EFFECTS OF CULTURE MEDIA ON THE KINETICS OF INFECTIVE JUVENILE PRODUCTION OF THE ENTOMOPATHOGENIC NEMATODE *Steinernema carpocapsae,* IN SUBMERGED MONOXENIC CULTURE

EFECTO DEL MEDIO DE CULTIVO SOBRE LA CINÉTICA DE PRODUCCIÓN DE FASES INFECTIVAS JUVENILES DEL NEMATODO ENTOMOPATÓGENO *Steinernema carpocapsae,* EN CULTIVO MONOXÉNICO SUMERGIDO

N. Chavarría-Hernández[1,*], M. A. Islas-López[2], G. Maciel-Vergara[1], B. R. Rodríguez Pastrana[1] and A. I. Rodríguez-Hernández[1]

[1] *Cuerpo Académico de Biotecnología Agroalimentaria. Centro de Investigaciones en Ciencia y Tecnología de los Alimentos del Instituto de Ciencias Agropecuarias, Universidad Autónoma del Estado de Hidalgo. Av. Universidad km 1, Rancho Universitario, Tulancingo de Bravo, Hidalgo. CP 43600. México.*
[2] *Universidad de la Cañada. Carretera Teotitlán-San Antonio Nanahuatipan km 1.7, Teotitlán de Flores Magón, Oaxaca. CP 68540. México.*

Recibido 16 de Septiembre 2006; Aceptado 4 de Abril 2008

Abstract

The effects of culture medium formulations on the kinetics of infective juvenile (IJ) production of the entomopathogenic nematode *Steinernema carpocapsae*, were studied in submerged monoxenic culture in orbitally agitated cylindrical bottles using four culture media containing agave juice from *Agave* spp. among other ingredients. The IJ production kinetics was well modelled through a re-parameterised 3-parameter Gompertz model with kinetic parameters: IJ-lag phase, λ_{IJ} (d), maximum IJ-stage conversion rate, m_{max} ($\equiv[d(C_{IJ}/C_{IJ,0})/dt]_{max}$) ($d^{-1}$), and IJ-multiplication factor, $(C_{IJ}/C_{IJ,0})_{max}$ (-), with values within the ranges 14 d < λ_{IJ} < 16 d; 33 d^{-1} < m_{max} < 241 d^{-1} and 66 (-) < $(C_{IJ}/C_{IJ,0})_{max}$ < 611 (-), respectively. It was apparent that maximum values of m_{max} and $(C_{IJ}/C_{IJ,0})_{max}$ were obtained in medium A4 (27.6 % (v/v) agave juice, 1.7 % (w/v) yeast extract, 1.2 % (w/v) dried egg yolk, 2.5 % (v/v) corn oil). Also, the maximum average IJ concentration (249,444 per mL) was achieved in A4-fermentations.

Keywords: entomopathogenic nematode, culture medium, fat/carbohydrates ratio, carbohydrates/protein ratio, agave juice, modelling.

Resumen

Se estudiaron los efectos de medios de cultivo en la cinética de producción de fases infectivas juveniles (IJ) del nematodo entomopatógeno *Steinernema carpocapsae* en cultivo monoxénico sumergido, mediante el desarrollo de cultivos en frascos cilíndricos agitados orbitalmente usando cuatro formulaciones de medio conteniendo aguamiel de *Agave* spp., entre otros ingredientes. La cinética de producción de fases IJ fue bien modelada mediante el modelo de Gompertz de 3 parámetros, reparametrizado, que involucró los siguientes parámetros cinéticos: fase lag IJ, λ_{IJ} (d), máxima velocidad de conversión a IJ, m_{max} ($\equiv[d(C_{IJ}/C_{IJ,0})/dt]_{max}$) ($d^{-1}$), y factor de multiplicación IJ, $(C_{IJ}/C_{IJ,0})_{max}$ (-), registrando variaciones en los intervalos 14 d < λ_{IJ} < 16 d; 33 d^{-1} < m_{max} < 241 d^{-1} y 66 (-) < $(C_{IJ}/C_{IJ,0})_{max}$ < 611 (-), respectivamente. Aparentemente, los máximos valores de m_{max} y $(C_{IJ}/C_{IJ,0})_{max}$ fueron determinados cuando se usó el medio A4 (27.6 % (v/v) aguamiel, 1.7 % (p/v) extracto de levadura, 1.2 % (p/v) yema de huevo deshidratada, 2.5 % (v/v) aceite de maíz). Además, la máxima concentración promedio de fases IJ (249,444 por mL) fue alcanzada usando el medio A4.

Palabras clave: nematodos entomopatógenos, medios de cultivo, relación grasa/carbohidratos, relación carbohidratos/proteína, aguamiel, modelado.

1. Introduction

Within the last 30 years, research concerning the bioinsecticide production has been conducted in view of the apparent negative ecological consequences due to the extensive use of chemical insecticides in the Agro-Industry (FAO, 2002). An average of 2.360×10^6 Ton/year of active ingredients (mainly herbicides, insecticides and fungicides) was supposed to be used all over the world in 2000 and

* *Corresponding author*: *E-mail: norberto@uaeh.reduaeh.mx*

2001 (Kiely *et al.*, 2004). Among the bioinsecticides, entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* are interesting in view of their success as biocontrollers of different insect pests (Shapiro-llan and Gaugler, 2002). Both steinernematids and heterorhabditids form a symbiosis with Gram-negative bacteria from the genera *Xenorhabdus* and *Photorhabdus*, respectively, which can occur in two forms, being only one of them –the so-called phase I- essential for effective killing of the insect host. The bacteria are carried by the infective juvenile nematode stage (IJ), a specialised free-living form which is able to seek out insect larvae within the soil, invade them and release their symbiotic bacteria within the haemolymph where they proliferate and transform the insect tissues into a nutrient soup so the nematodes can grow and reproduce until the nutrient supply becomes limiting at which time they develop into new generation IJ's that leave the insect cadaver and search for new hosts (Woodring and Kaya, 1988). IJ's have been used successfully for the biocontrol of some insect pests like the Black vine weevil, *Otiorhynchus sulcatus*, and Blue green weevils, *Pachnaeus* spp. (Shapiro-llan and Gaugler, 2002). Therefore, the IJ stage is of particular interest for field application so high quantities of nematodes are required that can be produced by in-vivo and in-vitro cultures. In this sense, according to different authors the submerged monoxenic culture is the most suitable technology for the large scale IJ-production since higher productivities can be achieved and processes are more easily controlled (Ehlers, 2001; Chavarría-Hernández *et al*., 2006). Although industrial production of IJ stages is already being used for some species (SIP, 2006), the production processes are partially empirical giving place to variable results. Thus, efforts must be done to improve such bioprocesses. More studies dealing with medium formulation, nematode population growth kinetics, bioreactor design and operating conditions are required for reliable IJ stage production processes. Particularly, research concerning the culture medium formulation effects on the nematode population growth kinetics is scarce. There are only two reports dealing with such aspects but focusing on the total nematode concentration evolution (i.e., Chavarría-Hernández and de la Torre (2001) modelled the *S. feltiae* nematode population growth by means of the Gompertz equation, and Chavarría-Hernández *et al.* (2006) did model the *S. carpocapsae* nematode population growth using a sigmoidal equation). Nevertheless, there are no published results concerning the modelling of IJ stage production kinetics although this is the only nematode stage that is useful for biocontrol applications.

The present work deals with the production of the entomopathogenic nematode *Steinernema carpocapsae* in presence of its symbiotic bacterium, *Xenorhabdus nematophila*, in orbitally agitated cylindrical bottles, using different culture media containing agave juice from *Agave* spp. as the main carbohydrates source. The IJ stage concentration time course is modelled using a re-parameterised 3-parameter Gompertz model (Chavarría-Hernández and de la Torre, 2001) and the effects of culture medium formulations on the resulting kinetic parameters are discussed.

2. Materials and methods.

2.1 Biological specimens.

Sanitised IJ stages of *S. carpocapsae* (originally donated by Dr. Alatorre-Rosas, *COLPOS, México*) were maintained in 50 mL sterile-distilled water suspensions (300,000 IJ/mL) within cell culture flasks (total volume 450 mL) at 4 ºC with shaking periods of 15 min every 15 d to provide the required oxygen.

X. nematophila-phase I was conserved in 2 mL-vials in 25 % (v/v) glycerol at -80 ºC (10^9 bacteria per vial). Symbiotic bacterium was isolated from NBTA plates (Akhurst, 1980) streaked with haemolymph from last instar larvae of *Galleria mellonella* previously infected (24 h) with IJ stages of *S. carpocapsae* (Woodring and Kaya, 1988). Identification of the bacterium using the 16S rRNA gene sequencing methodology with 99 % homology was kindly carried out by the group of Dr. Wacher-Rodarte (*FQ-UNAM, México*).

2.2 Culture media

TYB medium (Buecher and Popiel, 1989): 3 % (w/v) trypticase soy broth and 0.5 % (w/v) yeast extract.

NBTA medium (Akhurst, 1980): 2.3 % (w/v) nutrient agar, 0.025 % (w/v) Bromothymol Blue and 0.004 % (w/v) triphenyl-tetrazolium choride (TTC).

Production media (Table 1): four culture media (A1 to A4) containing agave juice from *Agave* spp. were tested. Medium formulations were based on previous work of different research groups (Surrey and Davies, 1996; Chavarría-Hernández and de la Torre, 2001; Islas-López *et al*., 2005; Chavarría-Hernández *et al*., 2006). The agave juice was provided as a refrigerated fresh-liquid which was autoclaved in 2 L bottles (1 L juice per bottle) immediately after reception in order to avoid undesirable fermentation. Then, sterile agave juice bottles were stored until medium preparation. The composition of production media based on the concentrations of total protein (P), total carbohydrates (CH) and total fat (F) were determined through mass balances on the basis of the corresponding contents of total nitrogen (N), CH and F for each medium ingredient that in turn were determined trough the Method 991.20 (AOAC, 1999), method of Dubois *et al*. (1956) and Method

963.15 (AOAC, 1999), respectively. All production media contained 0.5 % (w/v) NaCl and were adjusted to pH 7 before autoclaving.

2.3 Monoxenic cultures.

Cultures were carried out according to Chavarría-Hernández and de la Torre (2001) in orbitally agitated cylindrical bottles. One *X. nematophila*-phase I vial was inoculated into 50 mL of sterile TYB contained in a 250 mL-Erlenmeyer flask and incubated at 29 ºC and 150 rpm until a concentration of 10^9 bacteria/mL was reached (24 h approximately). Bacterium phase was then checked by streaking culture broth samples on NBTA plates. Thereafter, Erlenmeyer flasks (500 mL total volume) containing 150 mL of the corresponding sterile A-medium were inoculated with 5 % (v/v) *X. nematophila*-TYB culture broth, and incubated at 29 ºC and 150 rpm during 48 h. Afterwards, NBTA plates were streaked with culture broth samples to confirm the presence of *X. nematophila*-phase I. Subsequently, each culture broth was inoculated with an IJ stage-water suspension to give approximately an initial concentration ($C_{IJ,0}$) of 500 IJ/mL and after well mixing, the broth was poured into 3 sterile plastic-top glass cylindrical bottles (total volume 500 mL; inner diameter 9 cm) filled with 50 mL each one. Then, all cultures were incubated at 22 ºC and 150 rpm during 20 d taking samples of 0.1 mL-each bottle every 2 d.

2.4 Determination of the viable nematode concentration.

Depending on nematode concentration, monoxenic culture broth samples were diluted (1:10 to 1:1000) using an isotonic salt solution and 0.002 % (w/v) Methylene Blue as a contrast stain. Concentrations of total viable nematodes (C) and IJ stages (C_{IJ}) were determined by counts in 0.1 mL of diluted samples under the light microscope (40× and 100×) by triplicate.

2.5 Modelling of the kinetics of infective juvenile stage production.

The dimensionless IJ stage-nematode concentration ($C_{IJ}/C_{IJ,0}$) (-) (where $C_{IJ,0}$ is the initial IJ concentration) were fitted to a re-parameterised Gompertz model (Eq. (1)) (modified from Chavarría-Hernández and de la Torre, 2001) using the SigmaPlot 9.0 software package to determine the following kinetic parameters: 1) IJ stage-multiplication factor, $(C_{IJ}/C_{IJ,0})_{max}$ (-); 2) maximum IJ-stage conversion rate, $m_{max}=[d(C_{IJ}/C_{IJ,0})/dt]_{max}$ (d^{-1}), and 3) IJ-lag time, λ_{IJ} (d) (i.e., period of time necessary for the generation of new IJ stages).

$$\frac{C_{IJ}}{C_{IJ,0}} = \left(\frac{C_{IJ}}{C_{IJ,0}}\right)_{max} \exp\left\{-\exp\left[\frac{m_{max} e^{1}\left(\lambda_{IJ} - t\right)}{\left(C_{IJ}/C_{IJ,0}\right)_{max}} + 1\right]\right\} \quad (1)$$

Afterwards, the obtained kinetic parameters as well as the maximum IJ concentration ($C_{IJ,max}$) in all fermentations were compared among them through tests on means with variance known (Eq. (2)) with $\alpha = 0.05$ (Montgomery, 1991).

$$Z_0 = \frac{y_1 - y_2}{\sqrt{\frac{\sigma_1^2}{n_1} - \frac{\sigma_2^2}{n_2}}} \quad (2)$$

In Eq. (2), Z_0 is the test statistic; y would be $(C_{IJ}/C_{IJ,0})_{max}$, m_{max}, λ_{IJ} or $C_{IJ,max}$. The parameter σ^2/n is the square of the standard error value (SE = $\sigma/\sqrt{n}$). Sub-indices 1 and 2 refer to two different fermentations.

Table 1. Culture media used for the submerged culture of *Steinernema carpocapsae* and *Xenorhabdus nematophila* in orbitally agitated cylindrical bottles. All media contained 0.5% (w/v) NaCl and pH was adjusted to 7 before sterilisation. Medium formulations were based on previous work of different research groups[a].
The fat/carbohydrate (F/CH) and carbohydrate/protein (CH/P) ratios for each medium are presented.

Medium	Agave juice concentration (% v/v)	Yeast extract concentration (% w/v)	Dried egg yolk concentration (% w/v)	Corn oil concentration (% v/v)	[b]Total protein concentration, P (% w/v)	[b]Total carbohydrates concentration, CH (% w/v)	[b]Total fat concentration, F (% w/v)	F/CH ratio (g_F/g_{CH})	CH/P ratio (g_{CH}/g_P)
A1	8.2	0.4	0.5	5.3	0.57	0.94	5.44	5.8	1.6
A2	16.0	2.2	0.2	2.2	1.58	1.97	2.26	1.2	1.2
A3	22.2	0.2	1.7	0.7	1.07	2.40	1.29	0.5	2.2
A4	27.6	1.7	1.2	2.5	1.76	3.15	2.91	0.9	1.8

[a] Surrey and Davies, 1996; Chavarría-Hernández and de la Torre (2001); Islas-López *et al.* (2005); Chavarría-Hernández *et al.,* (2006).

[b]Values of P, CH and F were determined based on the ingredient analyses through the methods of 991.20 (AOAC, 1999) for total nitrogen –N- and the formula P=6.3×N; Dubois *et al.* (1956) for CH, and 963.15 (AOAC, 1999) for F, respectively.

3. Results and discussion.

All culture broth samples of *X. nematophila* streaked onto NBTA plates exhibited almost 100 % dark blue colonies, which suggest that *X. nematophila* was mainly in phase I. The phase I of the bacterium absorbs bromothymol Blue producing dark blue colonies whereas phase II-cells do not absorb bromothymol Blue, but reduce TTC producing red colonies (Akhurst, 1980).

$C_{IJ,0}$ values were from 430 IJ/mL to 550 IJ/mL and first generation adults were first observed in the period t = 4 d to t = 6 d, depending on the medium (figs. 1iii & iv). The release of fertilised eggs by the gravid first-generation females followed by hatching out of first stage juveniles (J1) (figs. 1v & vi) occurred after t=6 d and nematode population did start to grow up consequently (Fig. 2). The inoculated IJ recovery process (i.e., physiological and metabolic events that cause IJ stages to start feeding, giving place to J4 stage nematodes; Fig. 1ii) finished between t = 8 d to t = 10 d depending on the culture medium. Later on, new-generation IJ stages were first recorded between t = 14 d to t = 16 d, suggesting the beginning of non-favourable-conditions predominance which hinders any further nematode population growth. Under such conditions, J3 stage does transform into the resistant non-feeding IJ stage (Fig. 1i).

At t = 20 d, cultures exhibited maximal total nematode concentrations from the lowest 56,300 nematodes/mL (59 % IJ's) corresponding to A1 fermentations, to the highest 271,000 nematodes/mL (92 % IJ's) corresponding to A4 experiments (Fig. 2). It was apparent that the highest $C_{IJ,max}$ value was obtained in A4 medium (249,444 IJ/mL) ($C_{IJ,max-A2}$, $C_{IJ,max-A3}$ and $C_{IJ,max-A4}$ values exhibited non-significant differences among them; Fig. 3i), while the lowest corresponded to A1 fermentations (33,000 IJ/mL) (Table 2; Fig. 3i). Moreover, the maximum IJ stage concentration obtained in A4-medium was higher than the corresponding ones reported by other authors concerning the IJ production of steinernematids in orbitally agitated flasks (i.e., $C_{IJ,max}$=14,700 IJ/mL, Neves *et al*. (2001); $C_{IJ,max}$=134,000 to 190,000 IJ/mL, Chavarría-Hernández and de la Torre (2001)).

On the other hand, Table 2 presents results of kinetic modelling of IJ concentration time courses during the fermentations. There are no previous reports concerning the IJ concentration time course modelling of *S. carpocapsae* in submerged monoxenic culture. In fact, there are only two reports concerning total nematode population growth modelling of steinernematids (i.e., Chavarría-Hernández and de la Torre (2001) and Chavarría-Hernández *et al.* (2006) did model *S. feltiae* and *S. carpocapsae* population growth, respectively). In the present study, main attention is focused on IJ concentration time course modelling considering that this particular nematode stage is the only one that can be used for field applications. Concerning the IJ production of heterorhabditids, there are no reports dedicated to population growth modelling aspects, although other aspects have been exhaustively studied (Surrey and Davies, 1996; Yoo *et al.*, 2000; Ehlers, 2001).

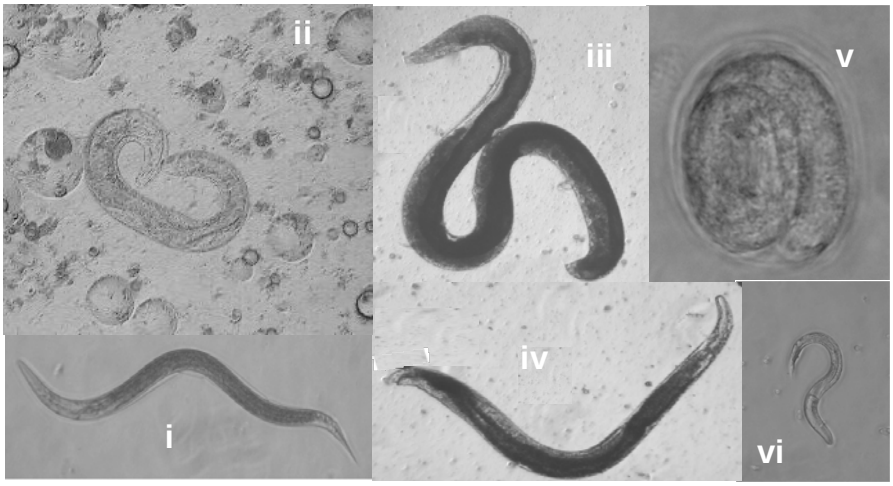

Fig. 1. Some developmental stages of the entomopathogenic nematode *Steinernema carpocapsae* in submerged monoxenic cultures in orbitally agitated cylindrical bottles using the A4-medium that contains 27.6 % (v/v) agave juice from *Agave* spp. i) Infective juvenile stage (length, L=537 μm; magnification, 100 ×); ii) fourth stage juvenile coming from the recovery of an IJ stage (L=880 μm; 100 ×); iii) first generation adult female (L=2,655 μm; 40 ×); iv) first generation adult male (L=1,226 μm; 40 ×); v) fertilised egg (L=22 μm; 1,000 ×), and vi) first stage juvenile (L=204 μm; 400 ×).

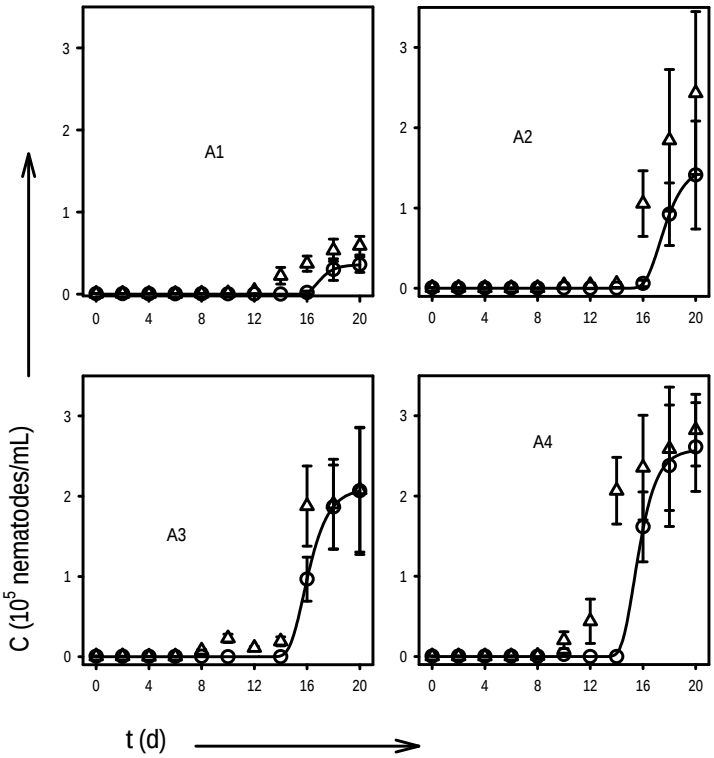

Fig. 2. Evolution of concentrations of both total viable nematodes (Δ) and infective juvenile stages (○) during the submerged monoxenic culture of *Steinernema carpocapsae* in orbitally agitated cylindrical bottles using four culture media containing agave juice from *Agave* spp. (A1 to A4). Best fits of infective juvenile concentration data to the Gompertz model (equation 1) are represented by line graphs. Each plot represents 3 independent runs with triplicate nematode counts per sample; error bars represent the standard deviation (σ).

Table 2. Kinetic parameters of the submerged culture of *Steinernema carpocapsae* and *Xenorhabdus nematophila* in orbitally agitated cylindrical bottles. For each treatment, the evolution of infective juvenile stage (IJ) concentration was fitted to a re-parameterised 3-parameter Gompertz model (Eq. (1)). The standard error values (SE)[a] are shown in parentheses.

Medium	Kinetic parameters			R^2	Maximum concentration of IJ stages $C_{IJ,max}$ (IJ/mL)
	IJ stage-multiplication factor $(C_{IJ}/C_{IJ,0})_{max}$ (-)	IJ-lag time λ_{IJ} (d)	Maximum IJ stage conversion rate (d^{-1})		
A1	66 (3)	16 (0.2)	33 (6)	0.88	33,000 (6,650)
A2	336 (37)	16 (0.6)	115 (38)	0.81	116,666 (34,100)
A3	571 (39)	15 (0.5)	200 (69)	0.88	196,666 (38,000)
A4	611 (25)	14 (0.4)	241 (62)	0.92	249,444 (37,750)

[a] SE = $\sigma / \sqrt{n}$

Based on data in Table 2, tendencies to differences are observed in parameters $(C_{IJ}/C_{IJ,0})_{max}$ and m_{max} among the experiments (figs. 3ii & iii). It was apparent that the maximum value of $(C_{IJ}/C_{IJ,0})_{max}$ (i.e., 611 (-)) was determined in A4 experiments, being 611/66=9.3 times the minimum one which corresponded to A1 fermentations. Also, it was apparent that the A4-m_{max} value was the highest determined during the present investigation and it was 241/33=7.3 times the lowest obtained value (i.e., A1-m_{max}). Both parameters $(C_{IJ}/C_{IJ,0})_{max}$ and m_{max} are directly proportional to the productivity of IJ stages in bioprocesses.

Concerning λ_{IJ} parameter, estimated values were similar among the fermentations (Fig. 3iv). It implies that, although there were important differences in medium formulations (i.e., 0.57 < P (% w/v) < 1.76; 0.94 < CH (% w/v) < 3.15 and 1.29 < F (% w/v) < 5.44; Table 1) during all fermentations the conditions became non-favourable to support further nematode population growth at relatively similar times (i.e., $\lambda_{IJ,mean}$=15.3 d, SE=1.0) being this circumstance indicated by the J3 stage conversion to new IJ stage.

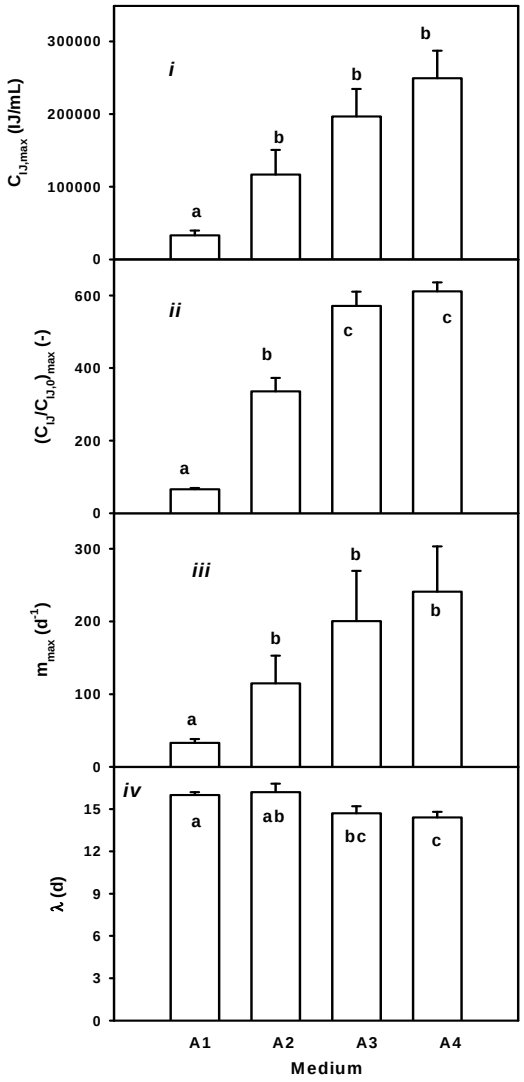

Fig. 3. Bar plots of i) Maximum infective juvenile concentration ($C_{IJ,max}$), and kinetic parameters: ii) Infective juvenile-multiplication factor $(C_{IJ}/C_{IJ,0})_{max}$, iii) maximum infective juvenile-conversion rate (m_{max}) and iv) infective juvenile-lag phase (λ_{IJ}), during the submerged monoxenic culture of *Steinernema carpocapsae* in orbitally agitated cylindrical bottles using for culture media containing agave juice from *Agave* spp. (A1 to A4). The same letters (a, b or c) on bars represent no significant differences.

Recently, Islas-López *et al*. (2005) demonstrated that increments in CH concentration can improve the IJ stage productivity during submerged monoxenic culture of *S. carpocapsae* using culture media containing agave juice, even though maintaining relatively low concentrations of both nitrogen and fat sources. Nonetheless, there are no studies dealing with the influence of variations of culture medium formulations on the IJ production kinetics, although some authors have discussed the importance of medium formulation during the production of entomopathogenic nematodes and have also published different results that pointed out the importance of the concentrations and quality of the sources of nitrogen (Chavarría-Hernández and de la Torre, 2001), fat (Yoo *et al*., 2000) and carbohydrates (Islas-López *et al*., 2005) involved within the experiments.

It was apparent that the maximum values of kinetic parameters $(C_{IJ}/C_{IJ,0})_{max}$ and m_{max} were obtained in A4 fermentations which also contained the highest P and CH concentrations among the tested media (Table 1). Besides, A4 medium exhibited values of F/CH=0.9 g_F/g_{CH} and CH/P=1.8 g_{CH}/g_P which would have also relation with the observed IJ kinetics and the achieved IJ concentration (Table 2; Fig. 3). In this sense, published information concerning effects of CH/P or F/CH ratios on the entomopathogenic nematode production is unavailable at the present time. In contrast, concerning the production of other multi-cellular organisms, there are some reports focusing on the effects of F/CH, P/energy and P/CH ratios on the growth and survival of different complex organisms like shrimps (Chuntapa *et al.*, 1999) and breams (Sá *et al.*, 2007).

On the other hand, some reported data concerning monoxenic production of steinernematids in orbitally agitated flasks were compared with the results here published. Chavarría-Hernández *et al.* (2006) propagated the same nematode species using a culture medium containing whey (ME: 50 % (w/v) whey, 2 % (w/v) yeast extract, 1 % (w/v) dried egg yolk and 3.7 % (w/v) corn oil) obtaining 126,666 IJ/mL in 24 d fermentations which were similar to A2-experiment results of the present work (i.e, $C_{IJ,max\text{-}A2}/C_{IJ,max\text{-}ME}$=116,666/126,666=0.92 times) at relatively similar values of F/CH and CH/P ratios (i.e., $(F/CH)_{A2}/(F/CH)_{ME}$ = 1.2/1.7 = 0.7 times; $(CH/P)_{A2}/(CH/P)_{ME}$ = 1.2/1.2 =1 times). Those similarities were found even though the mentioned bioprocesses exhibited important differences between them: medium A2 contains carbohydrates like sucrose, glucose, fructose and inuline (Sánchez-Marroquín and Hope, 1953) while ME-medium contains lactose as the main carbohydrates source (Chavarría-Hernández *et al*., 2006). In contrast, Neves *et al.* (2001) obtained 14,400 *S. carpocapsae* Az-20-IJ/mL with $(C_{IJ}/C_{IJ,0})_{max}$=29.4 (-) using a culture medium containing 2 % (w/v) soy flour, 1 % (w/v) yeast extract, 1 % (w/v) dried egg yolk and 2 % (w/v) corn oil, which implies F/CH=2.78 g_F/g_{CH} and CH/P=0.39 g_{CH}/g_P. The former differences would have relation with both the quality and the quantity of involved nutrient sources among other important factors. Particularly, it is possible that some components present in the agave juice would enhance the symbiotic bacterium growth. It has been published the prebiotic function of inuline in cultures of other microorganisms (Rycroft *et al.,* 2001).

Nonetheless, it is possible that the roles of both F/CH and CH/P ratios during the IJ production of steinernematids would be determinant on the performance of these bioprocesses. In order to determine such relations without any uncertainty, detail knowledge about specific nutrient requirements of both nematode and symbiotic bacterium species is essential. Unfortunately, at the present time our knowledge concerning the former aspects is scarce, particularly for the *S. carpocapsae*/*X. nematophila* complex (Wright and Perry, 2002).

Conclusions

Maximum *S. carpocapsae*-IJ concentrations of 249,444 individuals per mL were obtained in fermentations of 20 d using a culture medium containing 27.6 % (v/v) agave juice, among other ingredients. The evolution of IJ concentration was well modelled using a re-parameterised 3-parameter Gompertz equation, obtaining the kinetic parameters IJ-lag time λ_{IJ} (d), maximum IJ-stage conversion rate m_{max} (d^{-1}) and IJ stage-multiplication factor $(C_{IJ}/C_{IJ,0})_{max}$ (-), which showed to be useful to characterise the performance of each bioprocess.

Acknowledgments

Authors thank financial support of *Fondos Mixtos-Consejo Nacional de Ciencia y Tecnología-Gobierno del Estado de Hidalgo, México* (Grant 200201-9206), and PIFI P/CA-3 2006-14-04 *"Consolidación del Cuerpo Académico de Biotecnología Agroalimentaria"*. Many thanks to *Ometeotl-AC*, *Tlaxcala, México*, for the agave juice supply. Molecular identification of the symbiotic bacterium by the group of Dr. Wacher-Rodarte is deeply acknowledged.

References

Akhurst, R. J. (1980). Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. *Journal of General Microbiology 121,* 303-309.

AOAC International. (1999). Official Methods of Analysis of AOAC International. AOAC International, USA.

Buecher, E. J. and Popiel, I. (1989). Liquid culture of the entomogenous nematode *Steinernema feltiae* with its bacterial symbiont. *Journal of Nematology 21,* 500-504.

Chavarría-Hernández, N. and de la Torre, M. (2001). Population growth kinetics of the nematode, *Steinernema feltiae*, in submerged monoxenic culture. *Biotechnology Letters 23,* 311-315.

Chavarría-Hernández, N., Espino-García, J. J., Sanjuan-Galindo, R. and Rodríguez-Hernández, A. I. (2006). Monoxenic liquid culture of the entomopathogenic nematode *Steinernema carpocapsae* using a culture medium containing whey. Kinetics and modeling. *Journal of Biotechnology 19,* 405-409.

Chuntapa, B., Piyatiratitivorakul, S., Nitithamyong, C., Viyakarn, V. and Menasveta, P. (1999). Optimal lipid:carbohydrate and protein:energy ratios in semi-purified diets for juvenile black tiger shrimp *Penaeus monodon* Fabricius. *Aquaculture Research 30,* 825-830.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry 28,* 350-356.

Ehlers, R. U. (2001). Mass production of entomopathogenic nematodes for plant protection. *Applied Microbiology and Biotechnology 56,* 623-633.

FAO (2002). Early warning on hazardous pesticides. Agriculture 21. http://www.fao.org/ag/magazine/0205sp2test.htm. Cited 21 Oct 2007.

Islas-López, M. A., Sanjuan-Galindo, R., Hernández-Rodríguez, A. I. and Chavarría-Hernández, N. (2005). Monoxenic production of the entomopathogenic nematode *Steinernema carpocapsae* using culture media containing agave juice (aguamiel) from Mexican maguey-pulquero (*Agave* spp). Effects of the contents of nitrogen, carbohydrates and fat on infective juvenile production. *Applied Microbiology and Biotechnology 68,* 91-97.

Kiely, T., Donaldson, D. and Grube, A. (2004). Pesticides industry sales and usage. 2000 and 2001 market estimates. US-EPA, Washington, DC.

Montgomery, D. C. (1991). Design and analysis of experiments. John Wiley & Sons. Singapore.

Neves, J. M., Teixeira, J. A., Simões, N. and Mota, M. (2001). Effect or airflow rate on yields of *Steinernema carpocapsae* Az 20 in liquid culture in an external–loop airlift bioreactor. *Biotechnology and Bioengineering 72,* 369-373.

Rycroft, C. E., Jones, M. R., Gibson, G. R. and Rastall, R. A. (2001) A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. *Journal of Applied Microbiology 91*, 878-887.

Sá, R., Pousão-Ferreira, P. and Oliva-Teles, A. (2007). Growth performance and metabolic utilisation of diets with different protein:carbohydrate ratios by white sea bream (*Diplodus sargus,* L.) juveniles. *Aquaculture Research 38,* 100-105.

Sánchez-Marroquín, A. and Hope, P. H. (1953). Agave juice. Fermentation and chemical composition studies of some species. *Agricultural and Food Chemistry 1,* 246-249.

Shapiro-llan, D. I. and Gaugler, R. (2002). Production technology for entomopathogenic nematodes and their bacterial symbionts. *Journal of Industrial Microbiology and Biotechnology 28,* 137-146.

SIP-Society for Invertebrate Pathology. (2006). Nematodes. http://www.sipweg.org/ DirectoryMCP/nematodes.htm. Cited 21 Oct 2007.

Surrey, M. R. and Davies, R. J. (1996). Pilot-scale liquid culture and harvesting of an entomopathogenic nematode, *Heterorhabditis bacteriophora*. *Journal of Invertebrate Pathology 67,* 92-99.

Woodring, J. L. and Kaya, H. K. (1988). Steinernematid and Heterorhabditid Nematodes: a Handbook of Biology and Techniques. USA: Arkansas Agric. Exp. Stn.

Wright, D. J. and Perry, R. N. (2002). Phisiology and biochemistry. In: *Entomopathogenic nematology* (R. Gaugler, ed.). CABI Publishing. Cambridge MA.

Yoo, S. K., Brown, I. and Gaugler, R. (2000). Liquid media development for *Heterorhabditis bacteriophora:* lipid source and concentration. *Applied Microbiology and Biotechnology 54,* 759-763.