

Genetic determinism of ruminal microbiota in dairy sheep



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What is already known in sheep genetics rumen microbiota?

Some lines comparisons reported differences in bacteria abundances or diversity regarding feed efficiency, feeding rate, methane emission or fleece weight

(Kittelmann et al., 2014; de Barbieri et al., 2015; Ellison et al., 2017; Etancelin et al., 2018)

Few heritabilities estimates in sheep :

h^2 of CA 1^{rst} axis ~0.24

(Rowe et al., 2015)

Animals under study

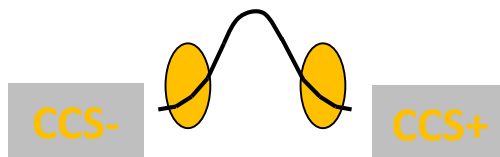
At INRA La Fage experimental farm

- ✓ 369 dairy Lacaune ewes (Roquefort cheese)
- ✓ Adult ewes (2nd lactation or more)
- ✓ Sampling at 3 months after lambing
- ✓ 4 days of sampling

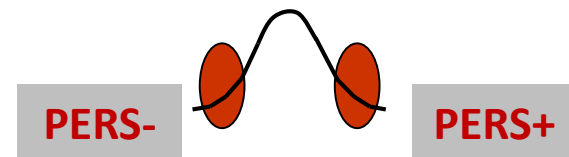


Flock genetically organized in 4 divergent lines

Somatic Cell Score

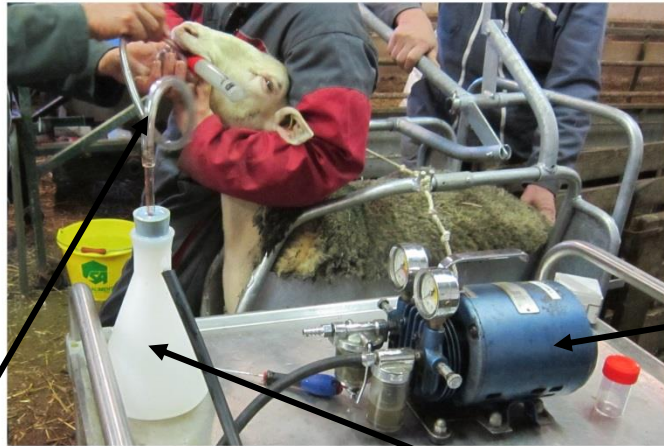


Milk persistency



Exploration of rumen microbiota

Expe. Agreement : APAFIS#6292-2016080214271984



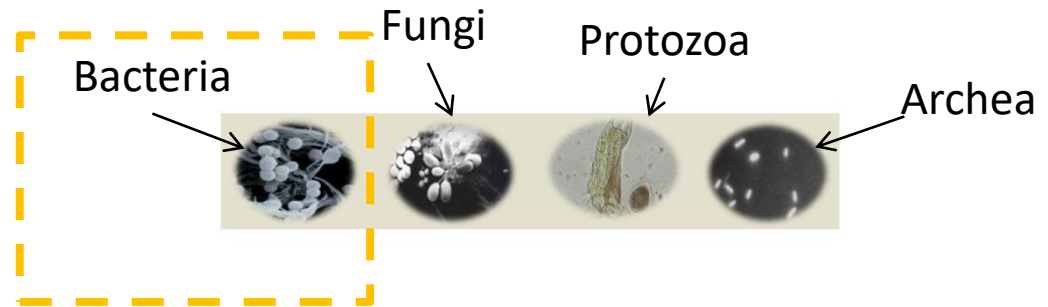
Rumen fluid sampling

Vacuum pump

Gastroesophageal tube

Erlenmeyer to collect rumen fluid

Rumen fluid microbiota composition



Bacterial microbiota quantification



Sequencing using 16s rRNA gene
(Illumina Miseq technology at Genopole, Toulouse, France)



Bioinformatics analysis of the microbiota sequences with FROGS software (Escudié et al., 2018)

8,267,982 sequences i.e. ~ 22,000 sequences / sample



- Removal of faulty sequences
- Swarm clustering with $d=3$ (Mahé et al., 2014)
 - Chimera removal
- Filters (abundances $> 5 \cdot 10^{-5}$)

4,944,307 sequences / 2,135 OTUs

- Affiliation with Silva 123

i.e. phyla 100% / classes 99,7% / orders 99.7% / families 99.4% / genera 84.5 %

Data analysis



- **Relative abundances** at the genus level
for each sample, sequences nb of genus X / total nb of sequences

- **Biodiversity estimates** (with R - phyloseq package)
 α -diversity of genera : Richness / Shannon entropy / Inv. Simpson

//
diversity
within a community

↙
Number of
observed genera

↓
Evenness of the
genera abundance
distribution

↘
Inverse probability that
two sequences sampled
at random come from the
same genera

Data analysis

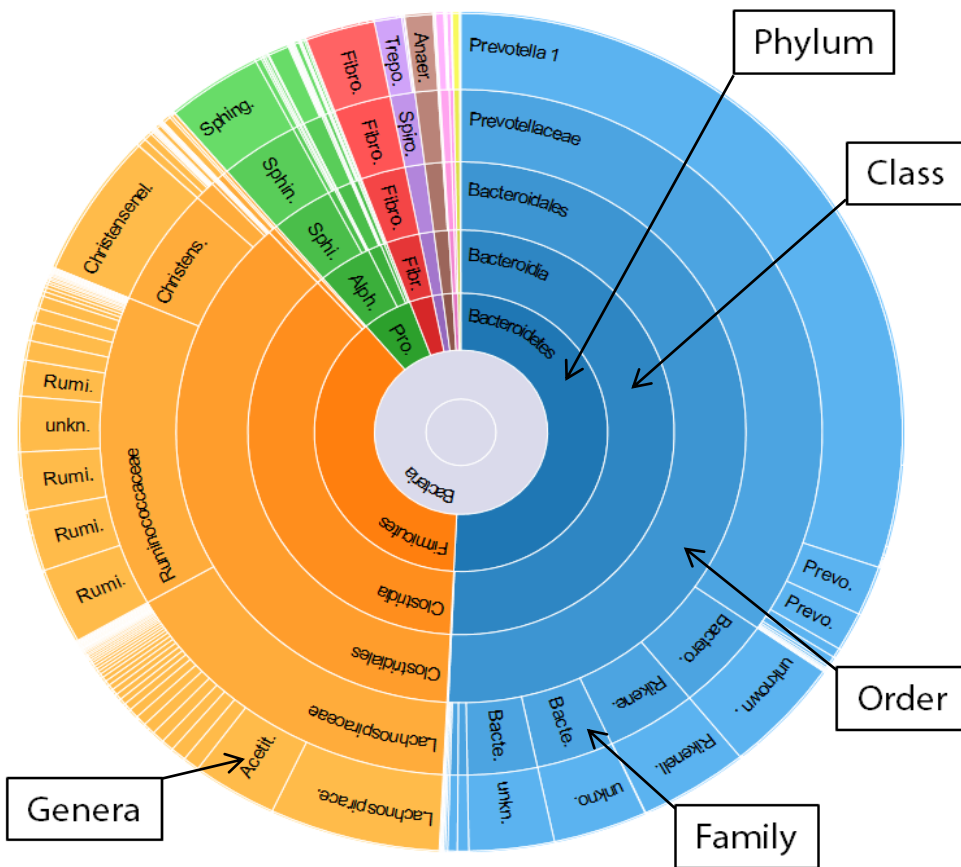
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Genera : Richness / Shannon entropy / Inv. Simpson

- 1) **ANOVA analysis** (with SAS - GLM proc.)
on square root of relative abundances and α -diversity indexes
Fixed effects:
Lines (4) Ewes parity (3) Sampling day (4)

- 2) **Heritabilities estimates** (with VCE software)
on single trait analysis of each relative abundances of genus and
 α -diversity indexes

Bacteria taxa distribution in rumen



| | |
|--------|-----|
| Phylum | 9 |
| Class | 17 |
| Order | 31 |
| Family | 50 |
| Genera | 139 |

Nb of different taxa

α-div.

Richness

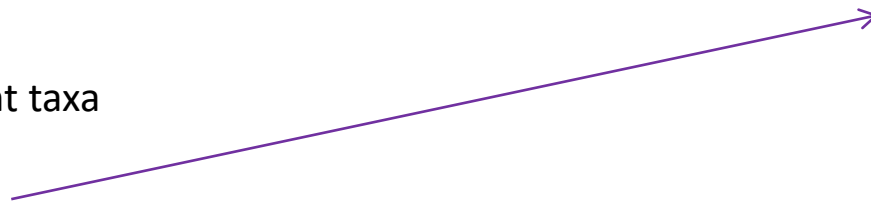
Shannon

Inv Simpson

Mean

Std

| | | |
|-------------|-------|------|
| Richness | 1432 | 262 |
| Shannon | 6.18 | 0.72 |
| Inv Simpson | 206.0 | 67.0 |



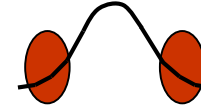
Differences between lines

Only genus taxa
and diversity criteria
with significant differences
are listed above

Somatic Cell Score



Milk persistency



P-value

CCS-

CCS+

PERS-

PERS+

Bacteria genera

Alpha diversity

Differences between lines

Only genera taxa
and diversity criteria
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Somatic Cell Score



| | P-value | CCS- | CCS+ |
|----------------------------|---------|-----------------|-----------------|
| Bacteria genera | | | |
| Alloprevotella | ** | | 0.014 |
| Coprococcus 1 | ** | | 0.122 |
| Olsenella | ** | 0.255 b | 0.331 a |
| Prevotella 1 | ** | 28.998 b | 33.039 a |
| Prevotellaceae Ga6A1 gr | *** | 0.067 b | 0.107 a |
| Succinivibrionaceae UCG002 | ** | | 0.646 |
| Syntrophococcus | * | 0.068 b | 0.099 a |
| Alpha diversity | | | |
| Shannon | 7% | 6.090 b | 6.392 a |

More Prevotella
in CCS+ line
...
linked to
udder inflammation ?

Little more evenness
in CCS+ line ...

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Differences between lines

Only genera taxa and diversity criteria with significant differences are listed above

| | P-value |
|----------------------------|---------|
| Bacteria genera | |
| Alloprevotella | ** |
| Coprococcus 1 | ** |
| Olsenella | ** |
| Prevotella 1 | ** |
| Prevotellaceae Ga6A1 gr | *** |
| Succinivibrionaceae UCG002 | ** |
| Syntrophococcus | * |
| Alpha diversity | |
| Shannon | 7% |

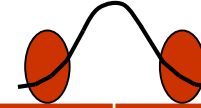
More bacteria with sugar lytic properties

in PERS+ line

....

better energy efficiency ?

Milk persistency



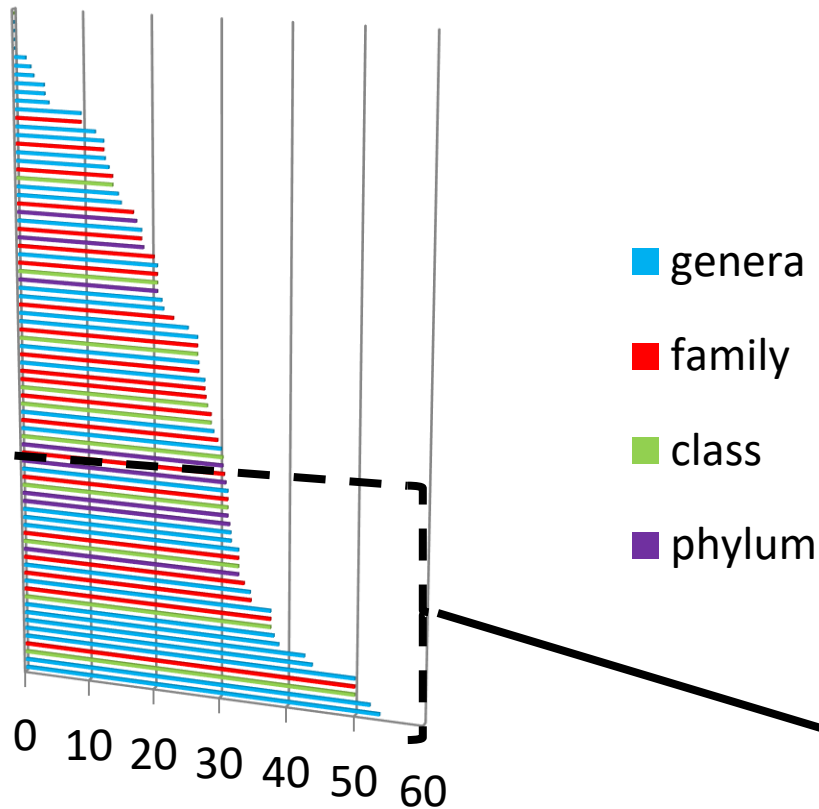
| PERS- | PERS+ |
|---------|----------|
| 0.007 b | 0.017 a |
| 0.141 a | 0.110 b |
| 0.294 a | 0.246 b |
| 29.507 | |
| 0.071 | |
| 0.294 b | 0.484 a |
| 0.062 c | 0.083 ab |
| 6.135 | |

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Repeatabilities' distribution

according to the taxa level

N=120 ewes at 1 week



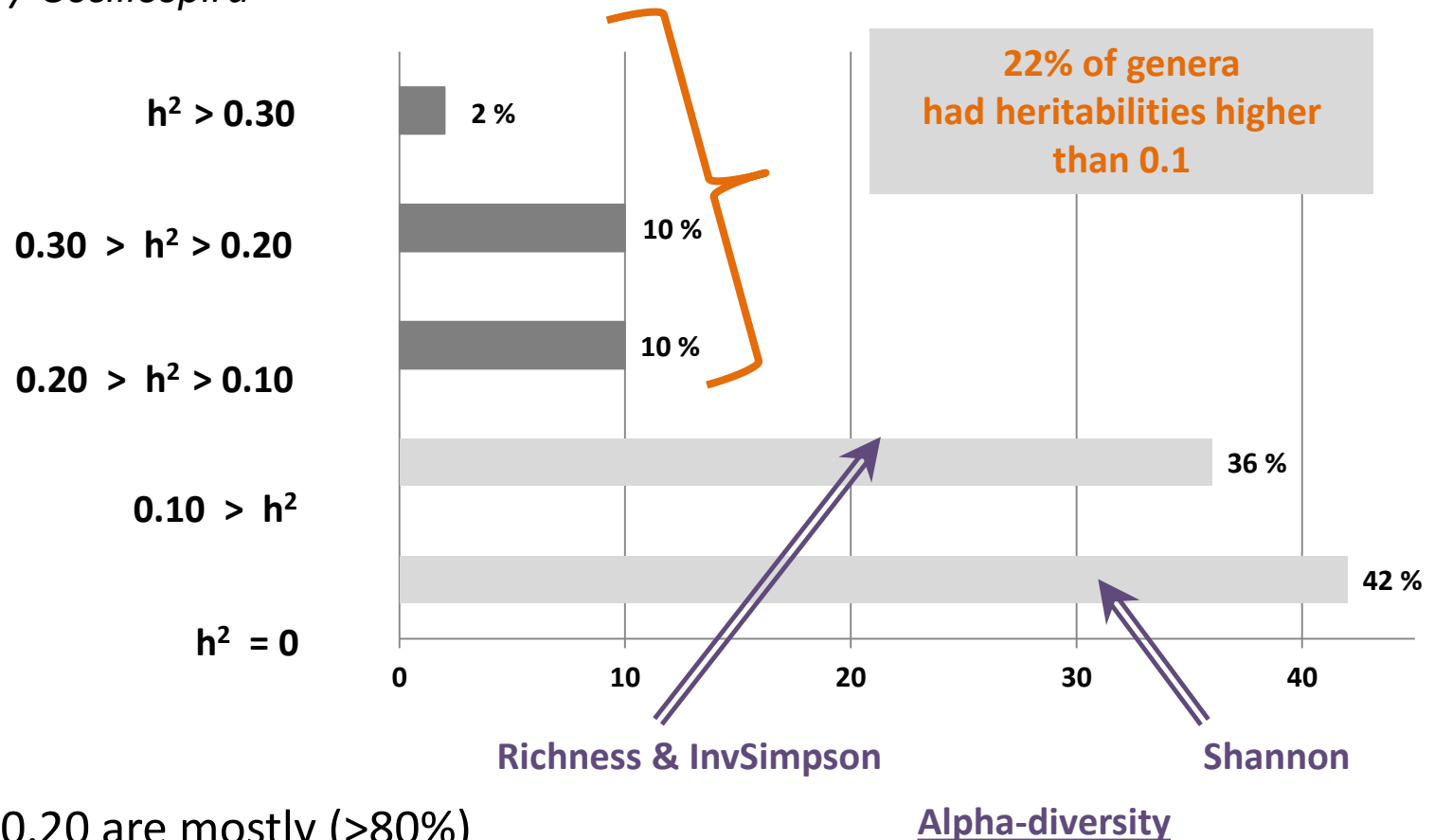
no clear classification
of repeatability values according
to the level of affiliation
(genera/family/class/phylum)

40% of taxa
have repeatabilities > 30%

Heritabilities' distribution of the genus abundances

Most heritable genera :

*RuminococcaceaeUCG002 / Lachnospira /
Atopobium / Oscillospira*



NB : $h^2 > 0.20$ are mostly (>80%)
obtained on genera taxa

Alpha-diversity

Take home message



Main bacteria in ewes' rumen belong to *Bacteroidetes* -51%- (mostly *Prevotella*) and *Firmicutes* -38%- (mostly *Laschnospiraceae*) phyla

H²

22% of bacteria genera have heritabilities of their abundancies higher than 0.10



Microbiome differences according to genetic lines:
ruminal Prevotella linked with udder inflammation?

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INRA Genetic Animal and Physiology departments
CRB-Anim
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INRA La Fage

Thank you for your attention

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