한국지하수토양환경학회 추계학술대회
2001년 9월 21-22일 전 주 대 학 교

MEDIA DEVELOPMENT FOR MASS PRODUCTION OF ENTOMOPATHOGENIC NEMTOIDE *HETERORHABDITIS BACTERIOPHORA* AS AN INSECTICIDE

Sun Kyun Yoo[1], Sung Young Cho[2], Seung Jai Kim[2], Randy Gaugler[3]
[1] *Engineering Research Institute, Chonnam National University. skyoo@chonnam.chonnam.ac.kr*
[2] *Department of Environmental Engineering, Chonnam National Univeristy*
[3] *Department of Entomology, Rutgers University, New Brunswick NJ 08901 USA*

KEY WORDS: *Heterorhabditis bacteriophora*; *Photorhabdus luminescens*; entomopathogenic nematode; lipids; fermentation; liquid culture

Abstract

The biological control potential of entomopathogenic nematodes (EPN) can be enhanced by improved culture efficiency. Optimization of media is a key factor for improving in vitro mass production of entomopathogenic nematodes. EPN yield was dependant of complex medium concentration, of which mixture is carbohydrates, lipids, proteins, salts, and growth factors, on the growth of *Heterorhabditis bacteriophora* and its symbiotic bacterium *Photorhabdus luminescensLipids.*

Introduction

Entomopathogenic nematodes (EPN) of the families Steinernematidae and Heterorhabditidae are recognized as effective biocontrol agents of insect pests (Kaya and Gaugler 1993). When they get into inside of insect, their arrested stage, so called infective juveniles, resume development. They release their symbiotic bacteria in hemolymph of insects and kill the host within 36 to 48 h. The bacteria create a suitable environment for nematode development and provide essential nutrients for nematode growth (Akhurst 1982). Entomopathogenic nematodes have been commercialized for twenty years, yet their full potential has hardly been realized, due to non-competitive costs compared with chemical insecticides and inconsistent nematode quality (Gaugler 1997). Two processes, in vivo and in vitro culture, have

been developed commercially for mass production of EPN. Culture in vivo has been used at the cottage industry level because minimal capital investment and expertise are required. In addition, a high yield coefficient further enhances the feasibility of in vivo culture for small-scale production . However, this process limits scale-up for mass production and is sensitive to the physiological condition and pathogen load of the insect host. Both issues may be addressed by improving liquid culture technology (Yoo *et al.* 2000, Yoo *et al.* 2001[a], Yoo *et al.* 2001[b]). Maximum nematode growth and reproduction can be achieved through optimizing. This paper reports the optimization of media such as lipids, proteins, carbohydrates, biomass of symbiotic bacteria and etc.

Materials and methods

H. bacteriophora were reared in last instar *Galleria mellonella* Dutky et al (1967). Infective juveniles, collected within four days of emergence and filtered to remove dead nematodes before use. Symbiotic bacteria were isolated using a modified method of Akhurst (1980). Established stock cultures kept at 4C until used. Monoxenic cultures were established on lipid agar plates, before transfer to liquid culture. Infective juveniles were surface-sterilized using 0.1 % (w/v) benzethonium chloride, washed three times with sterile distilled water and added to fresh bacterial lawns on lipid agar plates. After about 5 days at 25°C, the plate contained sufficient infective juveniles. The enriched liquid culture medium contained the following components: distilled water 1000mls, soy flour powder 25 g, yeast extract 5 g, lactalbumin hydrolyzate 10 g, canola: olive oil (50:50 w/v) 25g, cholesterol 0.2g, liver extract 0.1g, NaCl 4.0 g, MgSO4 0.5 g, CaCl2 0.3g, and KCl 0.3g. For all experiments nematode and bacterial yields were determined using the following methods. Nematode juvenile stages (J2, J3, J4, and infective juveniles), hermaphrodites, and gravid adults, were counted every two days, using a stereomicroscope, unless stated otherwise.

Results and discusion

The production of EPN was closely related to the size of adults *in vivo* culture and complex media concentration (Fig.A) Liquid media concentration of 84 g/l resulted in the highest size of nematodes, and surely produced the highest yield of nematodes. In vivo and vitro culture, the biomass of symbiotic bacteria and size of host

influenced final yield of infective juveniles. Linear relation ship appeared between yield of infective juveniles and the amount of biomass and size of *G. mellonella* (Fig. B). The yield of symbiotic bacteria and infective juveniles was dependant of different salt composition in media (Fig. C and E). Salt composition D supported the highest yield of nematode even though this salt composition did not ensure the same result in bacterial culture. The number of nematode was about 280,000 infective juveniles obtained within 12 days. Previous report addressed that different type of lipid make the yield to be varied in liquid culture (Yoo *et al.* 2000). The different type of protein sources also influenced the yield. Unhydrolyzed soybean flour supported the highest yield of nematodes (Fig. D).

References

Akhurst RJ (1980) Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. J Gen Microbiol 121: 303-309

Akhurst RJ (1982) Antibiotic activity of *Xenorhabdus* spp., bacteria symbiotically associated with insect pathogenic nematodes of the family Heterorhabditidae and Steinernematidae. J Gen Microbiol 128: 3061-3065

Dutky SR, Robbins WE, Thompson JV (1967) The demonstration of sterols as requirements for the growth, development and reproduction of the DD-136 nematode. Nematologica 15.

Gaugler R (1997) Alternative paradigms for commercializing biopesticides. Phytoparasitica 25:179-182.

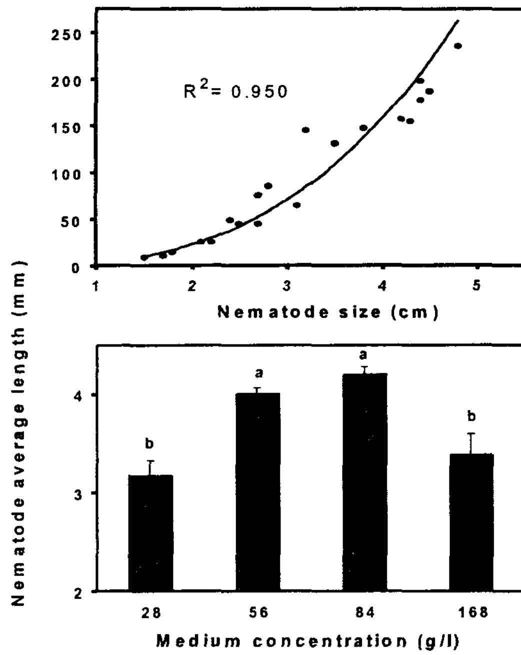
Hu K, Webster, JM (1998) *In vitro* and *in vivo* characterization of a small-colony variant of the primary form of *Photorhabdus luminescens* MD. Appl Environ Microbiol 64: 3214-3219

S.K.Yoo, Nancy Cohen, and Randy Gaugler. (2001) The Effect of Media Concentration on Growth of The Entomopathogenic Nematode *Heterorhabditis bacteriophora* in liquid culture. Journal of Applied and Microbiology (in press)

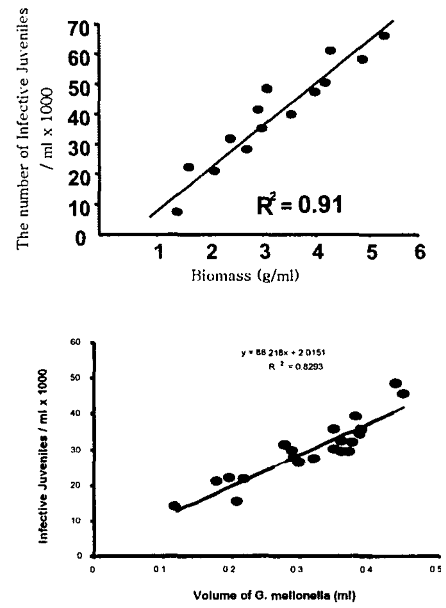
Sun Kyun Yoo, Randy Gaugler, and Christopher W. Brey. (2001). Entomopathogenic nematode *Heterorhabditis bacteriophora* production in relation to its symbiont *Photorhabdus* sp. strain TG growth in liquid culture. Korean Journal of Applied Microbiology and Biotechnology.p. 104-109.

S.K.Yoo, Ian Brown, and Randy Gaugler. (2001). Liquid media development for the *Heterorhabditis bacteriophora*: lipid source and concentration. Applied Microbiology biotechnology. 54: 759-763.

A

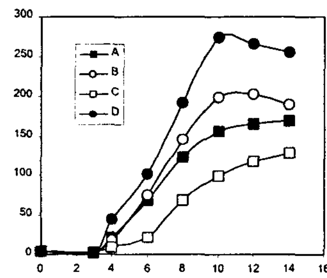


B.



C

E.



D.