Attachment tests of *Pasteuria penetrans* to the cuticle of plant and animal parasitic nematodes, free living nematodes and *srf* mutants of *Caenorhabditis elegans*

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**Abstract**

Populations of *Pasteuria penetrans* isolated from root-knot nematodes (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp.) were tested for their ability to adhere to a limited selection of sheathed and exsheathed animal parasitic nematodes, free living nematodes, including *Caenorhabditis elegans* wild type and several *srf* mutants, and plant parasitic nematodes. The attachment of spores of *Pasteuria* was restricted and no spores were observed adhering to any of the animal parasitic nematodes either with or without their sheath or to any of the free living nematodes including *C. elegans* and the *srf* mutants. All spore attachment was restricted to plant parasitic nematodes; however, spores isolated from cyst nematodes showed the ability to adhere to other genera of plant parasitic nematodes which was not the case with spores isolated from root-knot nematodes. The results are discussed in relationship to cuticular heterogeneity.

**Introduction**

The control of human and animal nematode infections is largely based on the administration of anthelmintic drugs. However, in response to the intensive use of anthelmintics, resistance has been reported (Waller, 1990; Jackson, 1993; De Clercq *et al.*, 1997; Reynoldson *et al.*, 1998) and this has led to a search for alternative strategies.

Nematophagous fungi are currently being evaluated for their potential to control plant parasitic (Kerry, 1993; Kerry & Bourne, 1996) and animal parasitic nematodes (Waller, 1993, Mendoza de Gives *et al.*, 1994; Wolstrup *et al.*, 1996; Morgan *et al.*, 1997; Llerandi-Juarez & Mendoza de Gives, 1998). However, to date, there are very few reports evaluating the use of bacteria to control these animal parasites. The *Pasteuria* group of Gram-positive endospore-forming bacteria are parasites of nematodes and water fleas (*Daphnia* spp.). Certain basic morphological types of spore, each with a variety of sub-types, have been identified and are found almost exclusively in Tylenchida and Dorylaimida; however, others have recently been found associated with Araeolaimida, Chromadorida, and Enoplida
Attachment of spores, of selected
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are available which differ solely in the surface character-
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represents the latter catagory where mutants
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Caenorhabditis
et al.,
and nematode cuticle (Davies
srf
nematodes and surface (sheath) and free living nematodes, to plant parasitic
different species of animal parasitic (with and without
proliferate throughout the pseudocoelom eventually
produce a germ tube which penetrates the nematode
spores have
germinated and penetrate the second-stage juvenile before the nematode has infected a plant
root, as in the case of spores adhering to the cuticle of
Heterodera avenae
(Sayre & Starr, 1985), or after
the nematode has infected a plant root and started feeding
(Sayre & Starr, 1985, 1988). In both cases, the spores each
produce a germ tube which penetrates the nematode
cuticle and produces a dichotomously branched micro-
colony. These microcolonies subsequently divide and
proliferate throughout the pseudocoelom eventually
killing the nematode and producing a cadaver filled with
spores (Sayre & Starr, 1988). Pasteuria spores have been
shown to differ in their ability to adhere to the
cuticle of plant parasitic nematodes (Davies et al., 1988; Stirling, 1991) and the interaction between the nematode
and Pasteuria is thought to involve a protein/carbo-
hydrate like mechanism between the spore and the
nematode cuticle (Davies et al., 1994). There are no reports
of isolates of the bacteria being tested against either
animal parasitic or free living nematodes, and Caenorhabditis
elegans represents the latter catagory where mutants
are available which differ solely in the surface character-
istics of their cuticle.

This paper reports the results of tests to study the
attachment of spores, of selected Pasteuria isolates, to
different species of animal parasitic (with and without
sheath) and free living nematodes, to plant parasitic
nematodes and surface (srf) mutants of Caenorhabditis
elegans, the cuticles of which react differently to either
antibodies and/or lectins.

Material and methods
Nematodes

The plant-parasitic nematodes Meloidogyne spp. and
Rotylenchulus reniformis were obtained from plant cultures
maintained in the glasshouse at 25°C on tomato plants,
cv. Pixie, grown in a peat/sand (1:1, v/v) compost.
Pratylenchus spp. and Radopholus similis were obtained
from axenic maize root cultures (Hooper, 1986b). Nema-
todes were hatched from infected root material by placing
small samples of infected root material in tap water on a
small sieve in a tray of water at room temperature
(Hooper, 1986a). The juveniles of cyst forming nematodes
were obtained by incubating cysts at optimum tempera-
tures in tap water and, in the case of the two species of
cyst nematode, Globodera pallida and G. rostochien-
sis, in the presence of potato root diffusate. Aphelenchoides
sp. and Ditylenchus sp were obtained from axenic Petri
dish cultures of Botrytis cinerea maintained at room
temperature in the laboratory by washing the surface of
the agar with water (Hooper, 1986b). Samples of Anguina
tritici were obtained by breaking open infected grains of
wheat in a small drop of tap water to release the
nematodes (Hooper, 1986a).

Cultures of the animal parasitic nematodes Haemonchus
contortus, Ostertagia (Teladorsagia) circumcincta and
Trichostrongylus axei were provided by Drs E. Munn,
Babraham Institute, Cambridge, and R. Coop, The
Moredun Research Institute, Edinburgh. Ancylostoma
ceylanicum and Heligmosomoides polygyrus were obtained
from the faecal material of infected hamsters and mice
respectively (Garside & Behnke, 1989). Steinernema and
Heterorhabditis were cultured in Galleria larvae. The free-
living nematodes Panagrellus redivivus, Pelodera strongy-
loides, Diplogaster sp., Mesodiplogaster sp., Panagrolaimus
sp., and Radutilus sp. were obtained from axenic Petri
dish cultures maintained at room temperature in the
laboratory. The wild type culture of Caenorhabditis
elegans (N2) was provided by Dr Julie Arhinger, Medical
Research Council, Cambridge and the surface mutants
AT6, AT10 and CL261 (table 2) were obtained from Dr
Theresa Stiernagle, Caenorhabditis Genetics Center, Uni-
versity of Minnesota and were maintained in Petri dishes
seeded with E. coli strain OP50 following the method of
Wood (1988).

Bacterial cultures

Populations of Pasteuria were obtained from the species
of nematode from which they were originally isolated
growing on a suitable host plant. Either, the infected roots
were dried and the powder produced following the
method of Stirling & Wachtel (1980), or Pasteuria infected
nematode cadavers were collected from field soils. The
latter were recognized using a dissecting microscope and
identifying females present on or in roots but not
producing egg masses (Sharma & Davies, 1996). Suspen-
sions of spores were prepared by grinding either Pasteuria
infested root powder, or infected cadavers, in tap water
with a pestle and mortar. The spores were filtered with a
10 μm sieve, counted using a haemocytometer slide and
the concentrations of suspensions were adjusted to 10^9
spores/ml. Stock suspensions were stored frozen at
−20°C.

Attachment tests

Samples (250 μl) of spore suspensions of each of the
stock Pasteuria populations were placed in separate
cleanized Eppendorf tubes together with a 250 μl of a
suspension of the test nematode population containing
approximately 500 individuals. The nematodes and
spores were thoroughly mixed and an attachment
test performed by centrifugation (10,000 g for 5 min)
following the method of Hewlett & Dickson (1993). A
semi-quantitative score (0, no spores per nematode; +, 1–10 spores per nematode; ++, 11–40 spores per nema-
tode) was given for each population of nematode tested,
assessing a minimum of 25 nematodes for each nematode
population, using a light microscope (×400).
Results and discussion

No Pasteuria spores were observed adhering to any of the 3rd stage infective larvae of the animal parasitic nematodes either with or without their sheath (table 1) or to any of the free living nematodes including C. elegans and three srf mutants (table 2). All populations of Pasteuria used in these experiments had been isolated from plant parasitic nematodes and their attachment was restricted to plant parasitic nematodes (table 3). Attachment of those populations of spores isolated from root-knot nematodes (Meloidogyne spp.) was similarly restricted to root-knot nematodes, however, those isolated from the genus Heterodera appeared to have a broader range of hosts and all three Pasteuria populations, PPC, PPN and PPW also attached to Globodera. One population of spores, PPC, was also observed attaching to Pratylenchus, Radopholus, Rotylenchulus and Aphelenchoides; the attachment of these spores also exhibited interspecific variation between species within genera (table 3). It is interesting to note that the populations of Pasteuria from the apomictic populations of nematodes, i.e. the root-knot populations, appear to have a more restricted host range than those isolated from the cyst nematode populations which are amphimictic.

There are two fundamental problems in the deployment of Pasteuria as a biological nematicide, firstly, the inability to culture large populations of spores (Williams et al., 1989; Bishop and Ellar, 1991) and secondly, its host specificity (Stirling, 1985; Channer & Gowen, 1992; Davies et al., 1988). Populations of Pasteuria are found which parasitize all the major genera of plant parasitic nematodes (Sayre and Starr, 1988) and there have recently been reports of other populations which parasitize nematodes in other families and even orders (Sturhan, 1996). Monoclonal antibodies have shown that the surface of the spores of a Pasteuria isolate originating from M. incognita race 2 was highly heterogeneous, and baiting experiments showed that different sub-populations of spores adhere to different species and races of nematode (Davies et al., 1994). These and subsequent studies (Davies & Redden, 1997) have suggested that the surface properties of the spore are responsible for the virulence of the bacterium and suggest that similar heterogeneity will also be present in the nematode cuticle. As the bacterium infects other invertebrates such as the cladoceran Moina

Table 1. Animal parasitic nematodes, third stage larvae with and without sheath, to which no spores of the bacterial hyperparasite Pasteuria penetrans adhered.

| Genus/species | Origin1 | Pasteuria populations tested |
|---------------|---------|-----------------------------|
| Haemonchus contortus | BI | PP1, PP3O |
| Heligmosomoides polygyrus | UN | PP1, PP3A, PP3O, B7, PA |
| Ostertagia circumcincta | MRI | PP1, PP3O |
| Trichostrongylus axei | MRI | PP1, PP3O |
| Anguilluloma ceplanicum | UN | PP1, PP3A, PP3O, B7, PA |
| Steinernema feltiae | IACR | PP1, PP1, PPC |
| Heterorhabditis megidis | IACR | PP1, PPJ, PPC |

1BI, Babraham Institute, Cambridge; UN, University of Nottingham; MRI, Moredun Research Institute, Edinburgh; IACR, Institute of Arable Crops Research.
2Spores originating from Meloidogyne incognita; 3spores originating from M. javanica; 4spores originating from M. arenaria; 5spores originating from Heterodera glycines.

Table 2. Free living nematodes, mixed stages, to which no spores of the bacterial hyperparasite Pasteuria penetrans adhered.

| Genus/species | Origin1 | Pasteuria populations tested |
|---------------|---------|-----------------------------|
| Caenorhabditis elegans N2 | MRC | PP1, PP3O, B7, PNG, PPN |
| Surface mutants | CGC | PP1, PP3O |
| AT6 | CGC | PP1, PP3O |
| AT10 | CGC | PP1, PP3O |
| CL261 | CGC | PP1, PP3O |
| Panagrellus redivivus | IACR | PP1, PP3O, B7, PNG, PPN |
| Pelodera strongyloides | IACR | PP1, PP3O, B7, PNG, PPN |
| Diplodaster sp. | IACR | PP1, PP3O, B7, PNG, PPN |
| Mesodiopodaster sp. | IACR | PP1, PP3O, B7, PNG, PPN |
| Panagrolaimus sp. | IACR | PP1, PP3O, B7, PNG, PPN |
| Rhabditis sp. | IACR | PP1, PP3O, B7, PNG, PPN |

1MRC, Medical Research Council, Cambridge; CGC, Caenorhabditis Genetics Center, Minnesota.
2Spores originating from Meloidogyne incognita; 3spores originating from M. javanica; 4spores originating from M. arenaria; 5spores originating from Heterodera glycines.
Table 3. Attachment of spores of six populations of Pasteuria (PP1, PPA, PPJ, PPC, PPN, PPW) to the cuticle of second-stage juveniles of plant parasitic nematodes (0, no attachment; +, 1–10 spores; ++, >10 spores; –, not available; based on a mean of 25 individual nematodes).

| Genus/species          | Origin1 | PP12 | PPA2 | PPJ3 | PPC4 | PPN5 | PPW6 |
|------------------------|---------|------|------|------|------|------|------|
| Meloidogyne incognita  | IACR    | ++   | ++   | ++   | 0    | 0    | 0    |
| M. javanica            | IACR    | +    | ++   | ++   | 0    | +    | +    |
| M. arenaria            | IACR    | +    | +    | +    | 0    | 0    | 0    |
| M. hapla               | IACR    | +    | +    | 0    | 0    | 0    | 0    |
| Heterodera avenae      | IACR    | 0    | 0    | 0    | –    | –    | ++   |
| H. schachtii           | IACR    | 0    | 0    | 0    | ++   | +    | +    |
| H. glycines            | IACR    | 0    | –    | +    | +    | +    | –    |
| H. cajani              | ICRISAT | 0    | 0    | 0    | ++   | +    | –    |
| Globodera rostochiensis| IACR    | 0    | 0    | 0    | ++   | 0    | 0    |
| G. pallida             | IACR    | 0    | 0    | 0    | ++   | +    | –    |
| Pratylenchus crenatus  | IACR    | 0    | 0    | 0    | 0    | 0    | 0    |
| P. neglectus           | IACR    | 0    | 0    | 0    | 0    | +    | 0    |
| P. coffeae             | IIP     | 0    | 0    | 0    | +    | 0    | –    |
| Radopholus similis     | IIP     | 0    | 0    | 0    | +    | –    | –    |
| Rotylenchulus reinformis| ICRISAT | 0    | 0    | 0    | –    | –    | –    |
| Anguina tritici        | IACR    | 0    | 0    | 0    | 0    | –    | –    |
| Aphelenchoides sp.     | IACR    | 0    | 0    | 0    | +    | –    | –    |
| Ditylenchus sp.        | IACR    | 0    | 0    | 0    | 0    | –    | –    |

1IACR, Institute of Arable Crops Research; ICRISAT, International Crop Research Institute for the Semi-Arid Tropics; IIP, International Institute of Parasitology.
2Spores originating from Meloidogyne incognita; 3spores originating from M. javanica; 4spores originating from H. cajani; 5spores, P. nishizawae, originating from H. avenae; 6spores originating from H. glycines.

(Sayre et al., 1977; Ebert et al., 1996) it would seem likely that similar bacteria will be found infecting animal parasitic nematodes, especially those which have to spend prolonged periods of their life cycle in soil before infecting their respective animal hosts, and these infective stages will also exhibit a high level of cuticular heterogeneity. The challenge for the future will therefore be to isolate such bacteria targeting animal and human parasitic nematodes, and to evaluate their potential as tools for the biological control of important human and livestock diseases.

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