentage attachment suggests the presence of different surface epitopes.

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