

**INRA**

**« Biocontrol Symbiosis »**

**Entomopathogenic Nematodes and Symbiotic Bacteria  
(4th International Symposium)**

**Landeskulturzentrum Schloss Salzau,  
June 1 -6, 2006, Kiel, Germany**

**COST 850 – Achievements in France –  
UMR EMIP n°1133 (UMII - INRA)  
UMR GPIA n°5171 (UMII – CNRS)**

# Symbiosis and Pathology

**Entomopathogenic  
nematodes**

**Insecticidal  
toxins**



**Invasion  
Hemocoel**

**Oral toxicity  
Midgut**

**Insect pathology**

**Septicaemia and  
nematode reproduction**

**Laboratory conditions**



# *Photorhabdus* genome sequencing

Écologie microbienne des insectes  
et interactions hôte-pathogène



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Génomique des micro-  
organismes pathogènes  
(GMP)



Paris

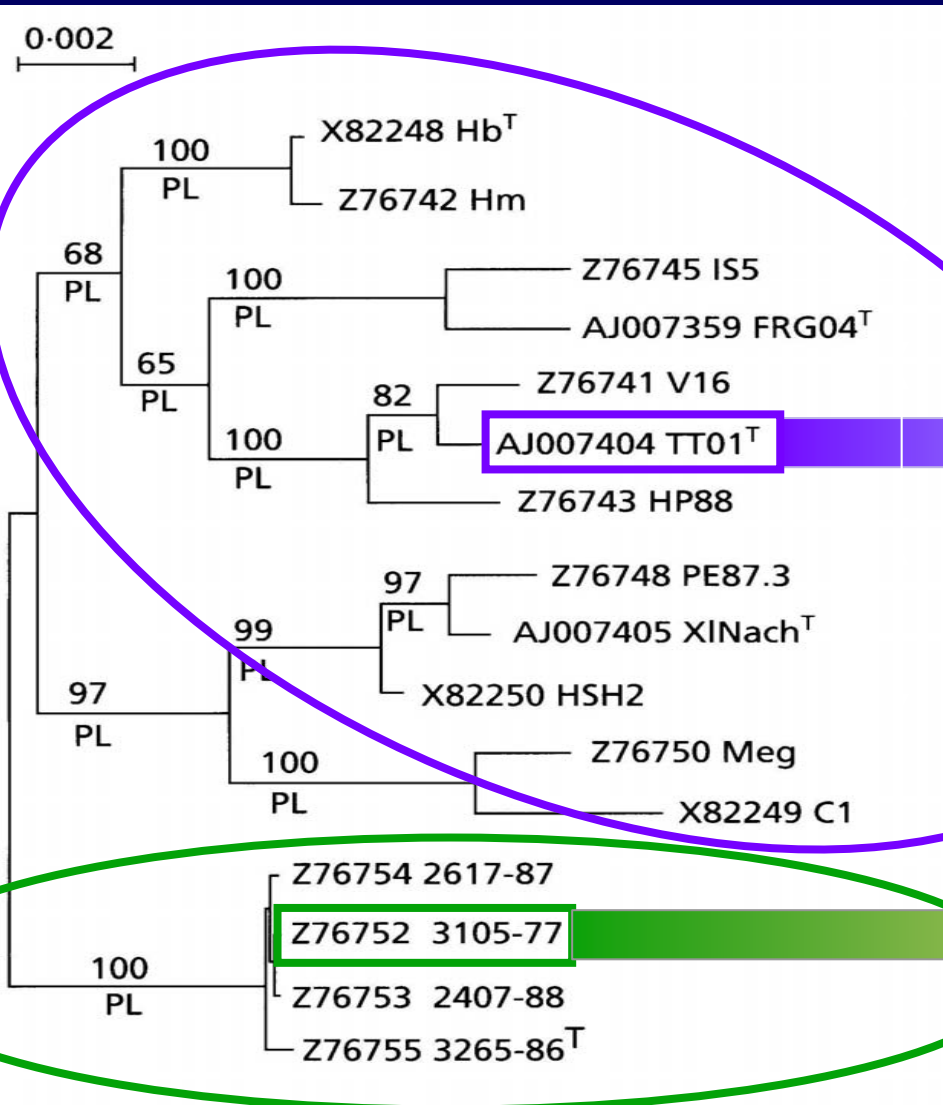
**Frank KUNST**  
**Antoine DANCHIN**

Nematode strains

Sub-species

Species

# Choice the strain



*luminescens*

*P. luminescens*

*akhurstii*

Strain sequenced by the  
INRA- Pasteur Institute  
-BayerCropScience  
consortium

*laumondii*

*P. temperata*

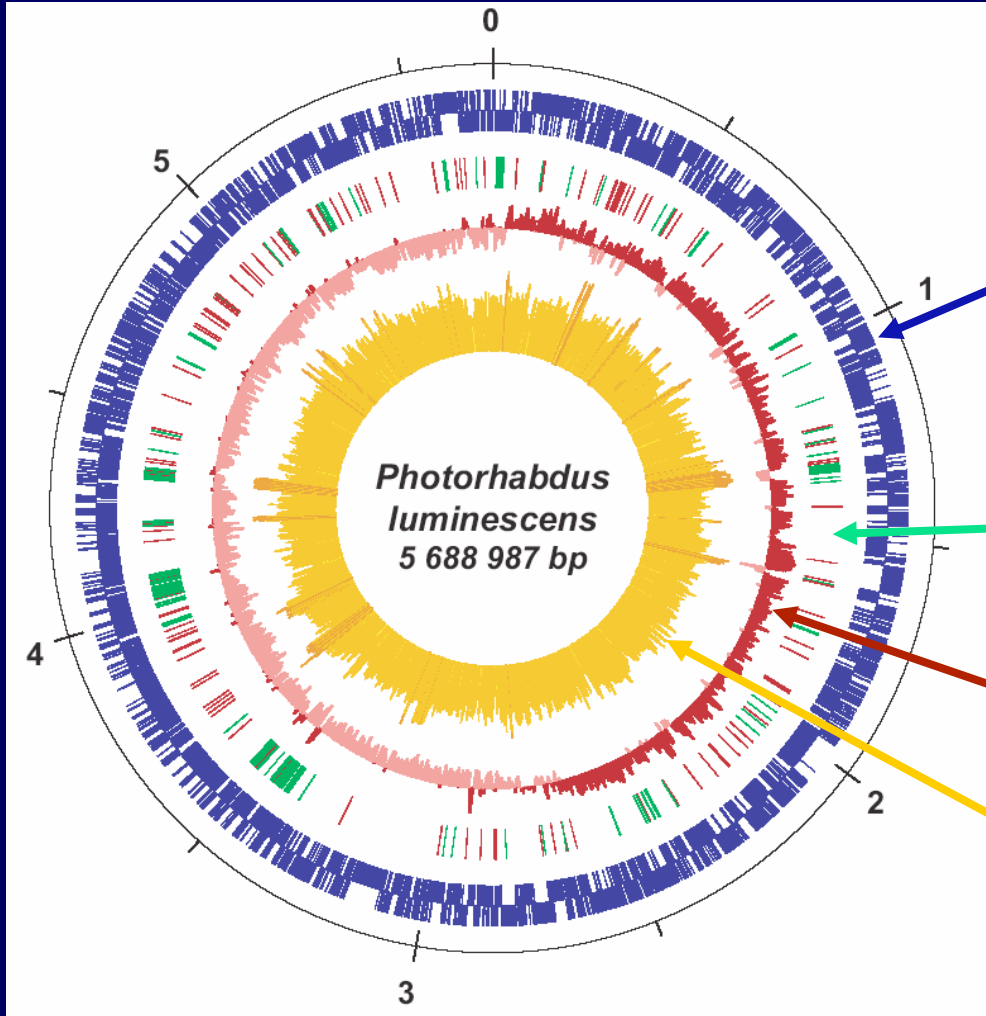
*temperata*

Strain under  
sequencing  
(Sanger Institute)

*P. asymbiotica*

Clinical strains

# THE TT01 GENOME



Gene density and orientation on the leading and lagging strands

Insertion sequences and phage sequences

« GC bias »  
(G-C/G+C)

GC content  
Average :42,8%

# *Photorhabdus* TT01 genome sequencing

**1. 5,66 Mbases genome, one unique replicon, 5000 ORFs**

**2. Genes potentially involved in interaction with invertebrate host**

- Entomotoxins of the group Tc
- Entomotoxin JHE-like
- proteases, lipases
- type III secretion system
- iron uptake systems
- hemolysins
- antibiotics biosynthesis pathway

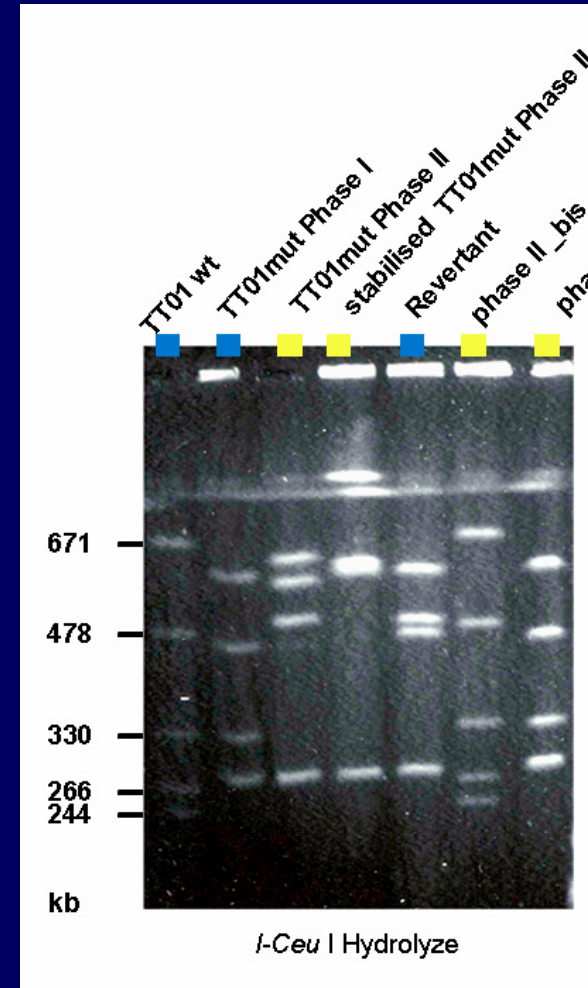
**3. Mobile and repeated genetic elements**

- - phage remnants : 4% of the genome
- - 195 insertion sequences (IS) or transposons
- - 711 ERIC (Enterobacteriaceae repeated and intergenic consensus) sequences (versus 21 in *E. coli* K12 genome).
- - over-represented gene classes (*rhs* elements, *tcc* genes, *vgrG* genes, *tpsAB* secretion system, colicins-like, iron uptake, recombinases, etc).

# The mobile and repeated genetic elements a support for unusual genomic variability in the *Photorhabdus* genome

## Important variation in genomic patterns !

- Identical phenotypes = Different genomic patterns
- Identical patterns = Different phenotypes



# The PhIA hemolysin which belongs to the *Serratia* type of hemolysins

1000 2000 3000 4000 5000 6000 pb



*Photorhabdus luminescens*



*Serratia marcescens*



*Edwardsiella tarda*



*Proteus mirabilis*



*Hemophilus ducreyi*

On blood agar *Photorhabdus luminescens* displays a halo of hemolysis around colonies

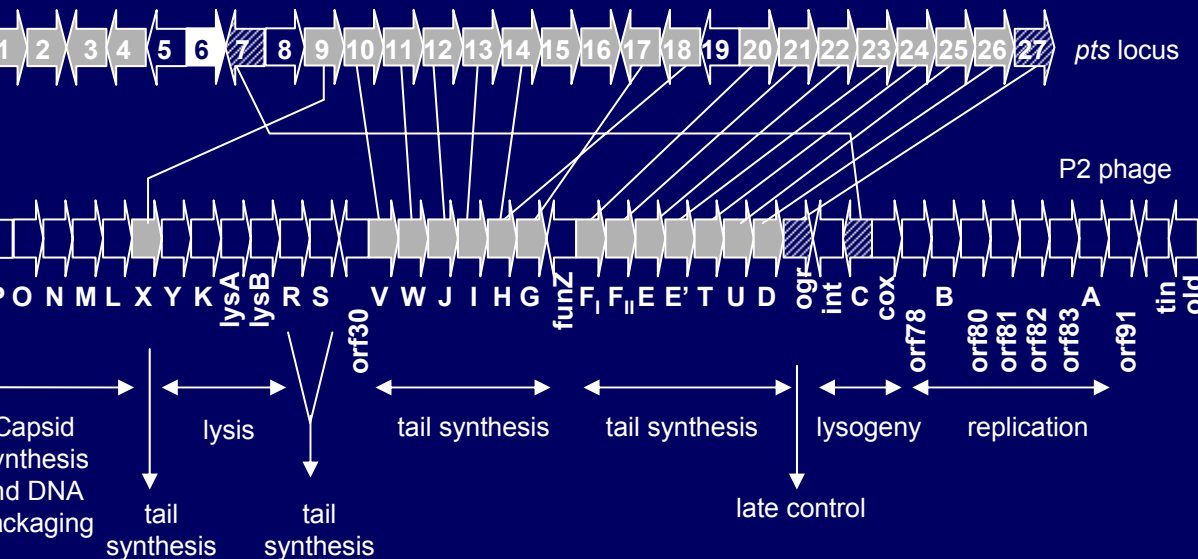


ation / Secretion system

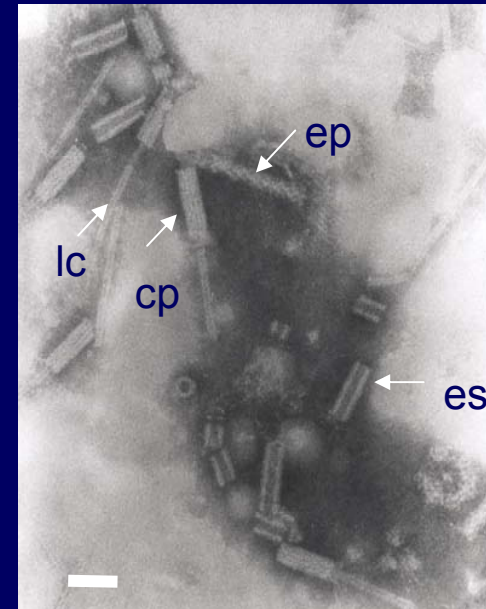
Hemolysin

# The *pts* locus is a phage P2 remnant that encodes a phage tail-like particle

*pts* locus (phage tail synthesis)

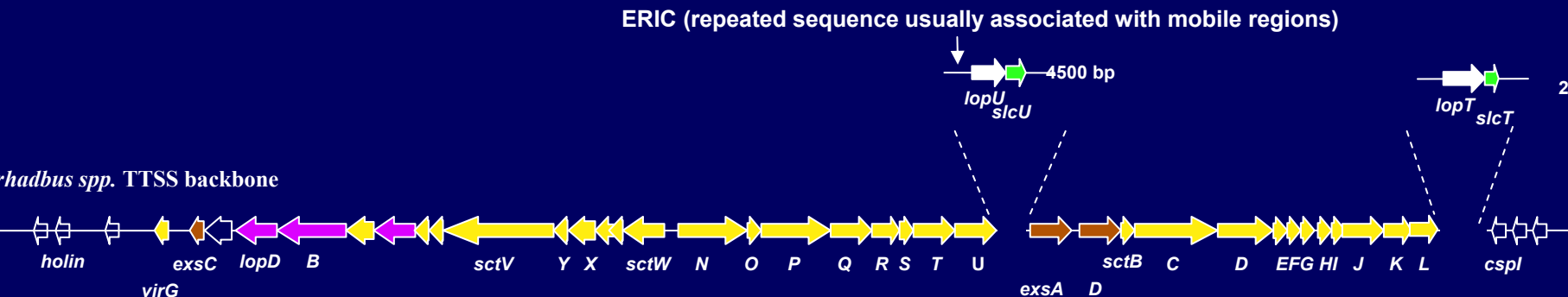
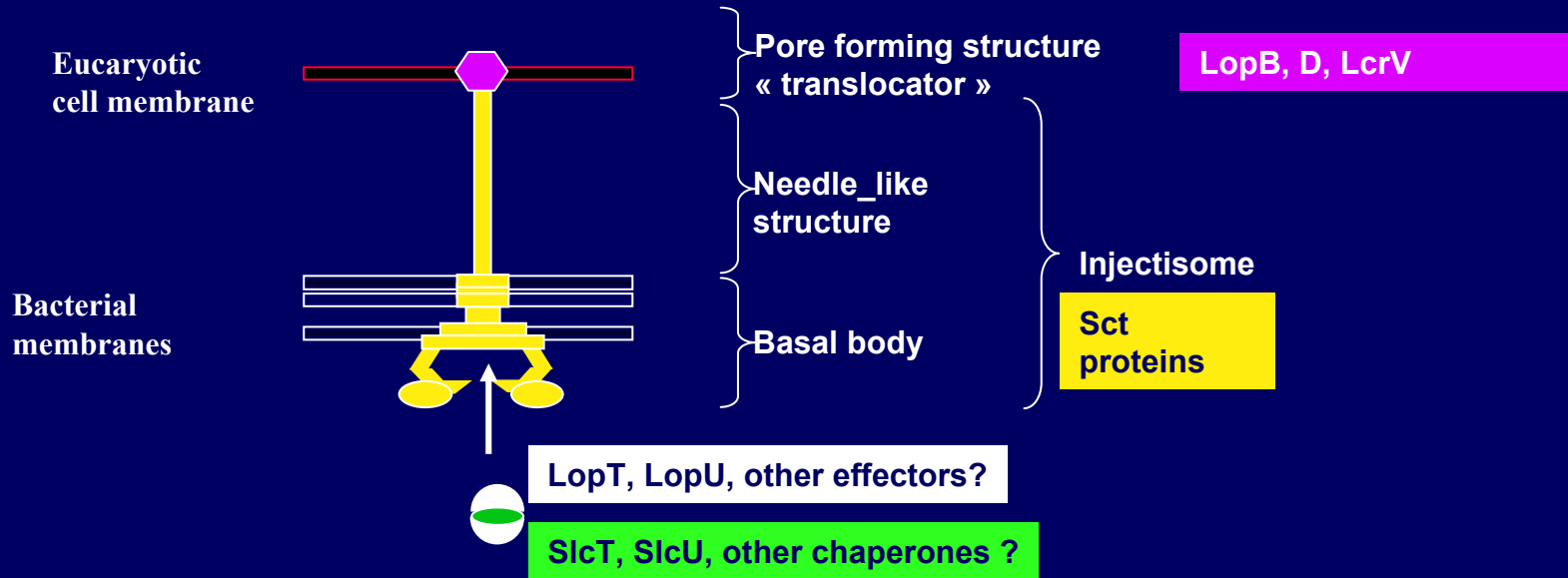


R-Photorhabdycin



ep : extended particles  
 cp : contracted particles  
 es : empty contracted sheath  
 lc : loose cores

# Type III secretion system in *Photobacterium*



# Physiopathology and Gnotobiology

**Écologie microbienne des insectes et interactions hôte-pathogène (EMIP)**

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- Marie Hélène BOYER
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- Bernard DUVIC
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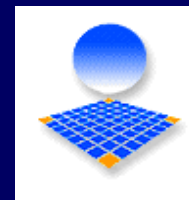
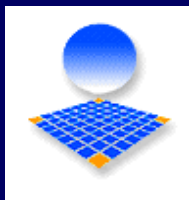
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- Nathalie LE BRUN
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# TAXONOMY

## *Steinernema*

- *S. abbasi*
- *S. arenarium* (synonym : *S. anomalae*)
- *S. affine*
- *S. bicornutum*
- *S. carpocapsae*
- *S. ceratophorum*
- *S. cubanum*
- *S. diaprepesi*
- *S. feltiae*
- *S. glaseri*
- *S. hermaphroditum*
- *S. intermedium*
- *S. karii*

## *Xenorhabdus*

- *Xenorhabdus indica.*
- *Xenorhabdus kozodoii*
- *Xenorhabdus bovienii*
- *Xenorhabdus budapestensis*
- *Xenorhabdus nematophila*
- *Xenorhabdus budapestensis*
- *Xenorhabdus poinarii*
- *Xenorahbdus doucetii*
- *Xenorhabdus bovienii*
- *Xenorhabdus poinarii*
- *Xenorhabdus griffinii*
- *Xenorhabdus bovienii*
- *Xenorhabdus hominickii*

# Taxonomy

## *Steinernema*

- *S. kraussei*
- *S. kushidai*
- *S. monticolum*
- *S. puertoricense*
- *S. rarum*
- *S. riobrave*
- *S. scapterisci*
- *S. scarabei*
- *S. serratum* (syn *S. longicaudum*)
- *S. siamkayai*
- *Steinernema* (Queensland, strain Q58)
- *Steinernema* (Queensland, strain Q1)
- *Steinernema* (St-Vincent, strain VC01)

## *Xenorhabdus*

- Xenorhabdus bovienii*
- Xenorhabdus japonica*
- Xenorhabdus hominickii*
- Xenorhabdus romanii*
- Xenorhabdus szentirmaii*
- Xenorhabdus cabanillasii*
- Xenorhabdus innexii*
- Xenorhabdus koppenhoferii*
- Xenorhabdus ehlersii*
- Xenorhabdus stockii*
- Xenorhabdus beddingii*
- Xenorhabdus miranidis*
- Xenorhabdus mauleonii*

# Taxonomy

## *Heterorhabdus*

- *H. bacteriophora* subgroup Brecon
  - *H. bacteriophora* subgroup HP88
  - *H. bacteriophora*
  - *H. bacteriophora*
  - *H. bacteriophora* subgroup NC
  - *H. indica*
  - *H. megidis* Nearctic group (Ohio, Wisconsin)
  - *H. megidis* Palaearctic group
  - *H. zealandica*
- 
- **Clinical strains (US)**
  - **Clinical strains (Australia)**

## *Photorhabdus*

- Photorhabdus luminescens luminescens*
  - Photorhabdus luminescens laumondii*
  - Photorhabdus luminescens kayaii*
  - Photorhabdus luminescens thracensis*
  - Photorhabdus temperata*
  - Photorhabdus luminescens akhurstii*
  - Photorhabdus temperata*
  - Photorhabdus temperata temperata*
  - Photorhabdus temperata*
- 
- Photorhabdus asymbiotica asymbiotica*
  - Photorhabdus asymbiotica australis*

# GNOTO BIOLOGY

**1°/ Analytical way = Qualitative and quantitative bacteriological analyses** of the *dauer larvae* from soil samplings

= Bacteriological definition of the residential microflora

# Gnotobiology : The Concept of monoxenic associations in EPNs

- **A. Natural intestinal symbionts**
- *Xenorhabdus* from *Steinernema* spp.
- *Photorhabdus* from *Heterorhabditis* spp.

**Sources** : ALL DESCRIPTIONS REPORTED UNTIL TODAY

# However...

- **B. Occasional bacteria from nematodes**
  - *Pseudomonas* spp. from *S. carpocapsae* (1) (2) (7)
  - *Vibrio* spp. from *S. carpocapsae* (2) (7)
  - *Enterobacter* spp. from *S. carpocapsae* (2) (7)
  - *Serratia marcescens* from *S. carpocapsae* (7)
  - *Serratia proteomaculans* (= *S. liquefaciens*) (2) (7)
  - *Citrobacter freundii* from *S. carpocapsae* (7)
  - *Alcaligenes* sp. from *S. carpocapsae* (1)
  - *Ochrobactrum* spp. from *S. scapterisci*, *H. indica* (3) (5)
  - *Paracoccus denitrificans* from *S. scapterisci* (3)
  - *Providencia rettgeri* from *Heterorhabditis* spp. (4)
  - *Paenibacillus nematophilus* from *Heterorhabditis* spp. (6)

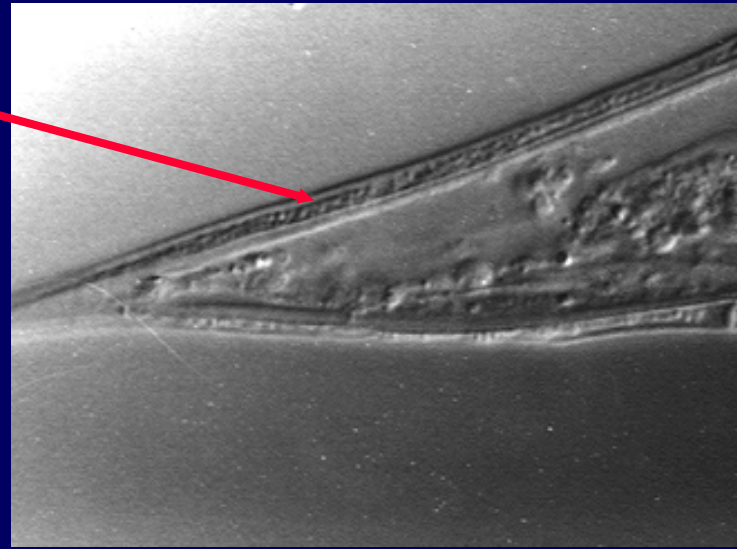


**Sources:** (2) Boemare (1983); (1) Lysenko (1974); (3) Aguilera *et al.* (1993); (4) Jackson *et al.* (1995); (5) Babic *et al.*, (2000); (6) Enright *et al.*, (2003); (7) Gouge & Snyder (2006)

# Gnotobiology

## Example : case of *Steinernema scapterisci* :

- Abundant microflora between the old cuticle L2 and the L3 cuticle of dauer larvae
  - Presence of the bacteria inside the Bird and Akhurst's intestinal vesicle
- = Several genera between the cuticles
- = **but only *Xenorhabdus* sp. in the gut**



# Gnotobiology

- **Direct isolation from the nature**
  - Transmission of *Xenorhabdus* and *Photorhabdus* is determined at a high level of specificity (antimicrobial barriers, specific attachment)
  - Possible occurrence of other micro-organisms mainly on the tégument (Bonifassi *et al.*, 1999; Enright *et al.*, 2003) but never involved in the nematode development and sometimes detrimental.
- **Mainly from lab samples**
  - occurrence of other bacteria occupying the same microbial niche (Boemare, 1983; Jackson *et al.*, 1995, Babic *et al.* , 2000, Gouge and Sydner, 2006)

# Gnotobiology

## 2° / Synthetic way = Gnotobiological experiments

- Axenization of eggs and/or germ free rearing
- Gnotoxenic re-associations defining the most suitable associations in terms of :
- **Qualitative and quantitative *in vitro* and *in vivo* production of nematodes**
- **Perenniality of the association during the generations**

# Parasitic success

(number of infestations with emergence on total number of infestations)

	<i>S. scapterisci</i> (160 insects)	<i>S. carpocapsae</i> (160 insects)
Aposymbiotics	37.5%	46%
Symbiotics	59%	80%
P value ( $\chi^2$ test)	P = 0.003	P = 0.001

**symbiotics > aposymbiotics**

# Statistical analysis of the reproduction rate (total number of offspring)

Median	<i>S. scapterisci</i>	<i>S. carpocapsae</i>
symbiotic	39100	111200
aposymbiotic	30500	15500
Symbiotic on aposymbiotic	1.3	7.2
Mann-Whitney test	P = 0.0358	P < 0.0001

# Transmission of the bacteria

(number of bacteria into 500 L3 [5 repeats])

	<i>S. carpocapsae</i>	<i>S. scapterisci</i>
holoxenic	21880 ± 2940 <i>X. nematophila</i>	34 ± 7 <i>X. innexii</i>
heteroxenic	0	0
aposymbiotic	0	0

In comparison with *S. carpocapsae* - *X. nematophilus*  
what is the mutualism of *S. scapterisci* – *X. innexii*

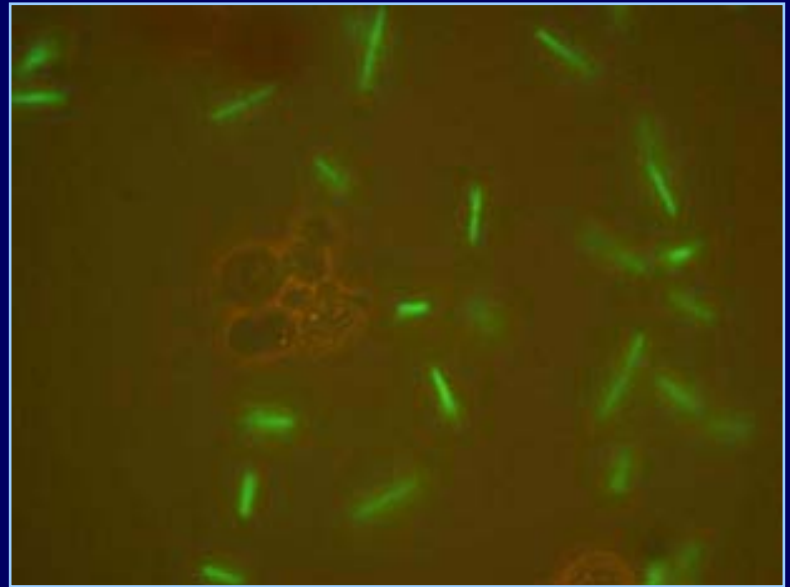
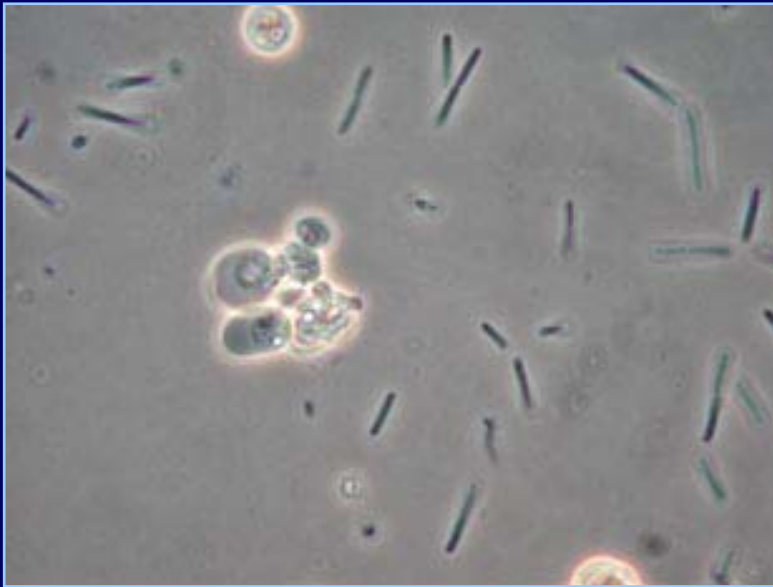
- Lesser transmission of bacteria by *S. scapterisci*
- Lesser impact on *S. scapterisci* development
- An important proportion of natural population of these nematodes is aposymbiotic
- *S. scapterisci* – *X. innexii* is probably a younger association



**The mutualistic association manages optimally the installation in the insect and provides a better reproduction**

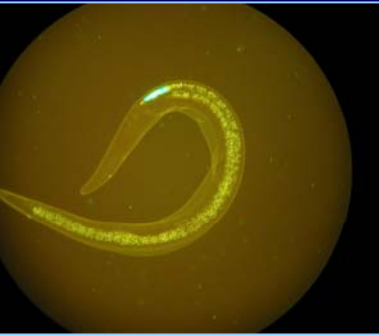
# **PATHOLOGY : Details on the bacteria life cycle**

**Bacteria were labelled with Green Fluorescent Protein (GFP) to follow the migration**

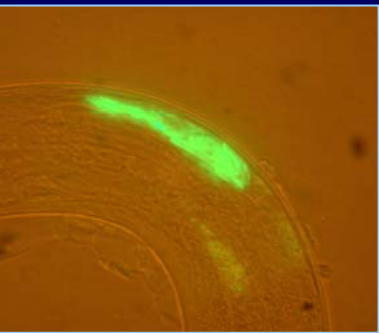


# Infective juveniles in the soil

= the seclusion of the bacteria

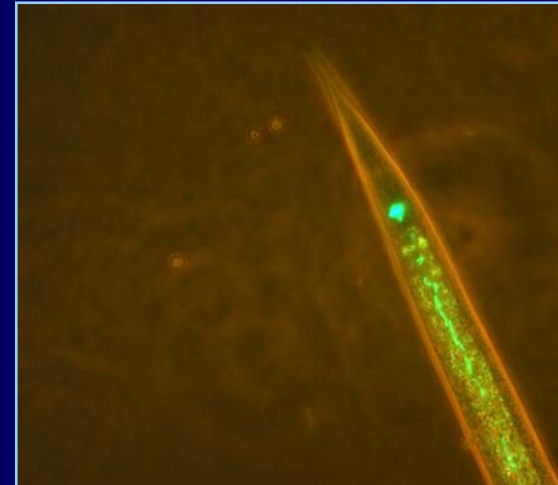
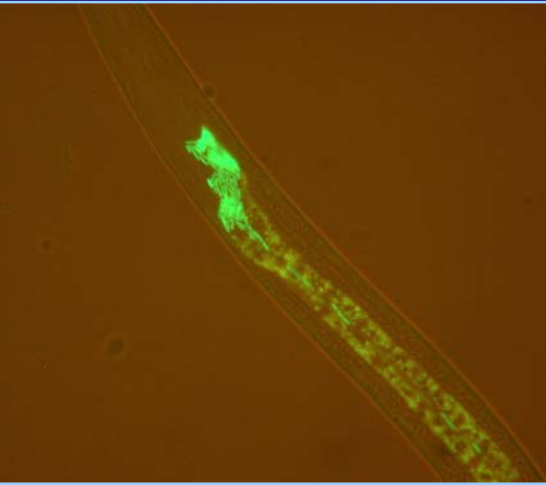


The nematodes carry *Xenorhabdus* cells in a « vesicle ». Is it the result of a co-adaptation or a pre-adaptation ?



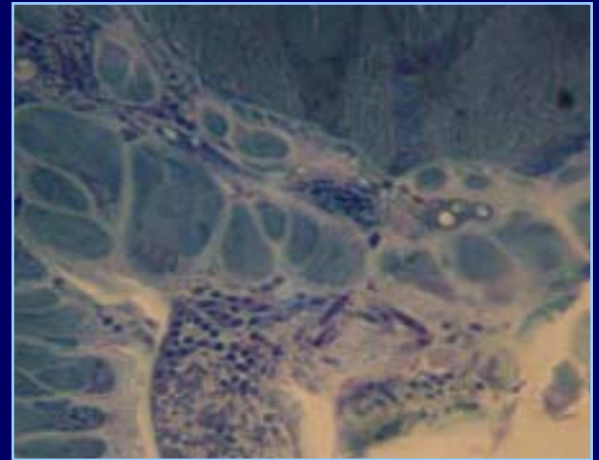
Is the vesicle a protection against other bacteria or a housing structure ?

# Symbionts release in the insect hemolymph



The bacteria migrate within the gut of the worm towards the anus

# The bacteria multiply within the Insect



Preferential sites of multiplication are connective tissues

Septicaemia



Insect's death

*Description de divers cycles de parasitisme du *Noelus boemarus* par trois sous-populations d'Emipiens*



*Cycle parasite fluctuant  
(mais non des moindres)*

*Premier cycle parasite décrit sur l'espèce (niche écologique = sous-sol)*

*Cycle parasite apparu secondairement*

