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Monoxenic culture of the slug parasite *Phasmarhabditis hermaphrodita* (Nematoda : Rhabditidae) with different bacteria in liquid and solid phase

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Summary – Sixteen bacterial isolates representing thirteen species were tested for their ability to support growth of *Phasmarhabditis hermaphrodita* in monoxenic cultures in Petri dishes of agar medium. Thirteen isolates were associated with the nematode in living or dead slugs *(Deroceras reticulatum)* or in xenic culture. Growth of *P. hermaphrodita* was obtained on all isolates but its reproductive capacity differed considerably when cultured with different bacteria. Five species of bacteria which supported vigorous growth of *P. hermaphrodita* on kidney agar were used to grow it in liquid cultures. All supported growth and reproduction but there were significant differences in final numbers of nematodes and numbers of dauer larvae formed. The differences in total numbers were not consistent with differences seen in Petri dish cultures. *Providencia rettgeri* was found to give the best yields of dauer larvae in liquid culture. In both liquid and solid phase, monoxenic growth of *P. hermaphrodita* was better on media based on pig kidney than on wholly soluble media.

Résumé – ***Élevage du parasite de limace* Phasmarhabditis hermaphrodita *(Nematoda : Rhabditidae) en milieu liquide et solide avec différentes bactéries*** – Seize isolats bactériens, représentant treize espèces, ont été testés pour leur capacité à maintenir la croissance de *Phasmarhabditis hermaphrodita* en culture monoxénique sur agar, en boîte de Petri. Tous les isolats permettent la croissance de *P. hermaphrodita,* mais la capacité de reproduction varie considérablement suivant les différentes bactéries. Cinq espèces de bactéries ayant permis une croissance vigoureuse de *P. hermaphrodita* ont été utilisées pour son élevage en milieu liquide. Toutes permettent la croissance et la reproduction du nématode mais des différences significatives se produisent en ce qui concerne le nombre total de nématodes et celui des *dauer larvae* formées. Les différences entre les chiffres totaux ne sont pas significativement différentes de celles observées dans le cas de culture en boîtes de Petri. En milieu liquide, *Providencia rettgeri* paraît produire les meilleurs rendements en *dauer larvae.* Tant en milieu solide que liquide, la croissance monoxénique de *P. hermaphrodita* est meilleure sur des milieux à base de rognon de porc que sur des milieux ne comportant que des éléments entièrement solubles.

Key-words : *Phasmarhabditis,* Rhabditida, nematodes, bacteria, monoxenic culture, solid phase, liquid phase, mass cultivation, biological control, slug parasite.

The rhabditid nematode *Phasmarhabditis hermaphrodita* (Schneider) is a parasite capable of killing several species of pest slugs from the genera *Deroceras, Arion* and *Tandonia,* and thus has potential for use as a biocontrol agent (Wilson *et al.,* 1993 *a*). The nematode is a bacterial feeder which has been grown in xenic cultures to study life cycles (Maupas, 1900). Recently, for field studies of its potential as a biocontrol agent (Wilson *et al.,* 1994), *P. hermaphrodita* has been mass produced in xenic culture using similar technologies to those developed for entomopathogenic nematodes (Heterorhabditidae and Steinernematidae) (Wilson *et al.,* 1993 *b*). Similarly, the rhabditid nematode *Rhabditis necromena* Sudhaus & Schulte has been grown in xenic culture for use in small plot field trials as a biocontrol agent for the millipede *Spinotarsus caboverdus* Pierrard (McKillup *et al.,* 1991). However, xenic cultures may contain bacteria which are pathogenic to nematodes, or which produce toxic metabolites and thus inhibit nematode growth. Growing nematodes in monoxenic culture with a single species of bacterium, gives more controlled conditions, in which it is possible to achieve more predictable and consistent yields than in xenic cultures (Poinar & Hansen, 1986).

Entomopathogenic nematodes, which are mutualistically associated with specific species of *Xenorhabdus* or

Photorhabdus bacteria, are always mass produced in monoxenic culture with the appropriate species of bacterium (Friedman, 1990). It is known that the growth rate and reproductive capacity of other rhabditid nematodes in monoxenic culture is strongly influenced by bacterial species (Sohlenius, 1968; Andrew & Nicholas, 1976; Grewal, 1990). Differing nematode growth and reproduction rates with different species of bacteria result from differences in bacterial attraction, ingestion, digestion and the presence or absence of toxic metabolites (Poinar & Hansen, 1986).

Friedman (1990) reviewed the successful commercial production and development of entomopathogenic nematodes and used mathematical models to analyze the economy of scale of various production systems. He concluded that liquid phase culture is the most robust method for development to large scale. Thus, for successful use of *P. hermaphrodita* as a biological molluscicide, a monoxenic bacterial/nematode combination capable of growth in liquid medium would need to be identified.

This study aimed to investigate the ability of sixteen bacterial isolates, representing thirteen species, to support growth of *P. hermaphrodita* and to identify specific bacteria which could be used to grow *P. hermaphrodita* in liquid-phase monoxenic culture. Initially bacteria were tested in solid-phase Petri dish cultures, then those found to support best growth of *P. hermaphrodita* were tested in shakeflask liquid cultures as used by Wilson *et al.* (1993 *b*) for xenic cultures. *P. hermaphrodita* forms dauer larvae which are the infective stage for slugs (Wilson *et al.*, 1993 *a*) and, thus, these are the desired end point of a system to produce *P. hermaphrodita* as a biocontrol agent. Therefore, in the liquid cultivation experiments, effects of different bacteria on numbers of dauer larvae were also studied. In both liquid phase and solid phase cultures, growth on media based on pig kidney was compared with growth on wholly soluble media.

Materials and methods

Source of test bacteria

Bacteria tested (Table 1) included species which were isolated from the intestine of surface-sterilised dauer larve of *P. hermaphrodita* (four isolates) and also from thriving xenic foam-chip cultures (Wilson *et al.*, 1993 *b*) of *P. hermaphrodita* (six isolates). Three isolates were obtained from slugs, *Deroceras reticulatum* (Müller), collected at Long Ashton Research Station and found to be infected with *P. hermaphrodita*. Also included were isolates of three species of bacteria which support growth of the free living nematode *Caenorhabditis elegans* (see Grewal, 1990) or the entomopathogenic nematode *Steinernema feltiae* (Table 1).

Bacteria were identified using the Analytical Profile Index (API) system (La Balme-les-Grottes, 38390 Montalieu-Vercieu, France) and other bacteriological tests. The other tests used followed the procedures recommended by Cruickshank *et al.* (1975) : metabolism of adonitol, trehalose and lactose, tested using 1 % (w/v) concentrations in peptone water and with bromocresol purple as a pH indicator in universal tubes; gas production during metabolism of carbohydrates was tested using similar universal tubes, containing a Durham tube to collect gas bubbles; sensitivity to the antibiotics novobiocin, streptomycin sulphate, penecillin G and 2,4,-diamino-6-7 diisopropyl pteridine (the vibriostat 0/129), were tested using disk inhibition tests on D.S.T. agar (Oxoid); the ability to produce pigments which fluorescence under ultra violet light (wavelength = 260 nm) was tested using the methods described by King *et al.* (1954); lecithinase production was tested with egg yolk

Table 1. *Species and source of bacteria used in monoxenic cultures with* Phasmarhabditis hermaphrodita. *All bacteria were isolated at Long Ashton Research Station unless stated otherwise.*

Bacterium	Isolation source
*Acinetobacter calcoaceticus**	Associated with *Caenorhabditis elegans* infesting mushroom compost
Aeromonas hydrophila	Intestine of dauer larvae *P. hermaphrodita*
Aeromonas sp.+	Cadaver of field collected slug *(D. reticulatum)* which had died following infection with *P. hermaphrodita*
Bacillus cereus	Intestine of dauer larvae *P. hermaphrodita*
Flavobacterium breve	Xenic foam-chip culture of *P. hermaphrodita*
Flavobacterium odoratum	Xenic foam-chip culture of *P. hermaphrodita*
Moraxella phenylpyruvica++	Xenic foam-chip culture of *P. hermaphrodita*
Providencia rettgeri	Xenic foam-chip culture of *P. hermaphrodita*
Pseudomonas fluorescens (isolate no. 1 a)	Intestine of dauer larvae *P. hermaphrodita*
Pseudomonas fluorescens (isolate no. 140)	Cadaver of field-collected slug *(D. reticulatum)* which had died following infection with *P. hermaphrodita*
Pseudomonas fluorescens (isolate no. 141)	Mantle cavity of living field-collected slug *(D. reticulatum)* infected with *P. hermaphrodita*
*Pseudomonas fluorescens** (PSG)	Associated with *Caenorhabditis elegans* infesting mushroom compost
Pseudomonas paucimobilis	Intestine of dauer larvae *P. hermaphrodita*
Serratia proteamaculans	Xenic foam-chip culture of *P. hermaphrodita*
Sphingobacterium spiritovorum	Xenic foam-chip culture of *P. hermaphrodita*
*Xenorhabdus bovienii***	Symbiont of *Steinernema feltiae*

* Obtained from P. S. Grewal, HRI, Littlehampton.
** Obtained from the Agricultural Genetics Company.
+ Similar to *Aeromonas salmonicida* (National Collection of Industrial and Marine Bacteria (NCIMB), pers. comm.).
++ Identified by NCIMB as an atypical isolate of *M. phenylpyruvica*, with a negative urease reaction, but the ability to produce urease in this species can be lost after frequent laboratory sub-culture.

suspension in liquid medium (Hayward, 1941); the ability to grow at 41 °C was tested by incubating nutrient broth cultures in a water bath for 5 days, ability to grow at 4 °C was tested by incubating nutrient broth cultures in a refrigerator; the ability to grow at different concentrations of NaCl was tested by adding NaCl to nutrient broth to make up the appropriate concentration.

Axenisation of nematodes

Gravid adult *P. hermaphrodita* were transferred, through two changes of sterile water, to a sterile watch glass containing water with 0.02 % sodium ethyl mercurithiosalicylate (" Thimerosal ", Sigma) where they were left for 16 h at 10 °C. After this period, the larvae (L1/L2) produced by the adults were transferred to centrifuge tubes containing 10 ml quarter strength Ringer's solution (Poinar & Thomas, 1984) with 500 units ml^{-1} of both benzyl penicillin and streptomycin sulphate (Sigma). The larvae were kept in this solution at 10 °C for 24 h then concentrated by centrifugation at 90 × G for 10 min, re-suspended in sterile quarter strength Ringer's solution and centrifuged again. Re-suspension and centrifugation were repeated once more to remove any traces of antibiotics, then sterile (axenic) larvae were transferred to a sterile watch glass, where they could be handled individually using sterile micro-pipettes.

Monoxenic growth in solid phase

All experiments were done using 3 cm diameter Petri dishes. Two media were used : Oxoid nutrient agar (2.8 %) which has previously been shown to support growth of *P. hermaphrodita* in xenic cultures (Wilson, 1992), and a kidney-based agar consisting of an aqueous solution of 10.0 % homogenised pig kidney, 2.5 % yeast extract, 3 % beef fat and 2 % Oxoid Agar technical No. 3 (% w/v). The kidney was first cut into small pieces then homogenised in a Waring blender. The beef fat was melted in a pan and the ground kidney added and cooked until brown. The water was added and the mixture was autoclaved for 10 min then passed through a sieve to remove remaining large pieces of kidney and congealed proteins. The agar was then added to the liquid and the mixture was sterilised by autoclaving for a further 30 min. The medium was poured into 3 cm Petri dishes which were used within one week of preparation. Petri dishes were inoculated with bacteria and nematodes as follows. Nutrient-broth cultures of the test bacteria were grown overnight at 25 °C then one bacteriological loopful (*ca* 0.03 ml) of bacterial culture was spread over one half of the agar surface of each Petri dish. Ten axenic larvae of *P. hermaphrodita* were placed on the agar surface, close to the edge of each Petri dish, in the half without added bacteria, so that nematodes had to move at least 15 mm across a bacteria-free surface before reaching the test bacterium. Plates were incubated at 15 °C for one week and any showing bacterial contamination in the half where nematodes were added were discarded. During this time in almost all cases the nematodes reached the half of the plate with added bacteria and they and their progeny remained there. If any nematodes did return to the bacteria-free half, the bacteria grew along the nematodes' trail, leaving characteristic lined growth, easily distinguished from contamination by other bacteria. After a further two weeks, nematodes were washed off the plates into a known volume of water and the resulting nematode suspensions counted. The remaining agar was removed from the Petri dishes, melted in a test tube and poured into a 5 cm diameter Petri dish to form a thin layer. This dish was placed over a counting grid so that numbers of nematodes which had burrowed into the agar could be counted. At least five replicate plates were prepared for each treatment.

Two experiments compared growth of *P. hermaphrodita* on several different bacterial isolates on kidney agar. A third experiment investigated growth of *P. hermaphrodita* in monoxenic culture with three different bacteria and in a xenic culture, with an unknown mix of bacteria, on both nutrient agar and kidney agar. The bacterial inoculum from xenic cultures was obtained by placing one foam chip from a thriving foam-chip culture (Wilson *et al.*, 1993 *b*) into a tube of nutrient broth and incubating overnight at 25 °C.

Monoxenic growth in liquid phase

Nematodes were cultured in 250 ml conical flasks containing 50 ml liquid medium incubated at 15 °C and rotated at 200 rpm in gyrotatory incubators/flask shakers (conditions suitable for growth of *P. hermaphrodita* in xenic culture, see Wilson *et al.*, 1993 *b*). Each flask was inoculated with the appropriate bacterium and incubated at 15 °C for 24 h before nematodes (3000 per flask), grown as previously described, in monoxenic culture with the appropriate bacterium on 3 cm Petri dishes, were added to each flask. As Wilson *et al.* (1993 *b*) found that peak numbers of dauer larvae were produced in liquid culture after 3 weeks, 1 ml of medium was removed from each flask after 3 weeks and nematodes (total numbers and dauer larvae) were counted in at least three sub-samples of each. A minimum of four replicate flasks were used for each treatment. Immediately prior to counting, the cultures were tested for bacterial contamination using the API system.

In the first two experiments, several bacterial isolates which had been found to be capable of supporting monoxenic growth of *P. hermaphrodita* in Petri dishes were tested for their capacity to support monoxenic growth of *P. hermaphrodita* in a liquid kidney (KYO) medium (10 % homogenised pigs kidney, 1 % yeast extract, 3.5 % corn oil). In a third experiment, *P. hermaphrodita* was grown in four different media in monoxenic cultures with each of three different species of bacteria which had been found to give good growth in the first two experiments. The media were as follows (all percentages

are w/v in aqueous solutions) : KYO medium, KYEO medium (5.95 % liquid egg, 3.53 % pig kidney, 0.78 % yeast extract and 3.5 % corn oil), TSB medium (2 % tryptic soya broth, 1 % yeast extract, 3.5 % corn oil) and AP medium (2 % yeast extract, 1 % animal peptone, and 3.5 % corn oil). The media containing kidney (KYO and KYEO) were prepared in a similar way to the solid kidney medium described above. TSB and AP medium used wholly soluble ingredients which were simply mixed together. All media were sterilised by autoclaving.

STATISTICAL ANALYSIS

Data were transformed to logarithms to stabilise the variance and subjected to analysis of variance.

Results

GROWTH IN SOLID PHASE

After three weeks in both experiments there were highly significant ($P < 0.001$) differences in numbers of *P. hermaphrodita* between cultures on kidney agar with different bacteria (Fig. 1). In the first experiment (Fig. 1 A), most nematodes were found on plates with *Providencia rettgeri* but numbers were not significantly greater than on plates with *Serratia proteamaculans, Moraxella phenylpyruvica, Aeromonas* sp. or *Pseudomonas fluorescens* (isolate no. 141). Nematodes had not reproduced on plates inoculated with *Xenorhabdus bovienii* or *Pseudomonas paucimobilis,* although some adults were found on these plates, indicating that *P. hermaphrodita* could develop but not reproduce by feeding on these bacteria. Nematodes had reproduced on one plate treated with *Bacillus cereus,* but not on any others. This plate was examined for contaminating bacteria, but none was found.

In the second experiment (Fig. 1 B), most nematodes were found on plates with *P. rettgeri* and *P. fluorescens* (isolate obtained from P.S. Grewal), but numbers were not significantly greater than on plates with *P. fluorescens* (isolate no. 140), *Acinetobacter calcoaceticus* and *Flavobacterium breve.* There was only limited growth and reproduction on plates inoculated with *P. paucimobilis* and *Aeromonas hydrophila.*

In the third experiment, both bacterial treatment and growth medium had highly significant ($P < 0.001$) effects on numbers of *P. hermaphrodita* recorded after three weeks and the two factors interacted significantly ($P < 0.001$). More nematodes ($P < 0.001$) were produced by all bacterial treatments on kidney agar than on nutrient agar (Fig. 2). The only nutrient agar plates on which *P. hermaphrodita* had grown were those with the xenic mix of bacteria. On kidney agar there were significantly ($P < 0.001$) more nematodes in all monoxenic cultures than the xenic culture. Differences in nematode numbers when grown in monoxenic cultures with the three different bacteria, but on the same medium (either nutrient agar or kidney agar), were not significant.

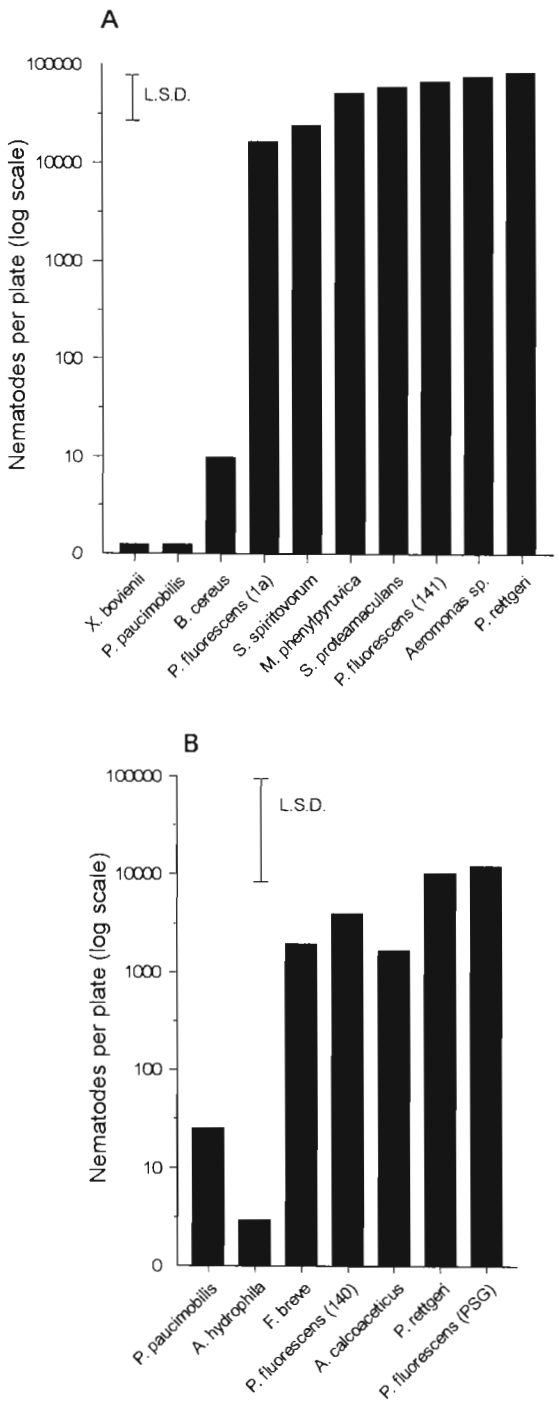


Fig. 1. *Numbers of* Phasmarhabditis hermaphrodita *(all stages) per 3 cm Petri dish after three weeks at 15 °C in monoxenic culture with different bacteria in (A) the first and (B) the second screening experiment. All plates were inoculated with ten axenic nematode larvae. L.S.D. = Least significant difference,* P = *0.05, 61 degrees of freedom (experiment 1) or 32 degrees of freedom, (experiment 2).*

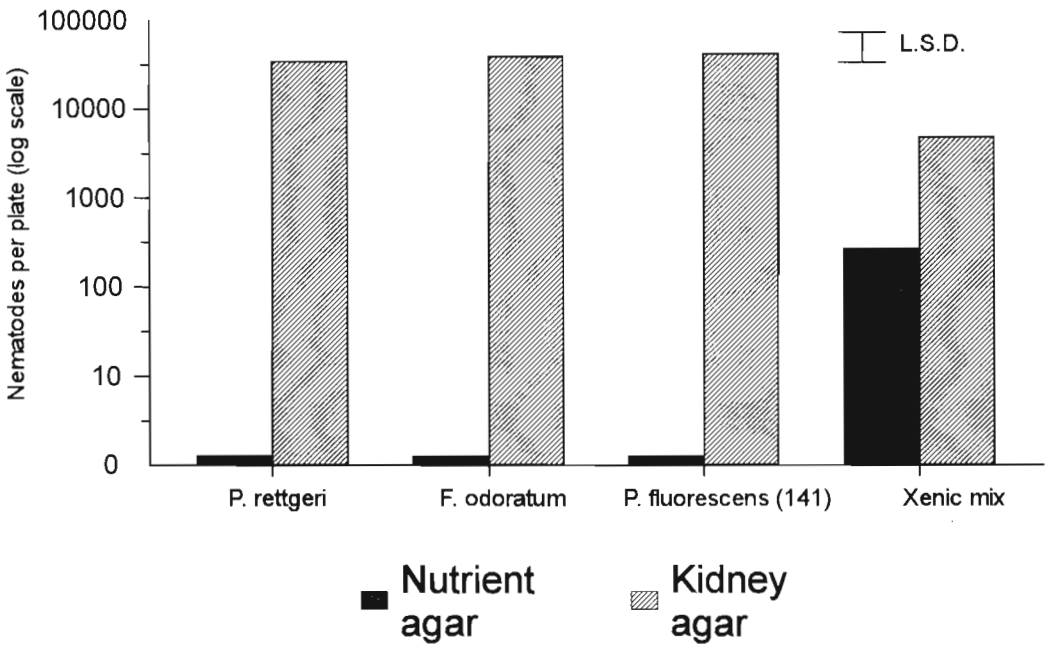


Fig. 2. *Numbers of* Phasmarhabditis hermaphrodita *(all stages) per 3 cm Petri dish after three weeks at 15 °C in xenic culture or monoxenic cultures with three different bacteria on nutrient agar or kidney agar. All plates were inoculated with ten axenic nematode larvae. L.S.D. = least significant difference (P = 0.05, 96 degrees of freedom).*

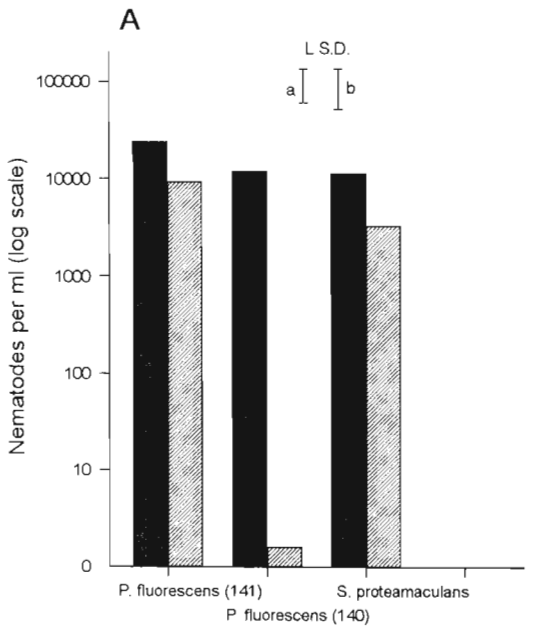
GROWTH IN THE LIQUID PHASE

In the first experiment (Fig. 3 A), there were no significant differences in total numbers of nematodes grown on different bacterial isolates, but there were highly significant ($P < 0.001$) differences in numbers of dauer larvae. Dauer larvae were present in similar abundance in cultures with *P. fluorescens* isolate no. 141 and *S. proteamaculans* but were scarce in cultures with *P. fluorescens* isolate no. 140. In the second experiment (Fig. 3 B), there were highly significant differences ($P < 0.001$) in total numbers of nematodes and numbers of dauer larvae. Cultures with *P. rettgeri* and *M. phenylpyruvica* produced significantly ($P < 0.001$) more nematodes than cultures with *S. proteamaculans* or *P. fluorescens* (isolate no. 141). Cultures with *P. rettgeri* produced significantly ($P < 0.05$) more dauer larvae than all other cultures.

In the experiment comparing growth of *P. hermaphrodita* in monoxenic culture with three different bacteria on four different growth media, both bacterium and medium had highly significant ($P < 0.001$) effects on total numbers of *P. hermaphrodita* and numbers of dauer larvae. The two factors did not interact and their effects are thus shown separately (Fig. 4). Total numbers of nematodes in cultures with *M. phenylpyruvica* and *P. rettgeri* were significantly ($P < 0.001$) greater than cultures with *S. proteamaculans* (Fig. 4 A). Numbers of dauer larvae in cultures with *P. rettgeri* were significantly ($P < 0.001$) greater than in cultures with *M. phenylpyruvica* and *S. proteamaculans*. The two media based on kidney (KYO and KYEO) gave similar yields of both total nematode numbers and dauer larvae (Fig. 4 B), greater ($P < 0.001$ and $P < 0.01$ respectively) than the two soluble media (TSB & AP) (Fig. 4 B).

Discussion

In this first study of the ability of *P. hermaphrodita* to grow in monoxenic culture, *P. hermaphrodita* was found to be capable of growing in the presence of a wide range of different bacteria, but its reproductive capacity differed between bacteria. In this respect, *P. hermaphrodita* is similar to other microbivorous nematodes including the free living nematodes *Caenorhabditis elegans* (Andrew & Nicholas, 1976; Grewal, 1990), *Rhabditis terricola, Diplogaster nudicapitatus* and *Mesodiplogaster biformis* (Sohlenius, 1968), and also *Rhabditis maupasi,* a nematode associated with earthworms (Sohlenius, 1968). Entomopathogenic rhabditid nematodes grow best in the presence of their mutualistically associated *Xenorhabdus* or *Photorhabdus* spp. bacteria, but they are also able to grow with other bacteria. Boemare *et al.* (1983) grew *Steinernema carpocapsae* in monoxenic culture in *Galleria mellonella* with *Serratia liquefaciens, Enterobacter agglomerans* and *Pseudomonas fluorescens* in addition to *X. nematophilus,* and Poinar (1979) reported that *S. glaseri* was capable of growth on *Alcaligenes faecalis, Providencia rettgeri* and *Pseudomonas aeruginosa. P. hermaphrodita* did not grow in the presence of *X. bovienii,* but cells of *X. bovienii* can alter between two phases, one of which is



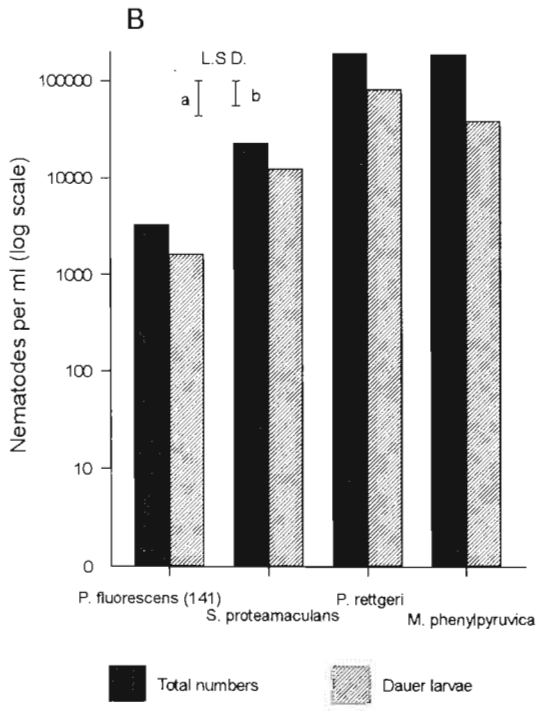
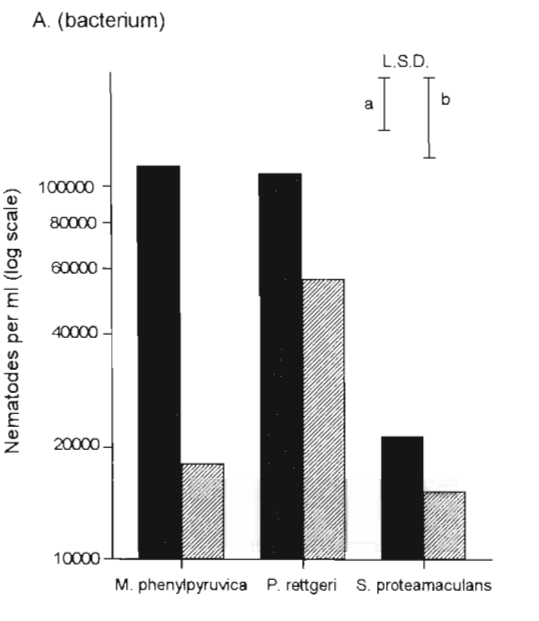


Fig. 3. *Numbers of* Phasmarhabditis hermaphrodita *(all stages) ml^{-1} and numbers of dauer larvae ml^{-1} after three weeks in 50 ml liquid KYO medium in 250 ml conical flasks rotated at 200 rpm at 15 °C in monoxenic culture with different bacteria in (A) the first and (B) the second screening experiment. L.S.D. = least significant difference for comparing* a) *total numbers and* b) *numbers of dauer larvae (P = 0.05, 9 degrees of freedom (d.f.) experiment 1 or 32 d.f. experiment 2).*



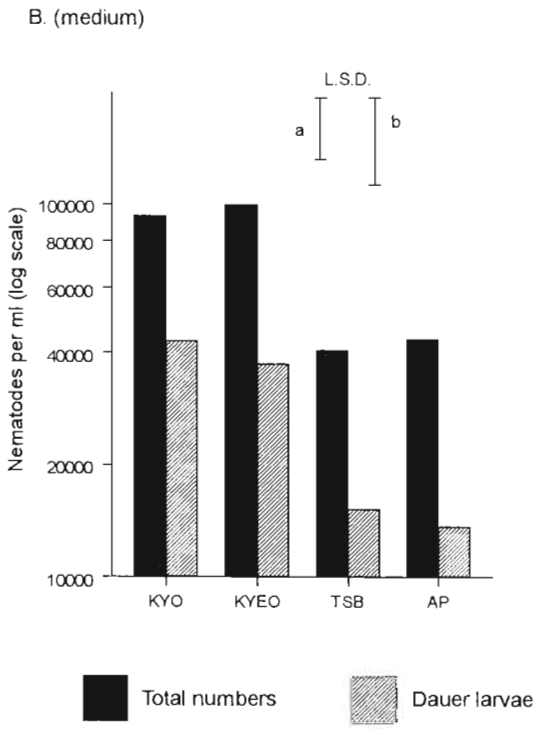


Fig. 4. *Mean numbers of* Phasmarhabditis hermaphrodita *(all stages) ml^{-1} and numbers of dauer larvae ml^{-1} after three weeks in 50 ml liquid medium in 250 ml conical flasks in monoxenic culture with (A) three bacteria* (M. phenylpyruvica, P. rettgeri *and* S. proteamaculans) *and (B) four different liquid media (KYO, KYEO, TSB and AP – see text for precise composition). L.S.D. = least significant difference (P = 0.05, 24 degrees of freedom) for comparing* a) *total numbers and* b) *numbers of dauer larvae.*

less efficient at supporting growth of entomopathogenic nematodes (Akhurst & Boemare, 1990). The phase of the *X. bovienii* culture tested with *P. hermaphrodita* was not determined.

In experiments using solid phase cultures, three species of bacteria which supported growth of *P. hermaphrodita* on kidney agar failed to support growth on nutrient agar. In this experiment the bacteria grew on the nutrient agar plates and the nematodes grew well on nutrient agar plates lawned with an unknown mix of bacteria. A similar medium/bacterium interaction has been observed before. Sohlenius (1968) found that *Escherichia coli* was capable of supporting monoxenic growth of *R. maupasi* on Nigon's agar, but not on nutrient agar. In our study it is possible that the xenic mix of bacteria contained species capable of synthesising nutrients essential for *P. hermaphrodita* from nutrient agar, whereas the three test bacterial species were not. On kidney agar, a much richer medium, it is likely that nutrients were more readily available to the nematodes in the presence of the test bacteria. Yields in liquid culture were higher in the rich kidney-based media (KYO and KYEO) than in TSB and AP media which are totally soluble and thus easier to handle in large scale fermenters.

In solid phase cultures there were no significant differences in total numbers of *P. hermaphrodita* between cultures with *P. fluorescens* isolate no. 140, *P. fluorescens* isolate no. 141, *S. proteamaculans, P. rettgeri* and *M. phenylpyruvica.* However, in liquid culture, *P. rettgeri* and *M. phenylpyruvica* both gave higher yields (> 100 000 nematodes ml^{-1}) than the other three species and *P. rettgeri* gave higher yields of dauer larvae than *M. phenylpyruvica.* The yields of dauer larvae obtained with *P. rettgeri* in these experiments were considerably greater than those achieved by Wilson *et al.* (1993 *b*) in xenic cultures of *P. hermaphrodita* grown under similar conditions in a less rich kidney-based liquid medium (approximately 85 000 ml^{-1} with *P. rettgeri* compared with approximately 15-20 000 ml^{-1} in xenic cultures).

Although these results indicate that *P. rettgeri* produces better yields of dauer larvae of *P. hermaphrodita* in liquid cultures than other bacteria tested, this does not necessarily mean that *P. rettgeri* would be the best bacterium for production of *P. hermaphrodita* as a biocontrol agent for slugs, since this study does not take account of possible differences in the quality of nematodes produced with different bacteria, in particular the ability to infect and kill slugs. It should be noted that these gnotobiotic studies were not done with the aim of identifying a specific bacterium which is mutualistically associated with *P. hermaphrodita,* as is the case with entomopathogenic nematodes, and these studies do not exclude the possible occurrence of such a bacterium.

Until now, most commercial development work on nematodes for biocontrol of invertebrate pests has concentrated on *Steinernema* spp. and *Heterorhabditis* spp. for which selection of the preferred bacterium relies on isolating the natural symbiont of these nematodes. However, in addition to *P. hermaphrodita* other microbivorous nematodes show promise as biocontrol agents for invertebrate pests, e.g. *Rhabditis necromena* to control the millipede *Spinotarsus caboverdus* (McKillup *et al.*, 1991) and *Chroniodiplogaster aerivora* (Cobb) to control white grubs, *Phyllophaga* Harris (Poprawski & Yule, 1991). If use of these nematodes is to become widespread it would be desirable to find bacteria which support optimum growth *in vitro.* It is likely that production of dauer larvae in these species will, like *P. hermaphrodita,* depend on interactions between media, bacteria and solid or liquid phase.

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