

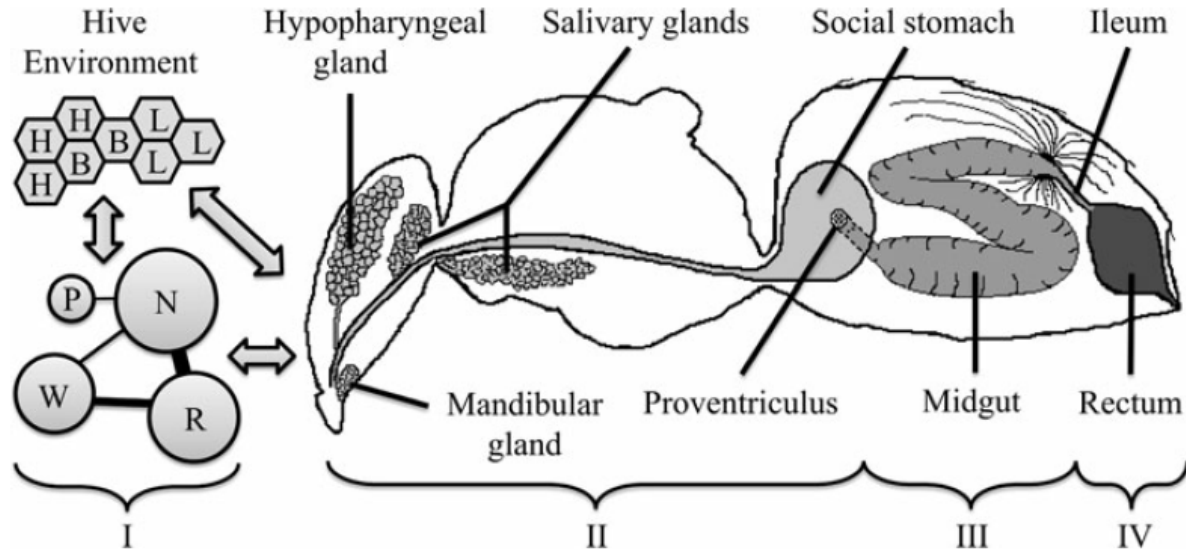
# BeeProbio: Improving honey bee health with probiotics

Andrée Rousseau, Sarah ElKhoury, Nicolas Derome and Pierre Giovenazzo



UNIVERSITÉ  
LAVAL

# Honey bee microbiota



**Fig. 1** The microbial niche of the hive and alimentary tract (gut). The relatively static hive environment (I) includes developing larva (L), stored honey (H) and beebread (B). The embedded network diagram (I) depicts a more dynamic niche generated through trophallactic interactions and the collection or processing by distinct worker sub-castes of nectar (N), pollen (P), water (W), or royal jelly (R). Two way arrows indicate the transfer of microbes or nutrients that may support

microbial growth. Niche II includes the mouth, esophagus, social stomach and associated secretory glands. The one-way valve of the proventriculus separates niche II from the digestive enzymes and more basic pH of the midgut (III). The hindgut niche (IV) is demarcated by another valve-like structure that separates the ileum and the rectum from the midgut. See text for details

# Honey bee gut microbiota

- Using 16S rRNA sequence variations:
  - 8 distinct species make up for up to 99% of variation
  - *γ-proteobacteria Lactobacilli* are present in every bee

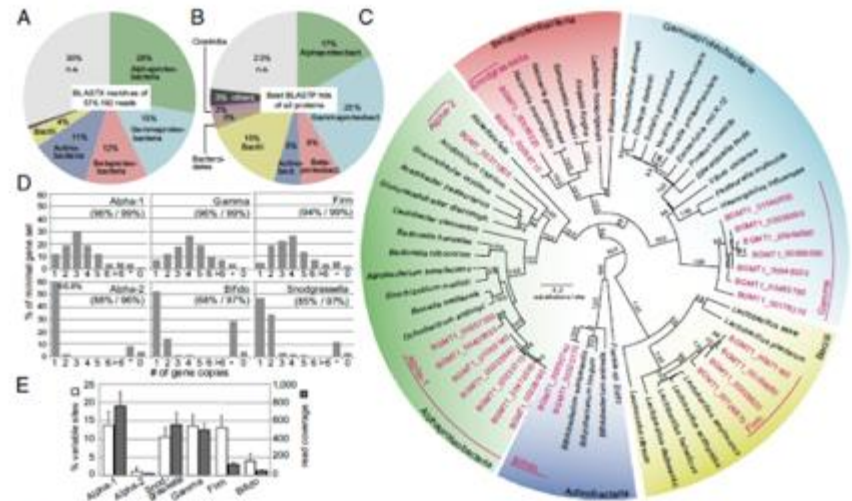
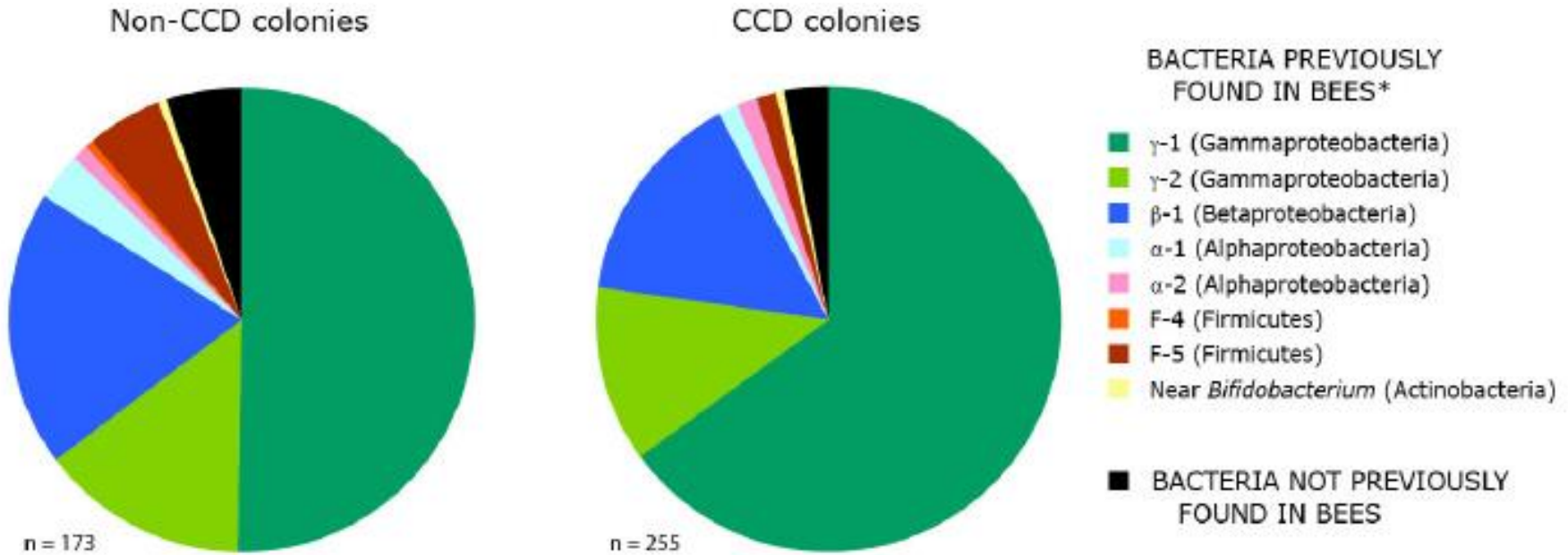


Fig. 1. Identification of bacterial species or species groups in the honey bee microbiome and analysis of their genetic diversity. Phylogenetic profile based on (A) classification of 576,152 reads mapping against 31 marker proteins with MetaPhyler (48) and (B) best BLASTP hit distribution of all 112,128 CDs; n.a., reads or CDs not assigned. (C) Maximum-likelihood protein phylogeny of UvrC. All eight phylogenies (see S1 Appendix, Fig. S1) for other trees revealed that most sequences from the honey bee microbiome (shown in pink) fall into the same six distinct clusters. These phylogenetic clusters are referred to as Alpha-1, Alpha-2, Snodgrassella, Gamma, Bifido, and Firm. We considered all closely related taxa with available genomes for this analysis. Bootstrap values >80 are shown. (D) Percentages of the minimal gene set present in each bin are depicted in parentheses (only full-length copies/including fragmented genes). Graphs show distribution of genes of the minimal gene set based on identified full-length copies per bin. Asterisks indicate fragmented genes. (E) Average percentage of variable sites and average read coverage for 27 ribosomal protein-encoding genes of each bin.

Philipp Engel, Vincent G. Martinson and Nancy A. Moran. 2012. Functional diversity within the simple gut microbiota of the honeyBee

Waldan K. Kwong and Nancy A. Moran. 2016. Gut microbial communities of social bees. Nature reviews

# Honey bee microbiota dysbiosis



« A trend towards increased abundance of one of the Gammaproteobacterial taxa in the CCD bees (Fig. 1) may reflect physiological changes accompanying CCD and impacting the commensal community. »

Cox-Foster D.L., Conlan S., Holmes E.C. et al. 2007. A metagenomic survey of microbes in honey bee colony collapse disorder. *Science* 318: 283-287

# Action of probiotic candidates?

- **Nosemosis is associated with a dysbiosis** of intestinal microbiome
  - “Interestingly, the increase in *Nosema spp.* spore loads was significantly correlated with the increase in ileum *F. perrara*. Thus, our results suggest that the early establishment of *Nosema spp.* is encouraged by blooms of *F. perrara*, bacterial dysbiosis and/or diet associated conditions in developing nurse bees” (Maes et al. 2016).

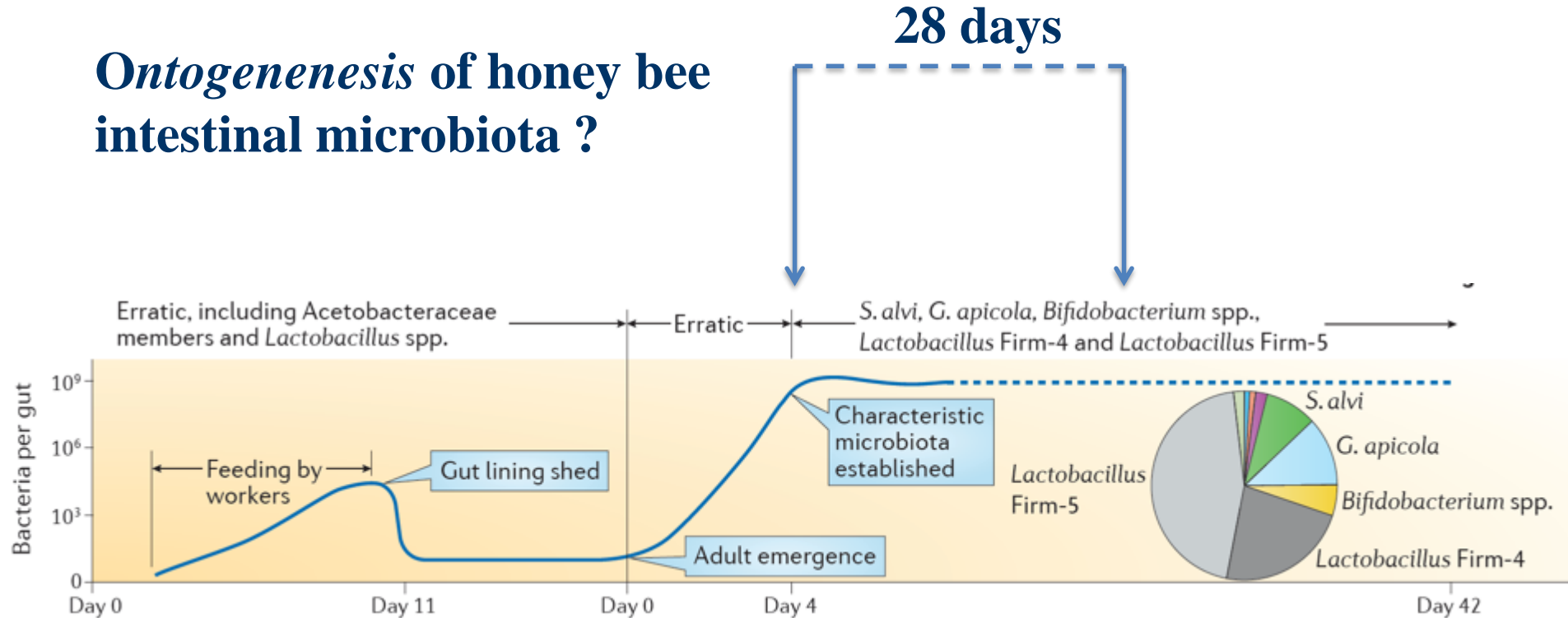
**Can candidate probiotics improve honey bee survival by reducing dysbiosis?**

# BeeProbio project

- Goal:
  - Test the efficiency of probiotics on honey bee health.
    - Two commercially available broad spectrum strains (Bactocell® and Levucell®, Lallemand Inc) and several honey bee gut isolated strains
    - Pathogen inoculation: caged bee trials
      - **Step 1. Honey bee survival**
      - Step 2. Metagenomic analysis (honey bee microbiota), Transcriptome (differential gene expression in the honey bee)

# Probiotic testing

## Ontogenenesis of honey bee intestinal microbiota ?



**Kwong & Moran Nat Rev 2016**

# Probiotic in vitro testing

- CRSAD: Honey bee center
  - Standard method for in situ tests
    - Good statistic power
    - Good honey bee survival
    - Young emergent bees 4 days old
    - *Nosema cerana* inoculation
  - Microbiota survival and concentration
    - In sugar solution
    - In honey bee gut
  - Microbiota isolation and culture
    - Aerobic strains
    - Two potential strains: *Acetobacter sp.* and *Bacillus sp.*



Evans, J., Chen, Y. P., di Prisco, G. D. P., Pettis, J., & Williams, V. (2009). Bee cups: single-use cages for honey bee experiments. *Journal of Apicultural Research*, 48(4), 300–302.



# Probiotic protocol



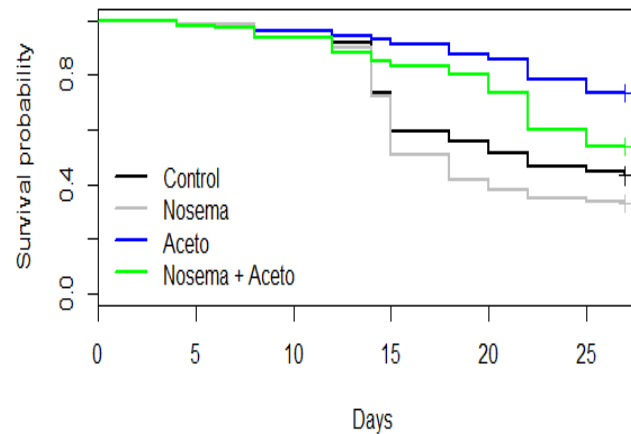
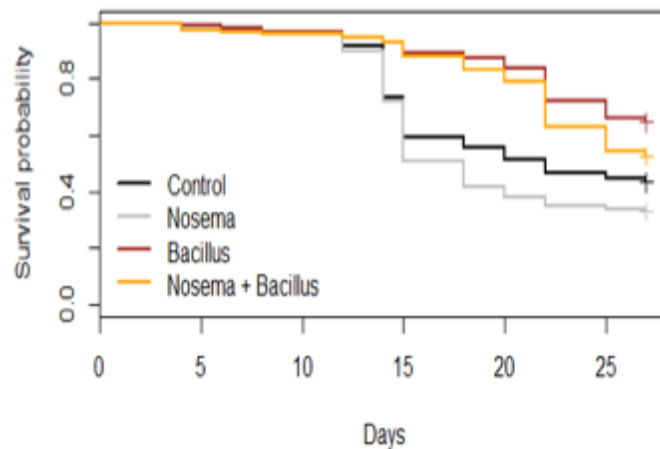
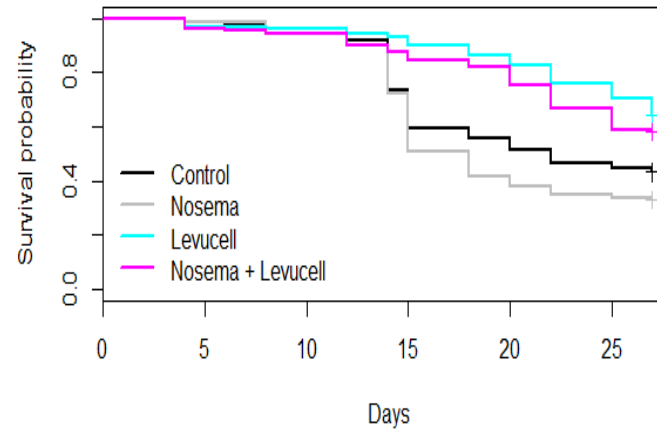
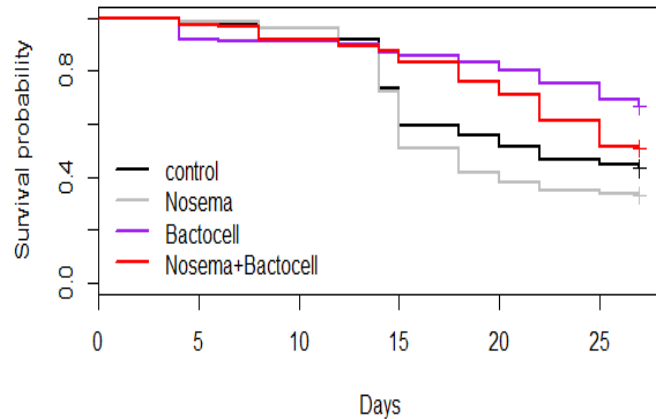
	<i>Endogenous</i>		Lallemand Inc.		
	<i>Acetobacter sp.</i>	<i>Bacillus sp.</i>	Bactocel®	Levucel®	Control
No <i>Nosema sp.</i>	20x	20x	20x	20x	20x
<i>Nosema sp.</i>	20x	20x	20x	20x	20x

- Bees 4 days old: cage trials during 27 days (D0-D27)
- 10 groups, 20 cages / group ; 20 bees / cage
- **Step 1. Honey bee Survival**
- Step 2. Metagenomics and transcriptome
  - Cage bee samples : D0, D14, D27
  - 5 replicates / group / sample

# Probiotic protocol

Group	N (cages)	1:1 Sugar syrup containing:	
		<i>Nosema sp.</i> inoculate (1 million spores/cage)	Probiotic (10 <sup>3</sup> CFU)
Control	20	-	-
<i>Nosema sp.</i>	20	Day 0	-
Bactocell®	20	-	Day 1-27 Bactocell®
<i>Nosema sp.</i> + Bactocell®	20	Day 0	Day 1-27 Bactocell®
<i>Acetobacter sp.</i>	20	-	Day 1-27 <i>Acetobacter sp.</i>
<i>Nosema sp.</i> + <i>Acetobacter sp.</i>	20	Day 0	Day 1-27 <i>Acetobacter sp.</i>
<i>Bacillus sp.</i>	20	-	Day 1-27 <i>Bacillus sp.</i>
<i>Nosema sp.</i> + <i>Bacillus sp.</i>	20	Day 0	Day 1-27 <i>Bacillus sp.</i>
Levucell®	20	-	Day 1-27 Levucell®
<i>Nosema sp.</i> + Levucell®	20	Day 0	Day 1-27 Levucell®

# Survival results



Kaplan-Meier Survival curves distribution of bees in each group during the 27 days in vivo experiment. Cox Proportional Hazard model comparisons after 27 days: control vs all groups  $p < 0.01$  ; *Nosema sp.* vs *Nosema sp.* + all probiotics  $p < 0.001$

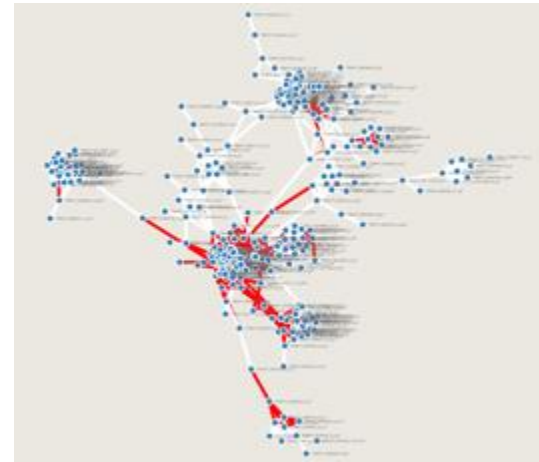
# Activity of intestinal microbiota

## Derome Lab

- Whole bee guts from ventriculus to rectum were dissected from the abdomen
- Taxonomic annotation database using BlastX and BlastN transcript mappings and transcript quantification data.
  - *Nosema sp.* infected bees:
    - Genus *Nosema* ranked second in both transcript activity and total unique transcripts, with *Nosema ceranae* accounting for 97% of all *Nosema* occurrences.
    - % of *Nosema sp.* activity of total transcript activity
      - Infected bees: 15-25%,
      - Non-infected bees: 0-3%.

# Current work

- Step 2. Metagenomics / transcriptome analysis
  - Cage bee samples : D0, D14, D27
    - 5 replicates / group / sample
- Colony trials 2016-2018
  - Probiotics during fall feeding



# Acknowledgements

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