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Diversity of the bacterial hyperparasite *Pasteuria penetrans* in relation to root-knot nematodes (*Meloidogyne* spp.) control on *Acacia holosericea*

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Summary – *Pasteuria penetrans* isolates sampled from different geographical areas were characterised both for the heterogeneity of the endospore surface using monoclonal antibodies and for the ability of spores to attach to different isolates of *Meloidogyne* spp. The efficacy of these different *Pasteuria* isolates as biological control agents was tested in a glasshouse experiment with *M. incognita* from Senegal on *Acacia holosericea*. The immunoprofiles divided the *P. penetrans* isolates broadly differently from the attachment tests. Isolate PP16 from Senegal was associated with better seedling development of *M. incognita*-inoculated *A. holoceria* than were other isolates. Substantial variation in root and shoot biomass was not related to the observed variation in spore attachment tests. The difficulties involved in obtaining consistent biological control with *Pasteuria* are discussed in relation to the high degree of variability of this bacterium.

Résumé – ***Diversité chez l'hyperparasite bactérien* Pasteuria penetrans *en relation avec le contrôle du nématode* Meloidogyne *spp. sur* Acacia holosericea** – Des isolats de *Pasteuria penetrans* provenant de différentes régions géographiques ont été caractérisés en ce qui concerne, d'une part l'hétérogénéité de la surface des endospores — par utilisation d'anticorps monoclonaux —, d'autre part la capacité des spores à s'attacher à différentes souches de *Meloidogyne* spp. L'efficacité de ces différents isolats de *P. penetrans* en tant qu'agent de contrôle a été testée lors d'une expérience en serre utilisant un *M. incognita* d'origine sénégalaise et *Acacia holosericea*. Les isolats de *P. penetrans* ont montré des différences dans les profils immunologiques, différences non corrélées avec celles observées lors des tests d'attachement. En comparaison avec d'autres isolats, l'isolat PP16 provenant du Sénégal était associé à une meilleure croissance des plants de *A. holosericea* infestés par *M. incognita*. La variabilité importante touchant les biomasses aérienne et souterraine ne montrait aucune relation avec les différences observées lors des tests d'attachement. Les difficultés rencontrées pour obtenir un contrôle valable à l'aide de *P. penetrans* sont discutées en tenant compte du degré important de variabilité chez cette bactérie.

Keywords – abiotic factors, biological control, plant parasitic nematodes.

Public concern over the toxicity of nematicides and their impact on the environment has focused research on alternative methods to control plant-parasitic nematodes (Thomason, 1987). A range of micro-organisms has been identified with potential to control nematodes; however, despite the number of studies, only a few products have been commercialised (Whipps & Davies, 2000). One reason for this is the inconsistent levels of nematode control obtained following their application. Two sources of variation are incompatibilities between nematodes and the potential parasites (Davies, 1998; Duponnois *et al.*, 1996), or an effect of indigenous microflora against the populations of parasites (Duponnois & Bâ, 1998).

Pasteuria penetrans (Thorne) Sayre & Starr, an obligate hyperparasite of root-knot nematodes (*Meloidogyne* spp.), has shown potential as a biological control agent for many years (Stirling, 1991). The use of this nematode hyperparasite is limited by the specificity of the in-

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teractions between *P. penetrans* spores and *Meloidogyne* juveniles (Davies & Redden, 1997). Some *P. penetrans* isolates adhere to a particular species of root-knot nematode while others are compatible with only individual populations within a species (Stirling, 1985; Davies & Danks, 1992). Compatible bacteria have been identified by screening them for their ability to adhere to second-stage juveniles (J2) of root-knot nematodes and for subsequent germination and infection of the females inside the roots. In fact, most reports of specificity of *P. penetrans* are based on attachment of endospores to the cuticle of nematodes rather than parasitism (production of endospores in the nematode pseudocoelom). Efficacy of a *P. penetrans* isolate is difficult to predict because not all individuals in a nematode population may be susceptible to all endospores (Chen & Dickson, 1998). Moreover, efficacy is difficult to predict even when all individuals in a population are susceptible (Chen & Dickson, 1998).

This work therefore aims *i)* to test the hypothesis that there is a phylogenetic relationship between the surface heterogeneity of the spores (reflected by immunological tests) of *P. penetrans* isolates from geographically distinct regions and their attachment to species and isolates of *Meloidogyne* from different geographical areas and *ii)* to identify *Pasteuria* isolates most suitable for deployment as a biological control agent against *M. incognita*, a common pathogen of *Acacia holosericea*, the Australian acacia frequently used in agroforestry systems in West Africa.

Materials and methods

Cultivation of *P. penetrans* isolates

A single egg mass isolate (MIS) of *M. incognita* from Senegal was reared on tomato (*Lycopersicon esculentum* Mill., cv. Roma) in a glasshouse (temperatures ranged from 25 to 35°C, day length about 15 h) in 1 dm^3 pots of soil, that had been autoclaved at 140°C for 40 min. The physicochemical characteristics of the soil were: pH_{H_2O} 7.1; clay 3.5%; fine silt 0.6%; coarse silt 1.4%; fine sand 61.6%; coarse sand 31.2%; total organic carbon 0.54%; total nitrogen 0.15%; soluble phosphorus 3.0%. Plants were uprooted after 1 month and the roots washed, cut into 1-2 cm lengths and placed in a mist chamber for 2 weeks to recover hatched J2 (Seinhorst, 1950).

Endospores of *P. penetrans* isolates from 25 geographical locations (Table 1) were stored for 1 year at room temperature as dry powders originally derived from infected nematodes from roots. Suspensions of spores were prepared by grinding *Pasteuria*-infested root powders in distilled water with a pestle and mortar and the resulting spore suspensions filtered through a 10 μm sieve to remove root debris. Spores were counted in a Mallassez counting cell at 145× magnification and the concentration adjusted to 10^6 spores ml^{-1}.

Five ml suspensions of 1000 J2 of the MIS root-knot isolate were exposed to 10^6 spores in glass tubes (1 × 10 cm). There were four replicates of each *Pasteuria* isolate. Tubes were placed on a rotary shaker for 12 h at 25°C. Sub-samples of 30 J2 were randomly taken from each tube and the percentages of J2 with *P. penetrans* spores and the number of spores per J2 were determined at 400× magnification. Data were statistically analysed by one-way analysis of variance with differences significant at $P \leqslant 0.05$.

After assessing the sub-samples, all the J2 in each suspension were inoculated to 1 month-old tobacco plants (*Nicotiana tabacum* L. cv. Paraguay × Claro) grown in 5 dm^3 pots of autoclaved soil, randomly placed in a glasshouse (25 to 35°C; 15 h daylength). After 2 months, the tobacco plants were uprooted and the root systems cut into short lengths. About 200 galls were collected per plant, dissected using a stereomicroscope at 145× magnification and females transferred into Eppendorf tubes. In each tube, females were crushed and the presence of endospores was recorded at 250× magnification. The extracted endospores were suspended in distilled water and stored at −80°C. These fresh endospores were used for the following experiments.

Spore attachment tests

Attachment tests were performed with 16 single egg mass isolates of *Meloidogyne* spp. (Table 2). One ml suspensions of 100 J2 were exposed to 10^4 fresh endospores in glass tubes (1 × 10 cm). There were four replicates per combination, a total of 16 *Meloidogyne* isolates and six isolates of freshly collected endospores. The suspensions were incubated for 12 h at 25°C. The numbers of J2 with *P. penetrans* spores and the number of spores per J2 were counted on 30 randomly chosen J2 at 400× magnification. All data were analysed by the one-way analysis of variance ($P \leqslant 0.05$). A similarity matrix was constructed and a hierarchical, simple linkage, cluster analysis was computed (Thioulouse *et al.*, 1997).

Table 1. *Geographic origins, host nematodes and sources of 25* Pasteuria penetrans *isolates and percentage of second stage juveniles (J2) of* Meloidogyne incognita *(Senegal) infected by the* P. penetrans *isolates and number of spores attached per J2.*

Isolates	Geographical origin	Nematode host	Sources	Number of J2 with spores attached (%)	Mean number of spores per J2
PP1	California. USA	*M. incognita*	1	60 ab[1]	0.9 d
PP2	Yogyakarta. Indonesia	*M. incognita*	2	90 ab	3.4 dc
PP3	Yogyakarta. Indonesia	*M. incognita*	2	100 a	8.0 c
PP4	Yogyakarta. Indonesia	*M. incognita*	2	100 a	6.2 c
PP5	Yogyakarta. Indonesia	*M. incognita*	2	100 a	22.1 b
PP6	Unknown	Unknown	5	100 a	3.5 dc
PP7	Yogyakarta. Indonesia	*M. incognita*	2	30 b	0.3 d
PP8	Yogyakarta. Indonesia	*M. incognita*	2	100 a	12.8 bc
PP9	North Sumatra. Indonesia	*M. incognita*	2	0 d	0 d
PP10	Klarangan. Indonesia	*M. incognita*	2	100 a	14.6 bc
PP11	Unknown	Unknown	5	100 a	14.4 bc
PP12	Kali Urang. Indonesia	*M. incognita*	2	100 a	3.8 dc
PP14	Unknown	Unknown	5	100 a	12.9 bc
PP15	Indonesia	*M. incognita*	2	100 a	15.4 bc
PP16	Thies. Senegal	*M. javanica*	4	100 a	5.8 c
PP17	Unknown	Unknown	5	100 a	25.2 b
PP18	South Africa	*M. javanica*	3	100 a	35.2 a
PP19	Brasil	*M. javanica*	4	60 ab	2.0 dc
PP20	Senegal	*M. javanica*	4	80 ab	1.0 d
PP21	Australia	*M. javanica*	4	0 d	0 d
PP22	South Africa	*M. javanica*	3	60 ab	1.0 d
PP23	West Indies. Martinique	*M. incognita*	4	100 a	23.1 b
PP24	New Caledonia	*M. incognita*	4	60 ab	1.0 d
PP25	Morocco	Unknown	5	0 d	0 d
PP26	Japan	Unknown	5	0 d	0 d

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[1] In each column, data followed by the same letter are not significantly different ($P \leqslant 0.05$).

Immunofluorescence with polyclonal antibody and monoclonal antibodies (Mabs)

A polyclonal antibody (PC/PP1) and Mabs, raised in mouse against endospores of *P. penetrans* (originating from California, USA; source: Dr S.R. Gowen, University of Reading, UK) were available (Davies *et al.*, 1994). Suspensions (15 μl) of endospores of six *P. penetrans* isolates, concentrated to 10^6 ml^{-1}, were spotted on multitest slides coated with poly-L-lysine (Harlow & Lane, 1988). Slides were washed three times in PBS (10 mM sodium phosphate buffer, pH 7.4, 0.9% sodium chloride) and endospores were blocked with 20-DMEM for 30 min. Slides were incubated for 2 h with specific monoclonal or polyclonal antibody suspended in tissue culture supernatant fluid or milk, respectively. Detection of the primary antibodies was performed by the incubation of the endospore preparations with either goat anti-mouse serum (1:50; v:v) for the monoclonal antibodies or goat anti-rabbit serum for the polyclonal antibodies, conjugated to FITC and washed three times. Endospores were then mounted in Citifluor™ (Agar Scientific) and examined with the aid of an Olympus BH-2 microscope fitted with epifluorescence illumination with a 455 nm excitation filter and a 520 nm barrier filter. The recognition of 20 endospores, randomly chosen from each isolate, by three monoclonal antibodies (PP1/12, PP1/117 and PP1/134) and the polyclonal antibody (PP1/PC) was evaluated as; no recognition (−), weak recognition (±), good recognition (+) or strong recognition (++). A similarity ma-

Table 2. *Designation of species, geographical origins of 16 single egg mass isolates of* Meloidogyne *used in the attachment tests with* Pasteuria penetrans *isolates and mean number of spores of the isolates of* Pasteuria *adhering to second-stage juveniles of* Meloidogyne.

Isolate	Species	Geographical origin	PP5	PP10	PP12	PP15	PP16	PP23
3	*M. mayaguensis*	Ivory Coast, Africa	0 c A[1]	0 c A	0 e A	0 a A	0 b A	0 d A
5	*M. mayaguensis*	Ivory Coast, Africa	0 c A	0 c A	0 e A	0 a A	0 b A	0 d A
10	*M. arenaria*	Ivory Coast, Africa	0 c A	0 c A	0 e A	0 a A	0 b A	0 d A
11	*M. incognita*	Louisiana, USA, North America	0 c A	0 c A	0 e A	0 a A	0 b A	0 d A
12	*M. incognita*	North Carolina, USA, North America	0 c A	0 c A	0 e A	0 a A	0 b A	0 d A
17	*M. incognita*	Burkina Faso, Africa	5.4 b B	1.1 b C	0.9 de CD	0 a D	0 b D	10.3 bc A
18	*M. incognita*	Tchad, Africa	4.7 b B	2.2 b C	2.3 cd C	0 a D	0.9 ab D	9.6 bc A
19	*M. incognita*	Martinique, French West Indies	5.4 b B	1.3 b C	1.8 d C	0 a D	1.5 a C	9.8 bc A
20	*M. incognita*	Guyanne, South America	11.3 a B	5.8 a C	5.7 b C	0 a D	0.9 ab D	20.1 b A
23	*M. javanica*	Burkina Faso, Africa	9.9 a A	2.6 b B	2.7 cd B	0 a C	0 C	9.2 c A
25	*M. javanica*	Portugal, Europe	11.7 a B	7.0 a B	3.6 c B	0 a C	0.6 b BC	38.7 a A
27	*M. incognita*	North Carolina, USA, North America	0 c A	0 c A	0 e A	0 a A	0 b A	0 d A
28	*M. arenaria*	Unknown, French West Indies	0 c A	0 c A	0 e A	0 a A	0 b A	0 d A
29	*M. arenaria*	Sainte Anne, French West Indies	11.0 a BC	5.7 a C	15.5 a AB	0 a D	0 b D	19.0 bc A
31	*M. arenaria*	Unknown, French West Indies	0 c A	0 c A	0 e A	0 a A	0 b A	0 d A
32	*M. arenaria*	Unknown, French West Indies	0 c A	0 c A	0 e A	0 a A	0 b A	0 d A

[1] Data in the same column or same row followed by the same lower or upper case letter respectively are not significantly different ($P \leqslant 0.05$).

trix was constructed and a hierarchical, cluster analysis computed using Ward's method (Thioulouse *et al.*, 1997).

Glasshouse experiment

Inoculum of *M. incognita* (MIS) was produced on tomato cv. Roma. After 2 months, tomato roots were harvested, cut into short lengths and placed in a mist chamber for 1 week to enable the nematode eggs to hatch (Seinhorst, 1950). Seeds of *A. holosericea* from Bel Air (Dakar, Senegal) were scarified with concentrated sulphuric acid for 60 min and washed for 12 h in sterile distilled water before sowing. Germinated seeds were grown for 1 month in 0.5 dm^3 polythene bags (5 cm diam) filled with autoclaved soil. Seedlings were placed in a glasshouse (27°C day, 20°C night, 12 h daylength) and watered twice weekly without fertiliser.

One month after sowing, the seedlings were inoculated with 5 ml suspensions of 1000 J2 of MIS and 5 ml of 10^6 *P. penetrans* endospore suspensions of isolates PP10, PP12, PP15, PP16, and PP23 (Table 1). Bags were arranged in a randomized design with ten replicates per treatment.

One month after nematode and *P. penetrans* inoculation, seedlings of *A. holosericea* were uprooted and their roots were washed. Shoots were oven-dried at 65°C for 1 week and weighed. Roots were cut into 1-2 cm pieces and placed in a mist chamber for 2 weeks to recover hatched J2 (Seinhorst, 1950). Total number of J2, number of J2 with *P. penetrans* endospores, and number of endospores per J2 were determined at 250× magnification. Then roots were oven dried at 65°C for 1 week and weighed.

The data were analysed by one-way analysis of variance and the mean values were compared by Student's t-test ($P \leqslant 0.05$). Nematode data were log(x + 1) transformed before analysis.

Results

Cultivation of *P. penetrans* isolates

Among the 25 *P. penetrans* isolates, no attachment occurred with PP9, PP21, PP25, and PP26 (Table 1). The proportion of J2 with attached endospores and the number of endospores per J2 were low (less than four spores per J2) with isolates PP1, PP2, PP7, PP12, PP19, PP20, PP22 and PP24. With the other isolates, all the J2 were encumbered with more than five endospores. Many endospores per J2 (more than 20) were observed with isolates PP5,

PP17, PP18, and PP23 (Table 1). After 2 months culture of tobacco plants inoculated with *P. penetrans* and *M. incognita* from Senegal, only six bacteria (PP5, PP10, PP12, PP15, PP16 and PP23) developed and produced endospores.

ATTACHMENT TESTS

Nine *Meloidogyne* isolates (*M. mayaguensis* 3, 5; *M. arenaria* 10, 28, 31, 32; *M. incognita* 11, 12, 27) were incompatible with the six *P. penetrans* isolates in the glasshouse experiment (Table 2). Isolate PP 15 did not attach to any of the *Meloidogyne* isolates. With other *Pasteuria* isolates, attachment only occurred on *M. incognita* 17, 18, 19; *M. javanica* 20, 23, 25; and *M. arenaria* 29. More spores of PP5, PP10 and PP12 attached to nematode isolates 20, 23, 25 and 29 (Table 2). PP23 had the greatest mean number of endospores per J2 (Table 2). PP16, originating from Senegal, was not very aggressive against these nematode isolates and was incompatible with *M. javanica* 23.

According to the results of these attachment tests, the *P. penetrans* isolates could be grouped in two different phenotypic clusters: PP16 to PP23 and PP5, PP10 to PP12 (Fig. 1A). Isolate PP15 was excluded from this analysis because of its incompatibility with all the nematode isolates.

IMMUNOLOGICAL STUDY OF *P. PENETRANS* SPORE SURFACE

The polyclonal antibody recognized endospores of each isolate tested (Table 3). Mab PP1/12 did not recognise PP5, PP10, PP12 and PP16, but had a good reactivity with PP23. Isolate PP23 was recognised by all the Mabs used in this experiment. Ability of the different Mabs to recognise the endospore surface of each *P. penetrans* isolate was used to distinguish them. The hierarchical cluster analysis represented by a dendrogram (Fig. 1B) showed that three populations PP10, PP5 and PP15 were closely related; this group was linked to PP12. The two other populations, PP23 and PP16, had similarities of less than 20% (Fig. 1B).

Compared to the cluster analysis of the attachment tests, the immunological tests showed different phenotypic clusters for PP16-PP23 which were grouped in the same cluster with the attachment tests but are very different according to the immunological analysis.

Table 3. *Indirect immunofluorescence of 20 spores of six isolates of* Pasteuria penetrans *with three monoclonal antibodies (PP1/12, PP1/117 and PP1/134) and the polyclonal antibody (PP1/PC) evaluated as no recognition (−), weak recognition (±), good recognition (+) or strong recognition (++).*

P. penetrans isolate	Antibody	Number of endospores in each recognition category			
		−	±	+	++
PP5	PP1/12	15	5	0	0
Indonesia	PP1/117	8	2	10	0
	PP1/134	2	1	11	6
	PP1/PC	1	7	10	2
PP10	PP1/12	18	2	0	0
Indonesia	PP1/117	1	3	10	6
	PP1/134	0	0	12	8
	PP1/PC	0	2	17	1
PP12	PP1/12	1	18	1	0
Indonesia	PP1/117	0	1	14	5
	PP1/134	0	0	0	20
	PP1/PC	0	1	18	1
PP15	PP1/12	12	6	2	0
Indonesia	PP1/117	2	6	12	0
	PP1/134	0	2	2	16
	PP1/PC	0	3	16	1
PP16	PP1/12	19	1	0	0
Senegal	PP1/117	20	0	0	0
	PP1/134	20	0	0	0
	PP1/PC	0	2	13	5
PP23	PP1/12	0	0	20	0
Martinique	PP1/117	0	0	20	0
	PP1/134	0	0	20	0
	PP1/PC	0	0	4	16

GLASSHOUSE EXPERIMENT

Nematodes greatly reduced shoot development of *A. holosericea* (Fig. 2). Inoculation with *P. penetrans* reduced the effect of the nematodes, significantly for PP16 and PP23 populations (Fig. 2). Isolate PP 15 significantly decreased nematode multiplication compared to the control (Fig. 3). *P. penetrans* was observed in all the treatments (Table 4). The larger infestations (% J2 with endospores and number of endospores per J2) were recorded with PP16 and PP23 and the smallest with PP12 (Table 4). Among the *P. penetrans* isolates, PP16 and PP23 showed the largest effects on both plant growth and nematode in-

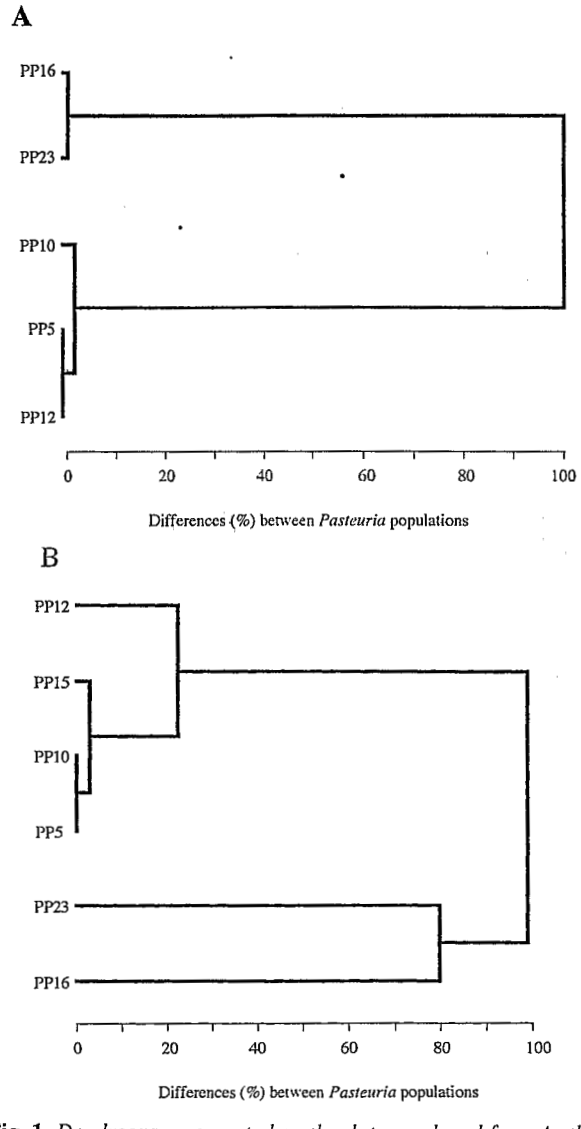

Fig. 1. *Dendrogram computed on the data produced from A: the attachment test performed with 16* Meloidogyne *isolates and five isolates of* Pasteuria penetrans *from different geographical regions; B: the immunofluorescence experiment performed with six isolates of spores of* Pasteuria penetrans *from different geographical regions.*

festation, which confirmed the cluster repartition of the immunological analysis.

Discussion

There was no clear relationship between surface heterogeneity of the endospores of the *P. penetrans* isolates, their ability to attach to the cuticle of different isolates

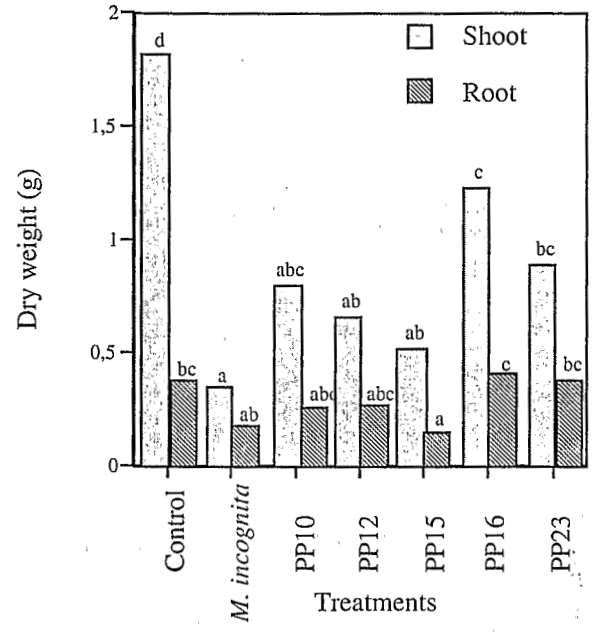

Fig. 2. *Effect of five* Pasteuria penetrans *isolates on the growth of* Acacia holosericea *seedlings infected by* Meloidogyne incognita. *Bars with the same letters are not significantly different (P ⩽ 0.05).*

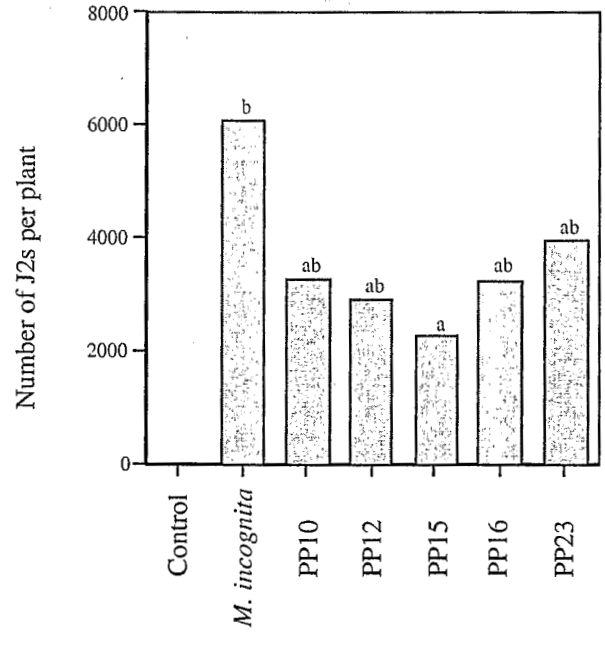

Fig. 3. *Effect of five* Pasteuria penetrans *isolates on the multiplication of* Meloidogyne incognita *on* Acacia holosericea *seedlings. Bars with the same letters are not significantly different (P ⩽ 0.05).*

Table 4. *Percentages of infested second-stage juveniles of* Meloidogyne incognita *(MIS) and the number of spores of five* Pasteuria penetrans *isolates per juvenile on* Acacia holosericea, *1 month after nematode and* P. penetrans *inoculation in the glasshouse experiment.*

P. penetrans isolates	Percentage of J2 with spores attached	Number of spores per J2
PP10	56 bc	1.7 ab
PP12	18 a	0.2 a
PP15	39.6 ab	1.6 ab
PP16	72.5 c	2.1 b
PP23	66.7 bc	2.3 b

Data in the same column followed by the same letter are not significantly different ($P \leqslant 0.05$).

of *Meloidogyne* and the level of control obtained. Neither was there a clear relationship between spore attachment and root-knot nematode phylogeny. Isolates PP16 and PP23, which gave best nematode control had different levels of attachment (5.8 and 23.1 endospores per J2, respectively). Although the majority of the *P. penetrans* isolates could attach to *M. incognita* originating from Senegal, only six isolates were able to infect the nematode and to multiply. It has been suggested that limited endospores attachment was associated with poor multiplication because spores do not always germinate and initiate infection (Stirling, 1984).

Our results confirm that spore attachment tests cannot be used as the only criteria for screening for the efficiency of the bacterial isolate against the nematodes. The attachment of the endospores is an obligatory step in the *P. penetrans* biological cycle but other factors are involved in the sporulation process of the bacterium. The surface of *P. penetrans* endospores is heterogeneous and this heterogeneity related to the ability of some isolates to adhere to different nematode species (Davies *et al.*, 1994). Our results showed a great diversity among the isolates; in contrast to the results of Davies and Redden (1997), and differences in the immunological reaction of the endospores were related to their geographical origin. Three geographical groups have been identified: West Indies, Africa and Indonesia.

The attachment tests with *Meloidogyne* isolates showed that *M. mayaguensis* was incompatible with *P. penetrans* isolates as demonstrated in previous studies (Fargette *et al.*, 1996; Phillips *et al.*, 1996). Specificity of the *P. penetrans* isolates was also noted within the species *M. incognita*. Some *P. penetrans* isolates adhered to *M. incognita* from Senegal but there was no attachment with other isolates of the same nematode species from the USA. The immunoprofiles of the *Pasteuria* endospore surface corresponded to similarity patterns different from those obtained with the attachment tests. This confirms that the biological variation expressed as differences in endospore attachment is a poor guide to explain differences in biochemical/molecular variation responsible for spore/cuticle recognition mechanisms (Frank, 1994). PP15 isolate was multiplied on *M. incognita* originating from Senegal but was incompatible with all other *Meloidogyne* isolates. The cuticle of the nematodes exhibits polymorphism that could explain variability in endospore attachment (Davies & Danks, 1992). However, this "all or nothing" result seems surprising: PP15 isolate, similar to PP5 and PP10 according to the immunoprofiles, has not the surface adhesins of the PP5 and PP10 spores that allow attachment to several *M. incognita* isolates (17, 18, 19 and 20). These three *P. penetrans* isolates must be studied to identify the compatible receptor with the *M. incognita* isolate from Senegal.

The immunoprofiles separated the bacterial isolates as did the attachment tests (*i.e.*, in relation to their geographical origin). Isolate PP16 from a Senegalese soil was one of the less aggressive isolates against *M. incognita* in the attachment tests but resulted in better plant growth. Moreover, the percentage of infested J2 and number of spores per J2 were larger than in the other treatments (except for PP23), suggesting that PP16 multiplication could be sustained. It has been demonstrated that the host plant and some biotic or abiotic factors could stimulate/repress the development of *P. penetrans* (Mateille *et al.*, 1995, 1996). In our study, *A. holosericea* and the type of soil appeared favourable for PP16 development. This *Acacia* species is often used in agroforestry systems in which trees and root-knot susceptible vegetable crops are associated. This leguminous tree may serve as a source for the bacterium and maintain a biological control of the root-knot nematodes.

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