

**COST 850 - WG 3 and WG 5 Meeting -
Kiel, November 5-7, 2004
Re-evaluation of the EPN risk
assessments**

**What about the risks of the
bacterial symbionts when associated
with EPN ?**

by N. Boemare, Convenor of WG 2

A – In natural conditions :

the intestinal microorganisms associated with entomopathogenic nematodes are:

- *Xenorhabdus* spp. from *Steinernema* spp.
- *Photorhabdus* spp. from *Heterorhabditis* spp.

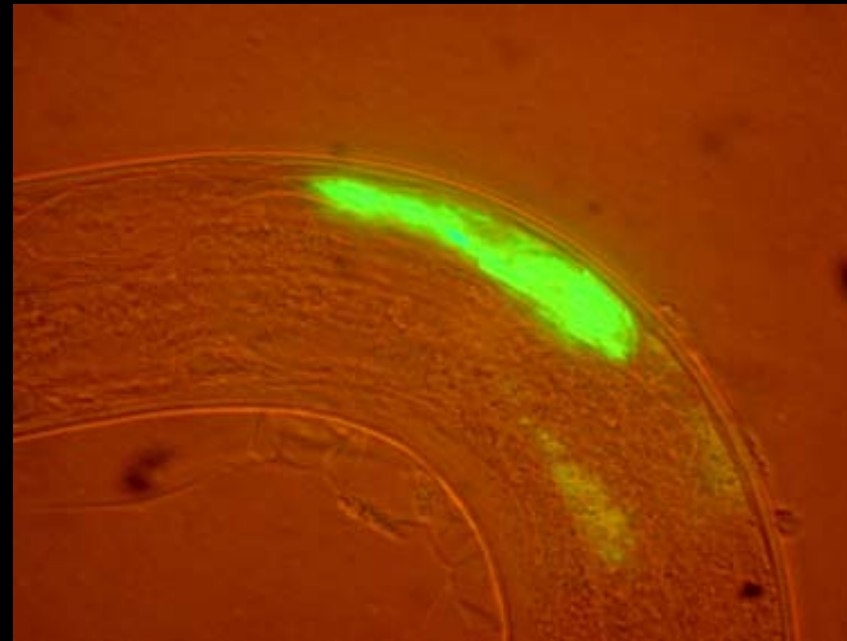
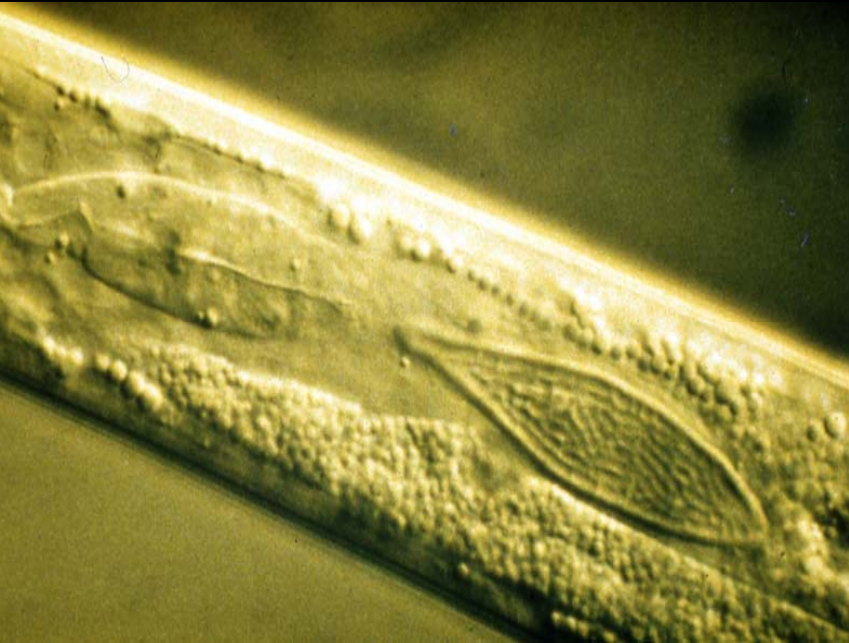
(Sources : All descriptions reported until to day)

There is a long time ago, that this natural bacterial monoxenic association with EPN was described

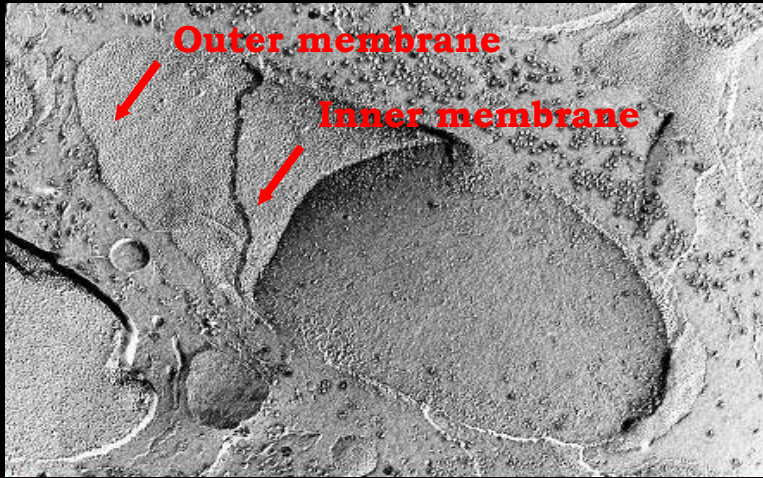
- Dutky, Thompson and Hough (1956), spoke about a specific bacterium useful for DD136 nematodes rearing
- Poinar (1966), described *Achromobacter nematophilus*, then proposed *Xenorhabdus nematophilus* (Thomas et Poinar, 1979) with a description corresponding to *Enterobacter agglomerans*
- Akhurst (1982) established definitively the monoxenic association and Boemare and Akhurst (1988) defined several ones.

Xenorhabdus are harbored by *Steinernema* infective juveniles in a special vesicle

X. nematophila expressing GFP within the vesicle
of *S. carpocapsae* infective juvenile



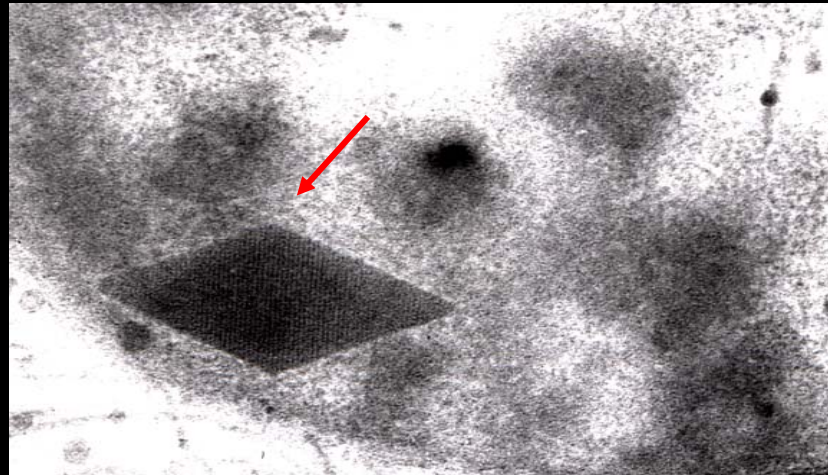
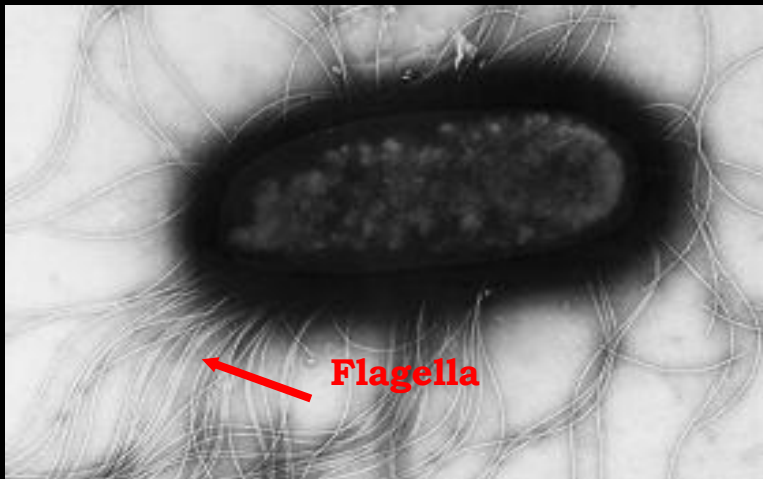
Exceptionally for such Gram negative bacteria, these symbionts have some specific morphological characters



Enterobacteriaceae, Gram negative,
with peritrichous flagella



Rods with **protoplasmic inclusions**

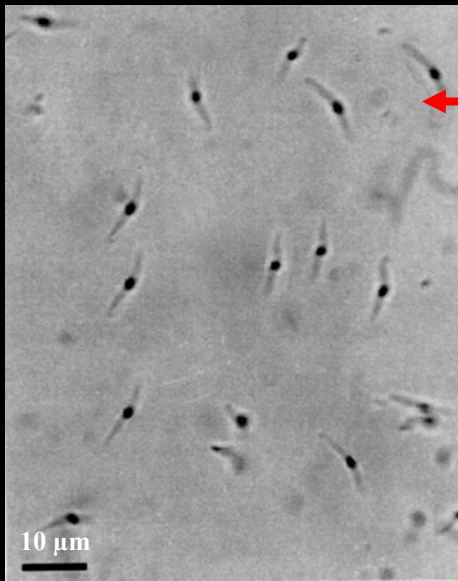


B – But occasional bacteria have been also isolated from nematodes

- - from *Steinernema carpocapsae* DD136 (Boemare, 1983)
- *Pseudomonas aureofaciens*, *Pseudomonas fluorescens* biovar B
- *Enterobacter agglomerans*, *Serratia proteomaculans* (= *Serratia liquefaciens*)
- - from *Steinernema carpocapsae* (Lysenko and Weiser, 1974)
- *Alcaligenes* sp., *Pseudomonas* sp., *Acinetobacter* spp.
- - from *Steinernema scapterisci* (Aguillera et al., 1993)
- *Ochrobactrum anthropi*, *Paracoccus denitrificans*, *Xanthomonas maltophilia*
- - from *Heterorhabditis* spp. (Jackson et al., 1995; Babic et al. 2000)
- *Providencia rettgeri* (= *Proteus rettgeri*)
- *Ochrobactrum* spp

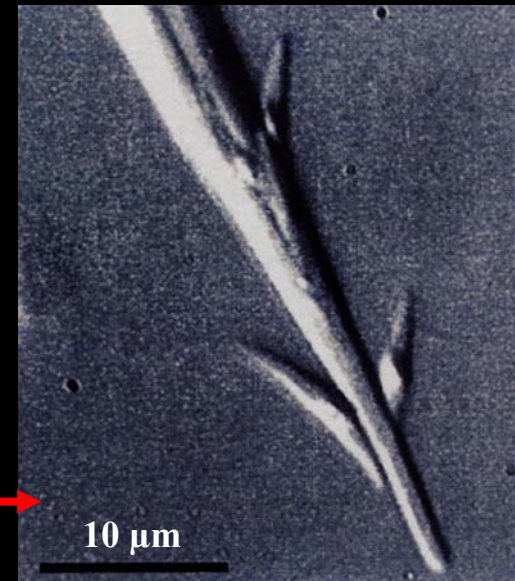
In addition, recently an « ectosymbiont » was characterized in *Heterorhabditis*

- *Paenibacillus nematophilus*, endospore-forming bacterium associated with *Heterorhabditis megidis* (Enright *et al.*, 2003; 2004).



Sporangia (5 days old-cultures)
with central spores

Sporangia on the tegument of
infective juveniles



In addition, miscellaneous *Pseudomonas* or so-called coliforms, and heteroxenic symbionts may provide nematode nutritional requirements

With *Steinernema*: (Lysenko & Weiser, 1974), (Poinar, 1979), (Boemare, 1983), (Ehlers et al., 1990), (Aguillera et al., 1993).

Heteroxenic association with “foreign” symbionts are possible depending on the nematode species :

- *Xenorhabdus* with *S. riobrave* et *S. scapterisci* (Grewal et al., 1993, 1997)**
- *Photorhabdus* with some Dutch strains of *Heterorhabditis* (Gerritsen et al., 1993)**

Gnotobiological experiments

1°/ Conditions :

- Axenic rearing of *Steinernema* (germ free animals) (Boemare *et al.*, 1983)
- Monoxenic combination from axenized eggs of *Heterorhabditis* (Lunau *et al.*, 1993)
- Pure culture of micro-organisms well identified

2°/ Results :

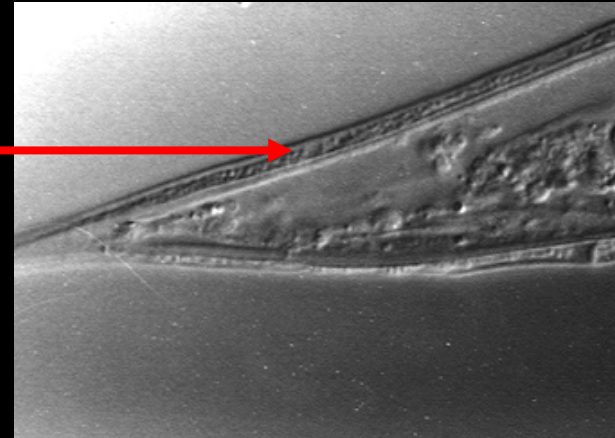
- Monoxenic growth with several foreign bacteria, but in many cases a better reproduction with the natural symbionts (Poinar, 1979; Boemare *et al.*, 1983; Ehlers *et al.*, 1990, Aguilera and Smart, 1993)

Entomopathogenic nematodes have a microbivorous diet

Careful disinfection of the integument with control of the exsheathing L2 cuticle : case of *S. scapterisci*

**Bacteria of Aguilera *et al.*
(1993) = contaminants
of the integument.**

Antagonists of the production *in vitro* and *in vivo*



**Production *in vitro* on sponge with
Xenorhabdus UY61**

(Bonifassi *et al.* 1999)



SAFETY TESTS (around the eighties)

Pathogenic effects

- *Xenorhabdus bovienii* (Obendorf *et al.* 1983) 0

(guinea pig, rat, mouse, rabbit)

- *Xenorhabdus nematophila* (Poinar *et al.* 1982) 0

(mouse, chicken)

- *Photorhabdus luminescens* (Poinar *et al.* 1982) 0

(mouse, chicken)

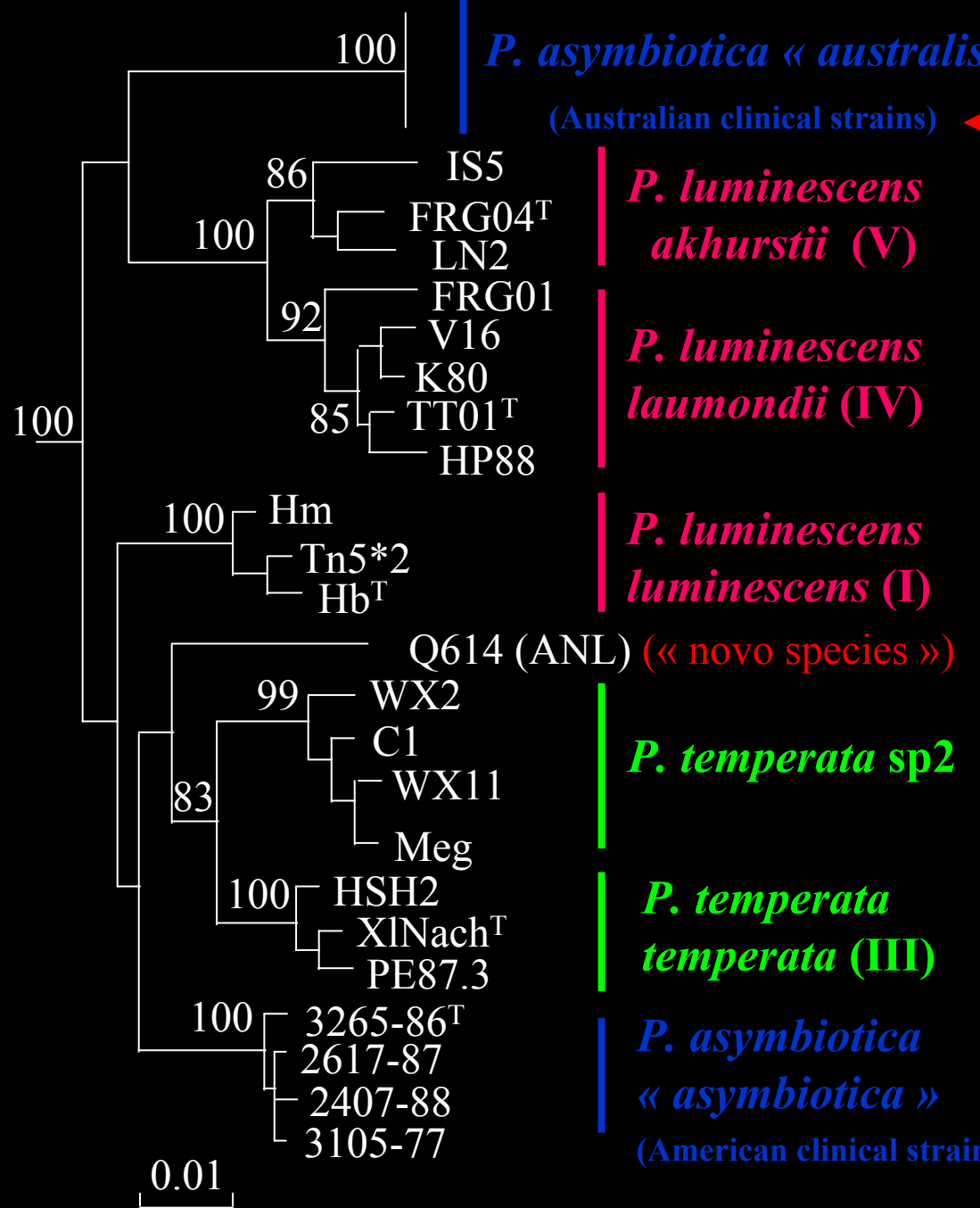
Taxonomic comparisons with vertebrate pathogens

- No *Xenorhabdus* known as pathogenic for plants, vertebrates or human.
- In contrast, some *Photorhabdus* have been found as responsible of some human infections.

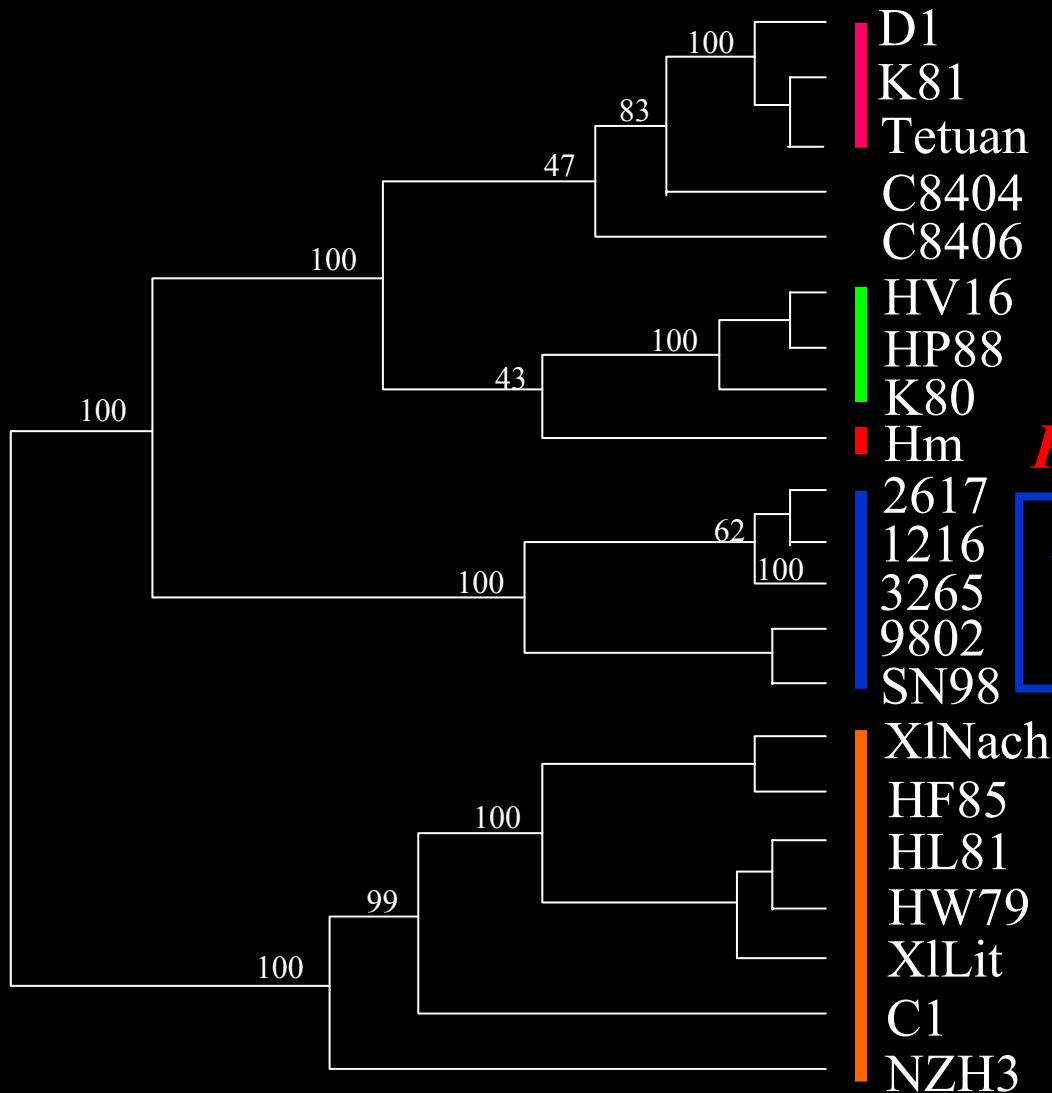
Phylogeny from 6S rRNA gene sequences



« » = proposed species and subspecies
 Akhurst, Boemare, *et al.*



Photorhabdus spp. phylogeny from *gyrB* gene



P. luminescens akhurstii

P. luminescens n. sp.

P. luminescens laumanni

P. luminescens luminescens

P. asymbiotica « *asymbiotica* »

P. asymbiotica « *australis* »

P. temperata

« » = proposed subspecies
 Akhurst, Boemare, et al.

Photorhabdus species cultivating at $t^{\circ} \geq 37^{\circ}\text{C}$

Bacterial species/subspecies	T°C max	Nematode host
<i>Photorhabdus asymbiotica</i>	$\leq 38^{\circ}\text{C}$	none (opportunist)
<i>Photorhabdus australis</i>	$\leq 38^{\circ}\text{C}$	none (opportunist)
<i>Photorhabdus akhurstii</i>	$\leq 39^{\circ}\text{C}$	<i>H. indica</i>
<i>Photorhabdus luminescens</i>	$\leq 39^{\circ}\text{C}$	<i>H. bacteriophora</i>
<i>Photorhabdus laumondii</i>	$\leq 37^{\circ}\text{C}$	<i>H. bacteriophora</i>
<i>Photorhabdus temperata</i>	$\leq 35^{\circ}\text{C}$	<i>H. megidis</i> <i>H. downesi</i> <i>H.zealandica</i>

Xenorhabdus species cultivating at $t^{\circ} \geq 37^{\circ}\text{C}$

Bacterial species	T°C max	Nematode host
<i>Xenorhabdus bovienii</i>	$\leq 33^{\circ}\text{C}$	<i>S. feltiae</i> , <i>affine</i> , <i>S. kraussei</i> , <i>intermedium</i>
<i>Xenorhabdus sp.</i>	$\leq 34^{\circ}\text{C}$	<i>S. monticolum</i> , <i>karii</i>
<i>X. nematophila</i>	$\leq 35^{\circ}\text{C}$	<i>S. carpocapsae</i>
<i>X. japonica</i>	$\leq 36^{\circ}\text{C}$	<i>S. kushidai</i>
<i>X. beddingii</i>	$\leq 39^{\circ}\text{C}$	<i>S. longicaudum</i> ??
<i>Xenorhabdus spp.</i>	$\leq 39^{\circ}\text{C}$	<i>S. rarum</i> , <i>ritteri</i> , <i>arenarium</i>
<i>Xenorhabdus spp.</i>	$\leq 40^{\circ}\text{C}$	<i>S. scapterisci</i> , <i>abbasi</i> , ...
<i>X. poinarii</i>	$\leq 41^{\circ}\text{C}$	<i>S. glaseri</i> , <i>cubanum</i>
<i>Xenorhabdus spp.</i>	$\leq 42^{\circ}\text{C}$	<i>S. riobrave</i> , ...

Other concerns : and the occasional bacteria ?

- The occasional bacteria have just some tiny similarities with other opportunistic pathogens of plants, animals or human patients.
- However the case of the entomopathovar *Providencia rettgeri* (Jackson *et al.*, 1995), or *Ochrobactrum* (Babic *et al.*, 2000), in relation with nosocomial infections invite to more carefulness.

Data from the symbiotic bacterial genomes

- More than 200 genes of toxins (hemolysin, cytolysin, genes coding for antimicrobials) have been identified in *Xenorhabdus* and *Photorhabdus*.
- These genes are more or less expressed depending on the strain, the bacterial species and the insect host. Most of them are very similar to the genes of human and vertebrate pathogens

CONCLUSION : to avoid the nosocomial and/or sporulated bacteria

- The industrial mass production should start from a carefully monoxenic association from the axenized eggs with the natural symbiont to prevent any development of nosocomial micro-organism for the safety of the manufacture personnel and later for the farmers.
- During the production, controls of contamination should be a pre-required safety regulation for the personnel and for delivering a final product well defined in terms of bacteria and nematodes according to the natural origin of these helminthic-bacterium complexes.

CONCLUSION : concerning *Xenorhabdus* and *Photorhabdus*.

- Some risk assessments should be undertaken this decade (after the pioneer trials of 1982 and 1983) to evaluate the risks of dissemination of the last warm strains recently identified from “tropical” nematodes.
- Even if the bacteria disseminated by the L3 are microbiologically ridiculous (100 to 200 cells by larvae) such nematodes may play a role of reservoir or sanctuary.
- If we think about the risk of pathogens possibly emerging from the insect world, such concern have to be solved.
- Additionnaly tests about the allergenicity should be probed every time with new products.