

# Genomic plasticity in the entomopathogenic bacterium, *Photorhabdus luminescens*.

***Sophie Gaudriault***

Laboratoire d'Écologie microbienne des insectes  
et interaction hôtes-pathogènes  
(EMIP)  
Montpellier  
France



# **Photorhabdus : symbiotic and pathogenic interaction with invertebrate hosts**

## **Nematode bacterium complexes (SYMBIOSIS)**

**Photorhabdus**  
an intestinal  
symbiont of  
**Caenorhabditis**  
nematode



**Nematode  
penetration and  
bacterial  
invasion in  
haemocoel**



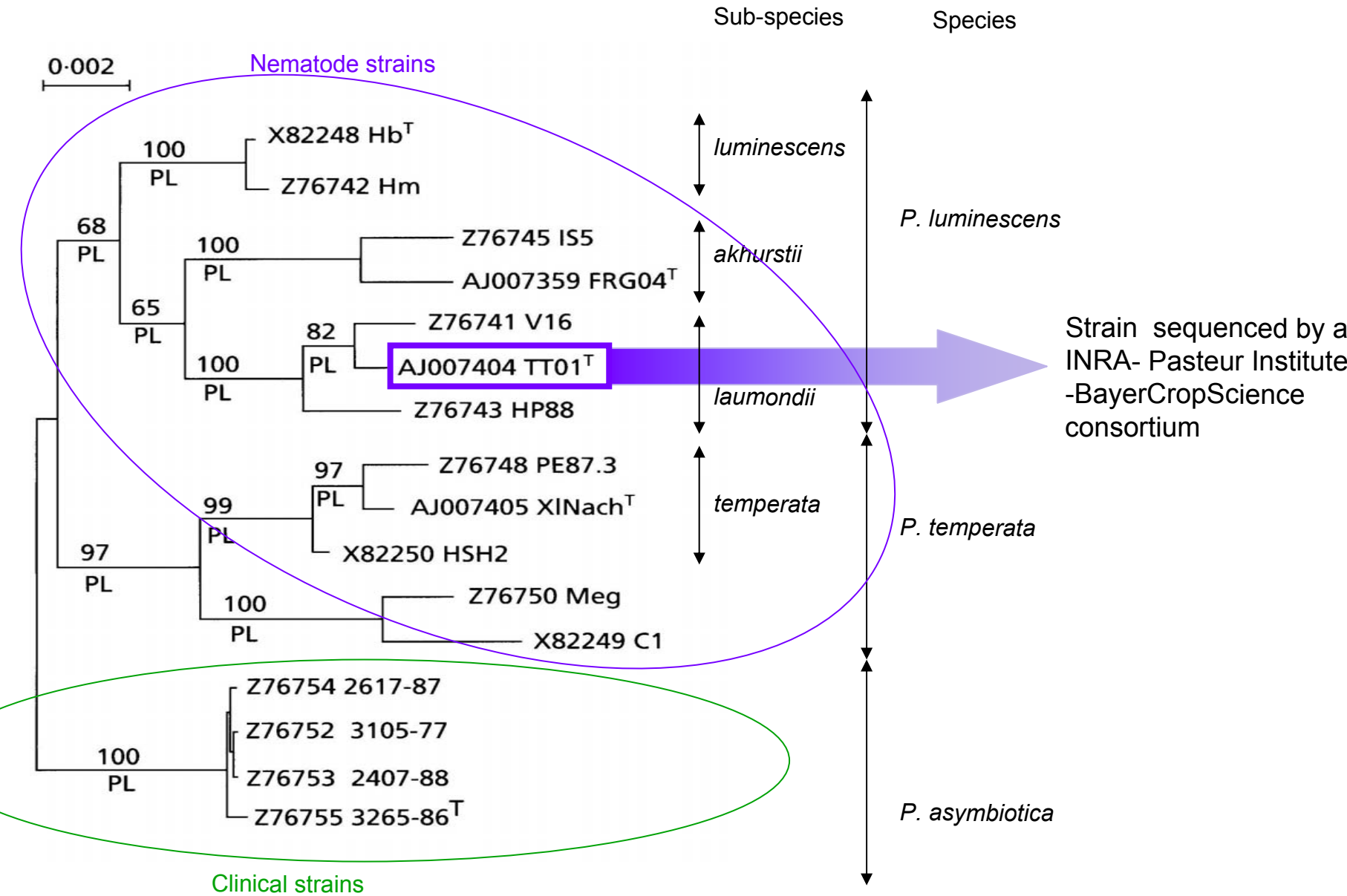
## **Insects (PATHOLOGY)**



**Infectious process and insect  
immune response; death of the  
insect**



# Photorhabdus phylogeny



# ***Photorhabdus* TT01 genome sequencing**

▶ **5,66 Mbases genome, one unique replicon, about 5000 genes**

▶ **genes potentially involved in interaction with invertebrate host**

- Tc toxins
- proteases, lipases
- type III secretion system
- iron uptake systems
- hemolysins
- antibiotics biosynthesis pathway

▶ **Mobile and/or repeated genetic elements**

- phage remnants : 4% of the genome
- 195 insertion sequences (IS) or transposons
- 711 ERIC (Enterobacteriaceae repeated and intergenic consensus) sequences
- over-represented families of paralogs (*rhs* elements, *tc* genes, *vgr* genes, *tpsAB* secretion system, colicins-like, iron uptake, recombinases, etc)

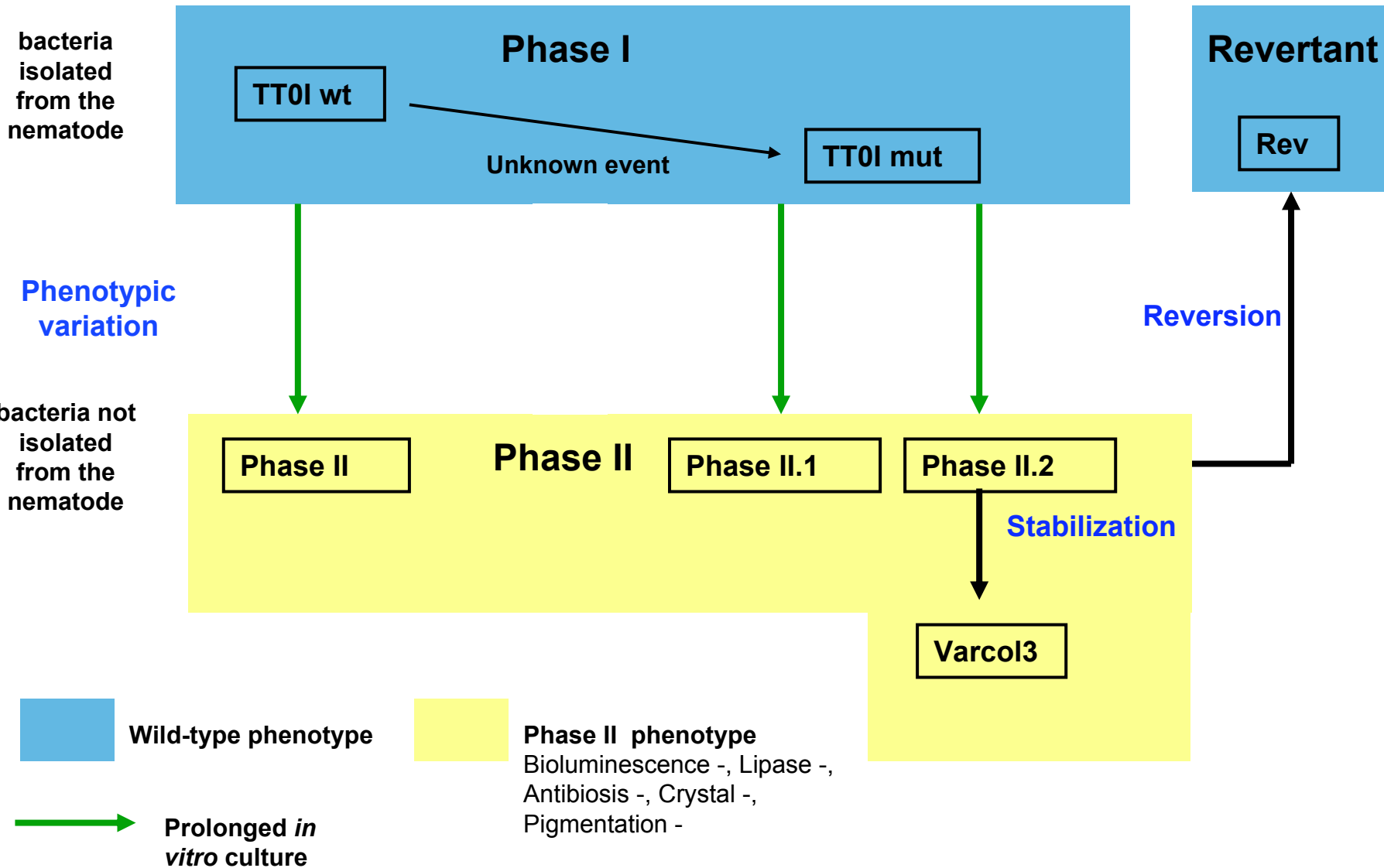
**Numerous potential mobile regions**

- 32 genomic islands
- Enterobacterial variable regions



**Genome plasticity and variants**

# Phenotypic variation in *Photorhabdus* genus



# Correlation between phenotypic variation and genomic variation ?

RFLP on whole TT01 variant genomes:

- *I-Ceu I* hydrolysis (7 predicted fragments in the TT01wt genome of 3660, 671, 478, 330, 266, 244 and 36 kbases)

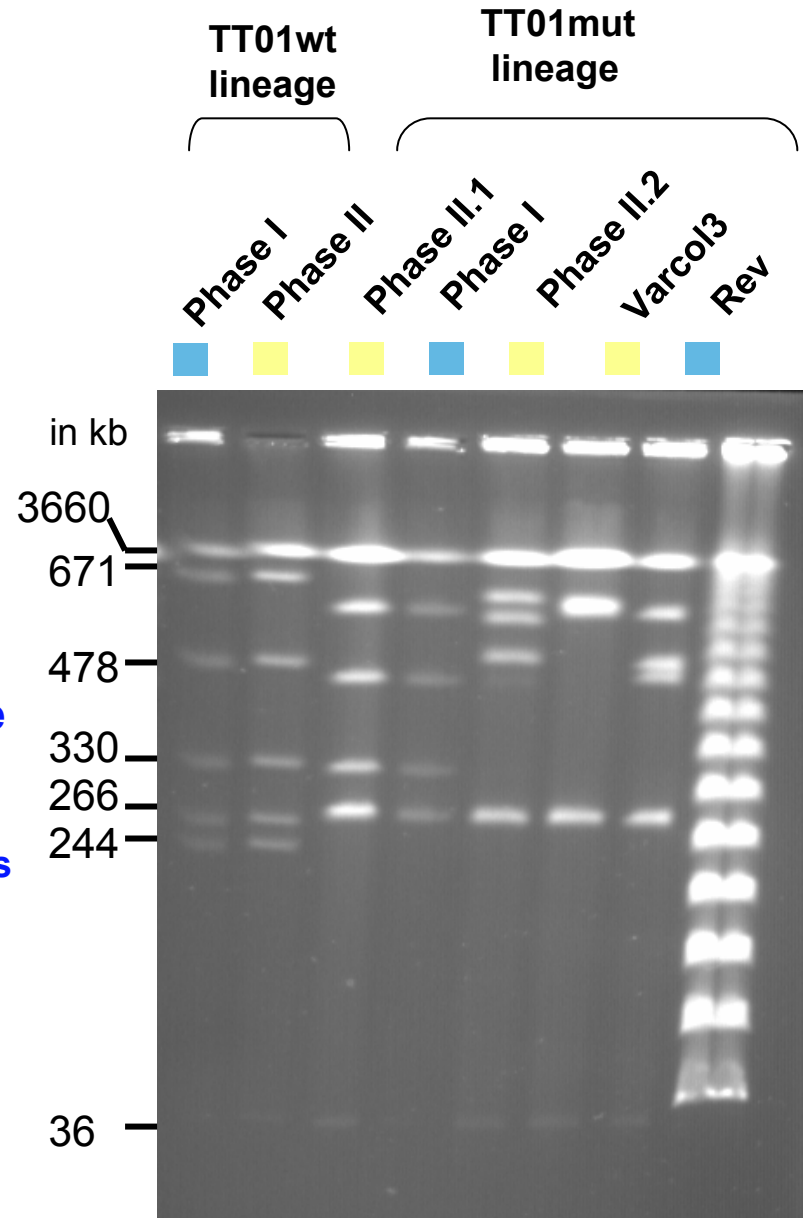
- Pulsed field gel electrophoresis that separates large fragments

Genomic variation is not the support for phenotypic variation

Independence between observable phenotype and genomic organization

- Identical phenotypes => Different genomic patterns

- Identical patterns => Different phenotypes



## ***Photorhabdus* DNA Micro-array**

**75 % of the whole genes**

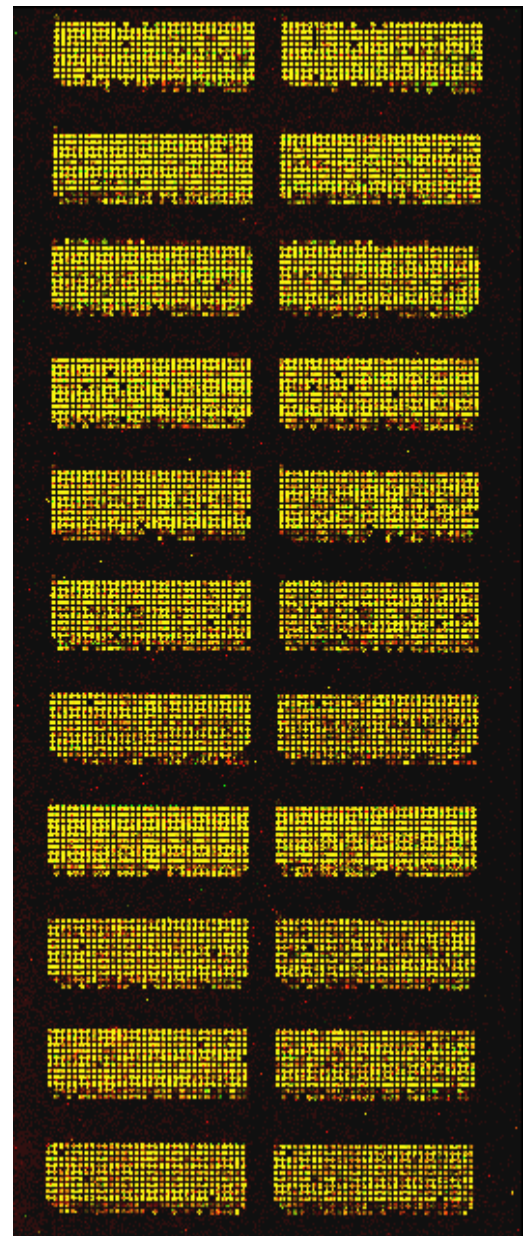
**90 % of the amplifiable genes**

**Each gene is represented by a PCR product of 500 bp**

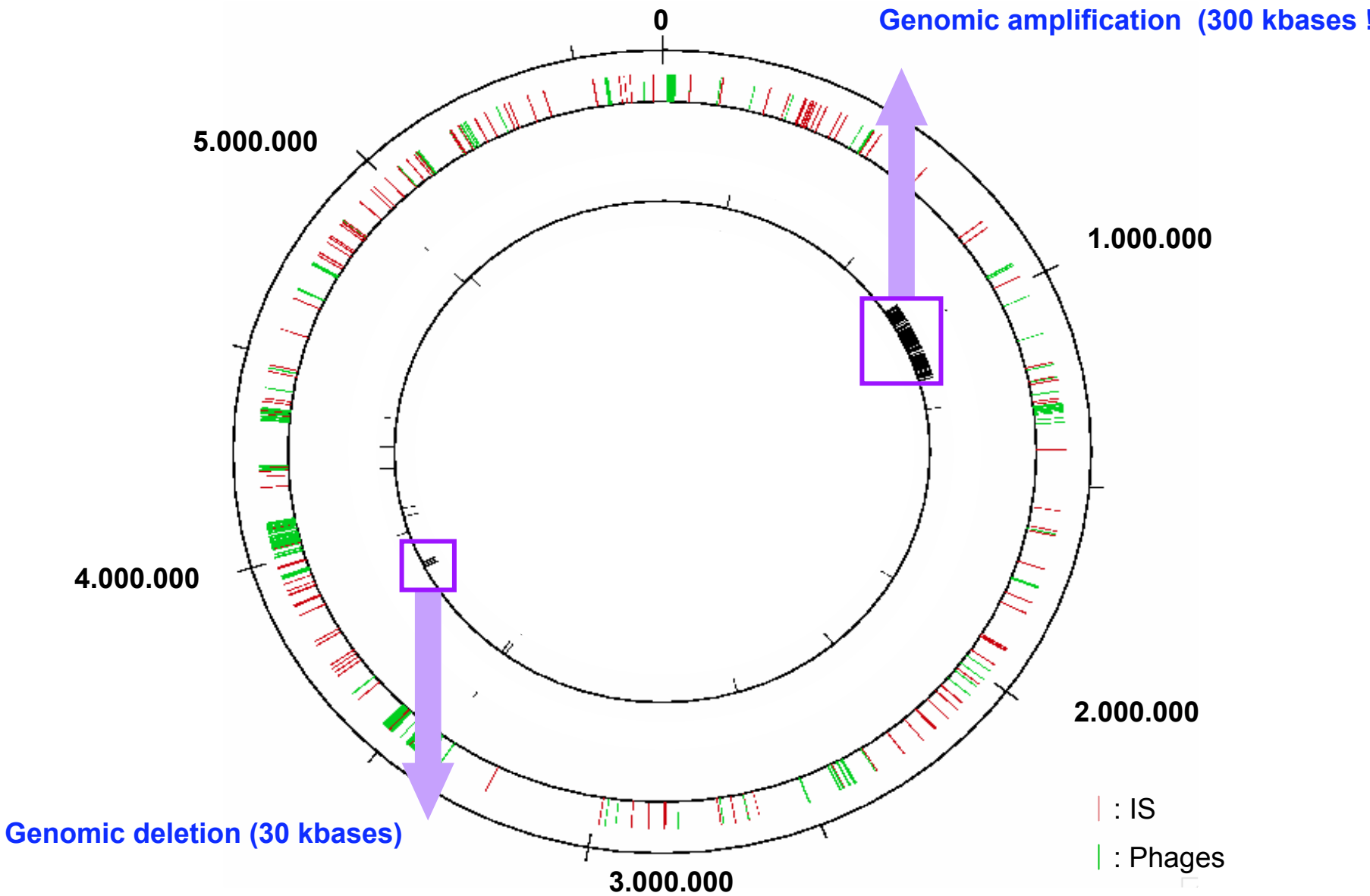
**Spotting of the PCR products on glass (type 7 star,  
Amersham)**

**Amersham)**

**2 replicates by micro-array**



# Genome content comparison between a TT01mut and Varcol3



# Genomic amplification

## → Genes concerned by amplification

- pili type I (*mrf genes*)
- insecticidal toxins (*tc genes*)
- colicins synthesis and transport
- antibiotic synthesis pathway (pyoverdine, actinomycin, etc.)
- amino acid metabolism and transport (tryptophan, serine, threonine)
- DNA metabolism (*dnaQ*)
- wall biosynthesis (*mltD*, *agaV*), ...

Host interaction

Basic metabolism

## → No remarkable mobile or repeated genetic elements

## → 300 kbases concerned the TT01wt genome; location in the TT01mut or Varcol3 genome?

# Genomic deletion (30 kbases)

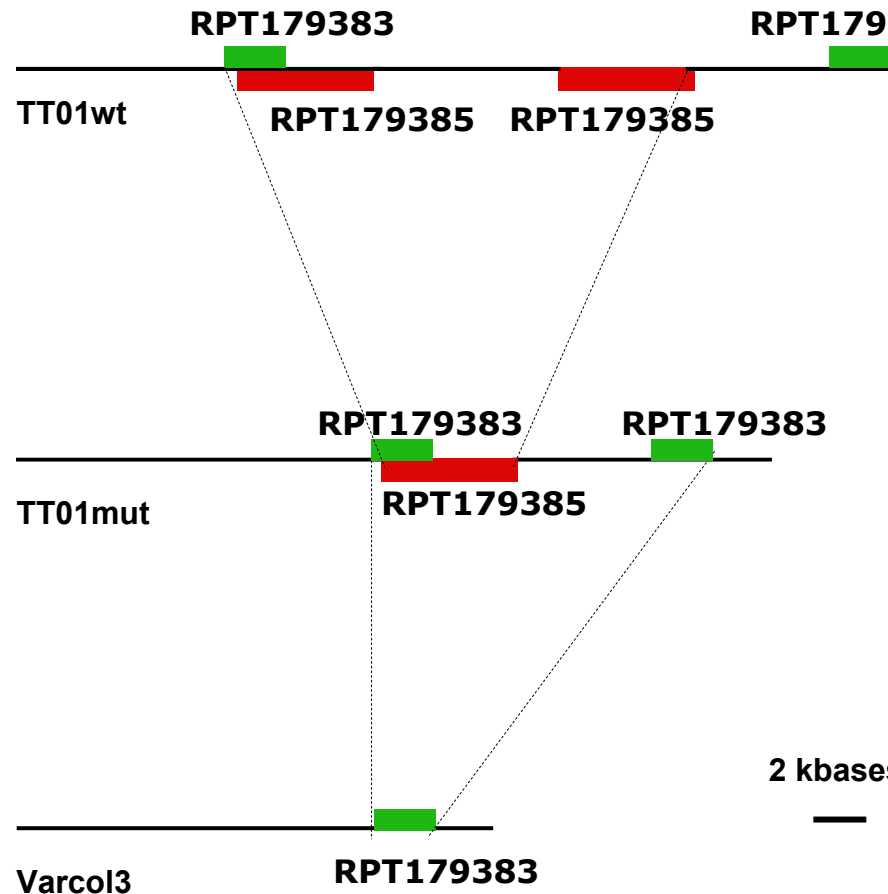
## Nucleotidic region in the TT01wt genome

- *vgr* related genes ( encoding valine-glycin rich proteins, located near of recombination hot spot motifs in *E. coli*)
- unknown genes
- pairs of repeated sequences (at least 1kbase)

## Location of the trace of deletion in the TT01mut and varcol3 genomes

- lacking regions match exactly with the inter-regions between two repeated sequences

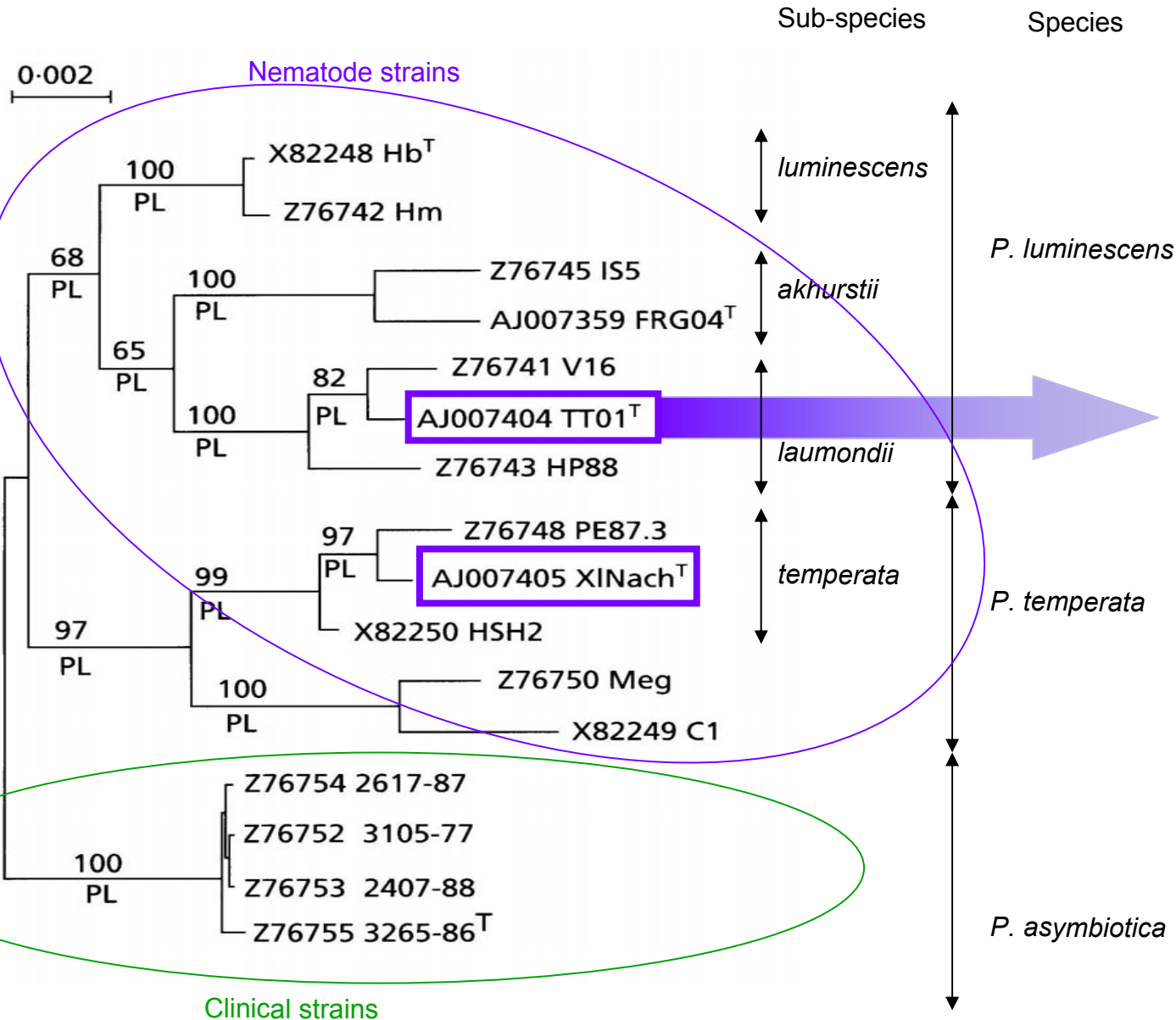
homologous recombination process  
between repeated sequences





**Genome plasticity and species**

# Photorhabdus phylogeny



# 31 TT01-specific regions

locus	Size of the region in TT01 (kbases)	plu	Remarkable products (similarity or function)
1	10	plu0125 - plu0132	X Unknown, <u>Sai integrase</u> , ...
2	18	plu0136- plu0156	X unknown, <u>transcriptional regulator</u> , CoA metabolism, <u>helicase</u> , ...
3	8	plu0263 - plu0269	X <u>Pili cluster VI** (Fim-like, type 1 pili)</u>
4	3	plu0280 - plu0282	X <u>Phage remnant</u>
5	12	plu0406 - plu0418	X <u>Phage remnant and Pili Cluster V*, (mrf-like, pili mannose-résistants)</u>
6	13	plu0567 - plu0577	X <u>sugar transport and metabolism, amino-acids synthesis</u>
7	5	plu0597 - plu0600	X unknown, <u>DNA-methyltransferase</u>
8	17	plu0752 - plu0764	X <u>peptide synthesis and transport</u> , CoA metabolism
9	16	plu0895 - plu0899	X <u>Cro/CI transcriptional regulator, antibiotic synthesis</u>
10	27	Plu0960 - plu0965	X <u>insecticidal toxins (loci tcd and tcc)</u>
11	4	Plu1002 - plu1005	X <u>deshydratase, dioxygenase, cyanate and benzoate transport</u>
12	14	plu1207 - plu1213	X <u>antibiotic synthesis</u>
13	11	plu1436 - plu1443	X <u>antibiotic synthesis</u>
14	3	plu2727 - plu2729	X <u>enterobactin synthetase (entABE)</u>
15	10	plu2792 - plu2799	X <u>antibiotic synthesis</u>
16	7	plu3135 - plu3139	X <u>citrate synthase, efflux transporter and unknown</u>
17	5	plu3144 - plu3146	<u>Isr (luxS synthesis regulated) operon, AI-2 import</u>
18	6	plu3398 - plu3405	X <u>phage remnant</u> , unknown proteins
19	5	plu3537 - plu3539	X <u>aminotransferase, propionate metabolism</u>
20	4	plu3724 - plu3726	<u>aminobenzoyl-glutamate uptake and utilization</u>
21	5	plu4077 - plu4081	X truncated aldolase, <u>deshydrogenase</u> , <u>transferase</u> , unknown proteins
22	19	plu4143 - plu4160	X ABC-transporter, <u>amino acids metabolism</u> , unknown
23	16	plu4205 - plu4219	X <u>transposase</u> , unknown proteins
24	5	plu4266 - plu4269	X <u>amino acid metabolism</u> , ABC-transporter
25	7	plu4324 - plu4328	X unknown proteins
26	14	plu4336 - plu4348	X <u>carotenoid biosynthesis</u> , unknown proteins
27	3	plu4589 - plu4591	X unknown, transcription regulator LysR
28	15	plu4621 - plu4630	X <u>ferric enterobactin biosynthesis and uptake</u>
29	15	plu4810- plu4823	X <u>lipopolysaccharide biosynthesis</u>
30	16	plu4873- plu4889	X <u>formate metabolism, O-methyltransferase, reverse transcriptase, macrolide-efflux protein, sugar kinase</u>
31	6	plu4892- plu4895	X <u>O-methyltransferase, transposase</u>

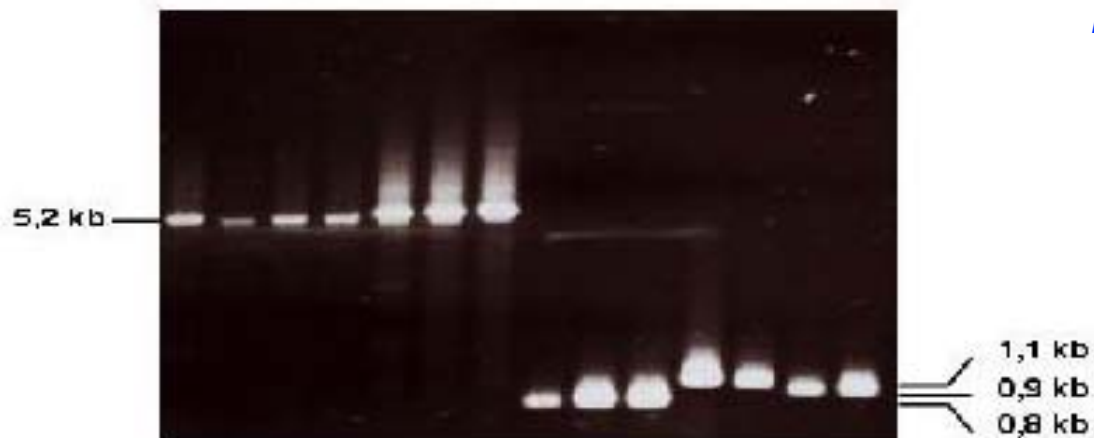
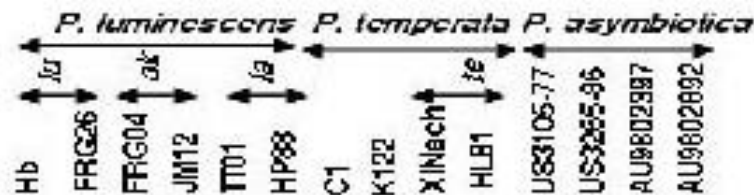
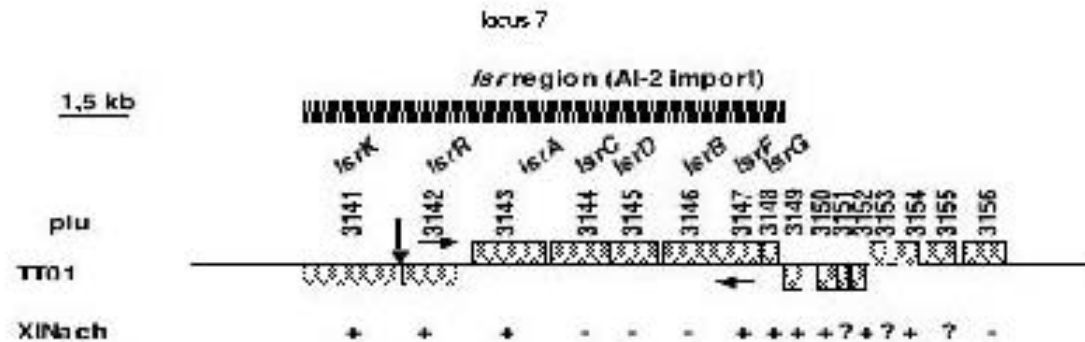
## Main functional classes

- phage remnants
- pilus biosynthesis
- antibiotic synthesis
- insecticidal toxins
- iron uptake
- amino acid metabolism

## Genomic islands : x

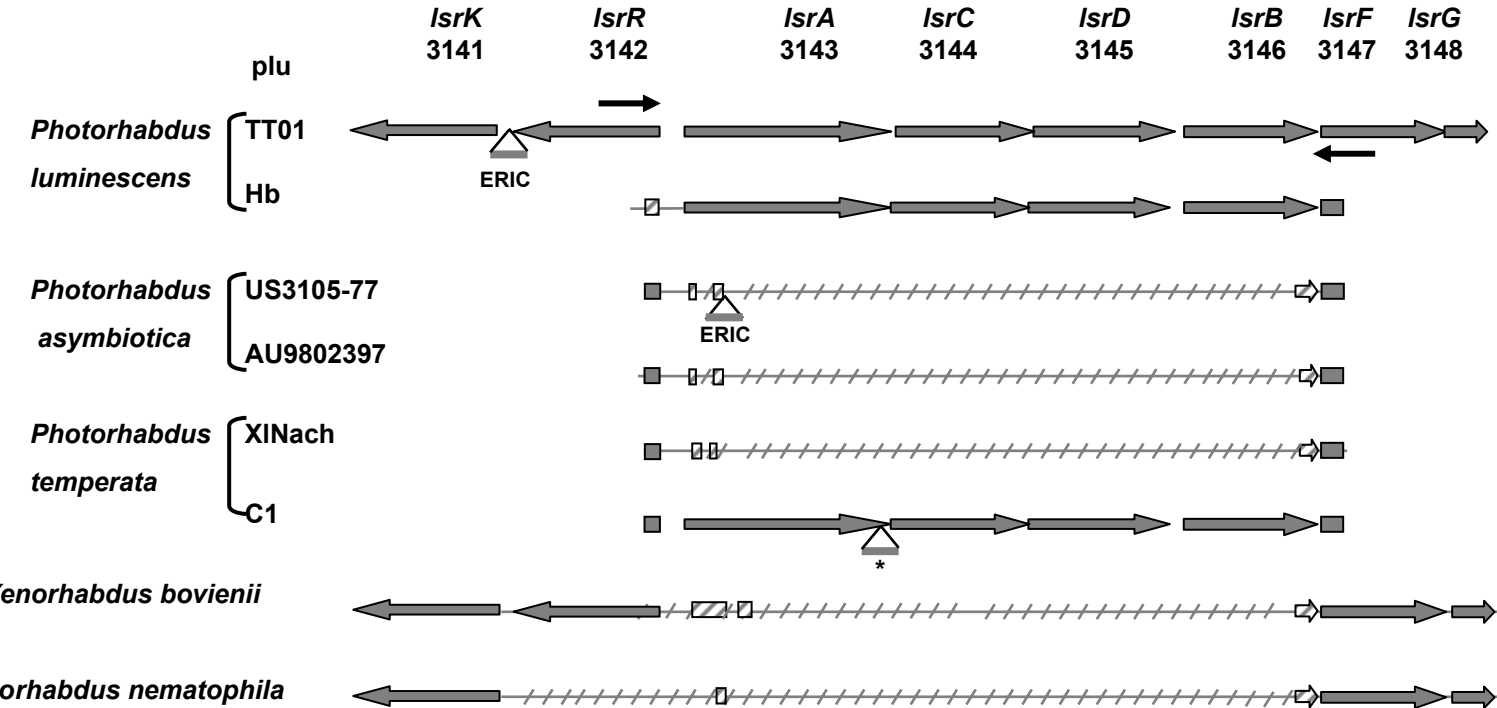
## Enterobacterial variable regions : x

# Distribution of the *Isr* region in *Photorhabdus* genus



TT01-specific regions belong to the flexible gene pool of the *Photorhabdus* genus !

# Sequences of *Isr* regions in *Photorhabdus* genus



➡ Reductive genomic events that occur several times in the *Photorhabdus* and *Xenorhabdus* evolutive history

➡ Case of *P. temperata* C1 strain : Horizontal transfert ?

# Conclusions

1. Confirmation of high genomic plasticity in *Photorhabdus* genus

2. At the strain level :

genomic variation is not correlated with phenotypic variation

BUT TT01 has a potential of genomic variation => genomic variants

3. At the species level :

identification of a part of the flexible gene pool of *Photorhabdus*

4. Local reductive genomic evolution

→ For the future :

- better understanding of the *Photorhabdus* evolutive history
- adaptation of the bacteria to its two hosts

→ Acknowledgement

# Écologie microbienne des insectes et interaction hôtes-pathogènes

(EMIP)  
N. BOEMARE



Montpellier



- **Eric DUCHAUD**
- **Sophie GAUDRIAULT**
- **Alain GIVAUDAN**
- **Anne LANOIS**
- **Sylvie PAGES**

Laboratoire de Bactériologie  
Faculté de Pharmacie  
Montpellier

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