The porcine gut microbiota: composition and links with host's genetics and phenotypes

Jordi Estellé & Claire Rogel-Gaillard

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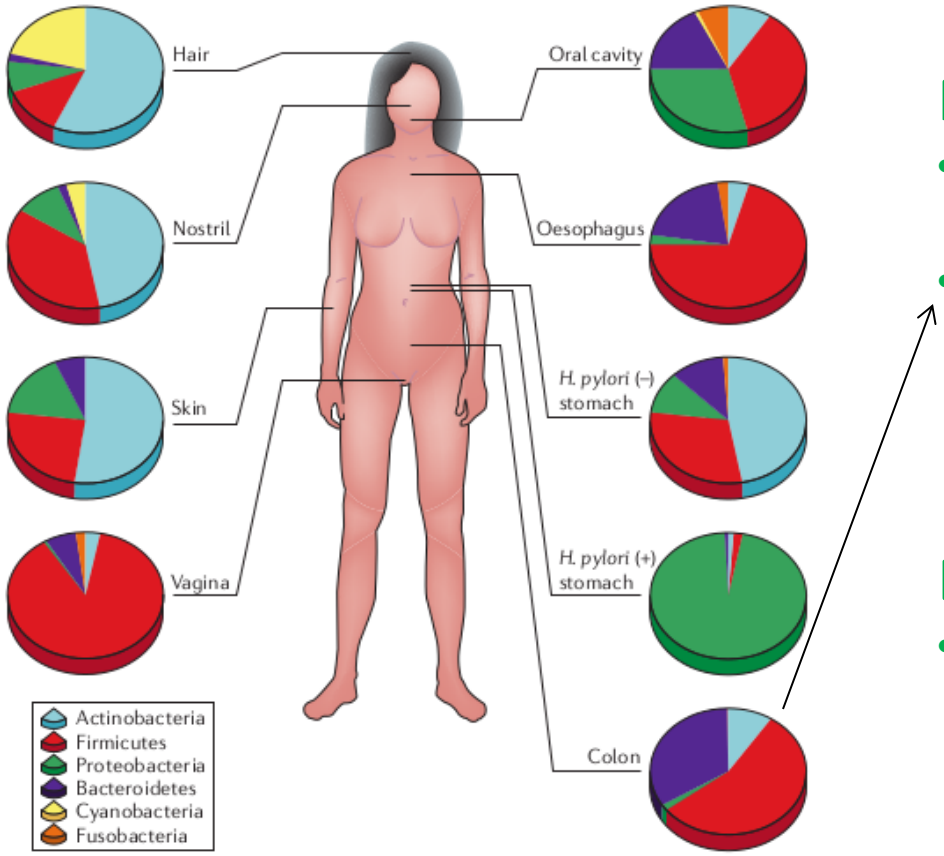
Who are we?

Simbiotic microbiota



Microbiota

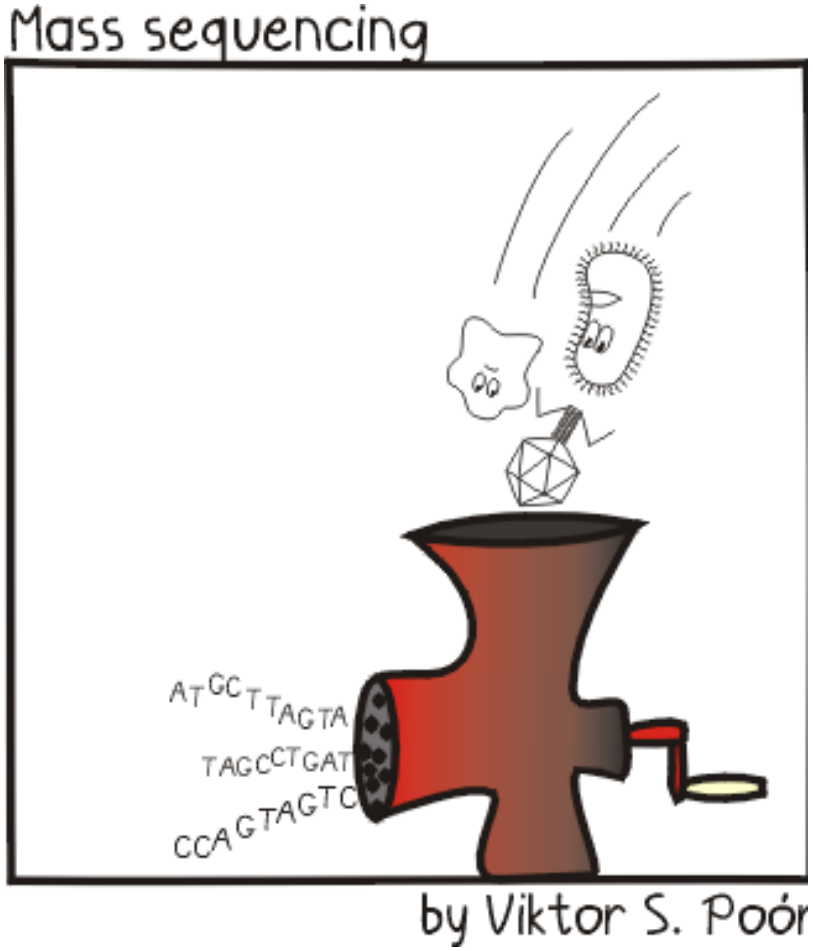
- Population of microorganisms living in symbiosis with its host
- Intestine:
  - 10^{14} microorganisms (~ 1,5 Kg)
  - Implantation at early age
  - Relative stability along host's life

Dysbiosis

- Disequilirium of microbiota composition that can let to pathology

How to look at microbiota?

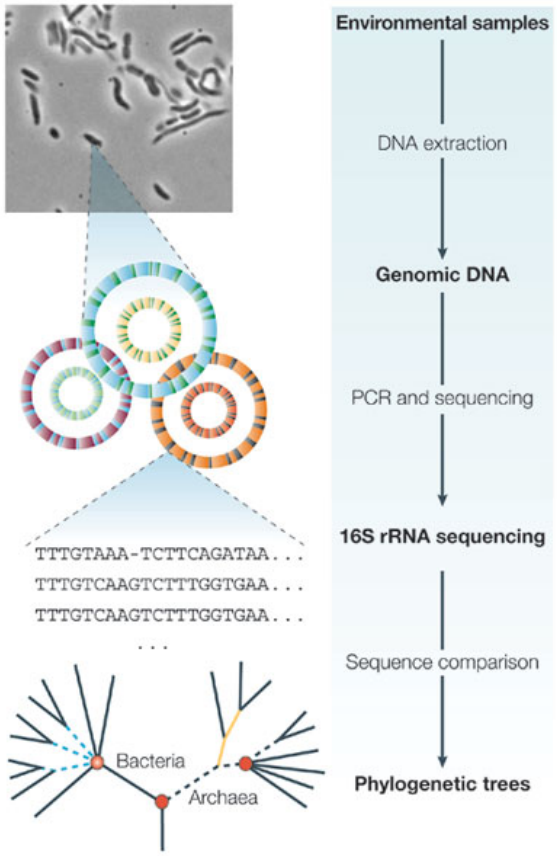
vs.



NGS approaches allow an unprecedented vision of microbiota

Targeted sequencing of 16S rDNA phylogenetic marker

- Multi-species PCR Amplification of 16S rDNA gene
- Roche 454 / Illumina MySeq sequencing with multiplexing (~8,000-10,000 sequences/sample)
- Diversity analyses and link with ecosystem (host!) parameters
- Main interest:
  - Ready to use for any ecosystem
  - Relatively cheap



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NGS approaches allow an unprecedented vision of microbiota

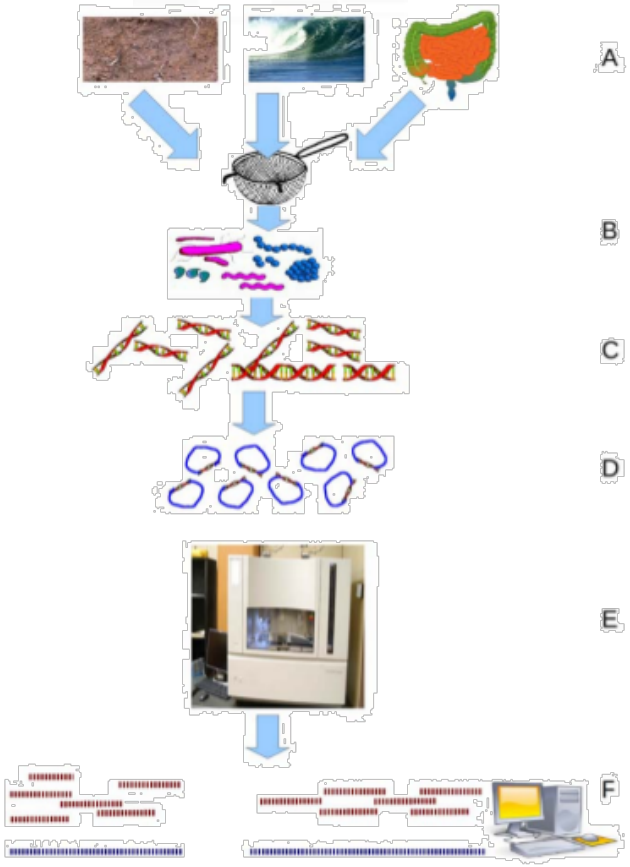
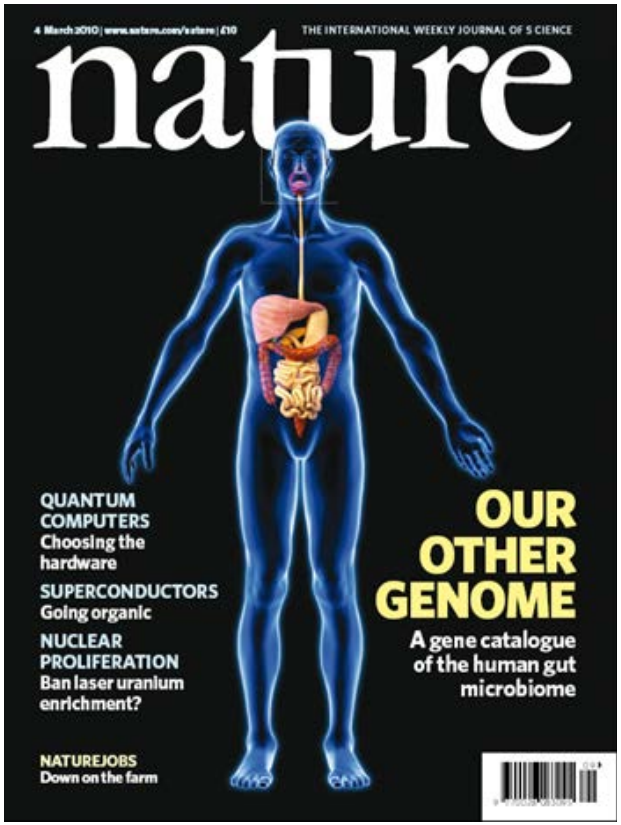


Figure 1. Environmental Shotgun Sequencing (ESS). (A) Sampling from habitat; (B) filtering particles, typically by size; (C) DNA extraction and lysis; (D) cloning and library; (E) sequence the clones; (F) sequence assembly.
doi:10.1371/journal.pcbi.1000667.g001

Whole metagenome sequencing: quantitative metagenomics

- Short-read NGS technology (Illumina HiSeq, SOLID)
- Alignment of reads to a catalog of known bacterial genes/genomes
- Diversity analyses at the gene level (function!) and species level
- Functional interpretation of microbiota-host interactions?
- Requires a known reference metagenome for the ecosystem!

The human metagenome



Qin et al, Nature. March 2010

Intestinal human microbiota:

- A catalogue including over 3.3 million bacterial genes (which represents150 times the human genome)
- A common «shared» core of 540000 bacterial genes
- At least three major enteroptypes (Arumugam et al., Nature, 2011)
- The possibility of quantitative metagenomics

At INRA: Dusko Ehrlich, Joël Doré
MetaGenoPolis platform

Who are we?

What about livestock?

A challenge in livestock production: the end of antibiotics (ab)use

- Livestock selection schemes have traditionally target production traits = growth, fatness, …
- New EU regulations promote welfare and limit the prophylactic use of antibiotics (and ban them as growth factors!)
  - CHALLENGE : how to breed robust animals against pathogens?

Our approach at INRA's GABI team :

– Integrative biology of host's genetics and microbiota

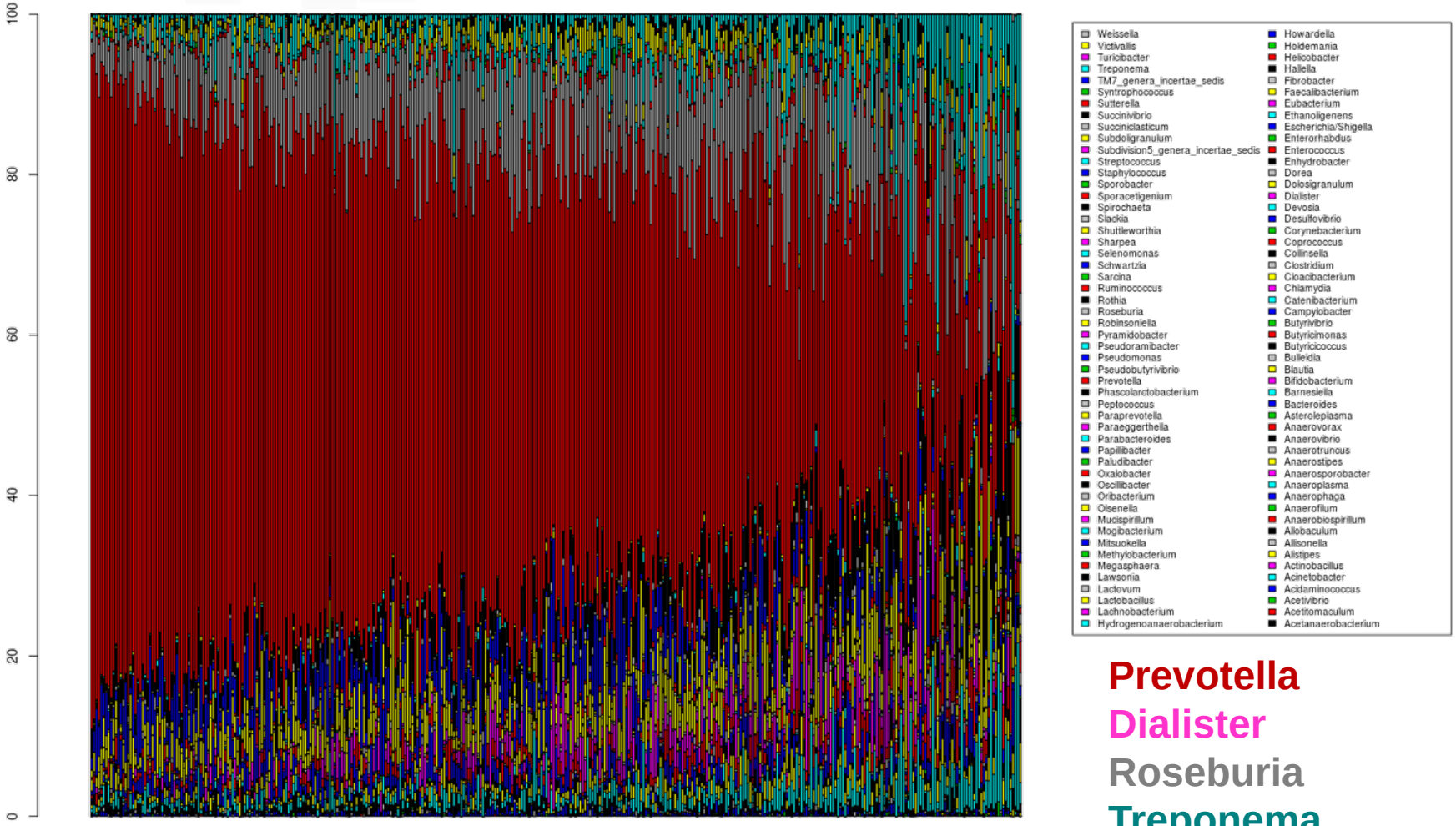
SUS_FLORA project

- INRA GABI, Jouy
  - **J. Estellé**, **Y Ramayo-Caldas**, **N Mach**, G Lemonnier, C Denis, S Bouet, F Andreoletti, JP Bidanel, **C Larzul**, **C Rogel-Gaillard**
- CEA LREG, Jouy
  - G Piton, JJ Leplat, **S Vincent-Naulleau**
- INRA MICALIS, Jouy
  - **P Lepage, N Mach**, F Levenez, **J Doré**
- INRA TOXALIM, Toulouse
  - L Guzylack, **IP Oswald**
- INRA ISP, Tours
  - **M Berri,** F Meurens
- INRA VIM, Jouy
  - **N Bertho**
- INRA GENESI, Le Magneraud
  - J Bailly, **Y Billon**
- BIOPORC, Le Rheu
  - **MJ Mercat**

SUS_FLORA: analysis of porcine intestinal microbiota

- >520 individuals scored for intestinal microbiota composition at 60 days of age
- 16S rDNA approach (V3-V4 region) coupled to massive parallel sequencing in a Roche-454
  - **Who is there?**
  - **Link with host phenotypes?**
  - **What about host genetics?**

SUS_FLORA: 16S fecal microbiota composition at the genus level



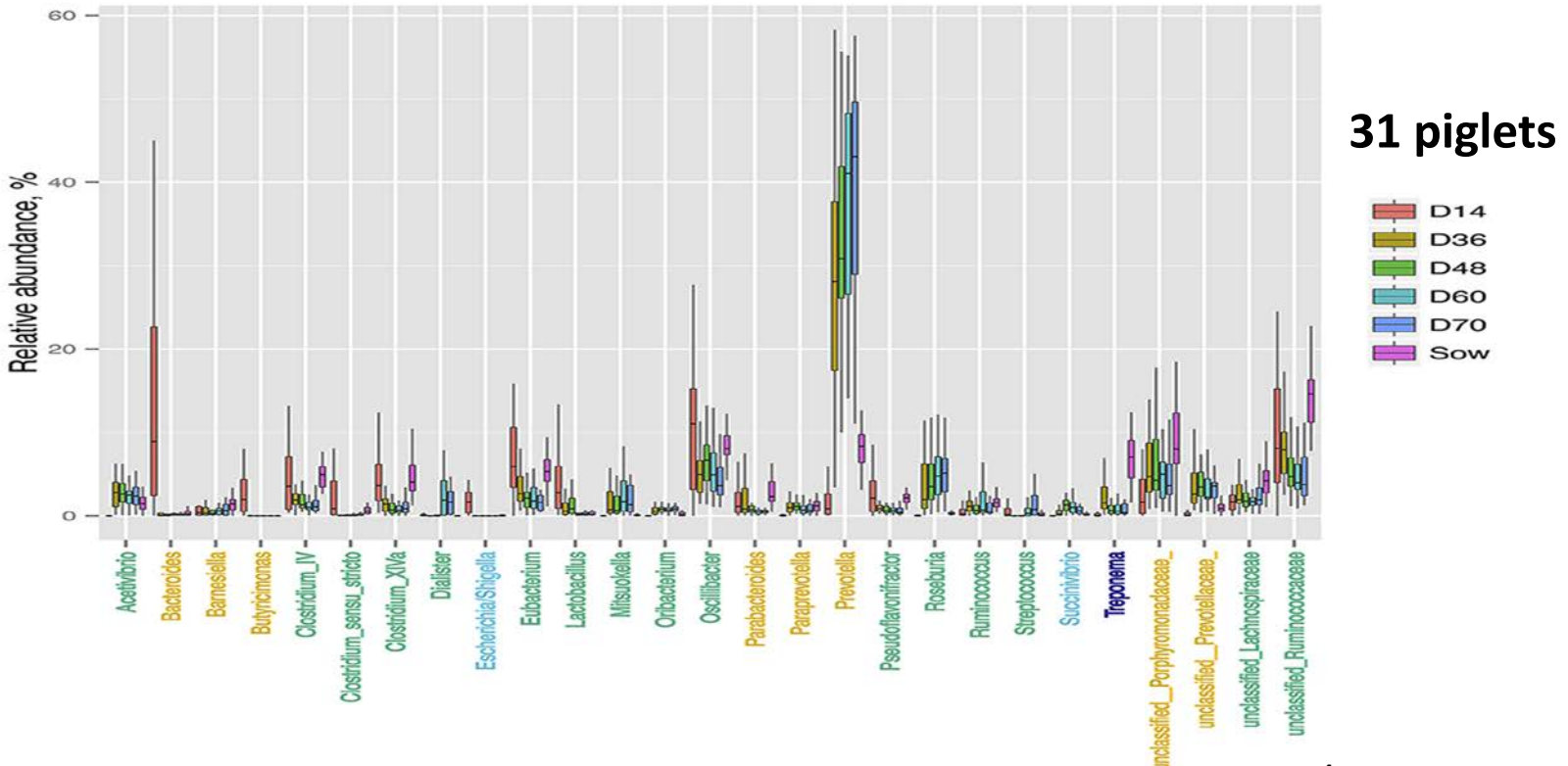
Prevotella

Dialister

Roseburia

Treponema

SUS_FLORA: dynamics of implantation



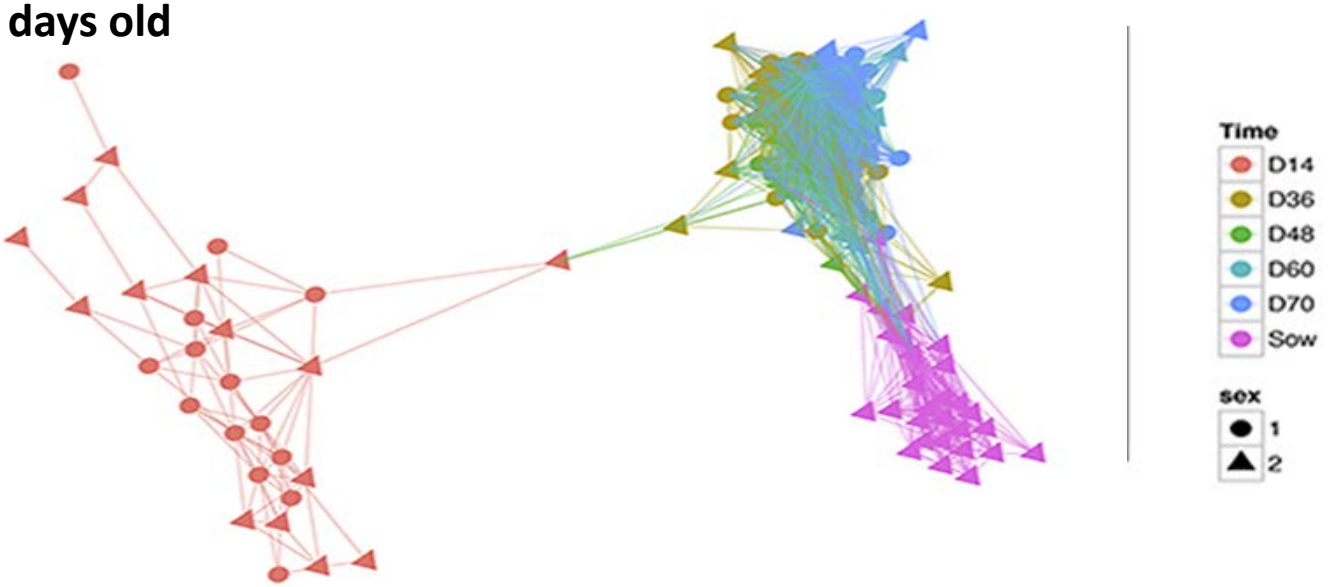
- **Suckling piglets**: predominance of *Bacteroides, Oscillibacter, Escherichia/Shigella, Lactobacillus* and *unclassified Ruminococcacae* genera
- **After weaning (28 days old)**: Start of stabilization after 36 days

Mach et al., 2015, Environ Microbiol Rep

SUS_FLORA: dynamics of implantation

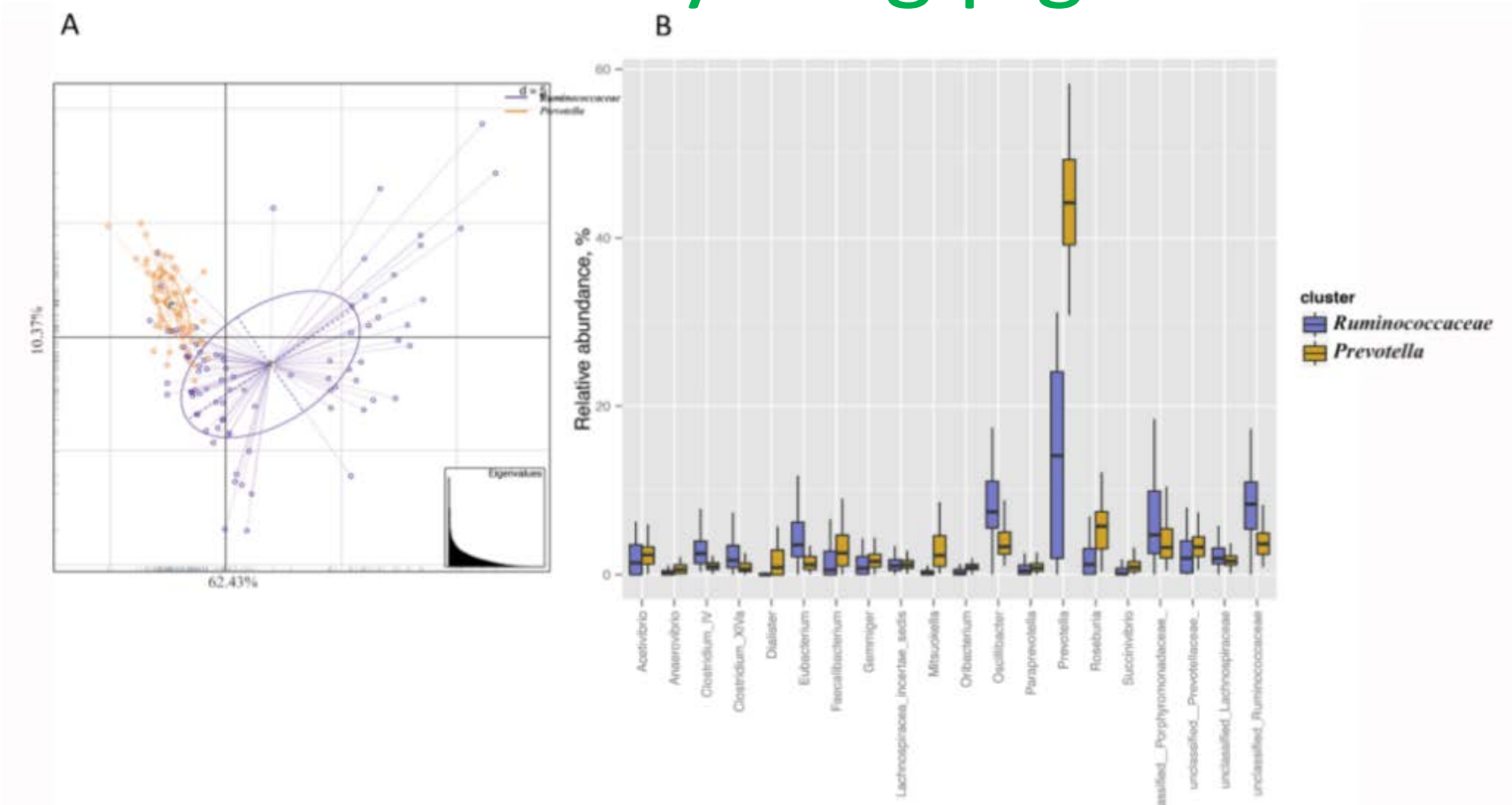
Older piglets and their mothers

Piglets at 14 days old



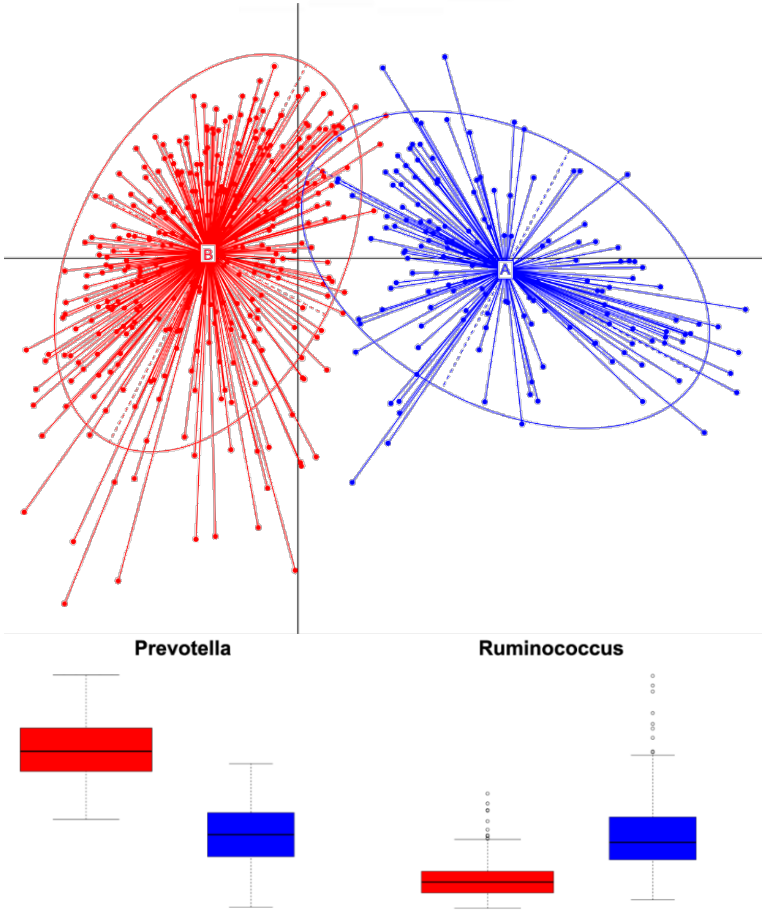
Mach et al., 2015, Environ Microbiol Rep

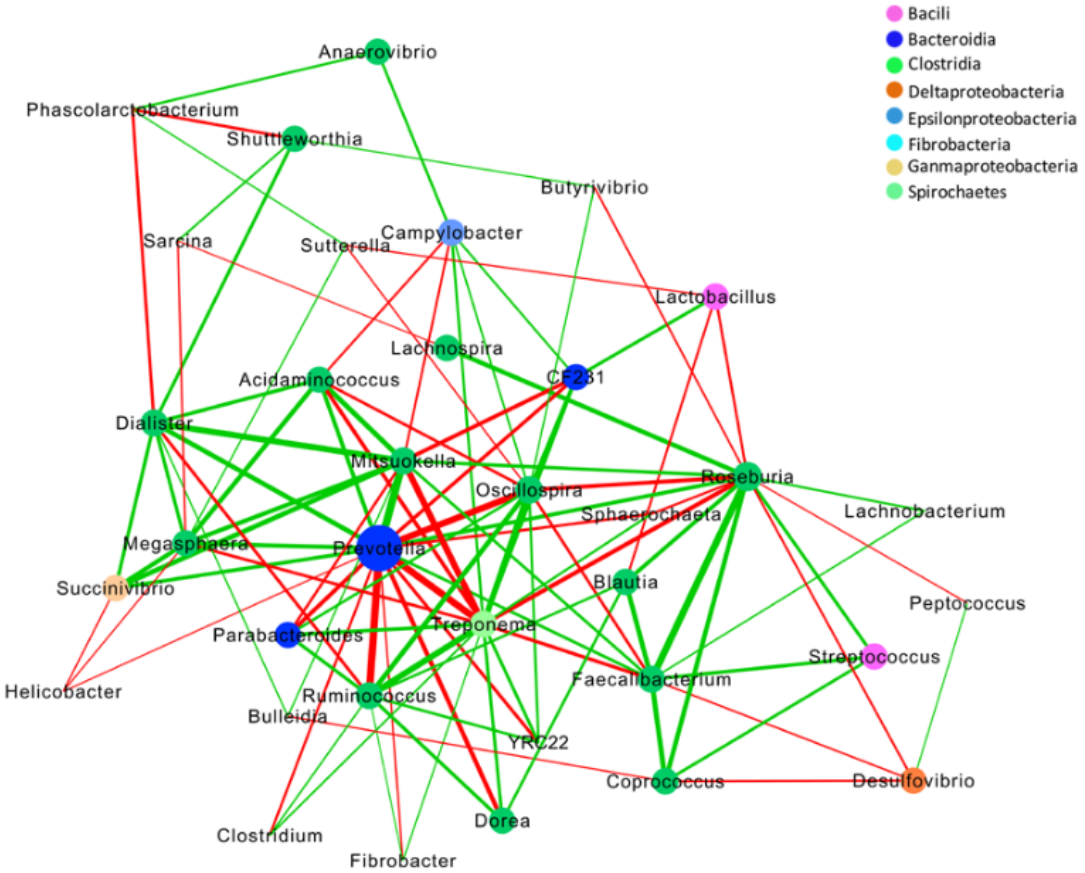
Two “enterotype-like” clusters detected in young piglets



Mach et al., 2015, Environ Microbiol Rep

Enterotype clusters and network analysis in the whole population (60 days old pigs)



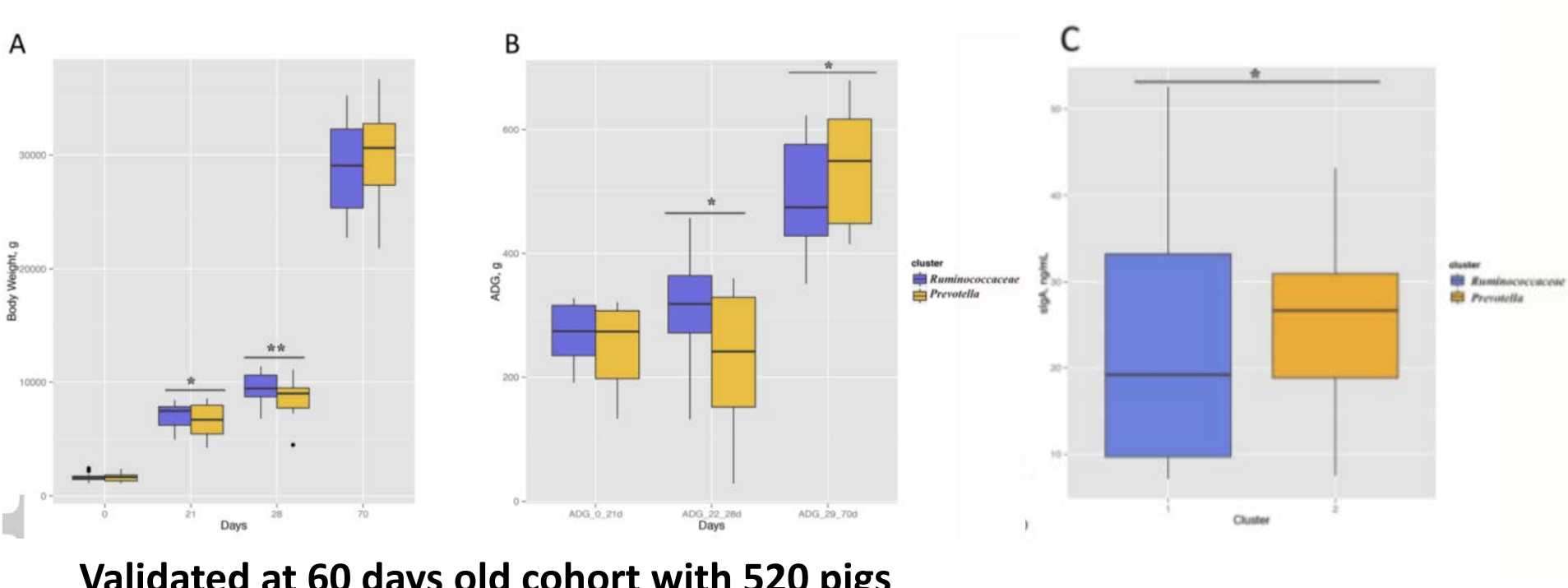


Ramayo-Caldas et al., 2016, ISME Journal

Pig gut microbiota and interactions

- Prevotella is dominant in porcine intestinal tract microbiota (genus and family) and the Rominococcacea family acts as the second main player:
  - Bacteroidetes & Firmicutes
- Strong interactions among genus and families at the composition level:
  - The enterotype concept applied to pig microbiota: **influence on growth performance?**

“Enterotypes” linked to growth and intestine luminal IgA abundance



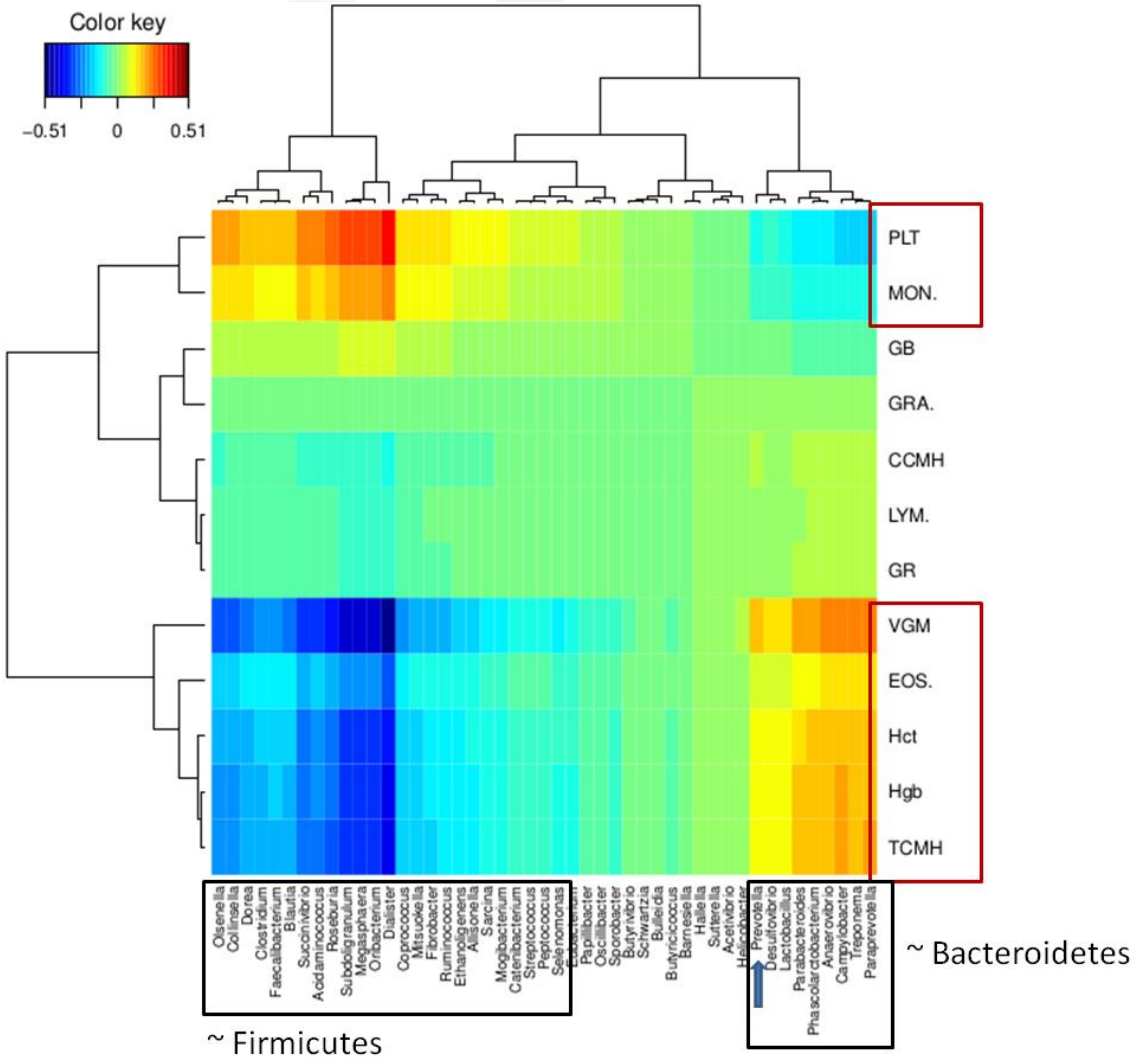
Validated at 60 days old cohort with 520 pigs

Mach et al., 2015, Environ Microbiol Rep
Ramayo-Caldas et al., 2016, ISME Journal

SUS_FLORA: analysis of porcine intestinal microbiota

- >520 individuals scored for intestinal microbiota composition at 60 days of age
- 16S rDNA approach (V3-V4 region) coupled to massive parallel sequencing in a Roche-454
  - Who is there?
  - **Links with health phenotypes?**

SUS_FLORA: links with health traits



rCCA ANALYSIS

PLT: platelets
MON: monocytes
GB: white blood cells
GRA: granulocytes;
CCMH: mean cell hemoglobin conc
LYM: lymphocytes
GR: red blood cells
VGM: red cell distribution width
EOS: eosinophils
Hct: hematocrit
Hgb: hemoglobin
TCMH: mean cell hemoglobin

rCCA analysis by N. Mach
Manuscript in preparation

SUS_FLORA: analysis of porcine intestinal microbiota

- 321 individuals scored for intestinal microbiota composition at 60 days of age
- 16S rDNA approach (V3-V4 region) coupled to massive parallel sequencing in a Roche-454
  - Who is there?
  - Link with host phenotypes?
  - Genetic studies: **a role of host genetics on the gut microbiota composition?**

SUS_FLORA: genetic control

Genre bactérien[1]	h2	stdh2
Ruminococcus	0,55	0,16
Prevotella	0,37	0,27
YRC22	0,36	0,10
Campylobacter	0,34	0,10
Dorea	0,31	0,23
Treponema	0,29	0,03
CF231	0,29	0,31
Anaerovibrio	0,26	0,17
Faecalibacterium	0,25	0,28
Blautia	0,24	0,09
Parabacteroides	0,22	0,30
Lactobacillus	0,18	0,23
rc4-4	0,17	0,07
Catenibacterium	0,16	0,08
Peptococcus	0,14	0,08
Fibrobacter	0,14	0,43
Helicobacter	0,13	0,08
Streptococcus	0,13	0,10
Anaerostipes	0,13	0,08

518 piglets, analysis in VCE with pedigree:

- Genera for which a count was found for more than 100 animals (a subset of 63 genera retained)
- Fixed effects: animal batch, weight at d21, sequencing run
- Random effect: pen, animal

VCE analysis by C. Larzul
Estellé et al, in preparation

SUS_FLORA: CONCLUSIONS

- Microbiota composition
  - Majority of the phyla *Bacteroidetes* and *Firmicutes*
  - High predominance of the genus *Prevotella* at 60 days, followed by the genera *Oscillibacter*, *Dialister*, *Roseburia* and *Treponema*
  - Diversification during early life and stabilization after 36 days

- Identification of two enterotype-like clusters dominated by *Prevotella* (and *Mitsuokella*) or *Ruminococcus* (and *Treponema*) = link with growth phenotypes!

- Covariations between the relative abundances of the microbiota composition, immunity and growth parameters

- A significant genetic control of the host on the relative abundances of bacterial genera of the gut microbiota

How to go further?
Whole metagenome sequencing!

The pig other genome (*à la* MetaHit): for a gut metagenome reference

- **INRA – Univ. Copenhagen – BGI consortium**
  - Whole porcine metagenome sequencing
  - *De novo* assembly and annotation by using:
    - 100 French pigs from different breeds and locations
    - 100 Danish pigs with different nutrition conditions
    - 87 Chinese pigs from intensive and extensive farms

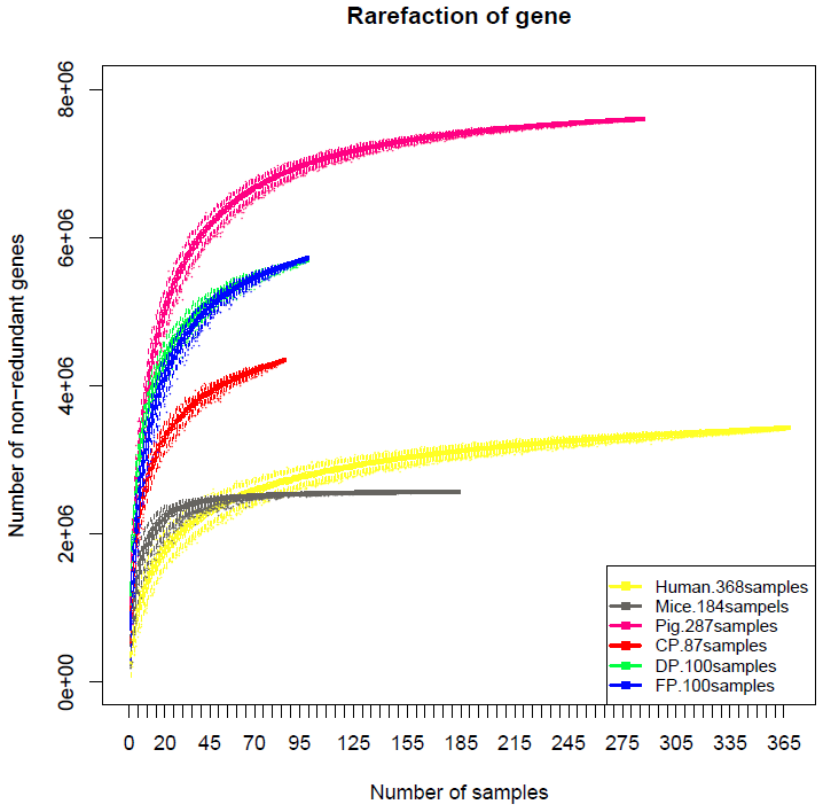
<u>to allow quantitative metagenomics in pigs</u>

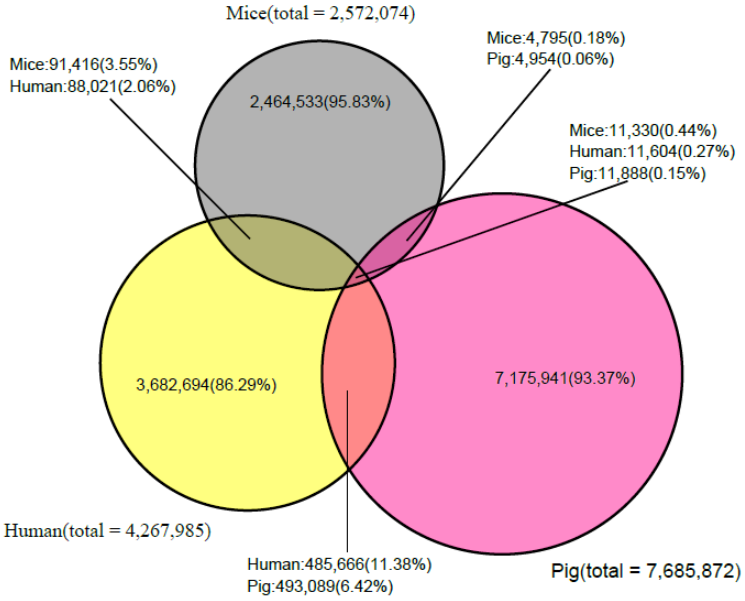
Pig gut metagenome consortium

- **France**
  - **INRA GABI**: J Estellé, Y Ramayo-Caldas, G Lemonnier, C Denis, F Andreoletti, JJ Leplat, C Rogel-Gaillard
  - **MetaGenoPolis:** D. Ehrlich, N. Pons, E. Le Chatellier, S. Kennedy, F. Levenez and coll.
  - **INRA MICALIS:** J. Doré, E. Maguin
  - Experimental and commercial pig farms
- **China**
  - **Bejing Genome Institute-Shenzhen:** Wang Jun, Liang Xiao, Zhongkui Xia, Qiang Feng, and coll.
  - Pig farm
- **Denmark**
  - **Department of Biology, University of Copenhagen:** Pia Kiilerich, Karsten Kristiansen and coll.
  - **Danish Pig Research Centre, Nutrition and Reproduction:** A.Ø. Pedersen, N.J. Kjeldsen
  - Commercial pig farms

The swine other genome

- A catalogue of 7.6M genes constructed with 287 pigs, with greater overlap with human than the mouse

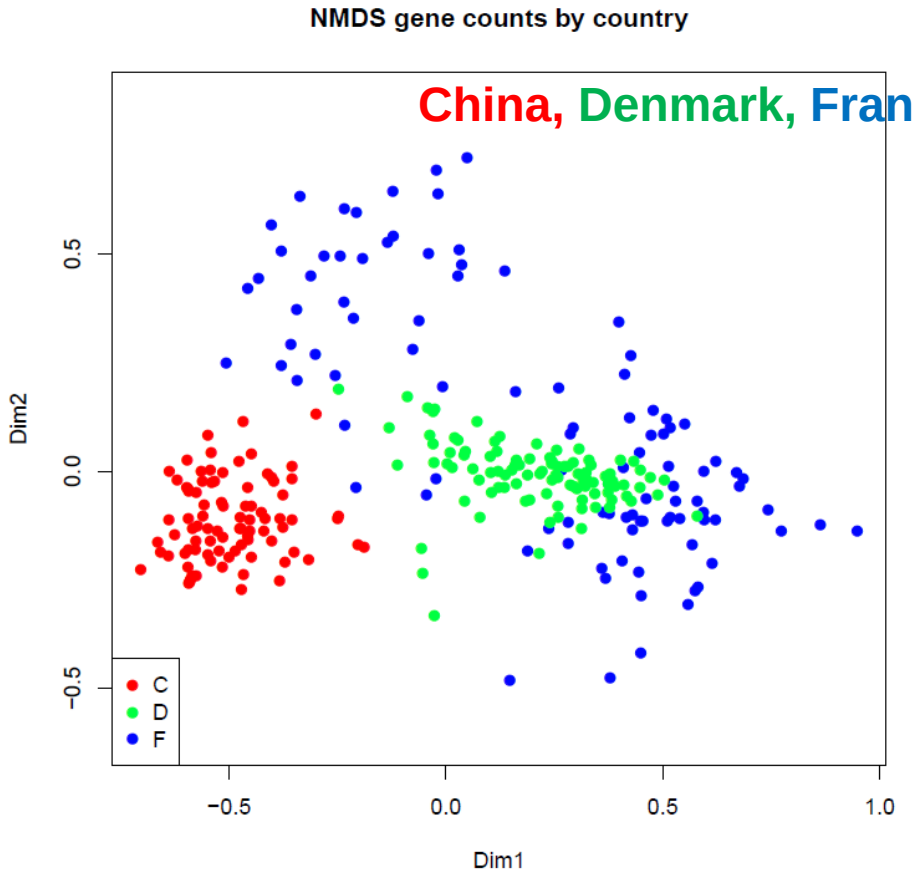


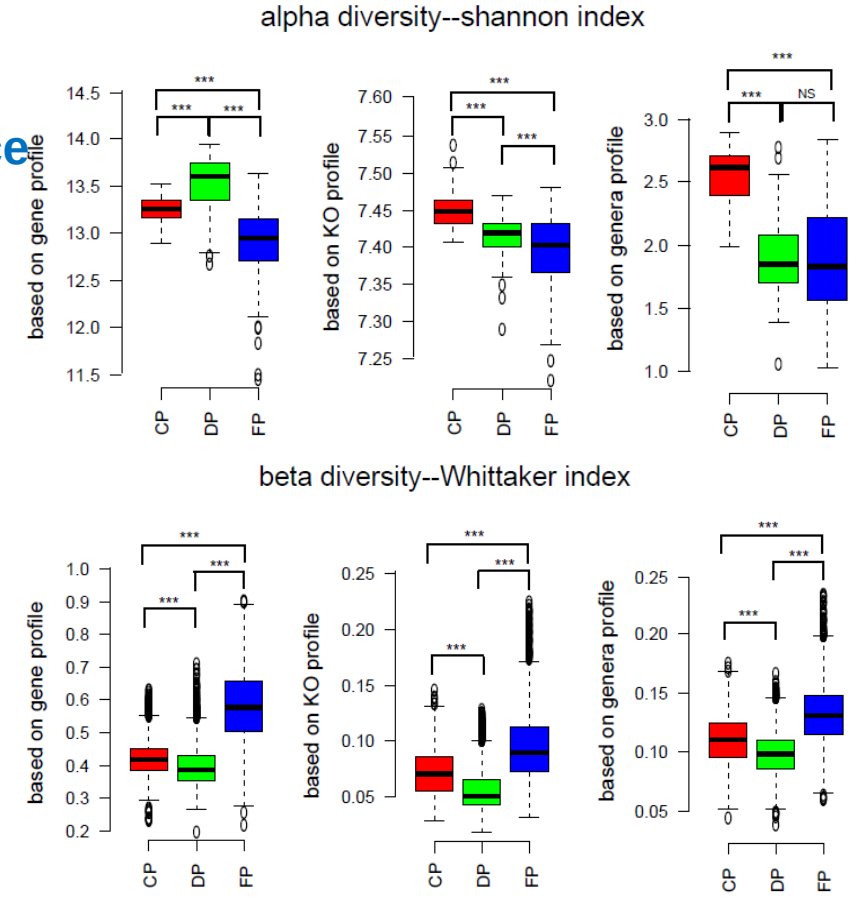


Xiao[1], Estellé[1], Kiilerich[1], et al., (accepted)

The swine other genome

- Stratification of samples according to country of origin





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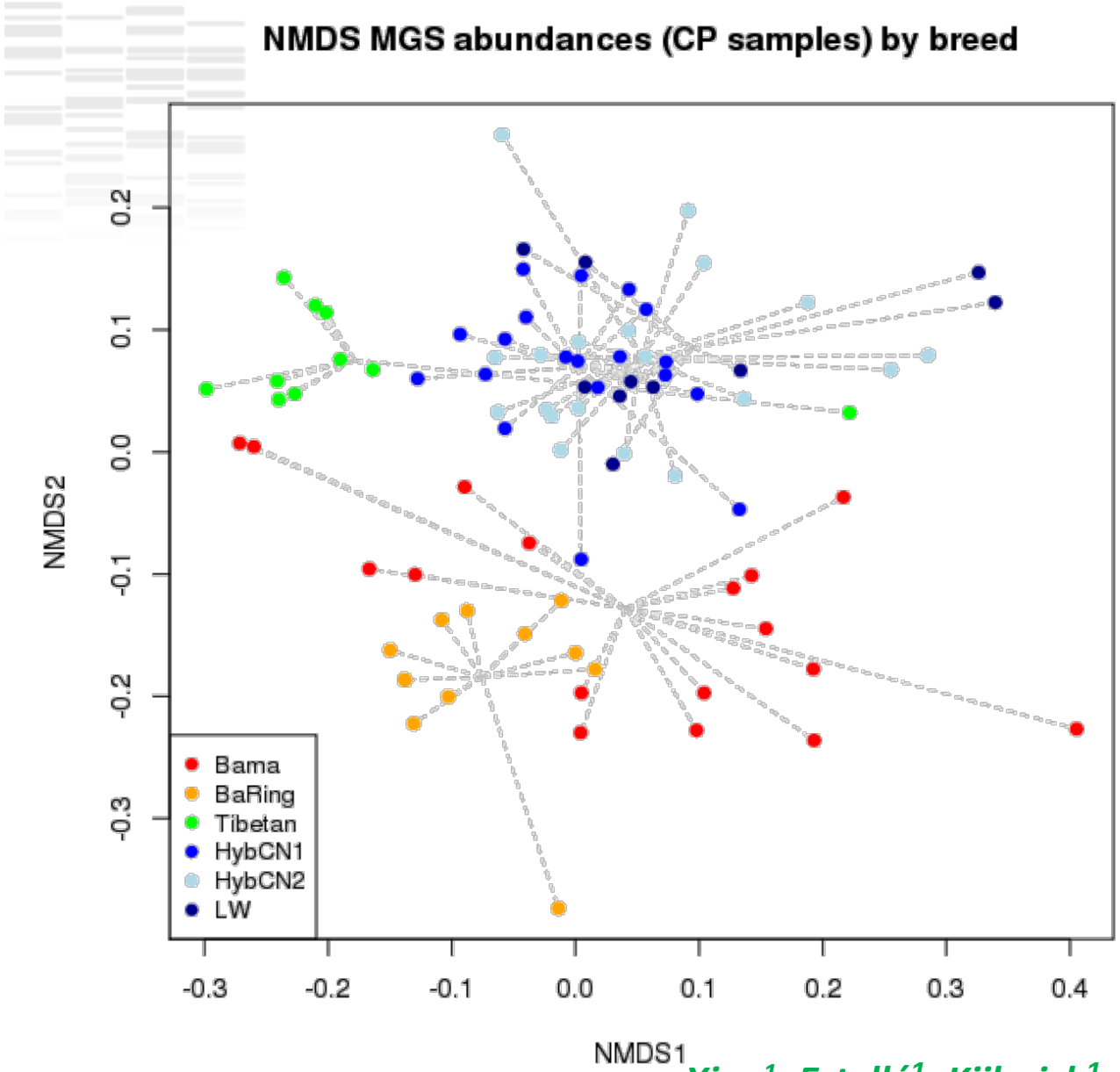
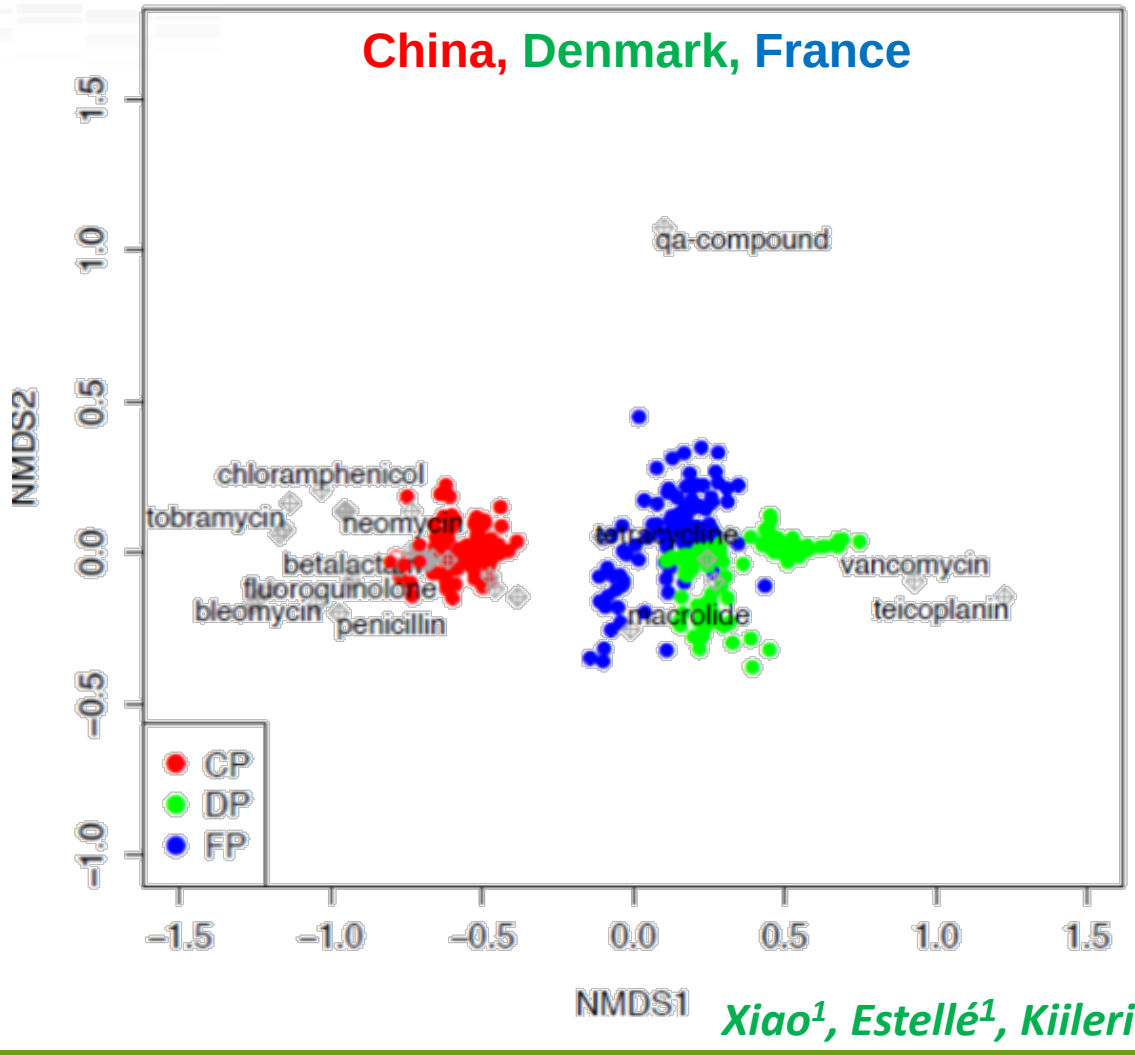


Xiao[1], Estellé[1], Kiilerich[1], et al., (accepted)

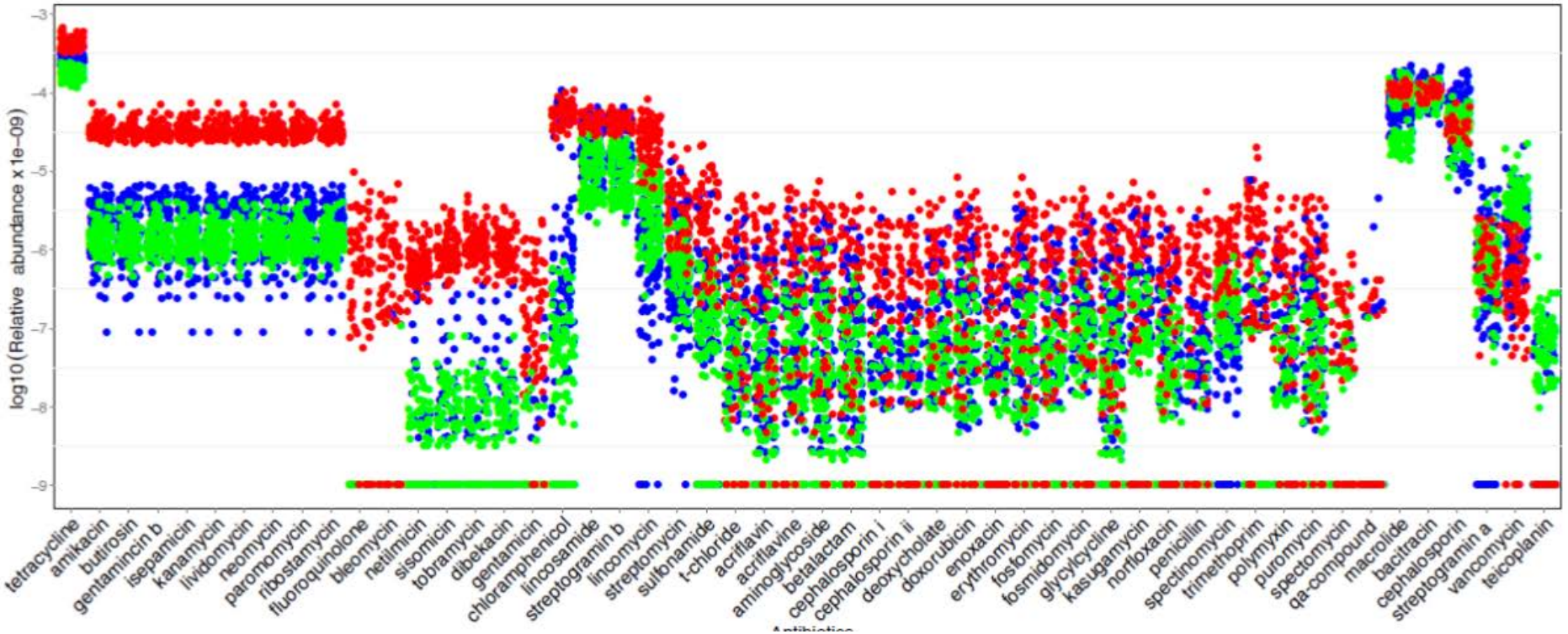
Prevalence of Ab resistance genes



Xiao[1], Estellé[1], Kiilerich[1], et al., (accepted)

Prevalence of Ab resistance genes

China, Denmark, France



Xiao[1], Estellé[1], Kiilerich[1], et al., (accepted)

Future direction: the best holobiont for a given environment?

- Integrative biology and advanced models will be necessary to define:
  - The best host (**genetics**) and the best intestinal microbiota (**metagenomics**) in a given environment (*e.g.* **nutrition**!) in order to optimize:
    - Production, immunocompetence, food efficiency, enviromental impact, robustness, …

Thanks for your attention!

INRA
SCIENCE & IMPACT

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