

Altered growth and development of *Steinernema carpocapsae* DD136 by *Xenorhabdus nematophilus* mutants

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Summary – The contribution of lipids to the growth and development of *Steinernema carpocapsae* DD136 in monoxenic culture with *Xenorhabdus nematophilus* ATCC 19061 using delipided Biosys egg yolk medium established that total egg yolk lipid extract and yields of infective juveniles (IJs). Phosphatidylcholine produced maximum total nematode and IJ yields at 0.05 g/100 ml. *X. nematophilus*, mutagenized with TN⁵, expressed elevated levels of lipase, reduced IJ yields and decreased the rate of nematode growth and development compared with the wild type. Medium initially conditioned by the wild type bacteria and subsequently with the mutant strain OP 1-7 produced fewer IJs but did not influence nematode growth rates compared with media with wild type bacteria only. Changes in medium total lipids, neutral and polar lipids, phosphatidylcholine and total protein did not affect nematode development. Changes in total medium carbohydrate did affect IJ yields. However, nematode development and IJ production may not be related to total carbohydrate since medium preconditioned by wild type bacteria, but subsequently supporting OP 1-7 growth, produced fewer IJs than the wild type control and both types of conditioned media contained similar carbohydrate levels.

Résumé – Modifications de la croissance et du développement de *Steinernema carpocapsae* DD 136 par des mutants de *Xenorhabdus nematophilus* – La part prise par les lipides dans la croissance et le développement de *Steinernema carpocapsae* DD 136 en culture monoxénique avec *Xenorhabditis nematophilus* ATCC 19061 a été étudiée en utilisant le milieu de culture « Biosys jaune d'œufs » délipidé. L'extrait lipidique total du jaune d'œuf et celui des phospholipides augmentent le taux de croissance et la production de juvéniles infestants (IJs). La phosphatidylcholine à une concentration de 0.05 g/100 ml, produit le maximum de nématodes et de IJs. Comparé au type sauvage, *X. nematophilus* « Tn⁵ muté » présente un niveau élevé en lipase, réduit la production de IJs et diminue le taux de croissance et le développement des nématodes. Le milieu de culture initialement conditionné par la bactérie sauvage, puis par la souche mutante OP 1-7, produit moins de IJs mais ne modifie pas le taux de croissance des nématodes. Les variations dans les quantités de lipides totaux, neutres et polaires, ainsi que de phosphatidylcholine et de protéines totales n'affectent pas le développement des nématodes. Les variations dans les hydrates de carbone totaux du milieu de culture influencent la production des IJs. Toutefois le développement des nématodes et la production des IJs ne peuvent être attribués à la quantité totale d'hydrates de carbone car le milieu de culture – préalablement conditionné par la souche bactérienne sauvage et permettant ensuite la croissance de OP 1-7 – produit moins de IJs que la souche sauvage témoin; de plus, les deux milieux de culture conditionnés contiennent des quantités similaires d'hydrates de carbone.

Key-words : *Steinernema carpocapsae*, phosphatidylcholine, *Xenorhabdus nematophilus*, transposon, infective juveniles.

The entomopathogenic Gram-negative bacterium *Xenorhabdus nematophilus* forms a mutualistic relationship with the nematode *Steinernema carpocapsae*. The non-feeding infective juvenile stage (IJ) of the nematode carries the bacteria in a vesicle associated with its alimentary tract (Bird & Akhurst, 1983). When a suitable insect is encountered, the nematode penetrates the host through the cuticle or natural orifices and enters the hemocoel where the bacteria are released (Mřáček *et al.*, 1988). The bacteria metabolize host tissues creating an environment conducive to nematode growth and reproduction.

X. nematophilus exists in two distinct phases which differ in several characteristics; the primary phase absorbs bromothymol blue and has protease, lecithinase and antimicrobial activity, whereas the secondary phase is deficient in these parameters (Boemare & Akhurst, 1988). The primary phase is associated with the nema-

tode and is necessary for optimal nematode growth and reproduction *in vitro* and on lipid-fortified nutrient agar (Akhurst, 1980, 1983; Boemare *et al.*, 1983; Dunphy & Webster, 1989). Bacterial species other than those of the genus *Xenorhabdus* influence steinernematid development; however, for *S. carpocapsae*, *X. nematophilus* is the ideal microorganism (Ehlers *et al.*, 1990; Aguilera & Smart, 1993).

There are numerous studies of the effects of dietary modification on the monoxenic culture of steinernematids (Dutky *et al.*, 1967; Wouts, 1981; Bedding, 1984; Ritter, 1988; Buecher & Popiel, 1989; Dunphy & Webster, 1989). However, the nutrients provided by the bacterial symbiont are not known although it seems likely that extracellular enzymes are involved. In the present study the novel use of Tn⁵ mutagenized *X. nematophilus* ATCC 19061, with alterations in extracellular enzyme production, will allow a more direct analysis of the bac-

terial contribution to nematode nutrition than would be possible using the phase two variant or chemically mutagenized bacteria.

Materials and methods

BACTERIAL STRAINS AND CULTURE CONDITIONS

Xenorhabdus nematophilus ATCC 19061 and its mutants were maintained on nutrient agar containing bromothymol blue (30 mg/l) and triphenyltetrazolium chloride (25 mg/l at 25 °C). Unless otherwise stated, bacterial cultures were grown in Luria broth (Xu *et al.*, 1991). Protease activity was assayed for 7 day old cultures on tryptic soy agar (TSA), Luria agar (LA) and nutrient agar (NA) supplemented with gelatin or casein (1 %, w/v). Cultures were flooded with 15 % (w/v) HgCl₂ in 20 % (v/v) HCl to detect the zones of clearance around the colonies which represent protein hydrolysis (Xu *et al.*, 1991). Lipase activity was detected using Tween 80 as a substrate (Sierra, 1957) and lecithinase and hemolytic activity on egg yolk agar and blood agar plates, respectively (Xu *et al.*, 1991).

Mutations of *X. nematophilus* were obtained by the method of Xu *et al.* (1991) using the transposon Tn5 (a 5.6 kilobase transposon coding for kanamycin resistance) with the positive selection vector pHX1. Approximately 700 putative mutants showing kanamycin resistance were screened for altered protease and lipase activity by replica plating. Three mutants showed diffuse calcium precipitates of fatty acids hydrolysed from Tween 80 compared with the tight halo associated with the wild type colonies. These mutants were called lipase overproducers (OP mutants, OP 1-7, OP 8-5 and OP 13-2). The mutants represented increased export of the enzyme into the extracellular milieu rather than an increase of the enzyme activity (unpubl.). Neither lipase negative nor protease negative mutants were obtained. The bacteria were additionally characterized for carbohydrase, protease, aminopeptidase and esterase activity using APIzym test strips (API, Plainsview, N.Y.). They had similar levels in carbohydrases, proteases, aminopeptidases but differed in esterase (OP 1-7, 10 nanomoles; OP 13-2, 20 nanomoles; OP 8-5, 5 nanomoles; wild type, 10 nanomoles) and esterase-lipase (OP 1-7, 10 nanomoles; OP 13-2, 10 nanomoles; OP 8-5, 5 nanomoles; wild type, 5 nanomoles).

Steinernema carpocapsae DD136 freshly isolated as IJs from the host insect, *Galleria mellonella* (Dunphy & Webster, 1989) were cultured monoxenically on nutrient agar enriched with sunflower oil (Wouts, 1981) to produce gravid females. Axenic eggs were obtained by dissolving the females in an alkaline hypochlorite solution (Popief *et al.*, 1989). Axenic juveniles were collected for use as inoculum after allowing the eggs to hatch overnight at 25 °C in 10 mM 4-morpholino-propane-sulfonic acid (MOPS) buffer, pH 7.0 (Sigma).

All studies were done using egg yolk medium [dry yeast, 2 g; soyflour, 4 g; dried egg yolk, 2 g; corn oil, 3.5 g; NaCl, 1 g; K₂HPO₄, 0.5 g; distilled water, 200 ml; pH 8.0 (BEM), Biosys Inc., California] and modifications thereof described elsewhere in the text. The contribution of lipids to nematode culture was determined by adding designated lipids to BEM previously rendered free of lipids by extraction with five volumes of chloroform-methanol (1:1, v/v) per gram of egg yolk. All cultures were incubated at 25 °C, the optimum temperature for nematode growth (Dunphy & Webster, 1989). Unless stated otherwise, nematode growth was assessed in terms of total nematode yields which were obtained by directly counting 25 replicates of 100 µl each per sample (100 samples of 20 µl volumes in the study on optimized phosphatidylcholine levels) of nematodes previously suspended in 10 ml of distilled water (Dunphy & Webster, 1989) and in terms of population doubling time from a graphic plot of log₁₀ of the total nematode yield per sampling time (days). The IJ levels were based upon direct counts of nematodes resistant to solubilization by 1 % (w/v) sodium dodecyl sulfate for 60 min and expressed as a percentage of the total nematode yields.

EFFECT OF BACTERIA ON NEMATODE GROWTH AND MEDIUM COMPOSITION

To determine if the mutants influenced nematode growth by either not releasing nutrients or by releasing toxins, BEM was conditioned initially by a given mutant spread on the surface of sterile filter discs (0.22 µm, Millipore) on BEM for 48h. The discs were removed and the medium subsequently inoculated with the indicated mutant (as a control) or with the wild type bacteria. Similarly, media initially conditioned by the wild type on discs were later inoculated with a test mutant strain. The yields of total nematodes and IJs were determined at designated times.

The change in BEM composition by the bacterial strains was determined using BEM as broth culture (100 ml) inoculated with bacteria (optical density of 0.1 at 600 nm) and shaken at 200 rpm on a horizontal gyrotary shaker at 25 °C for 21 days. Samples (10 ml) were removed at selected times and the bacteria removed by centrifugation (10 000 g, 4 °C, 10 min). The supernatant was analyzed for total protein (Bradford, 1976), with bovine serum albumin as a standard; total lipids using gravimetric measurements of pooled solute from five-50 ml chloroform-methanol (2:1 v/v) extracts (Ames, 1968) and total carbohydrates using the anthrone reagent with glucose as a standard (Hanson & Phillips, 1981). Total neutral lipids and phospholipids from the total lipid extracts were determined using acid-treated Florisil (BDH) chromatography (Hanson & Phillips, 1981). Phospholipids were quantified by phosphate determination (Hanson & Phillips, 1981).

Thin-layer chromatography of the conditioned media was used to determine semiquantitative changes in the neutral lipid and phospholipid classes. Lipid samples, extracted with a chloroform-methanol (2:1, v/v) solution, were stored in the solvent phase at -20°C until used. Samples were dried under nitrogen, dissolved in chloroform and analyzed by two-step one-dimensional chromatography on silica gel plates impregnated with CaSO_4 (Fisher Scientific). Phospholipids were resolved using chloroform : methanol : water (80:35:5 v/v/v) and subsequently neutral lipids were separated with a hexane:ether (4:1, v/v) solution. Plates were stained with either 2', 7' - dichlorofluorescein or Dragendorff's reagent (stains quaternary nitrogen compounds) and spots compared with authentic standards.

STATISTICAL ANALYSIS

Tabular data, except for IJ yields, were expressed as the mean \pm the standard error of the mean. IJ yields were recorded as the decoded mean of percentage data transformed as $\text{arc sin } \sqrt{p}$ (with the range of average IJ yields). Graphic data represented the mean with standard error of the mean. A minimum of eight replicas were made for each experiment, unless designated otherwise. All data were analyzed by the 95 % confidence overlap procedure (Natrella, 1972).

Results

EFFECT OF LIPIDS ON NEMATODE DEVELOPMENT

Lipid-free BEM supported low numbers of nematodes, extended the population doubling time and produced low yields of IJs (Table 1). The addition of total lipid extract from egg yolk to lipid-free BEM increased the total number of nematodes by 125 fold, and reduced the population doubling time by more than 16 fold, underscoring the importance of lipids on nematode development. Polar lipid extracts of egg yolk as opposed to neutral lipid extracts produced comparable results. The sterols, especially cholesterol, and the fat soluble vitamins enhanced nematode development by 50 to 100 fold compared with lipid-free BEM but to a lesser extent than did the polar lipid extract, the nematode yields increasing from 60 to 135 fold, depending on the supplement. Of the more common phospholipids in egg yolk, only phosphatidylcholine reduced nematode growth rates and increased nematode yields to the levels obtained with the polar lipid extract (Table 1) establishing phosphatidylcholine as a major nutrient in the monoxenic culture of the steinernematid. Interestingly, phosphatidylcholine also reduced the variation in total nematode and IJ yields. The optimum concentration of phosphatidylcholine for maximum total nematode and IJ yields was 0.05 g/100 ml (Fig. 1). The concentration of the lipid had a greater impact on the IJ yield above the optimum concentration than on total nematode levels, reducing IJ yields more extensively (by more than 70 %)

Table 1. Effect of lipids on the growth and development of *Steinernema carpocapsae* DD136 at 10 day in monoxenic culture on Biosys medium containing delipided egg yolk^(a).

Lipid	Population doubling time (days)	Total nematodes (pet plate $\times 10^3$)	IJ yield (%) ^(c)
Lipid-free medium	$56.2 \pm 0.2^{(b)d}$	102 ± 322^h	29(0-65) <i>k</i>
Egg yolk total lipid extract	3.4 ± 0.1^e	$12\,737 \pm 112^i$	76(35-81) <i>l</i>
Total polar egg yolk lipid extract	3.9 ± 0.1^e	$15\,521 \pm 123^i$	87(42-85) <i>l</i>
Total neutral egg yolk lipid extract	4.7 ± 0.7^f	$8\,712 \pm 100^h$	34(28-71) <i>k</i>
Phosphatidylcholine	3.4 ± 0.1^e	$13\,722 \pm 120^i$	81(61-92) <i>l</i>
Phosphatidylethanolamine	7.6 ± 0.1^g	$6\,512 \pm 260^j$	22(0-70) <i>k</i>
Phosphatidylserine	7.5 ± 0.2^g	$6\,228 \pm 100^j$	27(0-63) <i>k</i>
Phosphatidylinositol	7.3 ± 0.2^g	$5\,833 \pm 100^j$	25(15-75) <i>k</i>
Cholesterol	2.6 ± 0.2^h	$10\,003 \pm 330^h$	51(32-75) <i>m</i>
β -sitosterol	7.5 ± 0.3^g	$6\,216 \pm 800^j$	48(28-60) <i>m</i>
Ergosterol	7.9 ± 0.2^g	$5\,833 \pm 760^j$	25(15-75) <i>k</i>
Fat soluble vitamins ^(d)	7.1 ± 0.1^g	$6\,710 \pm 820^j$	23(18-22) <i>k</i>

^a Values with the same letter are not significantly different, $P > 0.05$.

^b Mean \pm standard error of the mean, $n \geq 10$ samples.

^c Decoded mean of $\text{arc sin } \sqrt{p}$ transformed data (range averages) of infective juvenile yields expressed as a percentage of the total nematode yields, $n \geq 10$ samples.

^d Pooled values of the vitamins A, E and K.

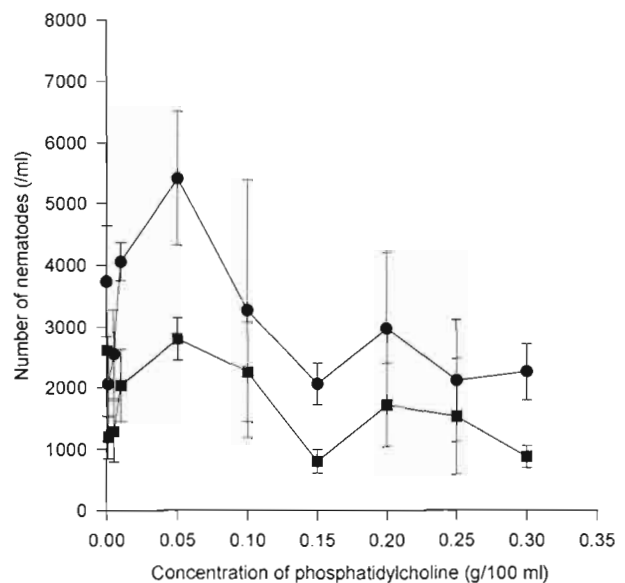


Fig. 1. Effect of phosphatidylcholine on the growth (●) and infective juvenile formation (■) of *Steinernema carpocapsae* DD136 in monoxenic culture on Biosys egg yolk medium. Points represent the mean \pm the standard error of the mean, $n \leq 8$ replicates.

Table 2. Influence of phosphatidylcholine derivatives on the growth and development of *Steinernema carpocapsae* DD136 during 10 day monoxenic culture on Biosys medium containing delipided egg yolk^(a)

Derivative	Total nematodes (per plate × 10 ³) ^(b)	IJ yield (%) ^(c)
Phosphatidylcholine dipalmitoyl	4 962 ± 700 c	54(22-81) f
Phosphatidylcholine dioleoyl	10 649 ± 320 c	73(52-93) g
Phosphatidylcholine distearyl	9 688 ± 312 d	49(36-64) f
Mix of the esters	13 474 ± 116 d	56(34-89) f
Phosphatidylcholine standard	12 672 ± 262 d	75(60-72) g
Choline	9 925 ± 275 d	67(31-72) g
Lipid-free medium	131 ± 151 e	23(33-98) h

^a Values with the same letter are not significantly different, $p > 0.05$.
^b Mean ± standard error of the mean, $n \geq 10$ samples.
^c Decoded mean of $\arcsin \sqrt{p}$ transformed data (range of averages) of infective juveniles yields expressed as a percentage of the total nematode yields, $n \geq 10$ samples.

than total nematode levels (by approximately 60%). The growth of the nematodes was not as sensitive to phosphatidylcholine in BEM that contained its normal complement of lipids [BEM, total nematodes = $1.2 \times 10^7 \pm 0.7 \times 10^7$ /plate; BEM + phosphatidylcholine (0.05 g/100 ml), total nematodes = $9.6 \times 10^6 \pm 1.1 \times 10^6$ /plate] as it was to choline supplementation (total nematodes = $3.7 \times 10^6 \pm 0.2 \times 10^6$). However, the level of IJs was substantially reduced by both phosphatidylcholine (IJ = 21% (15-72%)) and choline (IJ = 12% (0-61%)) compared with BEM (IJ = 83% (41-87%)).

Phosphatidylcholine is a class of lipids in which individual molecular species are esterified to a myriad of different fatty acids (Kuksis, 1992). The types of individual homogeneous fatty acid esters increased total nematode levels and IJ yields and reduced variation in IJ yields compared to those on lipid-free medium (Table 2). However, only the dioleoyl and distearyl esters produced IJ yields comparable to phosphatidylcholine mix made from individual esters. The mixture of the esters also produced high total nematode yields but low IJ yields. Choline alone produced total nematode and IJ yields similar to the standard phosphatidylcholine supplement.

EFFECT OF XENORHABDUS MUTANTS ON NEMATODE DEVELOPMENT

Nematodes grown with the wild type bacteria showed a continuous increase in number over a period of

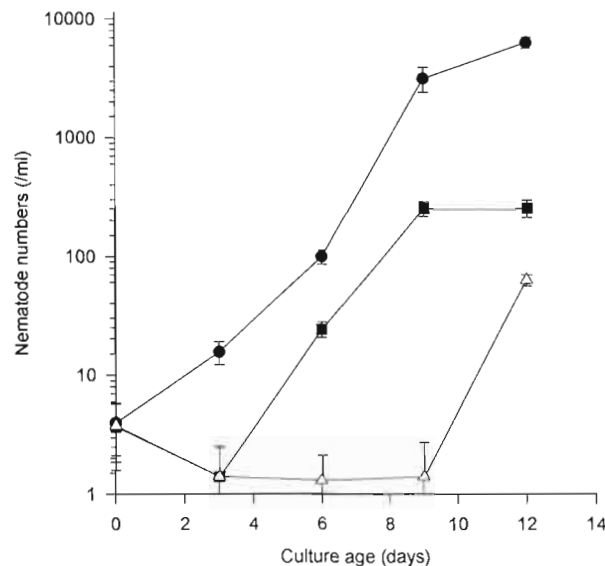


Fig. 2. The effect of wild type (●) and mutant OP 1-7 (■) and OP 13-2 (Δ) *Xenorhabdus nematophilus* on the monoxenic culture of *Steinernema carpocapsae* DD136 on Biosys egg yolk medium. Points represent the mean ± the standard error of the mean, $n = 10$ replicates.

12 days (Fig. 2). Nematodes cultured with OP 1-7 and OP 8-5 exhibited a comparable but 50% decrease in yield during the first 3 days and thereafter increased continuously. OP 13-2 caused a 70% decrease in nematode numbers during the initial 9 days of culture after which time the levels increased. The data implied that the metabolism of the mutants had been altered which may have influenced medium composition.

BEM conditioned by the mutants supported slower nematode growth as well as low and highly variable IJ yields compared with the wild type (Table 3). Media initially conditioned by the wild type bacteria and subsequently by the mutants supported growth rates of the juvenile stages and population doubling times comparable to media conditioned by the wild type bacteria alone but produced fewer IJs. However, the IJ yields were approximately double those on media conditioned by the OP mutants alone. Conditioning BEM with the mutants and then with the wild type produced comparable results (data not shown). Thus the mutants may have altered the composition of BEM reducing IJ formation.

The biochemical and physiological studies implied that isolates OP 1-7 and OP 8-5 were similar, although analysis of genomic DNA with a Tn5 probe indicated that the insert pattern was different (unpubl.). Because of this complexity, OP 8-5 was omitted from further study. Mutant OP 13-2 was also omitted from further study but due to its instability.

The wild type bacteria removed in excess of 80% of the total carbohydrates from BEM within 5 days of inoculation, whereas OP 1-7 did not reduce total carbo-

Table 3. Development of *Steinernema carpocapsae* DD136 on Biosys medium conditioned by individuals or combinations of wildtype and lipase overproducing mutants of *Xenorhabdus nematophilus*^a.

Bacteria	Rate of development (J1-J4, days) ^(b)	Population doubling time (days) ^(c)	IJ yields (%) ^(c)
Wildtype	1.2 ± 0.1 d	3.6 ± 0.1 g	70(50-78) g
OP 1-7	3.2 ± 0.2 e	7.1 ± 0.3 h	20(15-80) h
OP 8-5	2.5 ± 0.3 e	6.9 ± 0.2 h	31(18-85) h
OP 13-2	2.5 ± 0.2 e	15.2 ± 0.2 g	4(0-21) i
Wildtype replaced with wild-type	1.1 ± 0.2 d	3.3 ± 0.2 i	75(53-81) g
Wildtype replaced with OP 1-7	1.7 ± 0.2 f	3.9 ± 0.3 i	45(11-65) h
Wildtype replaced with OP 8-5	1.7 ± 0.2 f	4.1 ± 0.2 i	69(65-81) g
Wildtype replaced with OP 13-2	1.8 ± 0.2 f	3.9 ± 0.3 i	53(40-82) g

^a Values with the same letter are not significantly different, $P > 0.05$.

^b Mean ± standard error of the mean, $n \geq 10$.

^c Decoded mean of arc sin \sqrt{p} transformed data (range of averages) of infective juvenile yields expressed as a percentage of the total nematode yields, $n \geq 10$ samples.

hydrate levels over 21 days (Fig. 3). To test the assumption that the availability of a metabolizable sugar either directly or indirectly influenced nematode growth, the OP 1-7 conditioned medium was supplemented with 0.5 g/l of d/glucose. The medium produced total nematode yields ($15\,522 \pm 103$ nematodes/plate) comparable to medium conditioned by the wild type ($17\,237 \pm 320$ nematodes/plate) but did not enhance IJ levels (OP 1-7 conditioned medium = 22% (14-76) and wild type conditioned medium = (53-79) 68%).

Both strains removed about 60% of the total protein from the medium within 9 days and the wild type continued to remove protein for 21 days (Fig. 3). The OP 1-7 isolate, unlike the wild type, increased medium total protein within 10.5 days of incubation to a plateau level. Both strains removed more than 80% of the total lipids from the medium within 12 days, followed thereafter by an increase in total lipid. These changes parallel those of neutral and polar lipids (data not shown). The analysis of the total lipid extract revealed no semiquantitative differences in the extent of reduction in cholesterol, and phosphatidylethanolamine content in media conditioned by the isolates. However, the wild type totally removed detectable phosphatidylcholine from the medium by 2 days and the mutant by 5 days postinoculation

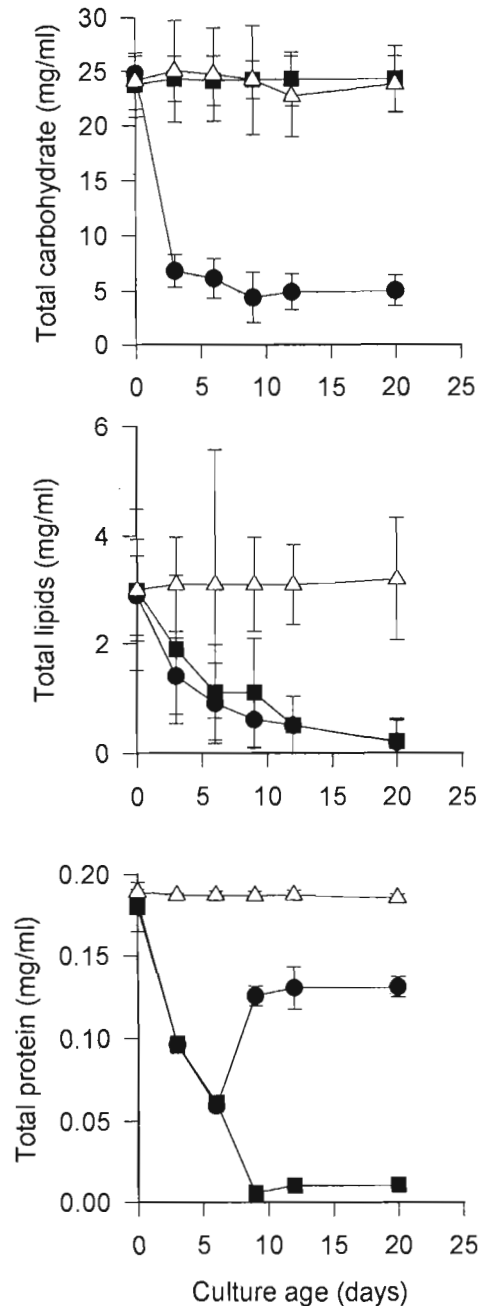


Fig. 3. Changes in total carbohydrates, lipids, and proteins of Biosys medium during the growth of the wild type (Δ) and OP 1-7 mutant (\blacksquare) compared with uninoculated control (\bullet) medium. Points represent the mean ± the standard error of the mean, $n = 10$. The change in total carbohydrates caused by OP 1-7 were not significantly different from the control values and are not shown.

(data not shown). In both cases, phosphatidylglycerol was detected in conditioned media at these times but not in uninoculated medium.

Discussion

Lipids are essential for the growth and reproduction of entomopathogenic nematodes (Gordon *et al.*, 1982; Dunphy & Webster, 1989), the infectivity of host insects by IJs of steinernematid (Vanninen, 1990) and IJ storage success (Selvan *et al.*, 1993 *a, b*). BEM contains egg yolk as a source of lipids, which were found to be essential for the growth, reproduction and IJ formation of *S. carpocapsae*, the absence of lipids being associated with low total nematode and IJ yields. The latter effect may be due to the IJ-inducing pheromone (Popiel *et al.*, 1989) being either bound to the agar and thus unavailable for activity or being diluted on the large surface area of the Petri dish. Egg yolk lipids could be replaced by a mixture of polar lipids and either cholesterol or phosphatidylcholine. Cholesterol is known to support the monoxenic culture of *S. carpocapsae* and *Heterorhabditis bacteriophora* (Ritter, 1988; Dunphy & Webster, 1989). Phosphatidylcholine supported the highest yields of IJs and reduced yield variability obtained with egg yolk lipids. In view of the absence of cholesterol and phosphatidylcholine among bacteria of the Enterobacteriaceae, the nematodes must either directly or indirectly utilize host sterols. The observation that *X. nematophilus* hydrolyses phosphatidylcholine suggests that the effect of the lipid on nematode growth may be due to the release of choline and/or fatty acids. Both phosphatidylcholine and choline (this study; Dunphy & Webster, 1989) and acetylcholine and the fatty acids stearic acid and oleic acid (at levels available in the phosphatidylcholine supplement) enhanced the monoxenic culture of *S. carpocapsae* (Dunphy & Webster, 1989). Phosphatidylcholine *per se* is not required for nematode growth, but can be replaced with choline. Choline is commonly found in egg yolk, corn oil, yeast and soyflour (Zeisel, 1990). The present results may indicate that the nematodes require higher levels of choline or a metabolic product related to choline than is provided by the normal growth medium.

The overproducing lipase/esterase mutants of *X. nematophilus* reduced both the rate of nematode development and IJ yields. Medium conditioning studies using sequential combinations of mutant and wild type bacteria implied that the effects were due to the mutants removing nutrients from the medium or releasing metabolites toxic to the nematodes. The wild type, as opposed to the OP 1-7 strain, removed carbohydrate from the medium yet the strains did not differ in the presence of α -glucosidase and N-acetyl-D-glucosamidase activity or the absence of α - or β -galactosidase, β -glucuronidase, β -glucosidase, α -mannosidase and α -fucosidase activity. Although the cause of the change in total carbohydrates is not known, monosaccharides are known to enhance steinernematid growth (Dunphy & Webster, 1989). In the present study *d*-glucose increased nematode development in medium conditioned by the OP 1-7 strain but did not elevate IJ yields. Popiel *et al.*

(1989) reported that soluble nutrients reduced IJ formation by preventing the production of the IJ-inducing pheromone under starvation and crowded conditions.

Both the wild type and OP 1-7 mutant decreased the total lipid, neutral and phospholipids during the initial 10.5 days of incubation in a manner not correlated with nematode growth. However, the mutant removed phosphatidylcholine more slowly than did the wild type. In view of the toxicity of the phospholipid to the nematodes, it is possible the different growth profile of the nematodes on media conditioned by the OP 1-7 strain represented phosphatidylcholine toxicity.

The total protein of the medium declined to equal levels in media conditioned by both the mutant and wild type and thus did not appear to be related to nematode development. The protein content of OP 1-7 conditioned medium, like the total lipids in the media containing both bacterial isolates, increased after 12 to 15 days of incubation. The nature of the increase is unknown but may have resulted from cell lysis. It is important to stress that in these studies no nematodes were present. Whether the same effect occurs in the presence of the nematodes is unknown.

In summary phosphatidylcholine, choline and cholesterol enhanced the growth rate and IJ yields of *S. carpocapsae* on BEM. The phospholipid also reduced the variability of IJ yields. Medium conditioning studies using lipase/esterase overproducing mutants of *X. nematophilus* revealed poor nematode growth until phosphatidylcholine was metabolized.

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References

- AGUILLERA, M. M. & SMART, G. C. JR. (1993). Development, reproduction and pathogenicity of *Steinernema scapterisci* in monoxenic culture with different species of bacteria. *J. Invert. Path.*, 62 : 289-294.
- AKHURST, R. J. (1980). Morphological and functional dimorphism in *Xenorhabdus* spp. bacteria symbiotically associated with the insect nematodes *Neoplectana* and *Heterorhabditis*. *J. gen. Microbiol.*, 121 : 303-309.
- AKHURST, R. J. (1983). *Neoplectana* species : Specificity of association with bacteria of the genus *Xenorhabdus*. *Expl Parasit.*, 55 : 258-263.
- AMES, G. F. (1968). Lipids of *Salmonella typhimurium* and *Escherichia coli* : Structure and metabolism. *J. Bacteriol.*, 95 : 833-843.
- AYMERIE, J.-L. & BOEMARE, N. E. (1990). Physiology and genetics of *Xenorhabdus nematophilus*. *Proc. Vth int. Colloq. Invert. Path. & microb. Control., Adelaide, Australia* : 1-7.
- BEDDING, R. A. (1984). Large scale production, storage and transport of the insect parasitic nematodes *Neoplectana* spp. and *Heterorhabditis* spp. *Ann. appl. Biol.*, 104 : 117-120.

- BIRD, A. F. & AKHURST, R. J. (1983). The nature of the intestinal vesicle in nematodes of the family Steinernematidae. *Int. J. Parasit.*, 13 : 599-606.
- BOEMARE, N. E. & AKHURST, R. J. (1988). Biochemical and physiological characterization of colony form variants in *Xenorhabdus* (Enterobacteriaceae). *J. gen. Microbiol.*, 134 : 751-756.
- BOEMARE, N. E., BONIFASSI, E., LAUMOND, C. & LUCIANI, J. (1983). Étude expérimentale de l'action pathogène du nématode *Neoplectana carpocapsae* Weiser; recherches gnotobiologiques chez l'insecte *Galleria mellonella*. *Agronomie*, 3 : 407-415.
- BRADFORD, M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analyt. Biochem.* 72 : 248-254.
- BUECHER, E. & POPIEL, I. (1989). Growth of *Steinernema feltiae* in liquid culture. *J. Nematol.*, 21 : 500-504.
- DUNPHY, G. B. & WEBSTER, J. M. (1989). Monoxenic culture of *Neoplectana carpocapsae* and *Heterorhabditis heliothidis*. *Revue Nématol.*, 12 : 113-123.
- DUTKY, S. R., ROBBINS, W. E. & THOMPSON, J. V. (1967). The demonstration of sterols as requirements for the growth, development, and reproduction of the DD-136 nematode. *Nematologica*, 13 : 140.
- EHLERS, R.-U., STOESSEL, S. & WYSS, U. (1990). The influence of phase variants of *Xenorhabdus* spp. and *Escherichia coli* (Enterobacteriaceae) and *Heterorhabditis*. *Revue Nématol.*, 13 : 417-424.
- GORDON, R., BURFORD, J. R. & YOUNG, T.-L. (1982). Uptake of lipids by the entomophilic nematodes. *J. Nematol.*, 14 : 492-495.
- HANSON, R. S. & PHILLIPS, J. A. (1981). Chemical composition. In: Gerhardt, P., Murray, R. G. E., Costilow, R. N., Nester, E. W., Wood, W. A., Krieg, N. R. & Phillips, G. B. (Eds). *Manual of methods for general bacteriology*. Washington, D.C., American Society for Microbiology : 328-364.
- KAYA, H. K. & GAUGLER, R. (1993). Entomopathogenic nematodes. *Ann. Rev. Entomol.*, 38 : 181-206.
- KUKSIS, A. (1992). Yolk lipids. *Biochim. Biophys. Acta*, 1124 : 205-222.
- MRAČEK, Z., HANZALYAUD R. & KODRIK, D. (1988). Sites of penetration of juvenile steinernematids and heterorhabditids (Nematoda) into the larvae of *Galleria mellonella* (Lepidoptera). *Invert. Path.*, 52 : 477-478.
- MORRISON, A. H. & RITTER, K. S. (1986). Effect of host insect sterols on the development and sterol composition of *Steinernema feltiae*. *Molec. Biochem. Parasit.*, 19 : 135-142.
- NATRELLA, M. G. (1972). The relation between confidence intervals and tests of significance. In: Kirk, R. E. (Ed.) *Statistical issues*. Monterey, USA, Brooks/Cole Publ. Co. : 113-117.
- POPIEL, I., FRIEDMAN, M. J. & GROVE, D. (1989). Infective juvenile formation in the insect parasitic nematode *Steinernema feltiae* strain All. *Parasitology*, 99 : 77-81.
- RITTER, K. S. (1988). *Steinernema feltiae* (= *Neoplectana carpocapsae*) : Effect of sterols and hypolipidemic agents on development. *Exp. Parasit.*, 67 : 257-267.
- SELVAN, S., GAUGLER, R. & GREWAL, P. S. (1933 a). Water content and fatty acid composition of infective juvenile entomopathogenic nematodes during storage. *J. Parasitol.*, 79 : 510-516.
- SELVAN, S., GAULER, R. & LEWIS, E. E. (1993 b). Biochemical energy reserves of entomopathogenic nematodes. *J. Parasitol.*, 79 : 167-172.
- SIERRA, G. (1957). A simple method for the detection of lipolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. *Antonie van Leeuwenhoek J. Microbiol. Serol.*, 23 : 15-22.
- STADLER, M., ANKE, H. & STERNER, O. (1993). Linoleic acid - The nematicidal principle of several nematophagous fungi and its production in transforming submerged cultures. *Arch. Mikrobiol.*, 160 : 401-405.
- VANNINEN, I. (1990). Depletion of endogenous lipid reserves in *Steinernema feltiae* and *Heterorhabditis bacteriophora* and effect on infectivity. *Proc. Vth int. Coll. Invert. Pathol. & microb. Control.*, Adelaide, Australia : 232.
- WOUTS, W. M. (1981). Mass production of the entomogenous nematode *Heterorhabditis heliothidis* (Nematoda : Heterorhabditidae) on artificial media. *J. Nematol.*, 13 : 467-469.
- XU, J., OLSON, M. E., KAHN, M. L. & HURLBERT, R. E. (1991). Characterization of Tn⁵-induced mutants of *Xenorhabdus nematophilus* ATCC 19061. *Appl. Envir. Microbiol.*, 57 : 1173-1180.
- ZEISEL, S. H. (1990). Biological consequences of choline deficiency. *Nutrition & Brain*, 8 : 75-99.