

# Relation of the entomopathogenic nematodes and their symbiotic bacteria during reproduction

**Richou Han**

*Guangdong Entomological Institute,  
Guangzhou 510260, China*

# Objective of this talk

- Try to summarize the progress on the relation of **Entomopathogenic nematodes and their symbiotic bacteria** during reproduction



# **Nematodes : symbiotic bacteria**

- **Food signals**
- **Nutrients**
- **Trans-specific toxins**
- **Bacterial colonization of nematodes**

# The facts about

- food signals .....

## The discovered parameters influencing the IJ recovery

Parameters	Effect on IJ recovery		References
	S	H	
Insect haemolymph	+	+	Strauch & Ehlers,1998 □ Ciche & Ensign, 2003
Insect tissue culture of			
<i>Drosophila melanogaster</i> DL-1	N	+	Ciche & Ensign, 2003
<i>Spodoptera litura</i> SL-zsu-1	+	-	Han et al., 2000
Symbiont culture in stationary phase	+	+	Strauch & Ehlers,1998
Compounds of artificial media	-	-	Strauch & Ehlers,1998 Ciche & Ensign, 2003
Fresh human blood	N	-	Ciche & Ensign, 2003
High bacterial density	+	+	Strauch & Ehlers,1998
pH within 6.5-9.0	N	+	Strauch & Ehlers,1998
pH<6.5	N	-	Strauch & Ehlers,1998
Increase of CO <sub>2</sub> concentration	N	+	Strauch & Ehlers,1998 Jessen et al., 2000
IJs originate from laid eggs	N	+	Ehlers & Shapiro-Ilan, 2004

Note: “+”,effect; “-”, no effect; N, data not available.

## The discovered parameters influencing the IJ recovery

Parameters	Effect on IJ recovery		References
	S	H	
IJs originate from <i>endotokia matricida</i>	N	-	Ehlers & Shapiro-Ilan,2004
Incompatible symbionts of <i>Photorhabdus</i>	-?	+	Han & Ehlers, 1998
Incompatible symbionts of <i>Xenorhabdus</i>	+	-?	Han & Ehlers, 1998
Other microorganisms <i>Escherichia coli</i>	+	-	Strauch & Ehlers, 1998
<i>Photobacterium phosphoreum</i>	+	-	Han et al.,2001
Insect hemolymph			Ciche & Ensign,2003
Centrifuged, filter sterilized	N	+	
Heat treatment (80 °C,30min)	N	+	
Dialyzed (10kDa)	N	-	
Centricon (10kDa) filtrate	N	+	
Through anion-exchange column (Sephacrose Q)	N	+	
Through cation-exchange column (Sephacrose S)	N	-	
Pronase (20ug/ml, 50 °C,2h)	N	+	

**But we do not know the exact  
compounds of the signals ...**

# **Nutritional significance of**

- **Insects**
- **Artificial media**
- **Symbiotic bacteria**
  - **Strains**
  - **Phase variation**
- **Expressed cipA or cipB proteins**

## Production specificity between the nematodes and bacteria

Nutrient suppliers	Nematode		
	<i>Steinernema</i> spp.	<i>Heterorhabditis</i> spp.	<i>Panagrellus redivivus</i>
Axenic <i>Galleria mellonella</i> larvae	+	-	N
(Han & Ehlers, 2000)	(Han & Ehlers, 2000)	(Han & Ehlers, 2000)	
Cell culture of <i>Spodoptera litura</i> SL-zsu	+	-	N
(Han et al., 2000)	(Han et al., 2000)	(Han et al., 2000)	
Artificial media with high nutrient factors	+	-	N
(Friedman 1990; Ehlers et al. 1990; Lunau et al., 1993)	(Friedman 1990; Ehlers et al. 1990; Lunau et al., 1993)	(Friedman 1990; Ehlers et al. 1990; Lunau et al., 1993)	
<i>Xenorhabdus</i> bacteria on lipid agar plates	(Han & Ehlers, 2000)	(Han & Ehlers, 2000)	
Phase I	+++ (indigenous) or – (some non-indigenous)	-	+
Phase II	+	-	N

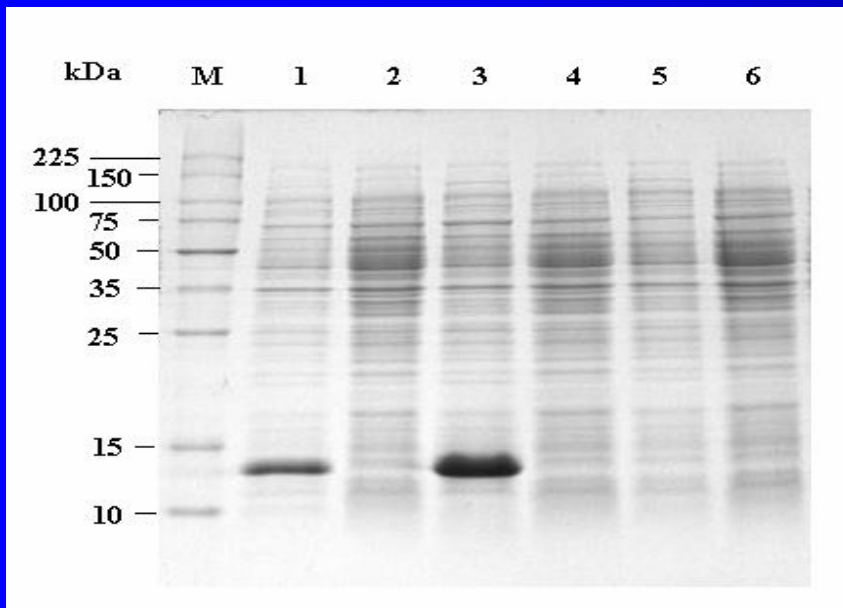
## Production specificity between the nematodes and bacteria

Nutrient suppliers	Nematode		
	<i>Steinernema</i> spp.	<i>Heterorhabditis</i> spp.	<i>Panagrellus redivivus</i>
<i>Photorhabdus</i> bacteria			
Phase I	-	+++ (indigenous) or – (some non-indigenous)	N
Phase II	+	+	N
Other bacteria, such as <i>E. coli</i> etc.	+ (Boemare, 1983; Ehlers <i>et al.</i> , 1990; Aguilera <i>et al.</i> , 1993; Grewal <i>et al.</i> , 1997)	- (Ehlers <i>et al.</i> , 1990; Han <i>et al.</i> , 2000)	+
<i>P. asymbiotica</i>	N	+ (Yoshiga <i>et al.</i> , 2005)	N

# **Nutritional significance of**

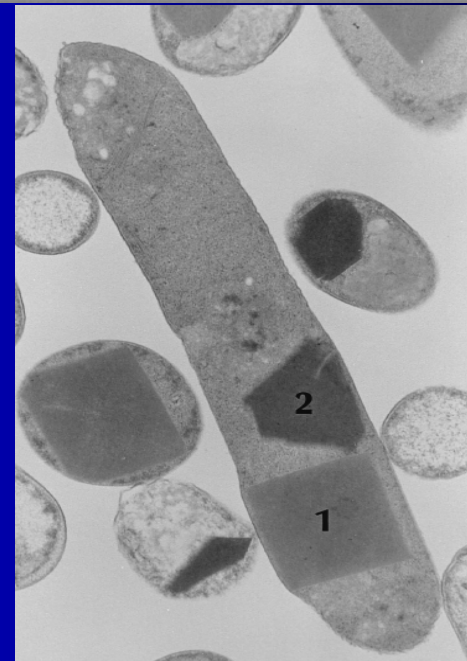
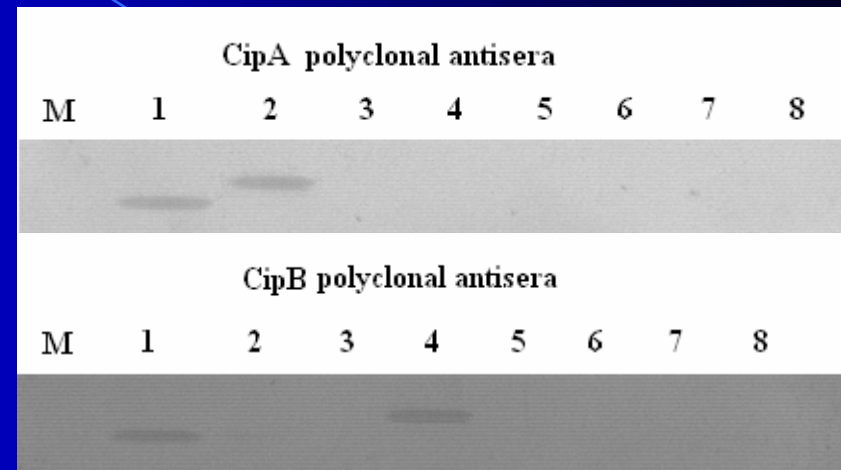
- **expressed cipA or cipB proteins**

# Expression of Crystalline inclusion proteins in *E. coli* for feeding experiments

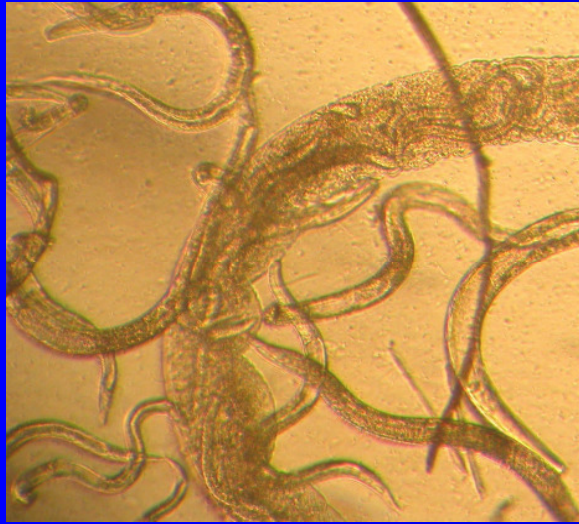


M. Marker

- 1.+IPTG BL21(DE3)/pET-15b-cipA
- 2.-IPTG □ BL21(DE3)/pET-15b-cipA
- 3.+IPTG BL21(DE3)/pET-15b-cipB
- 4.-IPTG □ BL21(DE3)/pET-15b-cipB
- 5.+IPTG BL21(DE3)/pET-15b
- 6.-IPTG □ BL21(DE3)/pET-15b



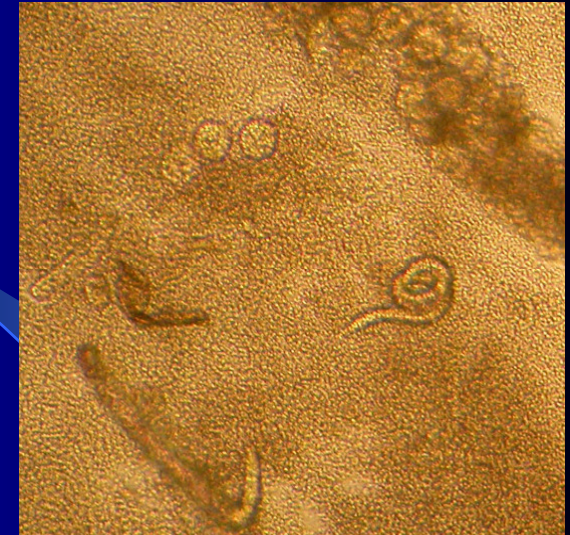
Bintrim *et al.*, 1998



A



B



C

- Development of *S. longicaudum* X-7 nematodes in the liquid *E. coli* cultures with (Fig. A and B) or without (Fig. C) expressed CipA or/and CipB proteins.
- It was concluded that Cip is very important for the formation of IJs.

# Nutritional significance

- Genes involved in the production symbiosis between *Heterorhabditis* spp. and *Photorhabdus* spp.
  - *cipA* or *cipB*
  - *ngrA*
  - *hexA*
  - *exbD*
- Genes involved in the production symbiosis between *Steinernema* spp. and *Xenorhabdus* spp. (?)

## **Trans-specific nematocidal activities of symbiotic bacteria**

- **Symbiotic bacteria may produce toxic effect on the non-symbiotic nematodes.**

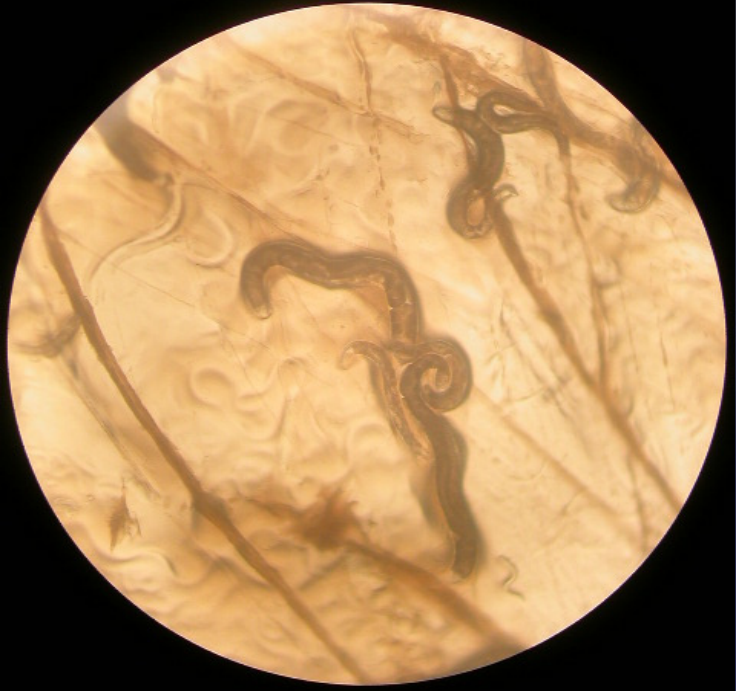
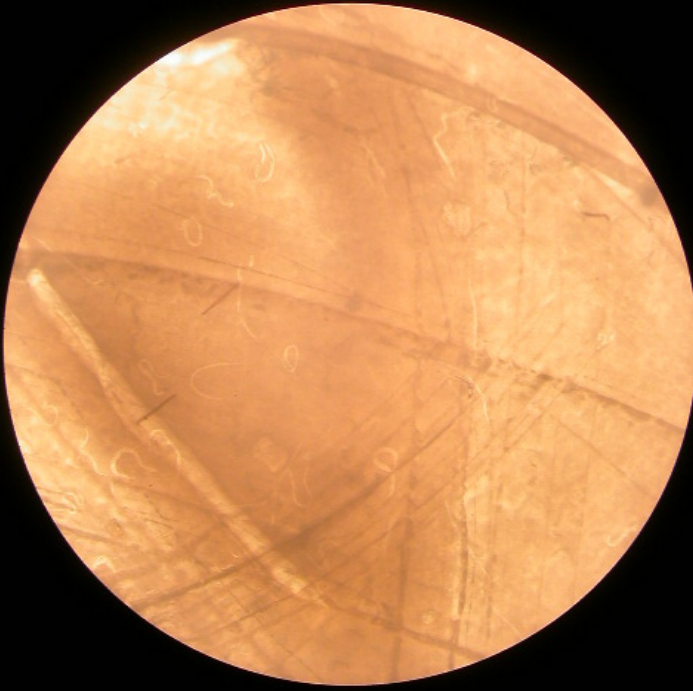
**Mortality of monoxenic *H. bacteriophora*, *H. indica* and *S. carpocapsae* 24h after inoculation into mono- or dixenic symbiont cultures of *P. luminescens* and *X. nematophila*.**

Nematode species	Symbiont cultures					
	H06	LN2	Q6	A24	H06+LN2	Q6+LN2
<i>H. bacteriophora</i>	-	+	-	-	+	+
<i>H. indica</i>	-	-	+	-	-	+
<i>S. carpocapsae</i>	+	-	-	-	-	-

“+”: mortality; “-”: No mortality

# Gene(s) involved in the nematocidal activity

- From more than 5,000 transposon mutants of *P. luminescens* LN2, one mutant was found to support the survival and reproduction of *H. bacteriophora* H06 nematodes.
- From this mutant, DNA sequencing identified an open reading frame of 1095bp that was flanked by the transposon.
- A novel gene was involved in the nematocidal activity of *P. luminescens* LN2 against *H. bacteriophora* H06 nematodes.



# **Bacterial colonization of nematodes**

- **Significant differences in the ability of the nematodes to retain the bacterial cells in the intestines of the IJs.**
- **Surface sterilization of the IJs obtained from heterologous bacterial isolates and free of bacteria in their intestines resulted in the establishment of a method of producing axenic IJs**

# One example:

Table 3

The percentage of the infectives with bacteria in *in vivo* culture of various combinations of *Heterorhabditis* spp. and *Xenorhabdus luminescens*

Nematode strain	Bacterium				
	X1HNA	X1H06	X1G12	X1H3	X1HNZ
<i>H. megidis</i>	94 (3.9) <sup>a</sup>	— <sup>b</sup>	—	—	41 (3.4)
<i>Heterorhabditis</i>					
sp. H06	0	98 (1.4)	—	—	83 (4.0)
sp. G12	11 (1.1)	0	92 (1.3)	98 (0.1)	—
sp. H3	27 (1.8)	n <sup>c</sup>	94 (3.9)	94 (0.9)	—
<i>H. heliothidis</i>					
HNZ	1 (0.01)	88 (2.3)	—	—	93 (2.3)

<sup>a</sup> Percentage with (SE).

<sup>b</sup> Not successful combination. See Table 2.

<sup>c</sup> n = No infectives available.

# Bacterial colonization of nematodes:

## Phase variation

Bacteria \ Nematodes	<i>Xenorhabdus</i>		<i>Photorhabdus</i>	
	Phase one	Phase two	Phase one	Phase two
<i>Steinernema</i> spp.	+	+	N	N
<i>H. bacteriophora</i>	N	N	+	-

# Bacterial colonization of nematodes

- Genes involved in the bacterial colonization of *Steinernema* nematodes
  - Four putative regulators required for nematode colonization: *RpoS*, *RpoE*, *Lrp* and *NilD RNA* (Vivas *et al.*, 2001; Heungens *et al.*, 2002)
  - Nutrient transport proteins: Nil proteins (Cowles *et al.*, 2004)
  - Metabolism related: *iscRSUA-hscBA-fdx* (Martens *et al.*, 2003)
  - Nutrient exchange: *serC-aroA* (Martens *et al.*, 2005; Orchard *et al.*, 2005)
- Genes involved in the bacterial colonization of *Heterorhabditis* nematodes: *pbgE1* (Bennett *et al.*, 2005) (Also looking forward to the talk from Dr Todd Ciche in Session 7)



Thank you!

□ □ !