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

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(Sturhan, 1996). Four species of *Pasteuria* have been described so far: (i) *P. penetrans* parasitic on *Meloidogyne incognita* and probably other root-knot nematodes (Sayre & Starr, 1985); (ii) *P. thornei* on *Pratylenchus brachyurus* (Sayre & Starr, 1988); (iii) *P. nishizawae* which parasitizes cyst nematodes (Sayre *et al*., 1991); and (iv) *P. ramosa*, a parasite of water fleas *Daphnia* spp. (Sayre *et al.*, 1983; Ebert *et al*., 1996).

Most research on *P. penetrans* has concentrated on its interaction with root-knot nematodes (*Meloidogyne* spp.) and it has been shown to have potential to control root-knot nematodes (Stirling, 1991). The life cycle of *P. penetrans* commences when endospores attach to the cuticle of motile larvae as they migrate through the soil. Spores either germinate 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* (Davies *et al.*, 1990), 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 microcolony. 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/carbohydrate 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 characteristics 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). Nematodes 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 temperatures in tap water and, in the case of the two species of potato cyst nematode, *Globodera pallida* and *G. rostochiensis*, 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 strongyloides, Diplogaster* sp., *Mesodiplogaster* sp., *Panagrolaimus* sp., and *Rhabditis* 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, University 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). Suspensions 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 10^6 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 siliconized 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 nematode) was given for each population of nematode tested, assessing a minimum of 25 nematodes for each nematode population, using a light microscope (× 400).

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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	Origin[1]	*Pasteuria* populations tested
Haemonchus contortus	BI	PP1[2], PP3O[3]
Heligmosomoides polygyrus	UN	PP1, PP3A[4], PP3O, B7[2], PA[4]
Ostertagia circumcincta	MRI	PP1, PP3O
Trichostrongylus axei	MRI	PP1, PP3O
Ancylostoma ceylanicum	UN	PP1, PP3A, PP3O, B7, PA
Steinernema feltiae	IACR	PP1, PPJ[3], PPC[5]
Heterorhabditis megidis	IACR	PP1, PPJ, PPC

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

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

Genus/species	Origin[1]	*Pasteuria* populations tested
Caenorhabditis elegans N2	MRC	PP1[2], PP3O[3], B7[2], PNG[3], PPN[5]
Surface mutants AT6	CGC	PP1, PP3O
AT10	CGC	PP1, PP3O
CL261	CGC	PP1, PP30
Panagrellus redivivus	IACR	PP1, PP3O, B7, PNG, PPN
Pelodera strongyloides	IACR	PP1, PP3O, B7, PNG, PPN
Diplogaster sp.	IACR	PP1, PP3O, B7, PNG, PPN
Mesodiplogaster sp.	IACR	PP1, PP3O, B7, PNG, PPN
Panagrolaimus sp.	IACR	PP1, PPA[4], B7, PNG, PPN
Rhabditis sp.	IACR	PP1, PPA, B7, PNG, PPN

[1]MRC. Medical Research Council, Cambridge; CGC, *Caenorhabditis* Genetics Center, Minnesota.
[2]Spores originating from *Meloidogyne incognita*; [3]spores originating from *M. javanica*; [4]spores originating from *M. arenaria*; [5]spores originating from *Heterodera glycines*.

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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	Origin[1]	PP1[2]	PPA[2]	PPJ[3]	PPC[4]	PPN[5]	PPW[6]
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	0	–	+	+	+
H. cajani	ICRISAT	0	0	0	++	+	–
Globodera rostochiensis	IACR	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	0
P. coffeae	IIP	0	0	0	+	0	–
Radopholus similis	IIP	0	0	0	+	–	–
Rotylenchulus reinformis	ICRISAT	0	0	0	+	–	–
Anguina trittici	IACR	0	0	0	0	–	–
Aphelenchoides sp.	IACR	0	0	0	+	–	–
Ditylenchus sp.	IACR	0	0	0	0	–	–

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

(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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