The monoxenic culture of *Neoaplectana carpocapsae* DD 136 and *Heterorhabditis heliothidis*

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SUMMARY

A series of experiments were done to determine the optimum physical and chemical components of the medium for maximum production of *Neoaplectana carpocapsae* DD136 and *Heterorhabditis heliothidis*. The associated bacteria, *Xenorhabdus nematophilus nematophilus* and *X. luminescens* of both nematode species had an optimum temperature for growth of 30°. However, maximum yields of *N. carpocapsae* DD136 and *H. heliothidis* in monoxenic culture occurred at 25° and 30°, respectively. In MOPS-buffered media *X. nematophilus nematophilus* and *X. luminescens* grew best at pH 6.5 and pH 6.5-7.5, respectively. *N. carpocapsae* and *H. heliothidis* cultured best at pH 6.0 and pH 6.5-7.5 respectively, using 2-[N-morpholino]ethanesulfonic acid-buffered media. Although nematode yields varied with the nematode species and type of lipid, both *N. carpocapsae* and *H. heliothidis* levels were stimulated by stearic acid. Industrial lipid sources supporting nematode culture included sunflower oil (0.1 % V/V) for *N. carpocapsae* and cod liver oil (0.5 % V/V) for *H. heliothidis*. Tryptic soy broth (15 g/l) and yeast extract (5 g/l) enhanced the yields of *N. carpocapsae* and *H. heliothidis*, respectively. D-glucose (14 mM) supported the growth of both nematode species. *N. carpocapsae* culture also was enhanced by D-fructose and D-galactose, whereas, D-sorbose and D-mannitol favoured *H. heliothidis* development at 14 mM levels. Both nematode species grew well with corn syrup as the carbon source 4 g/l for *N. carpocapsae* and 10 g/l for *H. heliothidis*. Vitamin supplements did not improve yields of these nematode species in monoxenic culture. *H. heliothidis* production was enhanced by magnesium chloride (10 mM), potassium chloride (10 mM) and potassium nitrate (1 mM).

RÉSUMÉ

La culture monoxénique de Neoaplectana carpocapsae *DD136*
et Heterorhabditis heliotidis

Une série d'expériences visait à déterminer les composantes physiques et chimiques optimales du milieu permettant une production maximale de *Neoaplectana carpocapsae* DD136 et de *Heterorhabditis heliothidis*. Il a été constaté que *Xenorhabdus luminescens* et *X. nematophilus nematophilus*, bactéries associées aux deux espèces de nématodes, ont une température de croissance optimale à 30°. Cependant, le rendement maximal de *N. carpocapsae* DD136 et *H. heliothidis* en élevage monoxénique est atteint à 25° et 30°, respectivement. Dans les milieux tampons MOPS, *X. nematophilus nematophilus* et *X. luminescens* ont un développement maximal lorsque les pH sont de 6,5 et de 6,5 à 7,5, respectivement. On a remarqué une multiplication maximale de *N. carpocapsae* et *H. heliothidis* à des pH de 6,0 et de 6,5 à 7,5 respectivement, lorsqu'on utilise des milieux tampons à l'acide 2-[N-morpholino] ethanosulfonate. Bien que les rendements des élevages de nématodes varient selon l'espèce et le type de lipide, ceux-ci sont stimulés par l'acide stéarique. Les sources de lipides industriels favorisant l'élevage des nématodes comprennent l'huile de tournesol (0,1 % V/V) pour *N. carpocapsae* et l'huile de foie de morue (0,5/5 V/V) pour *H. heliothidis*. Le bouillon de soja tryptique (15 g/l) et l'extrait de levure (5 g/l) augmentent le rendement de *N. carpocapsae* et *H. heliothidis*, respectivement. Le D-glucose (14 mM) entretient la croissance des deux espèces. Le D-fructose et le D-galactose sont également favorables à l'élevage de *N. carpocapsae*, alors qu'un taux de 14 mM de D-sorbose, et le D-mannitol, sont favorables au développement de *H. heliothidis*. Les deux espèces de nématodes se développent bien lorsque leur source de carbone est constituée de sirop de maïs (4 g/l pour *N. carpocapsae* et 10 g/l pour *H. heliothidis*). Des suppléments en vitamines n'améliorent pas le rendement des élevages monoxéniques des nématodes. En revanche, la production de *H. heliothidis* est améliorée par addition de chlorure de magnésium (10 mM), de chlorure de potassium (10 mM) et de nitrate de potassium (1 mM).

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The entomogenous nematodes *Neoaplectana carpocapsae* end *Heterorhabditis heliothidis* are of considerable interest as biological insecticides because of their ease of culture, rapid action, environmental safety (Bedding, 1981; Morris, 1985) and chemical pesticide tolerance (Poinar, 1979). Steinernematids can be cultured, in either a host insect (Dutky, Thompson & Cantwell, 1964) or on synthetic diet. In the latter case, nematode growth and reproduction may occur axenically (Stoll, 1948; Hansen *et al.*, 1968) or in the presence of its bacterial associate *Xenorhabdus* spp. (Bedding, 1981; Wouts, 1981) i.e., monoxenically. Under natural conditions the bacterium is vectored in the nematode gut (Bird & Akhurst, 1983), and is released into the insect hemocoel where it establishes conditions favorable for nematode growth and reproduction (Poinar & Thomas, 1966).

Monoxenic mass culture techniques with the goal of producing, cost effectively, large numbers of infective nematodes have been used for several years (Bedding, 1976, 1981, 1982, 1984; Hara, Lindegren & Kaya, 1981). However, the effects of dietary components on nematode culture under monoxenic conditions are insufficiently known to enable optimum culture of the nematodes. Dütky, Thompson and Cantwell (1967) documented the effects of sterols on *N. carpocapsae* DD136. Bedding (1976, 1981, 1984) described a range of animal-based substrates which *Xenorhabdus* spp. are able to convert into media suitable for nematode reproduction. Wouts (1981) ascertained the effect of selected oils on the production of *H. heliothidis* in culture. The present study examines the effects of selected physical factors and nutritional components of the diet on the growth and reproduction of *N. carpocapsae* DD136 and *H. heliothidis*. The data is used to formulate media appropriate for the industrial mass production of these two nematode species.

Material and methods

Cultures

Bacteria

The primary form of *Xenorhabdus nematophilus* subsp. *nematophilus* and *X. luminescens* (Akhurst, 1986) were isolated from *Neoaplectana carpocapsae* DD136* and *Heterorhabditis heliothidis*, respectively, on triphenyltetrazolium chloride supplemented tergitol-7-agar (Dunphy & Webster, 1985). Bacteria were sub-cultured at 14 d intervals and maintained at 25° on tergitol-7-agar.

Nematodes

Dauer stages of *N. carpocapsae* DD136 strain and of *H. heliothidis* NC15 were isolated from *Galleria mellonella* larvae on White's water traps, gravity washed three times with sterile, distilled water and surface sterilized with 0.1 % Thimersol (Dunphy & Webster, 1985). After washing twice in sterile, distilled water, 0.1 ml aliquots containing 1 × 10^3 nematodes were added to lipid agar in Petri dishes (Wouts, 1981) that had been inoculated 24 h previously with the nematode's corresponding bacterium to produce a confluent bacterial lawn (Dunphy & Webster, 1985). The nematodes were subcultured on bacterial laden lipid agar at 21 d intervals and incubated at 25°. New monoxenic cultures were initiated every two months using dauer juveniles.

Experimental procedures

Inoculum preparations

Bacterial inocula were produced by adding one loopful of 24 h old, stock bacterial culture to 250 ml of lipid broth (= lipid agar without the agar) in 500 ml capacity Erlenmeyer flasks. Cultures were shaken at 100 rpm on a horizontal, gyrotary shaker at 25° for 48 h.

Third stage juvenile nematodes were collected from monoxenic cultures, gravity washed in distilled water, disinfected in Thimersol and rinsed with sterile, distilled water (Dunphy & Webster, 1985).

Physical parameters

The optimum temperature, buffer type and pH of pure cultures of each bacterial species was determined by adding 0.1 ml of the bacterial inoculum to 10 ml of lipid broth in 20 ml sterile, polypropylene test tubes and incubating at 10-35°. Bacterial growth was measured spectrophotometrically at 540 nm at 8 h. Exponential growth occurred for more than 15 h under all treatment conditions.

To determine the optimum buffer type for bacterial cultures one of the following buffers, namely 2-[N-morpholino]ethanesulfonic acid (MES), 3-[N-morpholino]propanesulfonic acid (MOPS), N-[2-hydroxyethylpiperazine]-N -2- ethanesulfonic acid (HEPES) and bis [2-hydroxyethyl]imino-tris-[hydroxymethyl]methane : 2-bis [-hydroxyethyl]amino-2-[hydroxymethyl]-1,3- propanediol (Bis-Tris) were added, at 10 mM concentration to lipid broth cultures on a shaker (100 rpm). The pH of the broth was adjusted within the range of 5.5-8.0 with 1N HCl or 1 N KOH. The bacterial cultures were incubated at their respective optimum temperatures.

The same physical parameters were adjusted and assayed so as to maximize the monoxenic culture of *N. carpocapsae* and *H. heliothidis*. Petri dishes containing

* We have accepted the recommendation of Dr. G. O. Poinar that the name *Neoaplectana carpocapsae* is preferable to *Steinernema feltiae* (see Poinar, 1984).

30 ml of lipid agar previously inoculated with bacteria were incubated at specified temperatures and pH values 24 h prior to inoculation with nematodes. Preliminary pH measurements showed no change in medium pH during this time. Nematodes were harvested after 10 d of incubation.

*Nutritional study**

All experiments were conducted in 9 cm diameter Petri dishes of lipid agar. Based on nematode reproduction in specificed supplemented agar, industrial media containing growth promoting nutrients were selected for their possible ability to support monoxenic nematode culture.

The industrial nutrient most conducive to nematode reproduction for a given experiment was incorporated into the basal medium which will be referred to subsequently as modified medium. The modified medium then would be used for the next nutrient test.

Oils : Both *N. carpocopsae* and *H. heliothidis* were grown on the basal medium containing sunflower oil (Safflo, Concord, Ontario) supplemented with selected fatty acids, sterols or phospholipids at 0.03 mM. Oils screened included butter, halibut oil, cod liver oil, olive oil and corn oil at 0.1 ml to 1.0 ml per 100 ml of basal medium.

Nitrogen sources : Industrial fermentation products (= complex nitrogen sources) (e.g., lactalbumin hydrolysate, fish meal, yeast extract, casein), standard microbiological media [e.g., peptone, nutrient broth (Difco)] and tissue culture media [e.g., heat inactivated fetal calf serum (GIBCO, Burlington, Ont.), amino acids from Grace's insect tissue culture medium (Grace, 1962)] were compared with the modified medium containing sunflower oil at the optimum concentration of 0.1 ml/100 ml of medium. Nutrient broth was deleted from the media supplemented with complex nitrogen sources to prevent masking the effects of the nitrogen supplements. All nitrogen sources were adjusted to give 2.5 g of total nitrogen per liter. The optimum concentration of the most effective nitrogen source was determined and subsequently, replaced the nutrient broth source of the modified medium.

Carbohydrates : The monosaccharides D-fructose, D-glucose, D-galactose and D-sorbose; the disaccharides sucrose, maltose and trehalose; and the alcohols glycerol and D-mannitol were added separately in tests to the modified media to produce 14 mM total carbon levels. The industrial carbon sources molasses, corn syrup, cornstarch, malt extract and soluble starch were screened for their ability to support monoxenic nematode culture.

* Unless otherwise stated all chemical were purchased from Sigma (St. Louis, Mo, USA).

Vitamins : The modified media were individually suplemented with 0.1 or 1.0 mg/l of ascorbic acid, D-pantothenic acid, riboflavin, nicotinamide, biotin, m-inositol, choline chloride, folic acid, p-aminobenzoic acid. Also tested were combinations of these vitamins, the concentration of each vitamin being 0.1 mg/l or 1.0 mg/l. The vitamins were filter-sterilized in 0.2 μm Nalgene filters and aseptically added to the media at 45°.

Salts : The effects of selected salts were tested for *H. heliothidis* culture. Cation levels of 1.0 and 10 mM were separately added to the modified media. The pH of the media were adjusted to 6.5 prior to autoclaving.

Harvesting and data analysis : All nematode cultures were harvested 10 d post inoculation by flooding the Petri dishes with 10 ml of distilled water. Cultures were incubated at 25° for 10 min to dislodge juvenile nematode from the agar-bacterial matrix. A combination of scraping with a rubber policeman and pipetting ensured nematode dispersion (Dunphy, Rutherford & Webster, 1985). The total collective number of all stages of nematodes per dish was estimated based upon the average number of nematodes in five aliquot samples of 100 μl of suspension per dish.

A minimum of four replicates were used for each treatment. Data were analyzed using Natrella's (1972) 95 % confidence overlap procedure.

Results

Optimum physical parameters

Both *X. nematophilus nematophilus* and *X. luminescens* showed most rapid growth at the higher temperatures (Fig. 1). However, the temperature responses differed with the bacterial species in that *X. luminescens* grew more slowly and exhibited a more restricted profile than *X. nematophilus nematophilus.*

In monoxenic culture both nematode species exhibited sharp temperature optima; *N. carpocapsae* at 25° and *H. heliothidis* at 30° (Fig. 2). *H. heliothidis* did not grow at or below 15° and both species grew poorly at 35°.

X. nematophilus nematophilus was sensitive to buffer type and to pH, and only MOPS buffer permitted growth at pH 6.5 to the level of the unbuffered medium (Fig. 3). The degree of response of *X. luminescens* to pH also varied with the type of buffer. MOPS buffer resulted in a broad pH curve with optimum values of pH 6.5 to 7.5 for *X. luminescens,* and HEPES produced a sharper profile with an optimum pH of 7.0. The other buffers were extremely toxic to both bacterial species.

The least toxic buffers for both nematode species were BIS-TRIS and MOPS. The optimum pH of the monoxenic culture varied with the buffer type and the nematode-bacterium culture (Fig. 4). The greatest yields

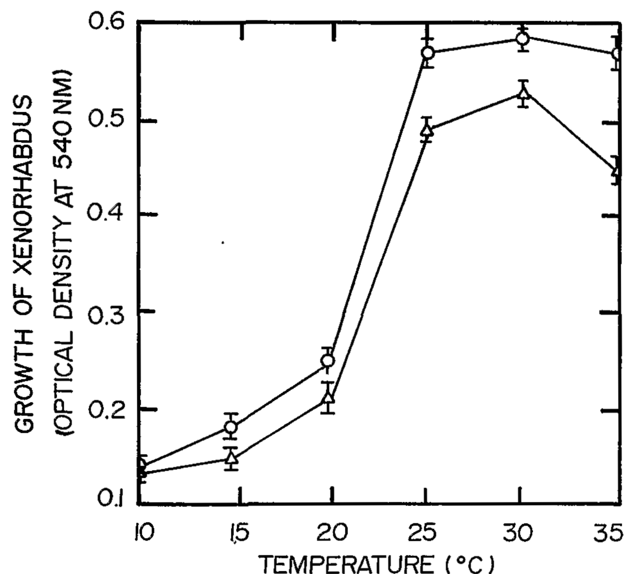

Fig. 1. Growth of *Xenorhabdus nematophilus* subsp. *Nematophilus* (o) and *X. luminescens* (Δ) in lipid broth at selected temperatures based on the optical density readings of the cultures at 540 nm.

of *N. carpocopsae* occurred with MOPS buffer at pH 6.0, whereas the medium containing BIS-TRIS showed greatest yield of *H. heliothidis* at pH 7.5

NUTRITIONAL STUDY

Lipids

Both sodium acetate and stearic acid enhanced the culture of *N. carpocapsae* above the basal medium (Tab. 1). Stearic acid also elevated the growth of *H. heliothidis*. The sterols and phospholipids produced *N. carpocapsae* levels comparable to those of the stimulatory fatty acids. Cholesterol elevated *H. heliothidis* levels but not to the value of stearic acid.

The industrial lipids most conducive to increased reproductive levels were Tween 60 (0.03 mM), sunflower oil (0.1 ml/100 ml) and Tween 80 (0.03 mM) for *N. carpocapsae* and cod liver oil (0.5 ml/100 ml) for *H. heliothidis* (Tab. 2). Olive oil inhibited *N. carpocapsae* and did not stimulate the growth of *H. heliothidis*. Sunflower oil at the routine level of 1 ml/100 ml of the basal medium was harmful to *N. carpocapsae*. A ten fold dilution of sunflower oil boosted nematode yield by more than a factor of three. Thus, the basal medium for *N. carpocapsae* was modified to lipid agar containing 0.1 % (V/V) sunflower oil and for *H. heliothidis*, lipid agar containing 0.5 % (V/V) cod liver oil.

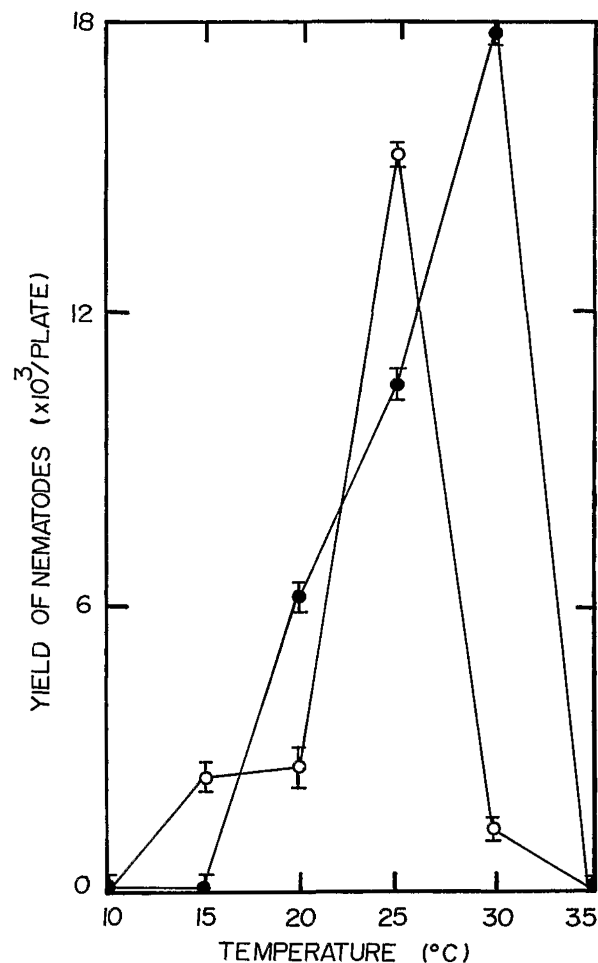

Fig. 2. Population increase of *Neoaplectana carpocapsae* DD136 (o) and *Heterorhabditis heliothidis* (•) in monoxenic culture on lipid agar at selected temperatures.

Nitrogen sources

The modified medium for both nematode species during these tests consisted of lipid agar with the lipid supplement appropriate for the nematode species and minus the nutrient broth. The latter was done to prevent masking of the nitrogen supplementation effects.

Tryptic soy broth (optimum concentration, 15 g/l) and yeast extract supported the greatest increase in numbers of *N. carpocapsae* (Fig. 5), and yeast extract (optimum concentration, 5 g/l) resulted in the largest yield of *H. heliothidis* (Tab. 3; Fig. 6). Supplementing the modified medium with nutrient broth i.e., restoring the media to its original composition, did not enhance yields of the nematode species above those in media with half the concentration of nutrient broth (Tab. 3).

Tryptic soy broth is a complex medium, the major components consisting of tryptone (casein digest) and

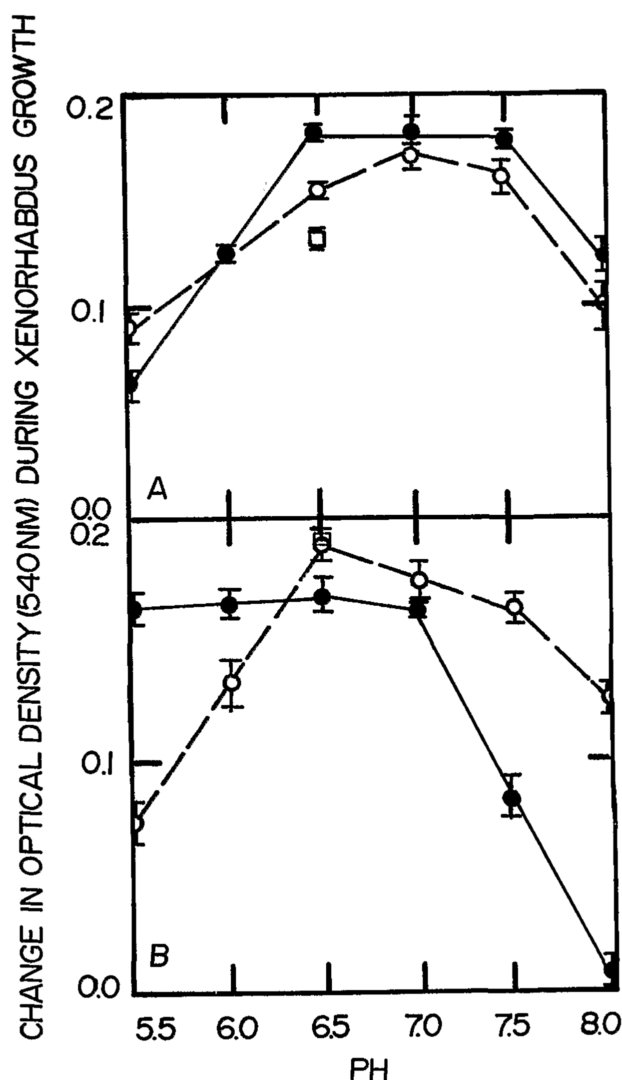

Fig. 3. Growth of *Xenorhabdus luminescens* (A) and *Xenorhabdus nematophilus* subsp. *nematophilus* (B) in lipid broth at selected pH values with MOPS (○), HEPES (●). (□) unbuffered control medium. Growth was based on optical density readings at 540 nm.

soyatone (soybean meal digest). The optimum concentration of tryptone and soyatone for *N. carpocapsae* cultures was 10 g/l and 15 g/l, respectively (Fig. 5). However, these digests did not support nematode yields comparable with that of tryptic soy broth.

Carbohydrates

Compared with the modified media, the medium together with one of the supplements D-galactose, D-fructose, D-glucose and glycerol supported significant *N. carpocapsae* reproduction and D-glucose, D-sorbose and D-mannitol significantly greater *H. heliothidis* reproduction in culture (Tab. 4). Trehalose did not increase the number of individuals of these species in culture.

Corn syrup was the preferred industrial carbon source for the culture of both nematode species (Tab. 5). The optimum corn syrup concentration for *N. carpocapsae* and *H. heliothidis* was 4 g/l and 10 g/l, respectively (Fig. 7). *H. heliothidis*, unlike *N. carpocapsae*, grew well in monoxenic culture containing soluble starch.

Table 1

Effect of different lipids (0.03 mM) on the production of *Neoaplectana carpocapsae* and *Heterorhabditis heliothidis* in monoxenic culture after incubation for 10 d

Lipid	*Total yield of nematodes (X 10^2/plate)*[a] N. carpocapsae *DD136*	H. heliothidis
FATTY ACIDS		
Oleic acid	63.5 ± 1.8	———
Stearic acid	109.3 ± 2.3	5.0 ± 1.6
Palmitic acid	28.3 ± 1.2	———
Palmitoleic acid	95.3 ± 2.2	———
Myristic acid	67.0 ± 1.8	———
Linoleic	57.3 ± 1.7	———
Pentadecaneic acid	34.4 ± 1.3	———
Sodium acetate	153.0 ± 2.8	———
STEROLS		
Cholesterol	109.3 ± 2.3	38.1 ± 1.3
B-sitosterol	129.4 ± 2.5	———
PHOSPHOLIPIDS		
Phosphatidycholine	146.0 ± 2.7	———
Acetylcholine	165.0 ± 2.9	———
Lipid agar basal medium	90.0 ± 2.1	27.3 ± 1.2

[a] Mean ± standard error of the mean; N = 5.
[b] Not determined.

Vitamins

Individual and pooled vitamins ranging in concentration from 0.1-1.0 mg/l were not conducive to elevating the reproduction of *N. carpocapsae* or *H. heliothidis* (data not shown). Ascorbic acid, m-inositol, choline chloride, riboflavin, folic acid and nicatinamide were toxic to both nematode species at the concentration of 1.0 mg/l.

Salts

Magnesium chloride (10 mM), potassium chloride (10 mM) and potassium nitrate (1 mM) enhanced *H. heliothidis* production (Tab. 6). Manganese chloride,

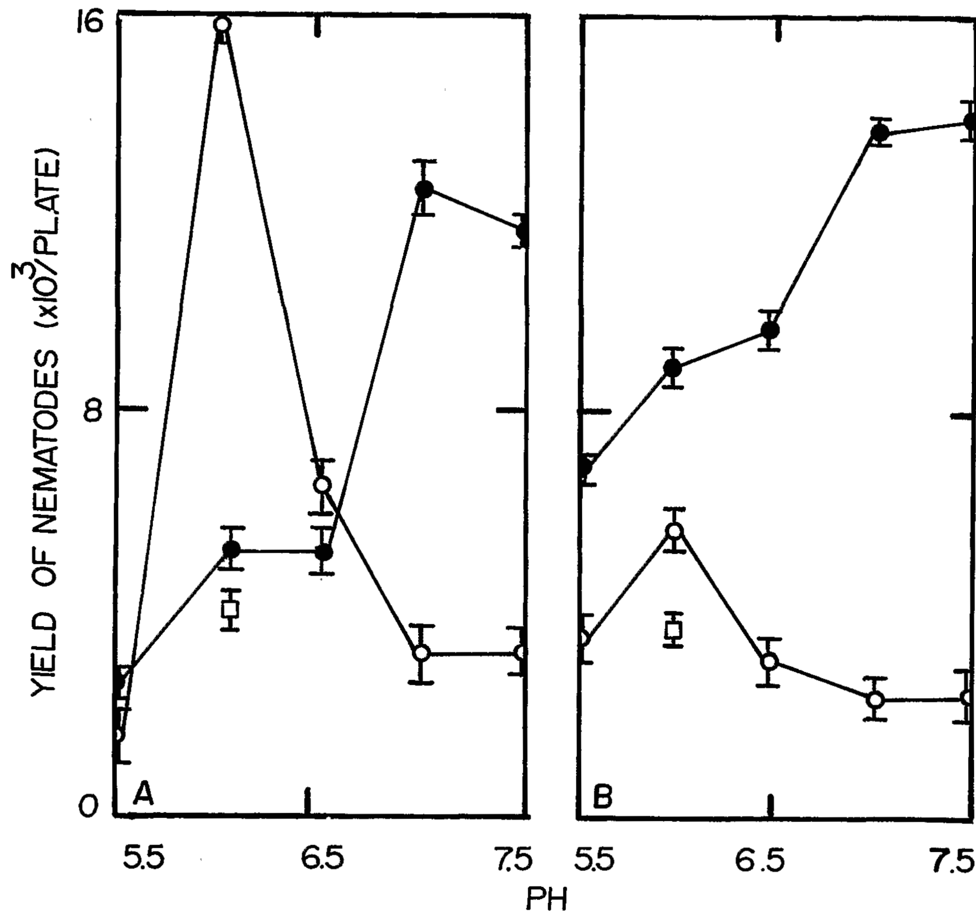

Fig. 4. Effect of MOPS (o) and BIS-TRIS (•) buffer on the growth of *Neoaplectana carpocapsae* DD136 (A) and *Heterorhabditis heliothidis* (B) in monoxenic culture at their optimum temperature. (□) unbuffered control medium.

ferric chloride and sodium bicarbonate were apparently detrimental to the nematode cultures.

Media comparison

Based on a comparison of nematode yields of each species on their new supplemented media (Tab. 7), both of these media supported significantly greater nematode reproduction than did the initial basic lipid agar medium used to culture both *N. carpocapsae* and *H. heliothidis* (Tab. 8).

Discussion

The physical parameters for optimum production of *N. carpocapsae* and *H. heliothidis* in this laboratory have been redefined. The crucial influences of temperature on steinernematid and heterorhabditid maturation and growth were identified by Kaya (1977) and Pye and Burman (1978), respectively. The optimum temperature for *X. nematophilus* subsp. *nematophilus* and *N. carpocapsae* DD136 reported by Kaya (1977) is confirmed, and the optimum temperature of 30° for *H. heliothidis* and *X. luminescens* is shown. Dunphy and Webster (1986) reported limited and good passaging of *H. heliothidis* and *N. carpocapsae* DD136 through *G. mellonella* at 15°. The discrepancy of the present data for *H. heliothidis* from those reported by Dunphy and Webster (1986) may reflect dietary differences and/or cultural artefact.

Both *Xenorhabdus* spp. and monoxenic nematode cultures are sensitive to buffers. The non-toxic buffers showed that pH 6.0-7.0 were optimal for nematode production and as pH 6.2 has been reported as the pH of *G. mellonella*'s hemolymph (Heimpel, 1955), it was chosen as the preferred value for nematode culture. The pH of unbuffered media did not change from 6.2 during monoxenic culture which precludes the need for adding costly buffers that only marginally increase nematode yield.

Throughout the study, as exemplified by the data from the lipid experiment, monoxenic cultures of *N. carpocapsae* and *H. heliothidis* exhibited qualitative and

Table 2

Effect of industrial lipids on the production of *Neoaplectana carpocapsae* and *Heterorhabditis heliothidis* in monoxenic culture after incubation for 10 d

Component	*Concentration*	*Total nematode yield (X 10³/plate)*[a]	
		N. carpocapsae	H. heliothidis
Tween 40	0.03 mM[b]	94.0 ± 2.8	——[d]
Tween 60	0.03 mM	145.7 ± 3.4	86.3 ± 2.7
Tween 80	0.03 mM	174.0 ± 3.8	23.0 ± 1.4
Butter	0.1 ml[c]	31.8 ± 1.6	31.3 ± 2.3
	1.0 ml	11.2 ± 1.0	——
Halibut oil	0.1 ml	42.7 ± 1.9	——
	0.2 ml	——	31.9 ± 1.6
	0.4 ml	——	29.7 ± 1.6
	0.8 ml	——	38.6 ± 1.6
	1.0 ml	91.5 ± 2.8	——
Cod liver oil	0.1 ml	93.6 ± 2.8	29.0 ± 1.6
	0.5 ml	——	42.1 ± 1.9
	1.0 ml	54.8 ± 2.1	24.3 ± 1.4
Olive oil	0.1 ml	13.1 ± 1.0	18.0 ± 1.2
	0.5 ml	——	23.0 ± 1.4
	1.0 ml	11.7 ± 1.0	22.1 ± 1.4
Corn oil	0.1 ml	——	5.1 ± 0.6
	0.5 ml	——	82.1 ± 2.6
	1.0 ml	——	9.5 ± 0.9
Lipid agar modified	0.1 ml	294.1 ± 5.0	——
medium with sunflower oil	1.0 ml	90.0 ± 2.7	27.3 ± 1.5

[a] Mean ± standard error of the mean; N = 4.
[b] Total fatty acids supplied by Tween compounds.
[c] Volume of oil added to make 100 ml of medium.
[d] Not determined.

quantitative differences in their responses to many of the nutrients. This may reflect species differences in : *i)* nematode metabolism, and/or *ii)* bacterial metabolism and/or *iii)* differences in the nematode-bacterium metabolic association. Thus, despite the significance of these experiments, it is difficult to draw unequivocal conclusions on nematode nutrition using monoxenic culture.

Tween 60 and 80 enhanced *N. carpocapsae*, and Tween 60 elevated *H. heliothidis* yields. However, *X. nematophilus nematophilus* does not produce Tween 80 active lipase (Akhurst, 1986), whereas *X. luminescens* does (Thomas & Poinar, 1979), which suggests that bacterial lipase is not a prerequisite for nematode production. Although lipase has not been detected in mermithids (Gordon, Burford & Young, 1982) it is

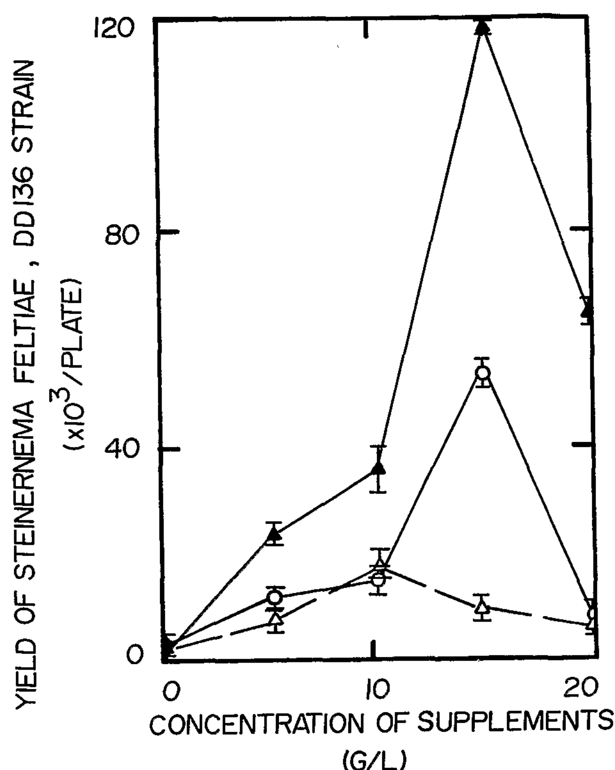

Fig. 5. Population increase of *Neoaplectana carpocapsae* on modified medium containing the nitrogen supplements typtic soy broth (▲), soyatone (○) and tryptone (△).

possible the Tween effect may reflect different lipase types or different mechanisms of lipid uptake between *N. carpocapsae* and *H. heliothidis.* In consonance with Dutky, Robbins and Thompson (1967), cholesterol and the plant sterol B-sitosterol were shown to enhance *N. carpocapsae* production. Cholesterol elevated the yield of *H. heliothidis.*

The basal medium was shown to contain more nutrient broth than was required for nematode reproduction of both species. Tryptic soy broth enhanced *N. carpocapsae* production but neither tryptone nor soyatone either individually or together in levels reflecting their tryptic soy broth concentrations produced nematode yields comparable to those on tryptic soy broth. Consequently, other dietary components in tryptic broth supplements must enhance *N. carpocapsae* culture.

Yeast extract stimulated the reproduction of both nematode species. The effect is not attributed to the vitamin content of yeast extract because (1) autoclaving the medium would have destroyed the vitamins and (2) individual and pooled vitamin supplements did not elevate nematode yields. The vitamin supplements were tested at concentrations spanning those encountered in yeast extract. Jcckson and Suddiqui (1965) and Jackson and Platzer (1974) established the need for folic acid and

Table 3

Effect of different nitrogen sources on the production of *Neoaplectana carpocapsae* and *Heterorhabditis heliothidis* in monoxenic culture after 10 d incubation

Nitrogen source[a]	*Total nematode yield (X 10^3/plate)*[b] N. carpocapsae	H. heliothidis
Grace's amino acids[c]	8.3 ± 0.8	——[f]
Fetal calf serum	23.9 ± 1.4	——
L-broth[d]	8.5 ± 0.8	——
Tryptic soy broth	588.0 ± 7.0	3.0 ± 0.5
Casein	62.7 ± 2.3	——
Casamino acids (vitamin free)	96.8 ± 2.8	6.5 ± 0.7
Lactalbumin hydrolysate	141.0 ± 3.4	4.2 ± 0.6
Liver	52.7 ± 2.1	——
Fishmeal	123.0 ± 3.2	——
Peptone	186.6 ± 3.9	2.5 ± 0.4
Proteose peptone		5.5 ± 0.7
Albumin	129.3 ± 3.2	
Yeast extract	241.5 ± 4.5	242.0 ± 4.5
Nutrient broth	166.3 ± 3.7	137.2 ± 3.4
Basal medium[e]	168.1 ± 3.7	124.3 ± 3.2

[a] Total nitrogen level = 2.5 g/L.
[b] Mean ± standard error of the mean, N = 4.
[c] From Grace, T.C.C. 1962. Nature *195* : 788-789.
[d] From Dillon, J.-A., Nasin, A. and E. R. Nestmann, 1985, Recombinant, DNA Methodology, John Wiley and Sons, N.Y., p. 82.
[e] For : *N. carpocapsae* : 2.3 g nutrient agar plus 0.1 ml sunflower oil/100 ml medium.
H. heliothidis : 2.3 g nutrient agar plus 0.5 ml cold liver oil/100 ml medium.
[f] Not determined.

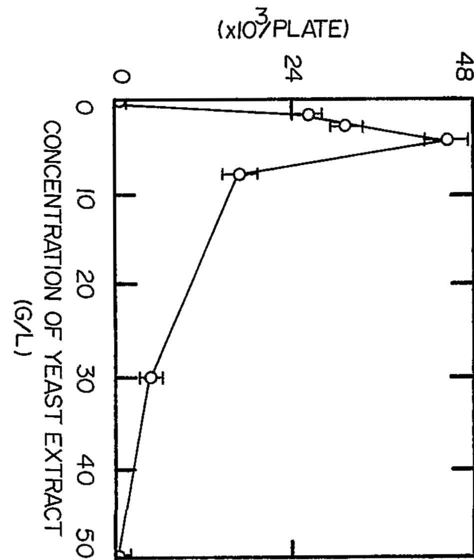

Fig. 6. Effect of yeast extract concentrations on the reproduction of *Heterorhabditis heliothidis* in lipid agar.

biotin in the axenic culture of *Steinernema glaseri*. Thus, our results do not show that nematodes do not require vitamins but rather that the bacteria probably act as a vitamin source for the nematodes.

There was no discernible correlation between stimulatory carbohydrate and bacterial fermentation ability suggesting that the sugar effects are complex. It was surprising that trehalose did not increase nematode yields because trehalose is a major hemolymph sugar (Wyatt, 1967) and readily fermented by *Xenorhabdus* species (Akhurst, 1983).

Pye and Burman (1981) reported positive chemotaxis by *N. carpocapsae* to Cl^-, MG^{2+}, Ca^{2+} and CO^{2-}_3 ions at 75 mM levels as a possible host locating mechanism. The present study is the first to establish the effects of salts on heterorhabditid growth and reproduction and their potential importance in mass production media. Thus, it will be possible to ensure nutrients with inhi-

Table 4

Effect of different carbon sources on the yield of monoxenic cultures of *Neoaplectana carpocapsae* and *Heterorhabditis heliothidis* after incubation for 10 d

Compound[a]	*Total nematode yield (X 10^3/plate)*[b] N. carpocapsae	H. heliothidis
D-glucose	63.6 ± 2.3	35.5 ± 1.7
D-fructose	70.5 ± 2.4	11.0 ± 1.0
D-galactose	160.0 ± 3.6	——[d]
D-mannose	33.2 ± 1.6	17.1 ± 1.2
D-sorbose	——	45.0 ± 1.9
Sucrose	——	3.0 ± 0.5
Trehalose	41.0 ± 1.8	6.0 ± 0.7
Maltose	——	6.2 ± 0.7
D-mannitol	39.2 ± 1.8	23.5 ± 1.4
Glycerol	133.8 ± 3.3	——
Modified medium[c]	38.5 ± 1.8	11.0 ± 0.9

[a] Total carbon added equalled 14 mM.
[b] Mean ± standard error of the mean, N = 4.
[c] Modified medium for : *N. carpocapsae* : 2.3 g nutrient agar + 0.1 ml sunflower oil 1.5 g soya tone/100 ml medium; *H. heliothidis* : 2.3 g nutrient agar + 0.5 ml cod liver oil + 0.5 g yeast extract/100 ml medium.
[d] Not determined.

Table 5

Effects of different industrial carbon sources on the yield of *Neoaplectana carpocapsae* and *Heterorhabditis heliothidis* in monoxenic culture after incubation for 10 d

Compound[a]	*Total nematode yield (X 10^3/plate)*[b]	
	N. carpocapsae	H. heliothidis
Molasses	93.2 ± 2.8	65.5 ± 2.3
Corn syrup	172.8 ± 3.8	148.0 ± 3.5
Corn starch	44.0 ± 1.9	———[d]
Malt extract	21.0 ± 1.3	———
Soluble starch	20.2 ± 1.3	129.5 ± 3.2
Modified medium[c]	38.5 ± 1.8	11.0 ± 1.0

[a] Total carbon supplied = 14 mM.
[b] Means ± standard errors of the mean, N = 4.
[c] Modified medium for : *N. carpocapsae* : 2.3 g nutrient agar + 0.1 ml sunflower oil + 1.5 g soyatone/100 ml medium; *H. heliothidis* : 2.3 g nutrient agar + 0.5 ml cod liver oil + 0.5 g yeast extract/100 ml medium.
[d] Not determined.

Table 7

Media supporting the monoxenic culture of *Neoaplectana carpocapsae* DD136 and *Heterorhabditis heliothidis*

Lipid agar medium for both nematode species	
Nutrient agar	23.0 g/l
Nutrient broth	8.0 g/l
Safflo oil	11 mL/l
N. carpocapsae culture medium	
Corn syrup	4 g/l
Soyatone	15 g/l
Sunflower oil	1 mL/l
Agar	15 g/l
H. heliothidis culture medium	
Corn syrup	10 g/l
Yeast extract	5 g/l
Nutrient agar	23 g/l
Cod liver oil	5 mL/l
$MgCl_2$ 6 H_2O	2 g/l

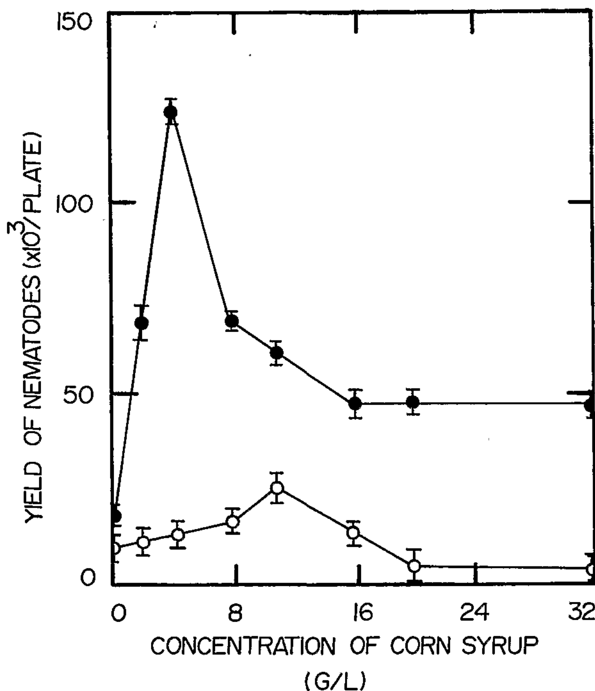

Fig. 7. Effect of various concentrations of corn syrup on the production of *Neoaplectana carpocapsae* DD136 (●) and *Heterorhabditis heliothidis* (o) on their respective culture media.

Table 8

Total yield of *Neoaplectana carpocapsae* and *Heterorhabditis heliothidis* after incubation for 10 d on different media

Medium	N. carpocapsae	H. heliothidis
Lipid agar	2,872 ± 27/plate	6,983 ± 42/plate
N. carpocapsae medium	29,750 ± 86/plate	———[b]
H. heliothidis medium	———[b]	31,000 ± 88/plate

[a] Mean ± standard error of the mean, N = 4.
[b] Not determined.

bitory salts or at inhibitory concentrations are not included in media formulation and that stimulatory salts are included to maximize nematode yields.

The data arising from this research enables the more reliable production and greater yields of all stages of *N. carpocapsae* and *H. heliothidis* in laboratory culture *in vitro* and is a step towards improved mass industrial production.

Table 6

Total Yield of *Heterorhabditis heliothidis* in monoxenic culture supplement with different salts after incubation for 10 d

Salt	*Concentration*[b] *(mM)*	*Nematode Yield*[a] *(X* 10^{-3}*/plate)*	*Salt*	*Concentration (mM)*	*Nematode Yield*[a] *(X* 10^{-3}*/plate)*
Na_2CO_3	1	50.0 ± 2.0	$CaCl_2$	1	68.8 ± 2.4
	10	158.8 ± 3.6		10	43.7 ± 1.9
NaH_2PO_4	1	53.8 ± 2.1	$CaSO_4$	1	100.8 ± 2.9
	10	8.0 ± 0.8		10	97.2 ± 2.8
$NaHCO_3$	1	16.0 ± 1.2	$FeCl_3$	1	0 ± 0
	10	6.5 ± 0.7		10	0 ± 0
$MgSO_4 \cdot 7\ H_2O$	1	180.0 ± 3.9	KNO_3	1	200.0 ± 4.1
	10	118.8 ± 3.1		10	103.8 ± 2.9
$MgCl_2 \cdot 6\ H_2O$	1	123.0 ± 3.2	KCl	1	78.0 ± 2.6
	10	217.5 ± 4.2		10	196.2 ± 4.0
$MnCl_2$	1	5.0 ± 0.6	Modified medium[c]		145.0 ± 3.5
	10	12.5 ± 1.0			

[a] Mean ± standard error of the mean, N = 4.
[b] The level of the cation.
[c] Modified medium : 2.3 g nutrient agar + 0.5 ml cod liver oil + 0.5 g. yeast extract/100 ml medium.

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