Microbiota of entomopathogenic nematodes: pathobiome et agronomical output

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biocontrol output)

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DGIMI
Diversité, Génomes & Interactions
Microorganismes Insectes

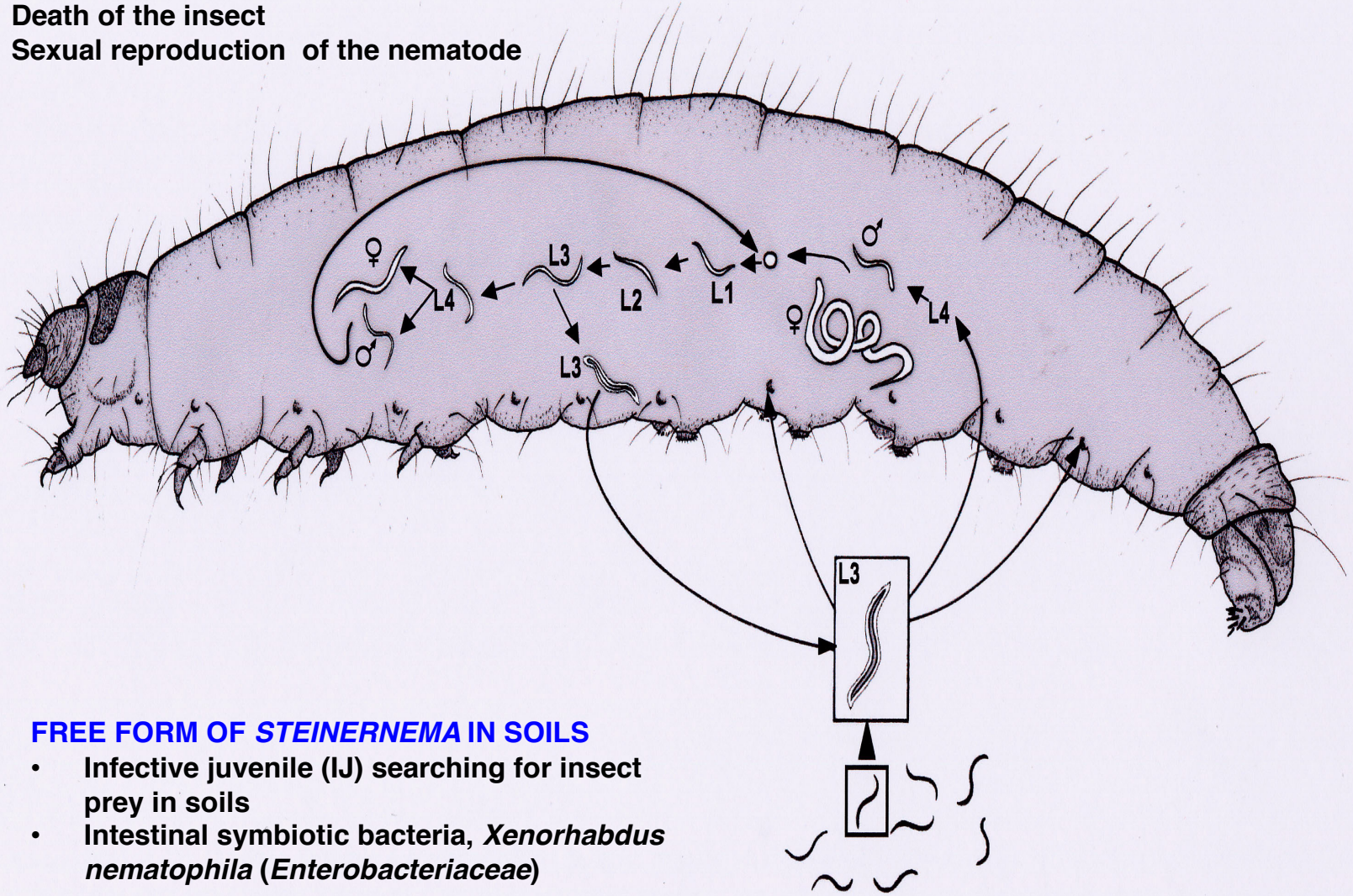
UMR INRA - UNIVERSITÉ DE MONTPELLIER


Steinernema carpocapsae
©S. Pagès, INRA, 2016
x400

Steinernema carpocapsae, biological cycle

PARASITIC FORM OF *STEINERNEMA* IN INSECT

- **Liberation of the symbiont in the insect body => bacterial multiplication**
- **Death of the insect**
- **Sexual reproduction of the nematode**



FREE FORM OF *STEINERNEMA* IN SOILS

- **Infective juvenile (IJ) searching for insect prey in soils**
- **Intestinal symbiotic bacteria, *Xenorhabdus nematophila* (*Enterobacteriaceae*)**

Entomopathogenic Nematodes (EPNs) and Biocontrol

A story that begins in the 1920s…

First field applications against the Japanese beetle (*Glaser & Farrell, 1935*)

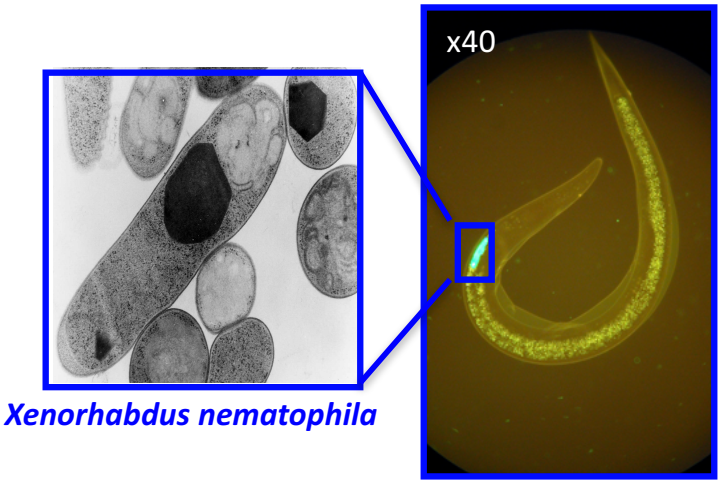
But current applications are limited

A few producers in Europe (England, Germany, Holland)

Application examples

Crops targeted	Pest name	EPNs
Vegetables	armyworm	Sc, Sf
turfs	Black cutworm	Sc
Ornamentals	Black wine weevil	Hb, Sc
Cranberries	Cranberry girdler	Sc

An old monoxenic hypothesis for *Steinernema*…



Xenorhabdus nematophila

Steinernema carpocapsae

X. nematophila

- **the only bacterial symbiotic partner**
- **located in a specialized part of the IJ gut**
- **kills the insect**
- **contributes to nematode maturation /reproduction**

Bonifassi et al., J Invertebr Pathol, 1999; Sicard et al., Appl Environ Microbiol, 2004; Richards and Goodrich-Blair, Cell Microbiol, 2009; Nielsen-Leroux et al., Current Opinion in Microbiology, 2012

… but this paradigm displays limits

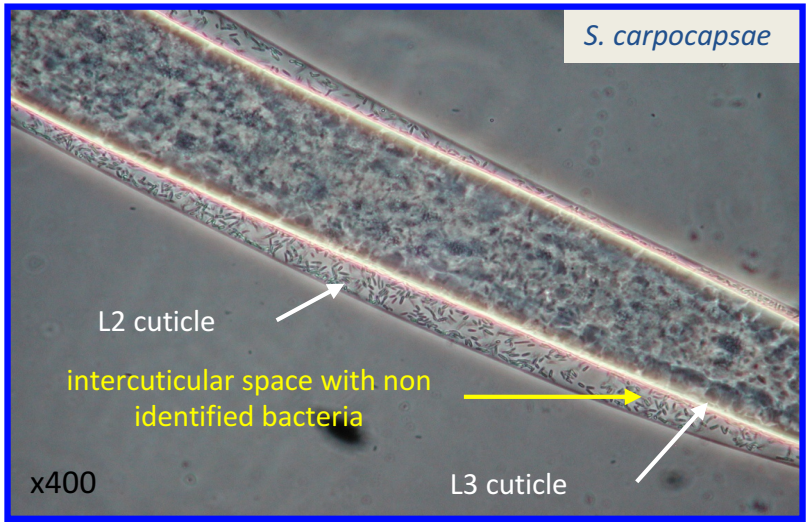
- **Plating crushed IJ or content of infested insect cadaver = > other taxa** (*Enterobacter, Vibrio, Pseudomonas, Serratia, Citrobacter, Bacillus* …)

Boemare, thesis, 1984; Bonifassi et al, J Inv Pathol, 1999; Gouge and Snyder, J Inv Pathol, 2006

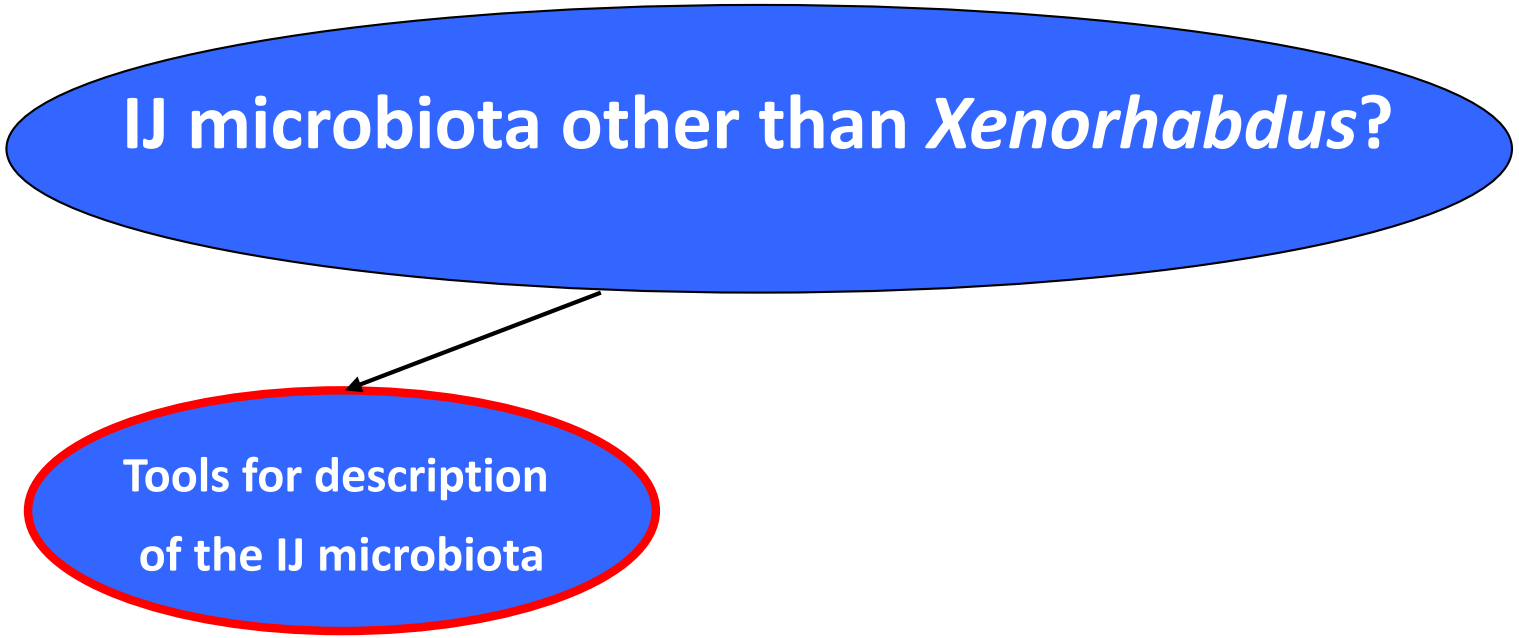
- **In *Xenorhabdus* genomes, remnant of recent HGT** (Firmicutes, Enterobacteriaceae, …)

Chaston et al, PloS ONE, 2011; Ogier et al, BMC Genomics, 2010; Ogier et al, Genome Biol Evol, 2014; Bisch et al, Genome Biol Evol, 2016

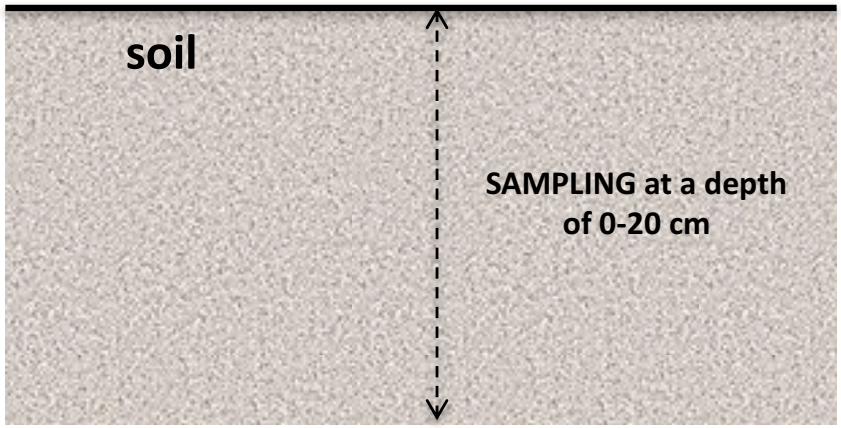
- **Microscopical data**

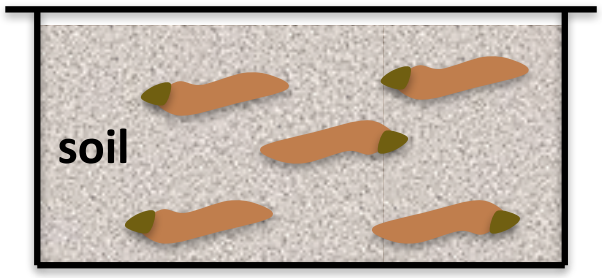


Sylvie Pagès, unpublished data

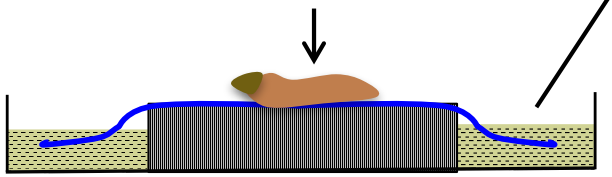

IJ microbiota other than *Xenorhabdus*?
Tools for description
of the IJ microbiota

Ex situ isolation, storage and laboratory multiplication of *Steinernema carpocapsae*



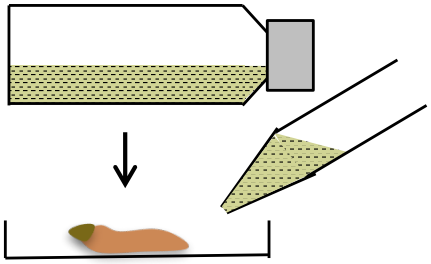


EX SITU GALLERIA **TRAP (1L plastic container)**
IJs from soil kill the insect larvae in 3-4 days

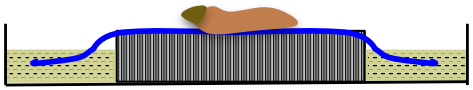


WHITE TRAP
Emergence of new IJs from the insect cadaver during 3-4 weeks post mortem

batch 1
storage in Ringer medium for 6 months

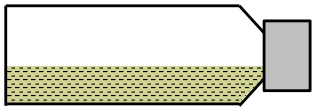


LABORATORY *GALLERIA* TRAP

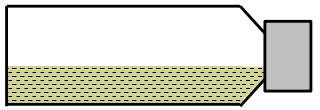


WHITE TRAP

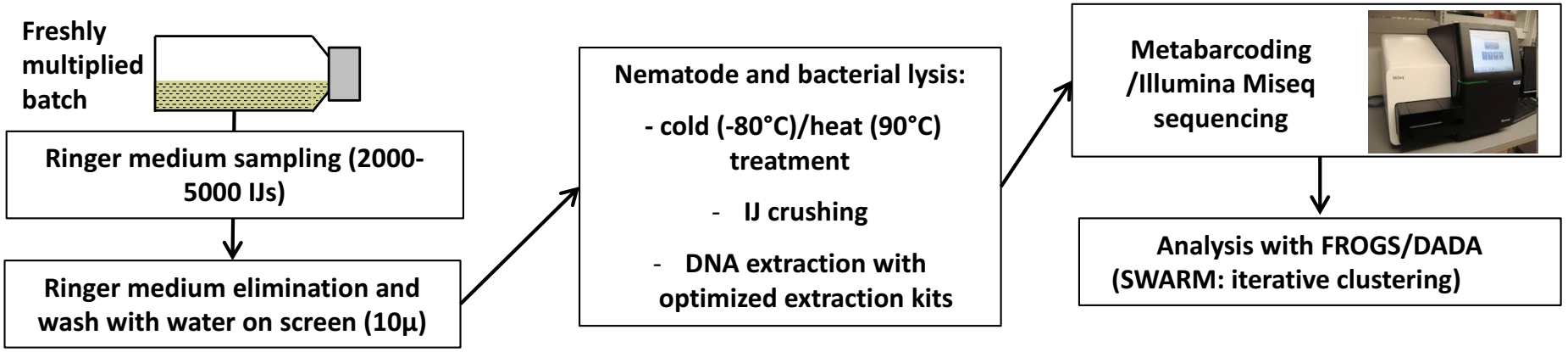
batch 2

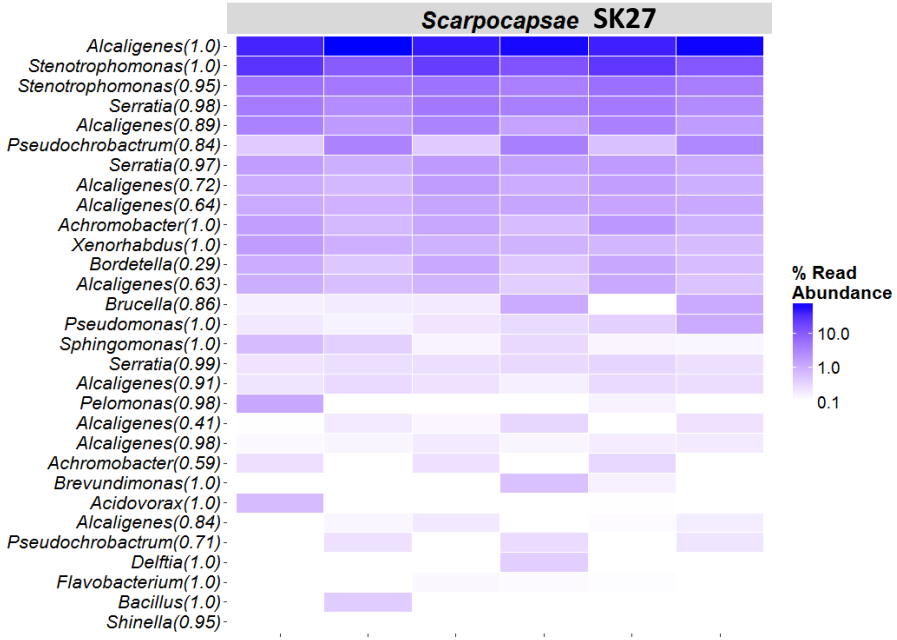


batch N (several years of storage)

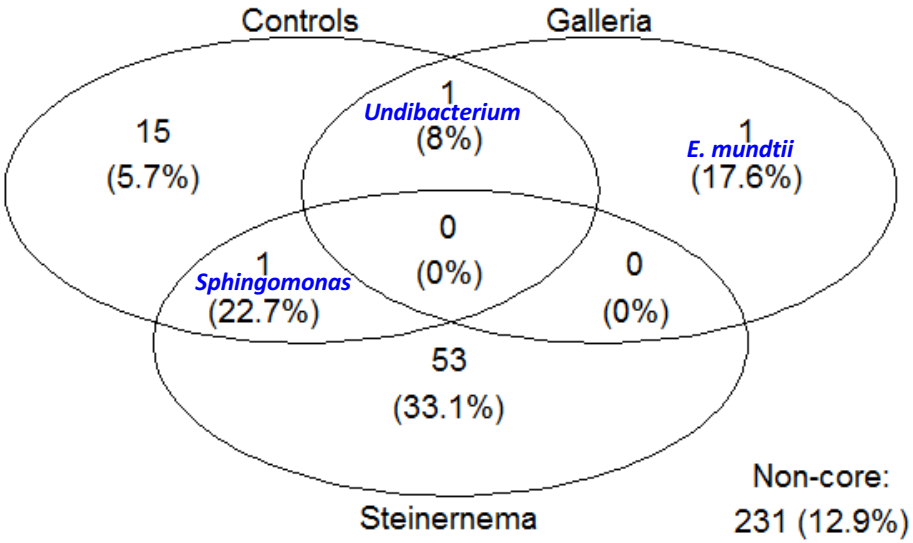


Impact of the laboratory manipulation on the IJ microbiota

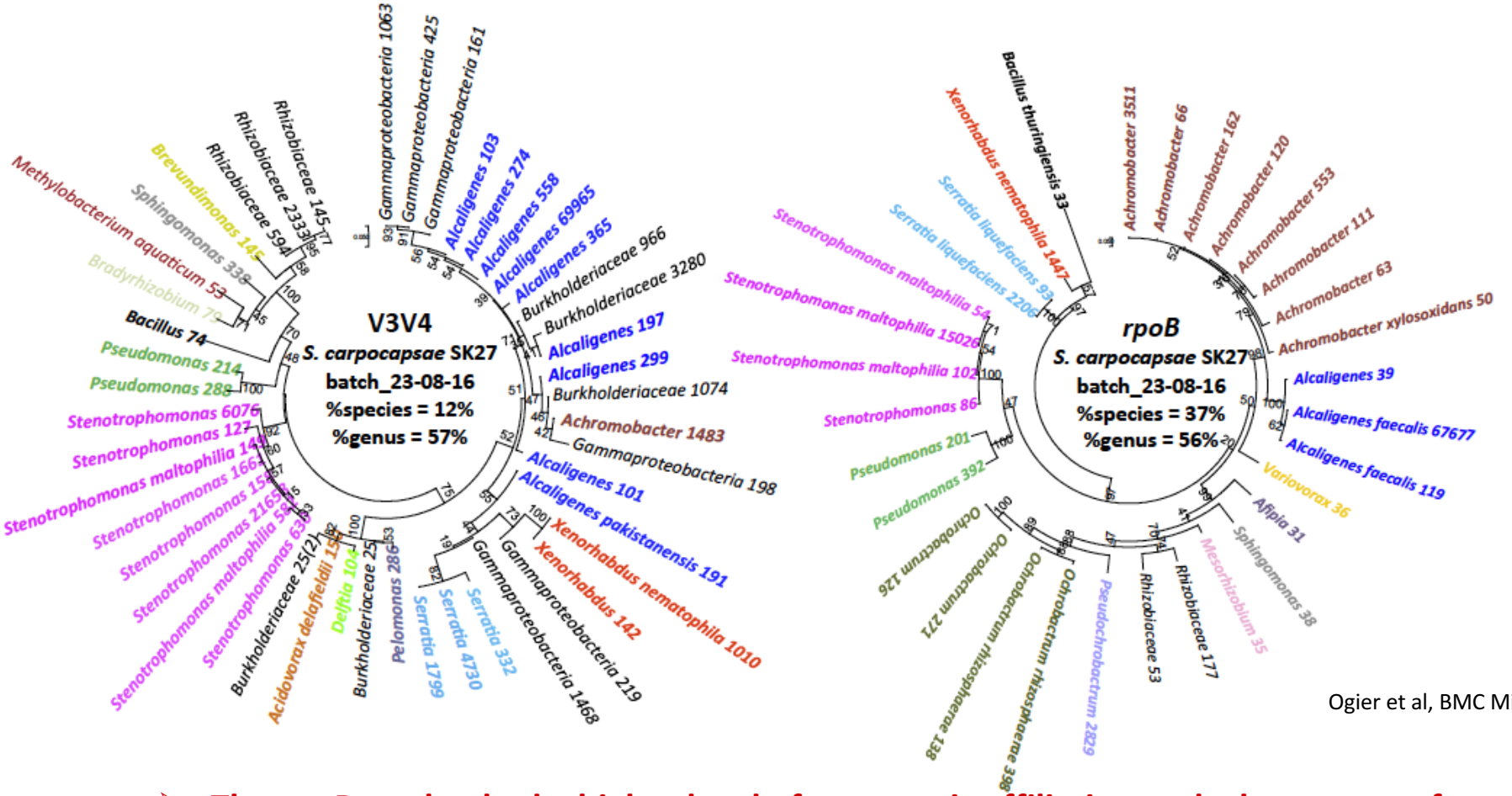




V3-V4 16S rRNA

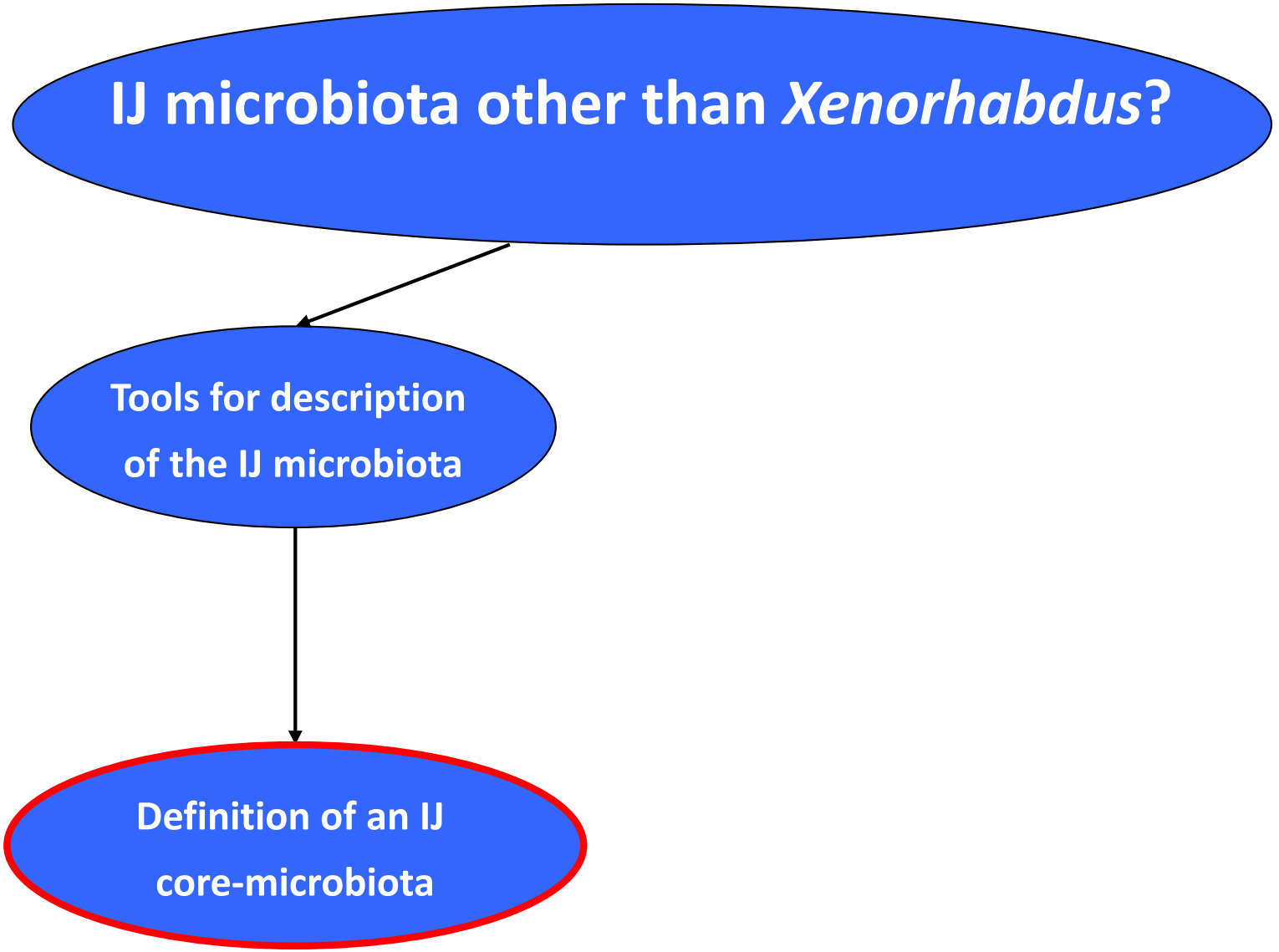


Impact of the metabarcoding marker on the molecular description of the IJ microbiota



Ogier et al, BMC Microbiol, 2019

- **The *rpoB* marker had a higher level of taxonomic affiliation and a lower rate of sequence variants than V3 V4**

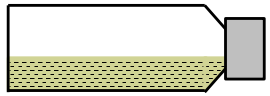

IJ microbiota other than *Xenorhabdus*?
Tools for description of the IJ microbiota
Definition of an IJ core-microbiota

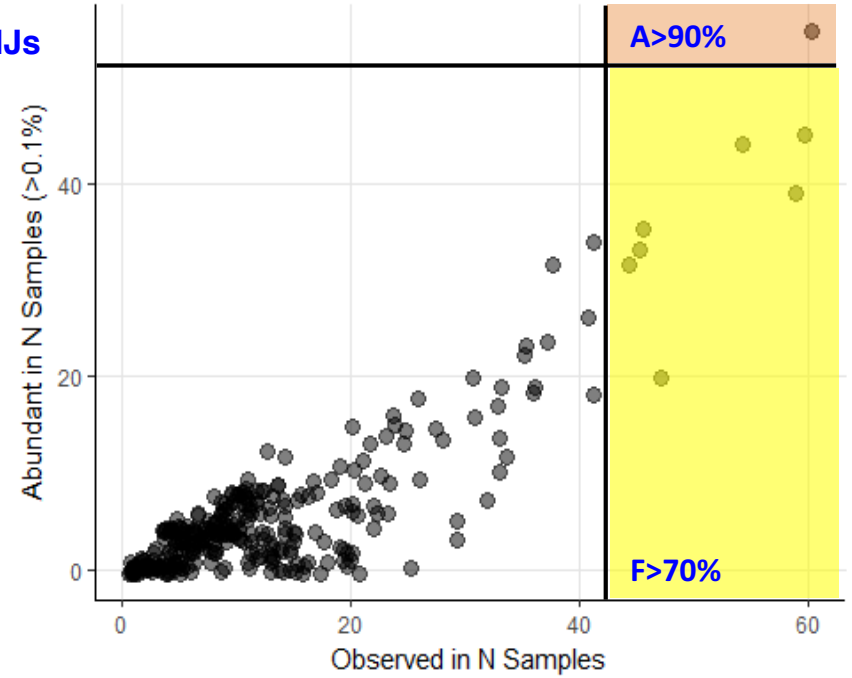
S. carpocapsae IJ molecular core-microbiota

1. LABORATORY-MULTIPLIED IJs

60 samples

- **6 different strains of *S. carpocapasae***
- **different multiplication batches**
- **different laboratory of storage (Montpellier, France/Byron, USA)**



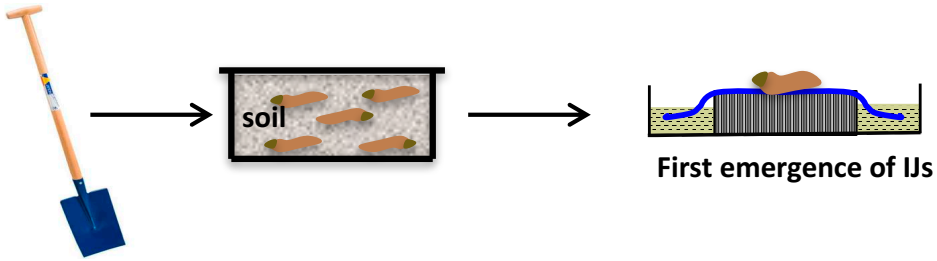


Core symbiont
Xenorhabdus nematophila

Frequently associated microbiota (FAM)

Alcaligenes faecalis
Stenotrophomonas maltophilia
Pseudomonas
***Rhizobiaceae* (*Ochrobactrum*, *Agrobacterium*, …)**

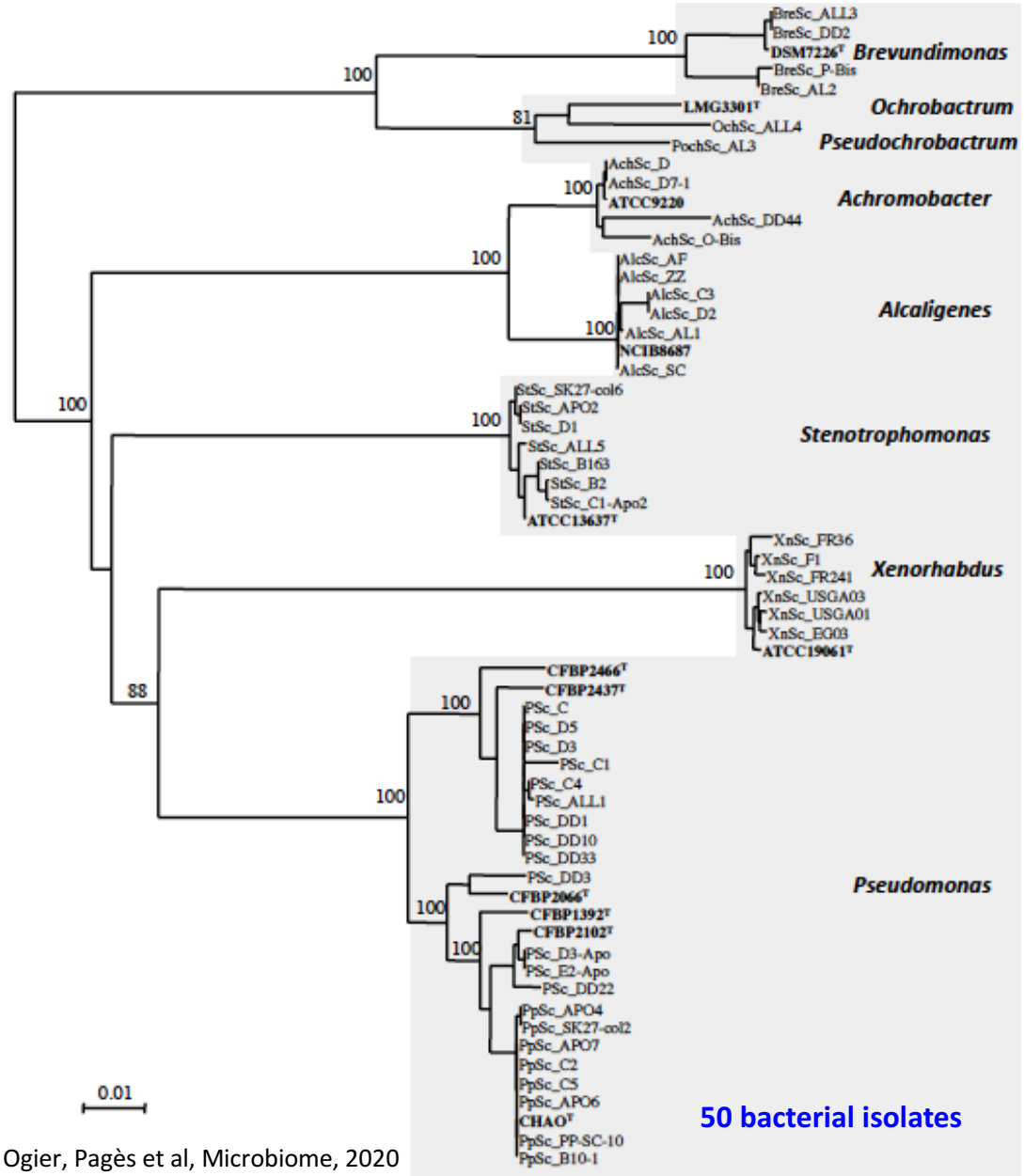
2. FRESHLY SAMPLED IJs



FAM is confirmed excepted *Alcaligenes faecalis* (laboratory drift ?)

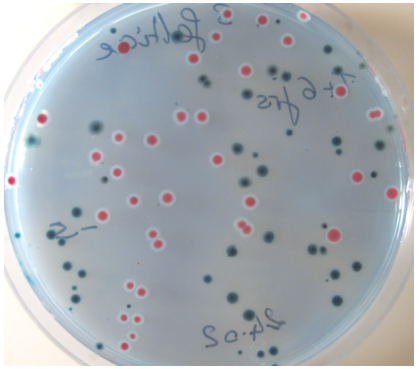
Ogier, Pagès et al, Microbiome, 2020

Culturomics:
taxa associated with *S. carpocapsae* IJs

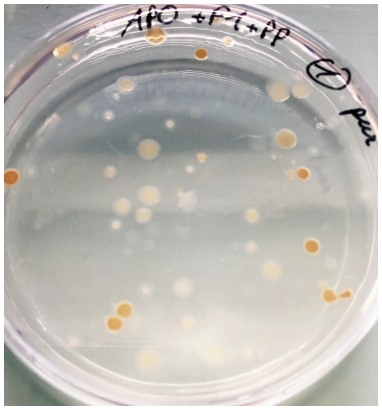


Ogier, Pagès et al, Microbiome, 2020

Selective medium for *Xenorhabdus*



NBTA agar medium

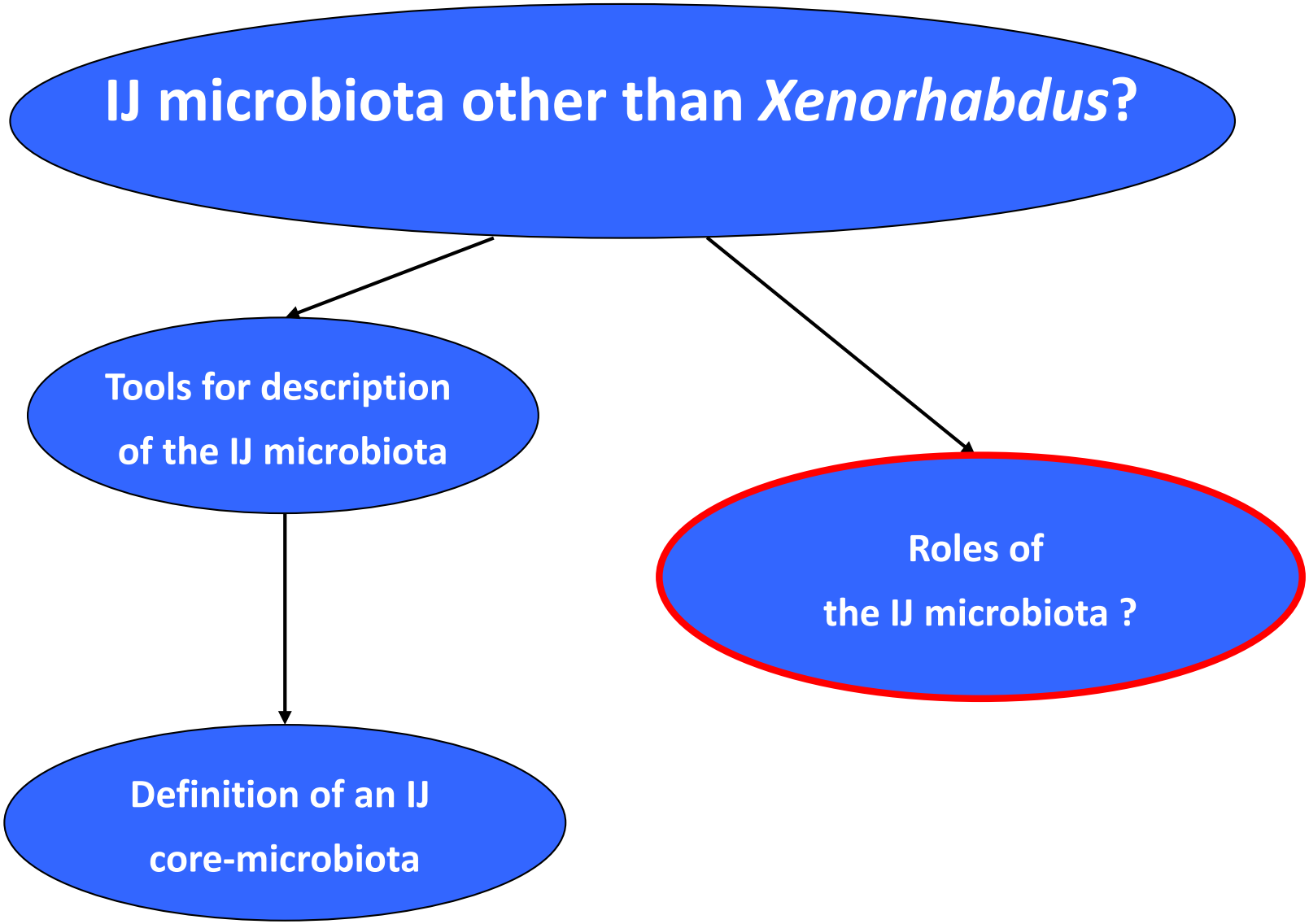


M9-PP agar medium

Pagès et al, J Microbiol Methods, 2020

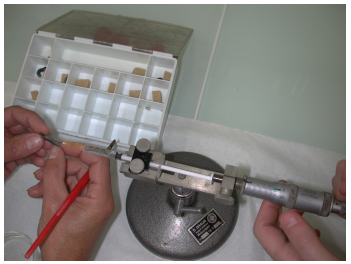
Bacteria associated to *S. carpocapsae* IJs

- **Proteobacteria**
- **Frequently encountered in organisms or compartments associated with soils**


IJ microbiota other than *Xenorhabdus*?
Tools for description of the IJ microbiota
Roles of the IJ microbiota ?
Definition of an IJ core-microbiota

Entomopathogenicity of members of the IJ microbiota

Pathoassay on *Spodoptera littoralis*, a lepidopteran pest



Injection assays:
10exp2 – 10exp3 bacterial cells / 20µl
Mortality measure:
20 insect larvae

Cultivable microbiota from	Strain	Dose range	LT50 (hours)
S. carpocapsae	*Xenorhabdus nematophila* XnSc_F1	10^2-10^3	27
	***Pseudomonas protegens* PpSc_PP-SC-10**	**10^2-10^3**	**29**
	Stenotrophomonas maltophilia StmSc_ALL5	10^5-10^6	no mortality
	Alcaligenes faecalis AlcfSc_SC	10^5-10^6	no mortality
	Pseudochrobactrum PochSc_AL3	10^5-10^6	no mortality
	Ochrobactrum sp OchSc_ALL4	10^5-10^6	no mortality
Rhizospheric *Pseudomonas* strains	*Pseudomonas protegens* CHAO^T	10^2-10^3	28
	Pseudomonas chlororaphis CFBP 2132^T	10^2-10^3	19
Negative control for pathogenicity	*Escherichia coli* CIP 7624	10^5-10^6	no mortality
***S. glaseri* SK39**	*Xenorhabdus poinarii* XpSg_G6	10^2-10^3	no mortality
	***Pseudomonas protegens* PpSg_SG6 Apo**	**10^2-10^3**	**28**
	***Pseudomonas chlororaphis* PcSg_SK39 ApoA**	**10^2-10^3**	**19**
	Stenotrophomonas maltophilia StmSg_SK39-2	10^5-10^6	no mortality
***S. weiseri* 583**	*Xenorhabdus bovienii* XbSw_CS03	10^5-10^6	no mortality
	***Pseudomonas protegens* PpSw_SW4**	**10^2-10^3**	**20**
	***Pseudomonas protegens* PpSw_TCH07 2-2**	**10^2-10^3**	**26**
	Stenotrophomonas maltophilia StmSw_SW1	10^5-10^6	no mortality
	Stenotrophomonas maltophilia StmSw_TCH07 2-3	10^5-10^6	no mortality
	Ochrobactrum anthropi OchaSw_SW2	10^5-10^6	no mortality

Ogier, Pagès et al, Microbiome, 2020

Antibiosis spectrum *X. nematophila* / *P. protegens*

OVERLAY ANTIBIOSIS ASSAY
-: negative
+: slightly positive
++: highly positive
v: variable
p: partial

		S carpocapsae microbiota						
Producer strain	Indicator strains	*Xenorhabdus nematophila* XnSc_F1	*Pseudomonas protegens* PpSc_PP-SC-10	*Achromobacter* sp AchSc_D7-1	*Alcaligenes faecalis* AlcfSc_SC	*Ochrobactrum* sp OchSc_ALL4	*Pseudochrobactrum* PochSc_AL3	*Stenotrophomonas* StSc_ALL5
Xenorhabdus nematophila XnSc_F1		ND	-	-	p	+	-	-

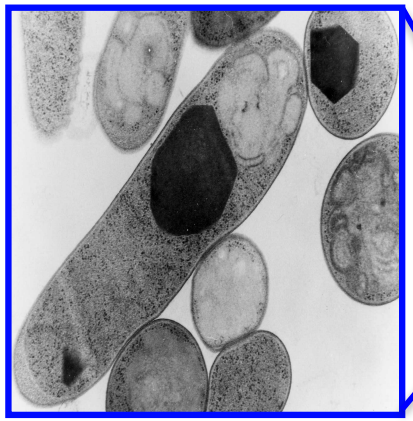
		S carpocapsae microbiota						
Producer strain	Indicator strains	*Xenorhabdus nematophila* XnSc_F1	*Pseudomonas protegens* PpSc_PP-SC-10	*Achromobacter* sp AchSc_D7-1	*Alcaligenes faecalis* AlcfSc_SC	*Ochrobactrum* sp OchSc_ALL4	*Pseudochrobactrum* PochSc_AL3	*Stenotrophomonas* StSc_ALL5
Pseudomonas protegens PpSc_PP-SC-10		v	ND	+	+	++	+	-

Ogier, Pagès et al, Microbiome, 2020

- **Different compartmentalization within the IJ?**
- **Different time succession within the insect cadaver?**

Conclusions

The IJ is not a monoxenic organism

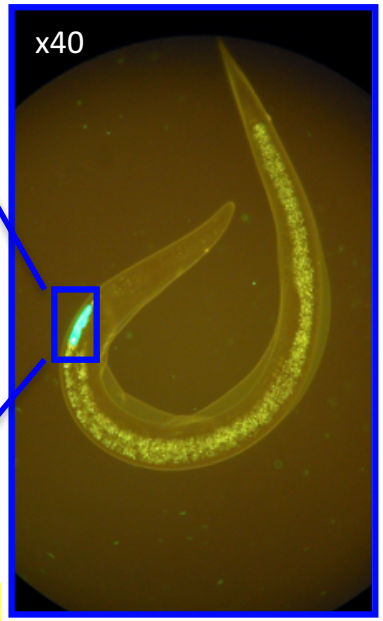


Xenorhabdus nematophila

+

Frequently associated microbiota (FAM)

Alcaligenes faecalis
Stenotrophomonas maltophilia
Pseudomonas
***Rhizobiaceae* (*Ochrobactrum*, *Agrobacterium*, …)**



Steinernema carpocapsae

IJ microbiota and nematode fitness?

- Participation to insect killing => ***P. protegens/P. chlororaphis***
- Preservation of insect cadaver from other microorganisms
- Nematode nutrition
- Nematode reproduction, …

Pathobiome

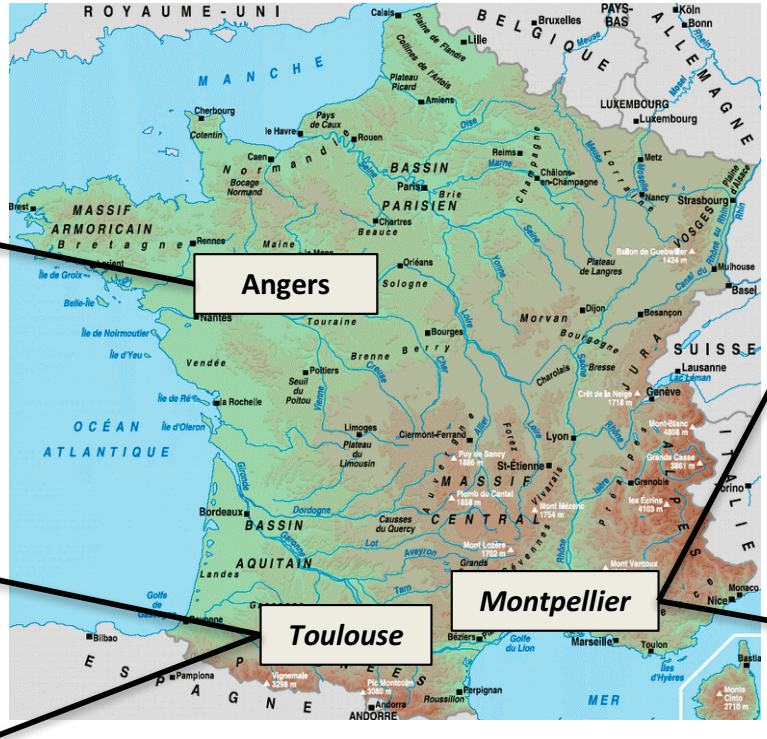
Pathogenic agent + biotic environment that influences infection process

- Methodological challenge: manipulation of this low-complexity microbiota (gnotobiology)

Holobiont?

Macro-organism + microbiota as evolution unit

- Transmission of the microbiota
- Co-evolution IJ/microbiota



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(*rpoB* database/ metabarcoding)

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GET-PLAGE Platform
(sequencing)

Maxime GALAN
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GenPhyse
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NEMATODE PROVIDERS:
David Shapiro (USA)
Vladimir Puza (Hungary)
Marie Berling (France)
Alexandre Jousset (France)
Jérome Lambion (France)

FUNDINGS
- ✓ **AAP Metaprogram "Meta-omics of Microbial Ecosystems" - INRA 2015-2016**
- ✓ **AAP Department "Health Plant and Environment" - INRA 2015-2017**
- ✓ **AAP Department "Health Plant and Environment" - INRA 2018-2019**

An operational output : promoting the vectorization of plant beneficial microorganisms by IJs

Frequently associated microbiota (FAM)

Alcaligenes faecalis
Stenotrophomonas maltophilia
Pseudomonas
***Rhizobiaceae* (*Ochrobactrum*, *Agrobacterium*, …)**

- **Entomopathogenic properties => *Pseudomonas protegens* and *Pseudomonas chlororaphis***
- **Plant Growth Promoting Rhizobia => *Pseudomonas sp, Stenotrophomonas sp, Alcaligenes sp, Rhizobium, etc…)***

Focus on *Pseudomonas* strains

Innovation funding from INRAE
Collaboration with : Christoph Keel on insecticidal activities
Marc Bardin on antifungal properties