Genetic approach of rumen metagenome: state of the art in small ruminant and perspectives

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Rumen metagenome

Central role in the nutrition of the ruminant host

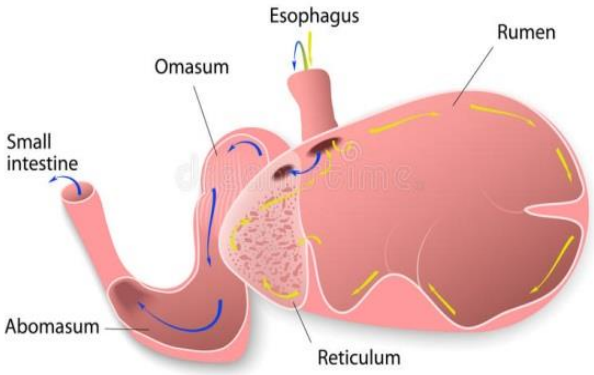
Bio-reactor

- Degradation of vegetable fibers (cellulose …),
- Degradation of non-structural carbohydrates (e.g. starch …),
- Fermentation of sugars,
- Bio-hydrogenation of unsaturated fatty acids,
- Production of short chain fatty acids, microbial proteins and vitamins,
- …

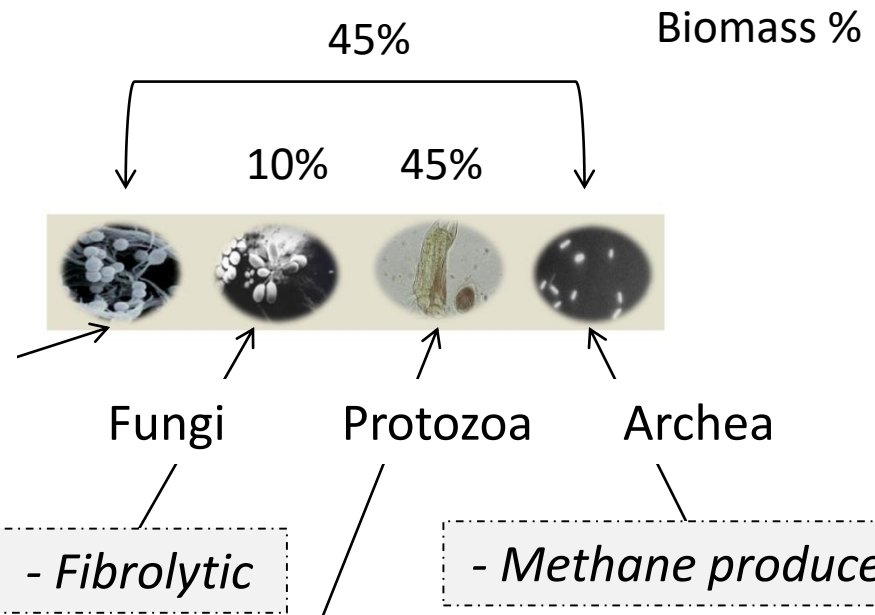
Affect animal production (meat, milk, health…)
Produces also undesirable by-products such as methane

Rumen specificity

Ruminant digestive tract

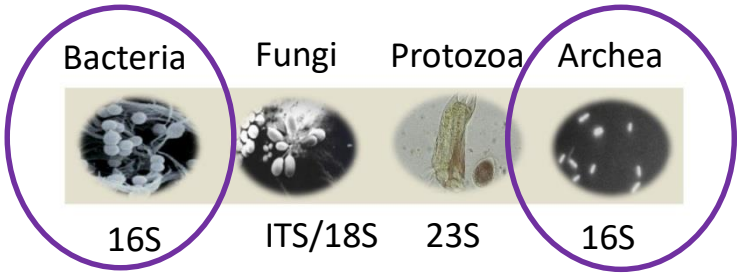


Rumen microbiota composition



How to characterize the rumen composition ?

- **Targeted rRNA sequencing:**



In sheep

→ determination of taxonomic abundance within bacteria/archea/fungi/protozoa after a blast on specific databases (☹ need long read sequences)

- **Whole genome shotgun sequencing (WGS):**

→ determination of abundance of total genes in the rumen (☹ including host or feed genome)

- **Genotyping by sequencing (GBS)** i.e. digestion of genomic DNA by restriction enzymes:

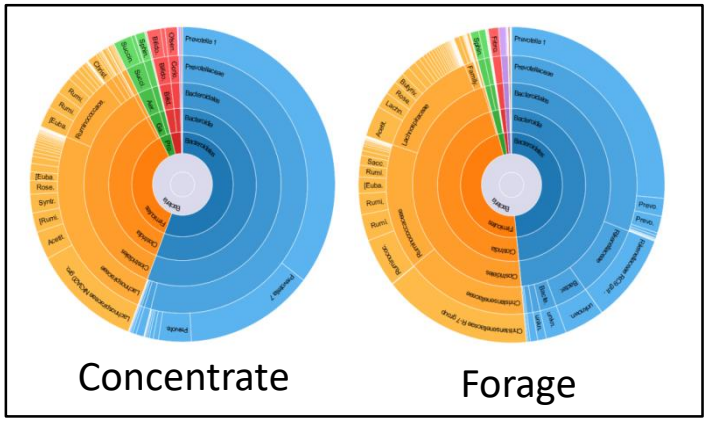
→ determination of taxonomic abundance for bacteria/archea/fungi/protozoa after a blast on Hungate1000 genomes database (☹ further evaluation needed ?)

Main factors of variation in the rumen composition

Feeding

➢ Lambs fed with concentrate or 66% hay mixed diet (Marie-Etancelin et al., 2018)

Among 228 OTUS identified, 221 were significantly different according to diet



More "bacteroidetes" abundances with C vs F

More fibrolytic bacteria with F
more amylolytic and lactolytic bacteria with C

➢ Fistulated lambs fed with 82% (/DM) of a wheat based concentrate to create a shift in RFA : t11-C18:1 ➔ t10-C18:1(*) (Meynadier et al., 2018)

➔ 2 types of animal's response :

* Animal A ➔
  ↗↗↗ t10-C18:1 (/ t11-C18:1) strongly linked (r=0.83) with
  ↗↗↗ % *Porphyromonadaceae*

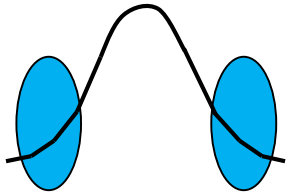
* Animal B ➔
  t10-C18:1 **remain <** t11-C18:1

Animal

(*) causing an alteration of fat quality in product (milk/meat)

How is sheep genetics taken into account ?

- Comparison of extreme animals
  (on phenotypic values, EBV , genetic divergent lines…)



- Genetic variability estimates of rumen microbiota

Until now, no publication in sheep on:

- GWAS on ruminal microbiota
- simultaneously host genomic and metagenomic contribution to variability of traits

Comparison of phenotypic extreme animals

- Selection of meat type wethers for extreme RFI phenotypes (Ellison et al., 2017). Interaction with feed type (concentrate vs forage-based pellet)

Groups comparison for relative abundances of 349 OTUs :

44 OTUs significantly different according to **Diet**

Greater abundance of « fiber degraders » bacteria and more diversity when lambs fed forage

11 OTUs significantly different according to **RFI**

Very little microbial species differences = difficult to determine which species contribute to feed efficiency

RESULTS DIFFICULT TO INTERPRET

Comparison of genetic extreme animals

for relative abundances of taxonomic classes/genus

➢ Selection of Merino wethers for fleece weight **EBV** (Barbieri et al., 2015)

➢ Selection of Romane lambs for feeding speed **EBV** (Marie-Etancelin et al., 2018)

Very few taxa significantly different (p>0.05) according to EBV

Wool Index

	WG+	**WG–**
Bacteroidia	71.9[a]	66.4[b]
Clostridia	26.4[b]	31.4[a]

Differences in 2 bacteria classes but no link with physiological or ruminal parameters

Feeding speed Index

	FS+	**FS-**
Syntrophococcus	1.44[b]	2.86[a]
Lachospiraceae NC2004	0.02[b]	0.10[a]
Ruminiclostridium 9	0.01[a]	0.00[b]

High FS had more *Ruminiclostridium* (cellulolytic) and less *Syntrophococcus*

RESULTS DIFFICULT TO INTERPRET

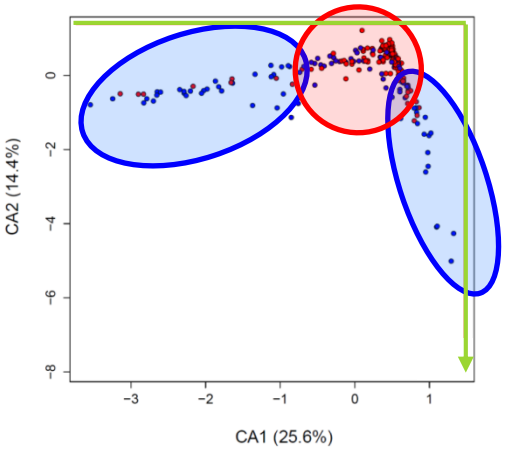
Comparison of genetic lines

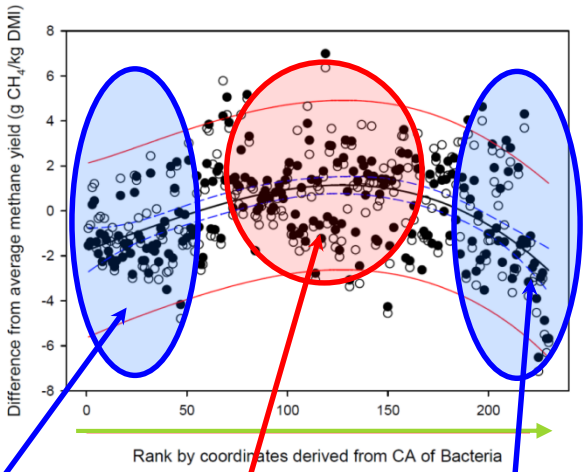
➢ From low and high methane emission*[1] NZ sheep (Kittelmann et al., 2014) :

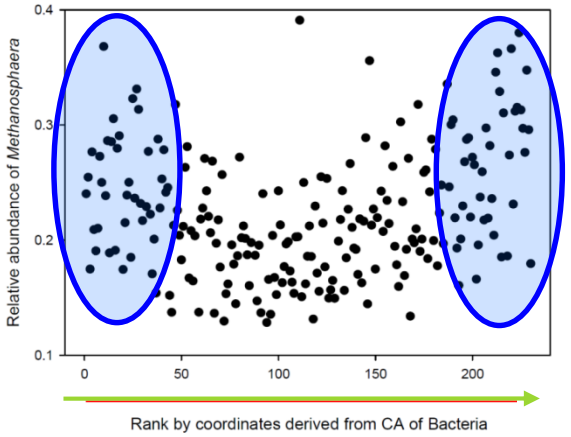
With a correspondence analysis of bacteria and archea,

2 "ruminotypes" linked to CH_4–

1 "ruminotype" linked to CH_4+







Lsmeans of *Quinella ovalis* : *32% v.s. 0.4-1.6%*

Lsmeans of *Sharpea azabuensis* *12% v.s. 1-1.7%*

No differences in densities of methanogenic archea between CH_4 lines but a higher relative abundances of *Methanosphaera*

More *Prevotella, Bacteroidales, Lachospiraceae, …*

*[1] g CH_4/ kg feed

Comparison of genetic lines

- Selection of Lacaune ewes for Somatic Cell Count (Marie-Etancelin et al., present congress)

From 139 genera taxa of bacteria

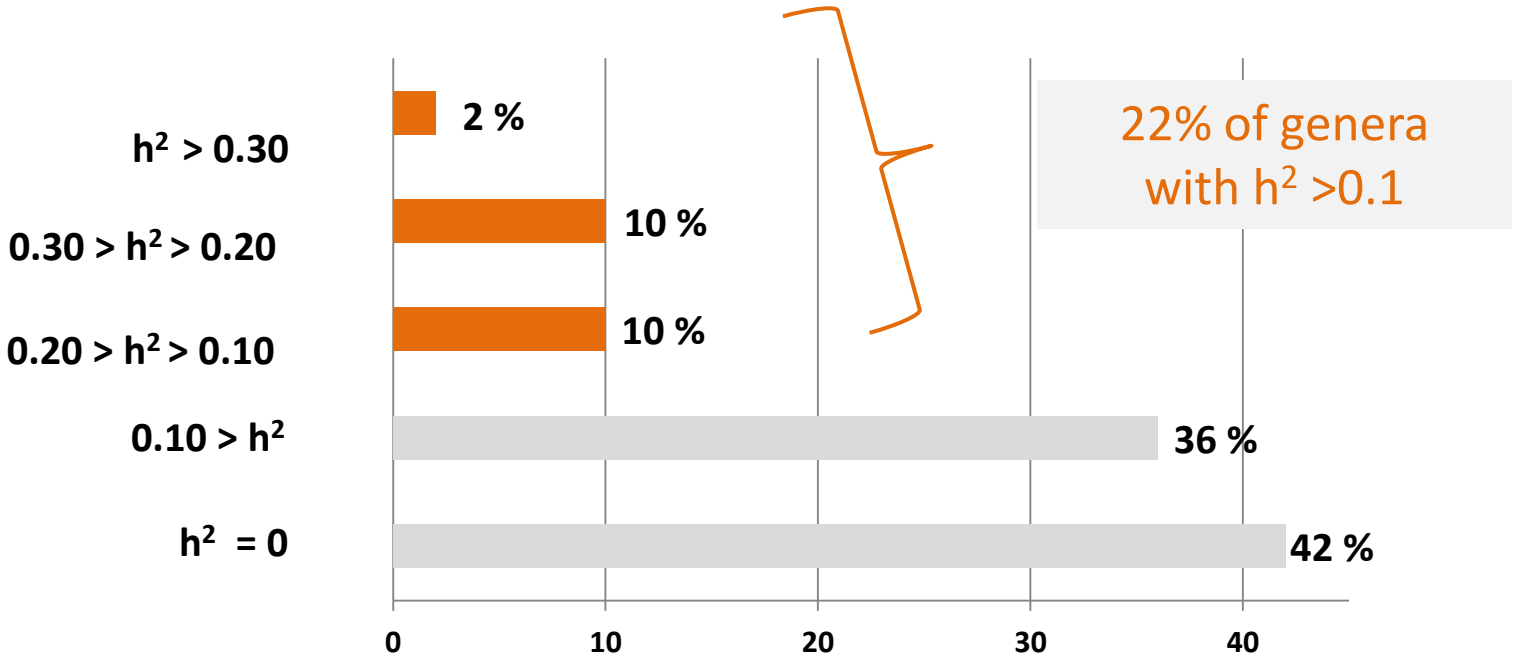
	P-value	CCS-	CCS+
Olsenella	>0.01	**0.255^{b}**	**0.331^{a}**
Prevotella 1	**>0.01**	**28.998^{b}**	**33.039^{a}**
Prevotellaceae Ga6A1 gr	>0.001	**0.067^{b}**	**0.107^{a}**
Syntrophococcus	>0.05	**0.068^{b}**	**0.099^{a}**

In human and mouse
links are made between an increase of Prevotella abundances and inflammatory diseases

Genetic parameters

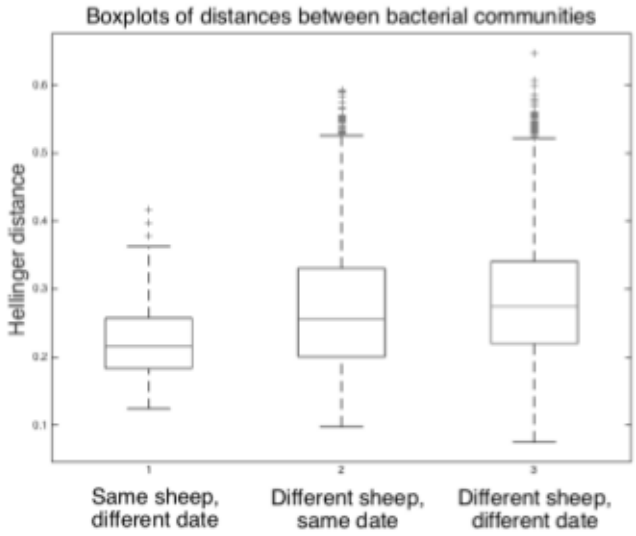
- On dairy Lacaune ewes (Marie-Etancelin et al., 2018)

Heritabiliy (n=369 ewes) on 139 genus rumen taxa



Genetic parameters

➢ On the same low and high methane emission NZ sheep (Rowe et al., 2015) :



Profiles obtain from
the same sheep at different times
were more similar
than different sheep at same date

		h^2
Correspondence analysis axis with moderate heritability	CA1	0.24 ± 0.12
	CA2	0.13 ± 0.09
	CH_4d	0.38 ± 0.18
	CH_4/DMId	0.42 ± 0.13

r_g	CA1	CA2
CH_4d	0.58 ± 0.42	0.77 ± 0.44
CH_4/DMId	0.06 ± 0.32	0.90 ± 0.35

In axis 2, 80% of the variation of methane yield could be explain by rumen microbial community

Genetic parameters using GBS/RE-RRS

- On the same low and high methane emission NZ sheep:
  - Reference-based (R): BLAST against Hungate 1000 Catalogue and assign to genus
  - Reference-free (RF): Count the number of times a set of common 65 bp tags appears

CA1	Heritability	Repeatability	$r_g(CH_4/DMI)$
16S	0.26 ± 0.23	0.45 ± 0.08	0.65 ± 0.47
ApeKI_R	0.62 ± 0.06	0.62 ± 0.06	0.59 ± 0.32
PstI_R	0.62 ± 0.06	0.62 ± 0.06	DNC
ApeKI_RF	0.07 ± 0.19	0.44 ± 0.07	DNC
PstI_RF	0.23 ± 0.26	0.62 ± 0.06	0.83 ± 0.31

Did Not Converge

≥ 16S

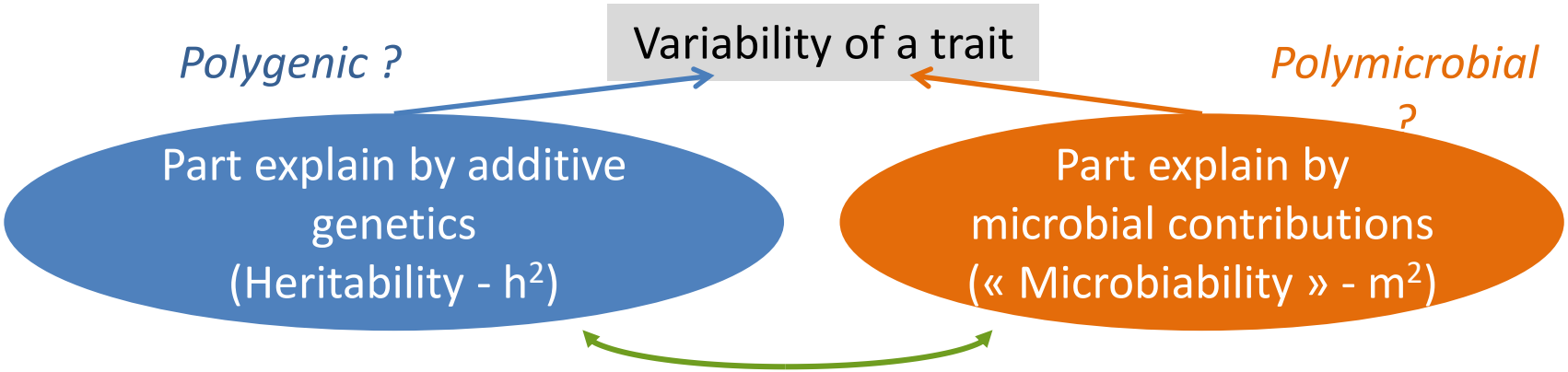
Best repeatability and correlation with methane yield

GBS/RE-RRS is an appropriate method to obtain information on the rumen microbial community

Some ideas to be developed ?

Camarinha-silva et al. (2017) in pig
Difford et al. (2018) in cattle

"compare the % of variance of a trait explain
either by the genetic additive or the microbiota"



But microbiota is partly under the control of host genetics…

→ for CH_4 : h^2 (0.18) ~ m^2 (0.15) (Difford et al.)
→ for ADG : h^2 (0.42) >> m^2 (0.28)
but for FCR h^2 (0.11)~< m^2 (0.16)

} (Camarinha-silva et al.)

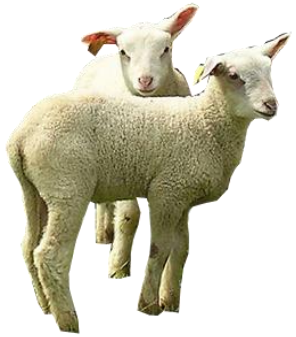
To conclude

➢

- **Host (genetic) selection**
- **Adequate feeding (feed additive ?)**

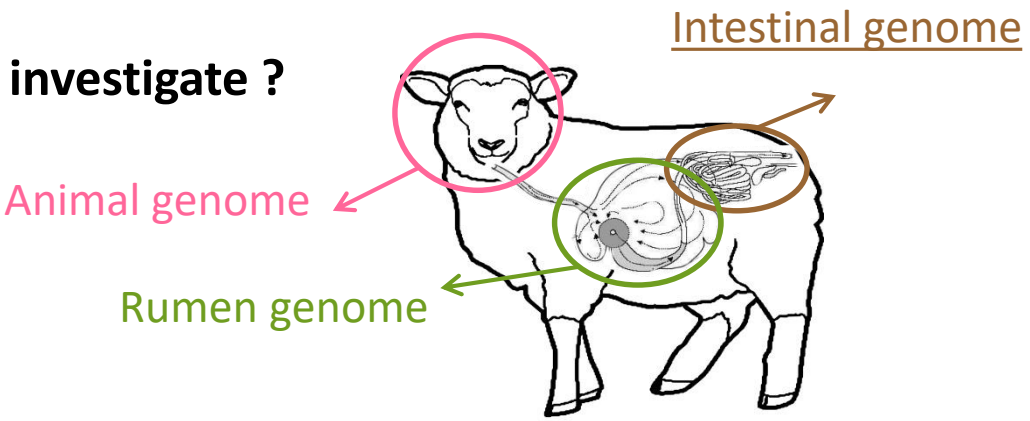
For an optimal microbiota

For a healthy



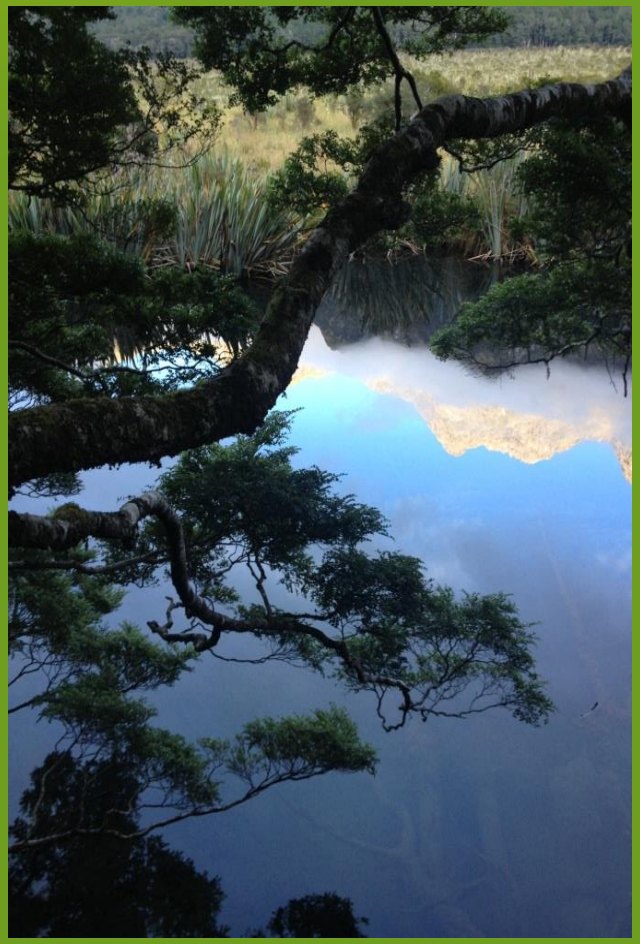
and efficient sheep

➢ **In ruminants, 3 genomes to investigate ?**



Thank you for your attention !

Pyrenean mountain (FR)



Mirror lake in Fiordland (NZ)