

Microbiome as a proxy of methane. Case of Spain



Oscar González-Recio



Why proxies?

All current methane-based traits **are** proxies but...

Measuring methane implies:

- -Specialized equipment, with specific training
- -High cost (labor and equipments)
- -Time consuming
- -Very challenging in pasture based or farms w/o AMS

Can we found other proxies, that complement methane-based traits?

Yield traits, BW, MIR, FI, Microbiome, ...





Strategy with proxies

Create an even larger reference population that can be used for genomic selection.

The proxy needs to be:

- 1. easier to measure
- 1. genetically correlated to methane
- 1. higher heritable









ROLE OF MICROBIOME IN

- Feed digestion
- Feed efficiency (DMI)
- Production traits



ROLE OF MICROBIOME IN

· Methane emissions





ROLE OF MICROBIOME IN

- Overall health
- Dysbiosis
- Pathogenes

ROLE OF HOST

- Symbiosis
- Microbiome composition
- Heritability, and genetic correlation



METHANE & GENETICS

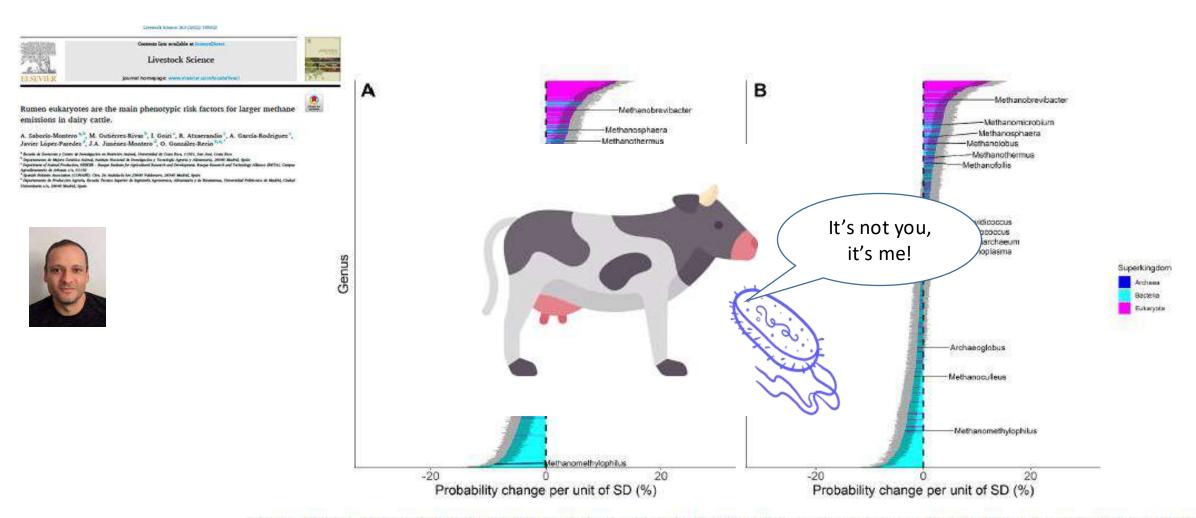


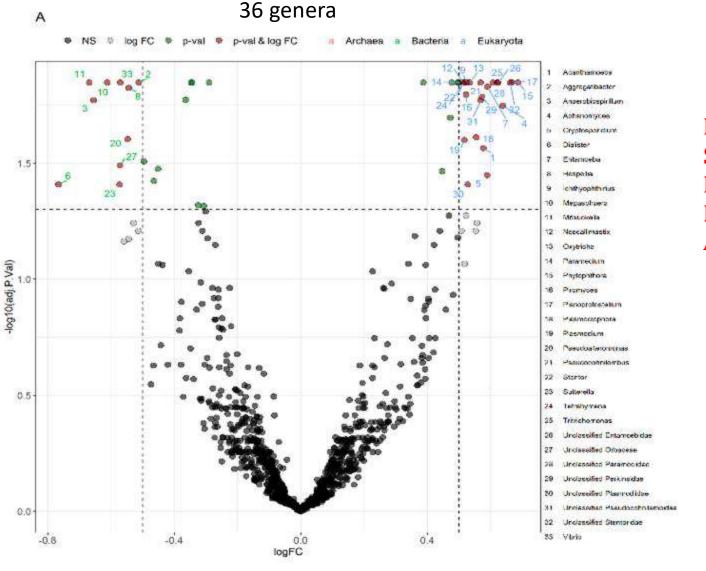
Fig. 3. Change in the probability of being classified in the upper quartile for (A) methane concentration (ppm CH₄) and (B) methane intensity (ppm CH₄/kg milk) per unit of standard deviation for relative abundance (%) of 1240 genera colored by superkingdom. Black dashed line indicates the baseline probability of being classified in the upper quartiles without any genus effect. All the archaea genera are explicitly indicated. Probability intervals based on posterior standard deviations are depicted in gray for all genera.



Differential abundance. Taxonomy associated with CH4

Betaproteobacteria (Sutterella)

Clostridiales (Hespellia)



Parameciidae Stentoridae Pseudocohnilembidae Fungi Amoebozoa





Differential abundance. Functionality associated with methane

Volatile fatty acid metabolism

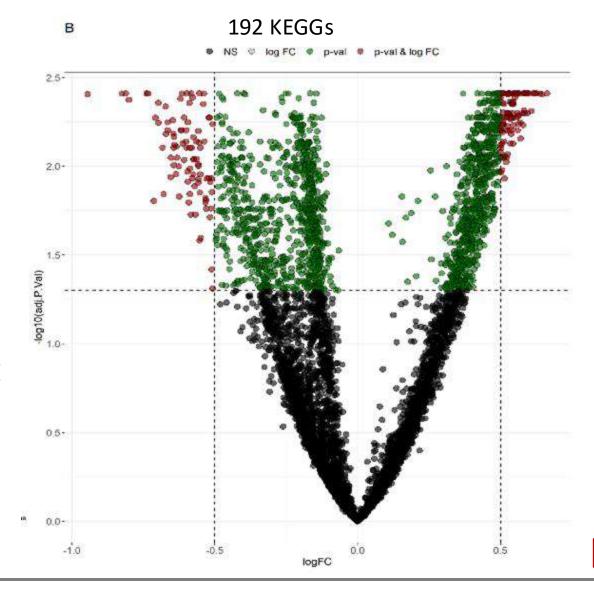
(K00209 enoyl-[acyl-carrier protein] reductase, K01902 succinyl-CoA synthetase alpha subunit, K01682 aconitate hydratase 2), K09251).

N metabolism

(K00370 and K00371 nitrate reductase subunits, K03885 oxidative phosphorylation, NADH dehydrogenase)

Carbohydrate, lipid or vitamin metabolism pathways.

Bacteria



Glutamate--cysteine ligase catalytic subunit (K11204)

Inositol-phosphate metabolism (K00889, K01110, K18082 and K20279)

Starch and sucrose metabolism (K01203)

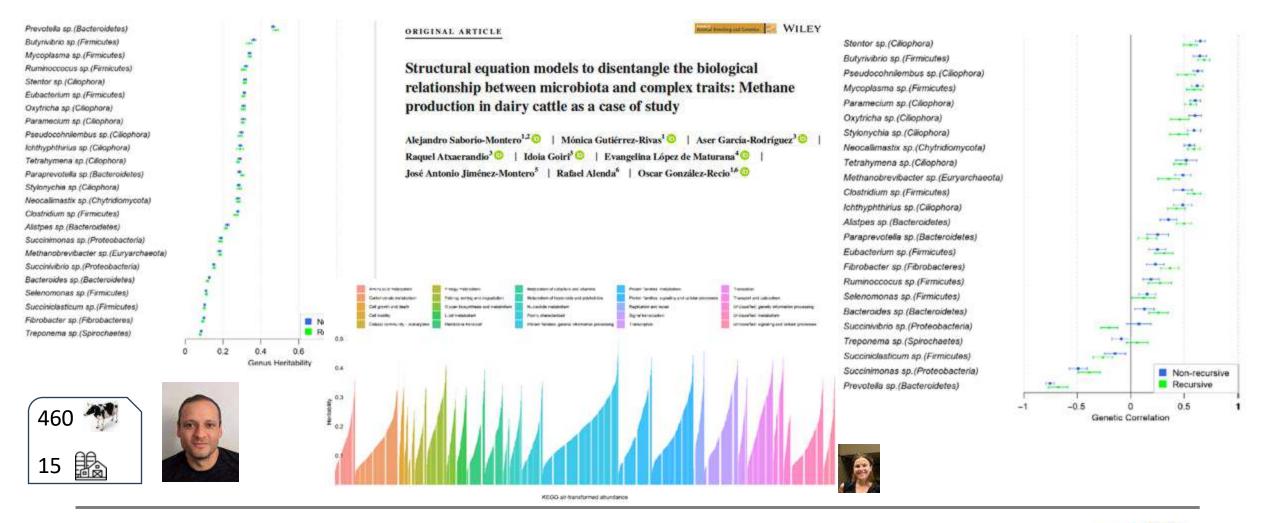
Lipid metabolism



Eukaryota

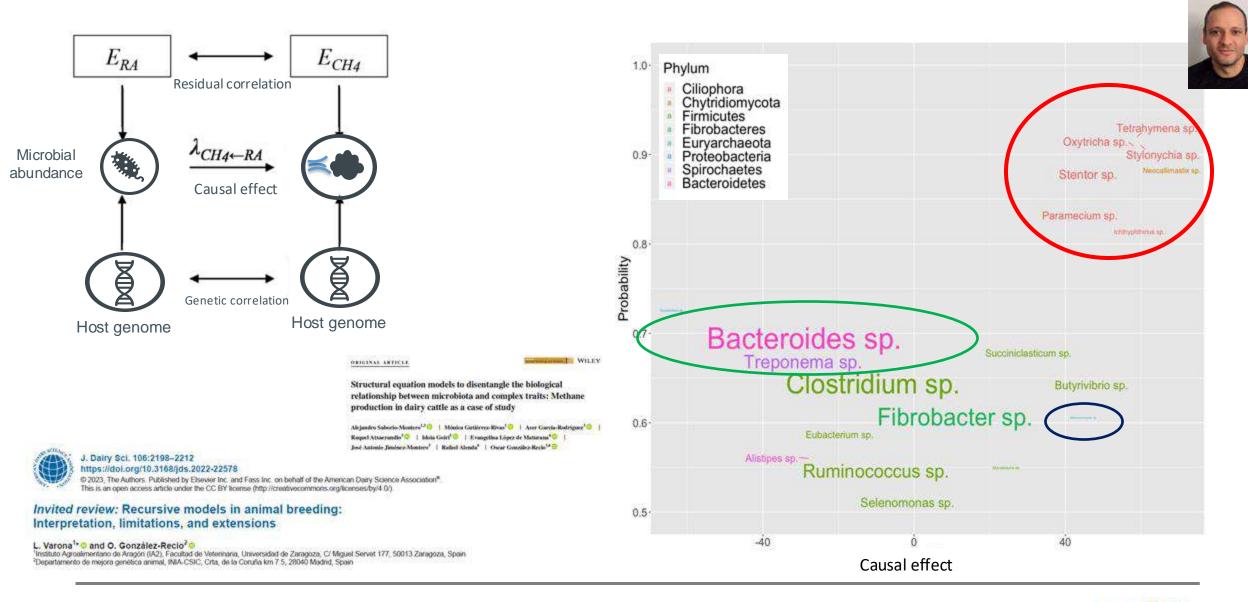


Microbiota composition is heritable and genetically correlated to methane emissions (mainly fungi and protozoa)



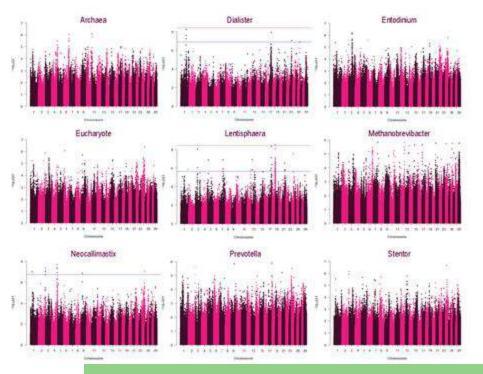


SEM capture causal effect of microbes on methane production



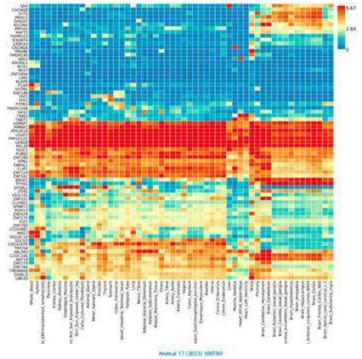


Genome-wide association analyses



GENES ASSOCIATED TO MICROBIOME COMPOSITION

- Expressed in brain (hypothalamus, basal ganglia, amigdala, hypocampus)
 - Satiety, appetite, intake levels
- Expressed in salivary glands
 - Rumen buffer



Contents lists available at ScienceDirect

Animal The international journal of animal biosciences



Review: Diving into the cow hologenome to reduce methane emissions and increase sustainability



Oscar Gonzalez-Recio **, Natalia Scrobota **, Javier López-Paredes *, Alejandro Saborio-Montero **, Almudena Fernández *. Evangelina López de Maturana b.f., Beatriz Villanueva *. Idoia Goiri *. Raquel Atxaerandio 1, Aser García-Rodríguez 11









scientific reports



OPEN

Rumen and lower gut microbiomes relationship with feed efficiency and production traits throughout the lactation of Holstein dairy cows

Hugo F. Monteiro¹, Ziyao Zhou², Marilia S. Gomes³, Phillip M. G. Peixoto⁴, Erika C. R. Bonsaglia¹, Igor F. Canisso³, Bart C. Weimer¹ & Fabio S. Lima¹⁰¹

Proc. Assoc. Advmt. Breed. Genet. 21: 89-92

PREDICTION OF RESIDUAL FEED INTAKE FROM GENOME AND METAGENOME PROFILES IN FIRST LACTATION HOLSTEIN-FRIESIAN DAIRY CATTLE

M. Wang1, J. E. Pryce1.2, Keith Savin1 and B. J. Hayes1.2

¹Biosciences Research Division, Department of Economic Development, Agribio, 5 Ring Road, Bundoora, VIC 3086, Australia ²La Trobe University, Bundoora, VIC 3086, Australia



OPEN

Whole rumen metagenome sequencing allows classifying and predicting feed efficiency and intake levels in cattle

Beatriz Delgado 31, Alex Bach 21, Isabel Guasch 4, Carmen González , Guillermo Elcoso, Jennie E. Pryce & Oscar Gonzalez-Recio 11, 15



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The oral microbiome as a proxy for feed intake in dairy cattle

C. N. Marcos, 1,2 A. Bach, 3 M. Gutiérrez-Rivas, 2 and O. González-Recio 4 Departamento de Producción Agraria, ETSIAAB, Universidad Politécnica de Madrid, Ciudad Universidaria, 28040 Madrid, Spain 4 Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria – CSIC, 28040 Madrid, Spain 3 ICREA, 08007 Barcelona, Spain

*Blanca from the Pyrenees, Hostalets de Tost, 25795 Lleida, Spain



SCIENTIFIC REPORTS

Previous results showed interesting predictive accuracy for Feed Efficiency and Dry Matter Intake using rumen microbiome

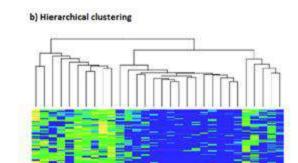


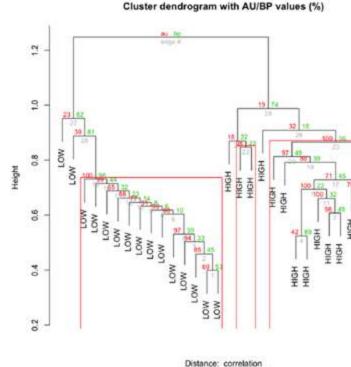
Received: 4 June 2018 Acopsed: 36 November 2018 Published online: 09 January 2019 Whole rumen metagenome sequencing allows classifying and predicting feed efficiency and intake levels in cattle

Beatriz Delgado ()³, Alex Bach ()^{2,3}, Isabel Guasch ()⁴, Carmen González ⁹, Gui Fermo Ekoso ⁴, Jennie E. Pryce⁴ & Oscar González Recio ()^{4,4}

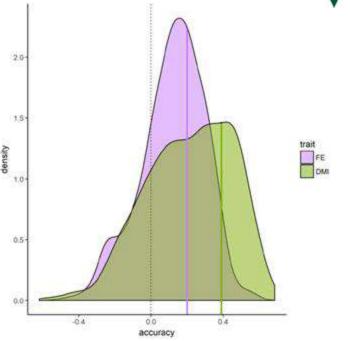








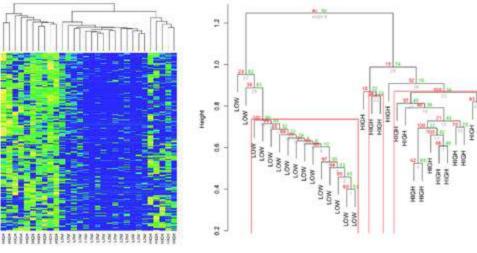
Cluster method: average



b) Hierarchical clustering

Cluster dendrogram with AU/BP values (%)

Distance: correlation Cluster method: average



SCIENTIFIC REPORTS

Corrected: Author Correction



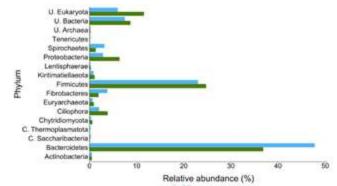


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⁴Blanca from the Pyrenees, Hostalets de Tost, 25795 Lleida, Spain



Predictive accuracy for DMI



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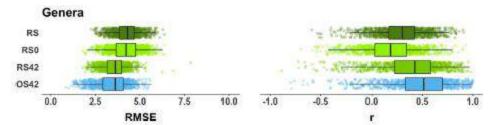


Figure 5. Estimated root mean square error (RMSE; kg of DM/d) and Pearson correlation (r) between the observed and estimated feed intake from out-of-bag validation. The dots are point bootstrapped estimates. The horizontal line displays the confidence interval around the median (first quartile = 1.5 × interquartile range [IQR] and third quartile + 1.5 × IQR). The box is drawn from first to third quartiles with the vertical line denoting

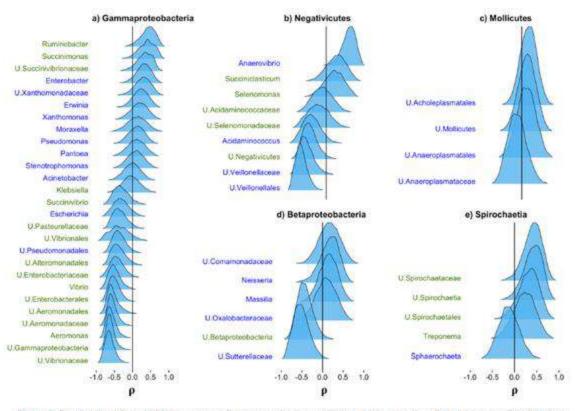
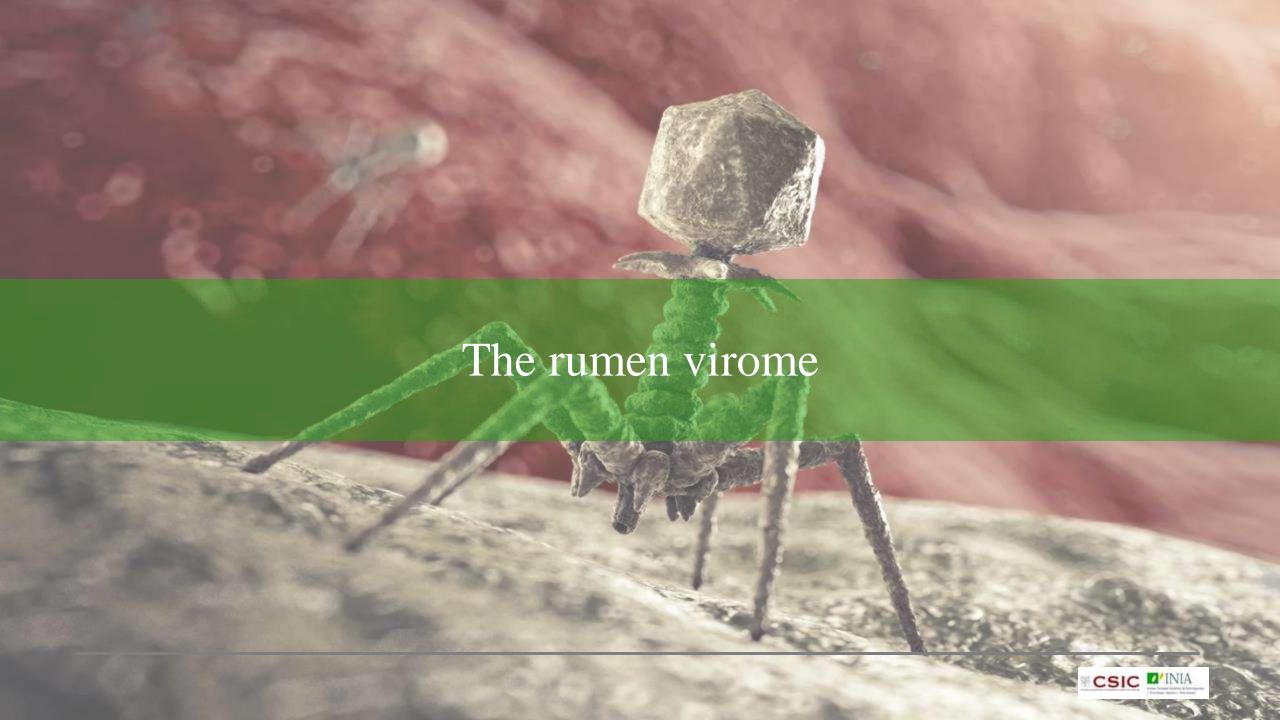


Figure 7. Density plots of the 10,000 bootstrapped Spearman correlation coefficients (p) between the relative abundance (RA; %) of (a) Gammaroteobacteria, (b) Negativicutes, (c) Mollicutes, (d) Betaproteobacteria, and (e) Spirochaetia and feed intake (kg of DM/d) in oral samples sampled after 42 d. Taxonomical features; names in green were found to be overabundant in rumen samples compared with OS42 and names in blue were found to be overabundant in OS42 compared with rumen samples. Density plots are sorted by the average \(\rho \) value in OS42 in increasing order. U. = unclassified





Important fraction of rumen ecosystem

10⁷-10⁹ particles/mL (10x number of bacteria in the rumen fluid; Ritchie et al., 1970)

Virus

- Infect bacteria
- Transfer MGE (including AMR)

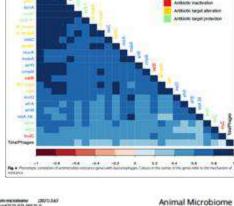
But still much uncertainty about its relevance

Rumen virome: an assessment of viral communities and their functions in the rumen of an Indian buffalo

Nidhi R. Parmar, Subhash J. Jakhesara, Amitbikram Mohapatra and Chaitanya G. Joshi*

Department of Animal Biotechnology, College of Veterinary Science and Animal Husbandry, Anand Agricultural University, Anand 380 001, India



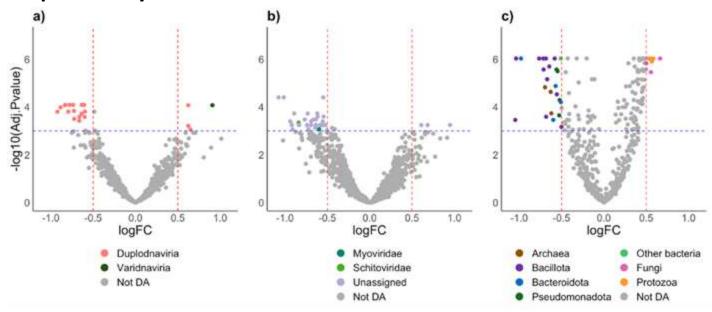


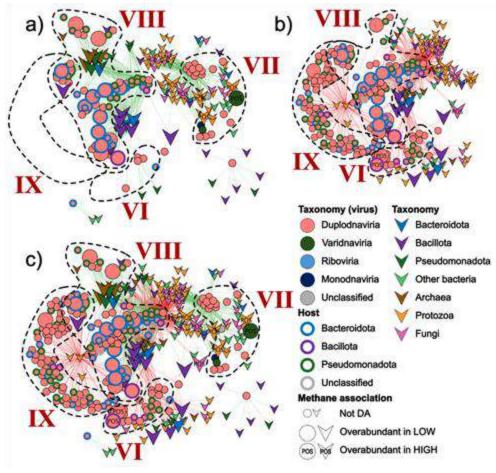




• A comprehensive exploration of the rumen virome unveiled a noteworthy population of phages and megaviruses

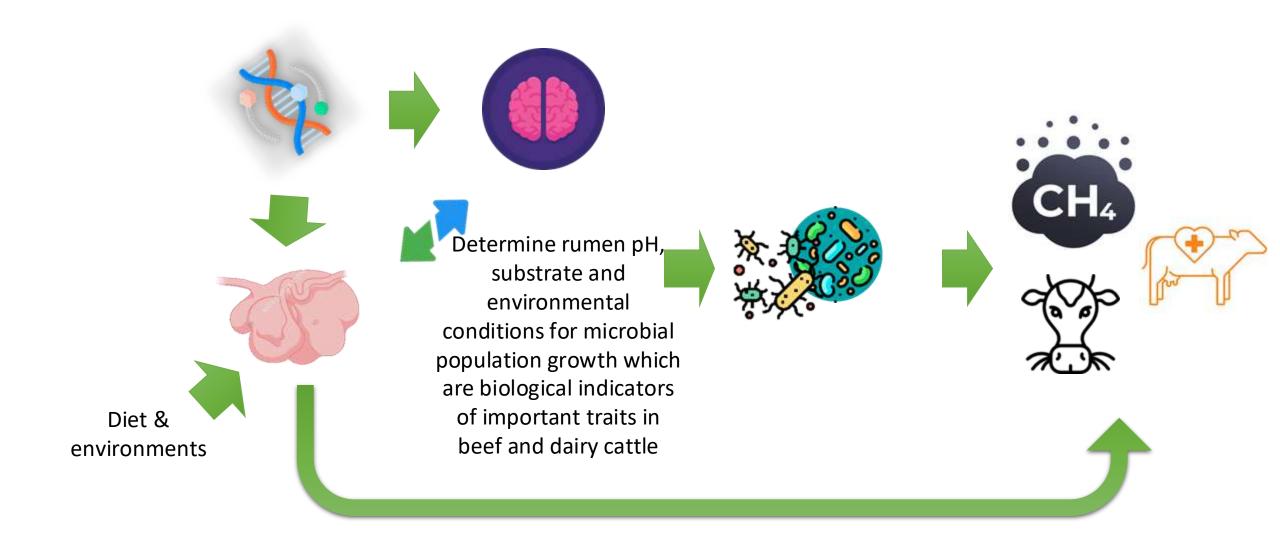
potentially associated with methane emissions.











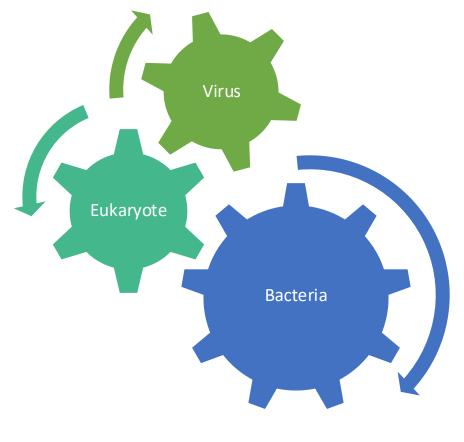
Rumen microbiome community

How to deal statistically in animal breeding?

Large dimensionality, redundancy and interplay

Including microbiome information in a multi-trait genomic evaluation: a case study on longitudinal growth performance in beef cattle

Marina Martinez-Alvaro^{1,27} G., Jennifer Mattock², Öscar Gonzälez-Recio³, Alejandro Saborio-Montero⁴, Ziqing Weng³, Joana Lima², Carol-Anne Duthie², Richard Dewhurst², Matthew A. Cleveland⁶, Mick Watson² and Rainer Roehe²⁷ Selection of features
based on genetic
correlations with traits
of interest



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https://doi.org/10.3188/ds.2020-20005
e.2021 American Day Science Associated* Published by Essever Inc. and Pass Inc. As optis Inserved.

A dimensional reduction approach to modulate the core ruminal microbiome associated with methane emissions via selective breeding

Alejandro Saborio-Montero, 10 Adrian López-Garcia, 10 Mónica Gutiérrez-Rivas, 10 Raquel Atxaerandio, 10 dois Goin, 10 Aser Garcia-Rodriguez, 10 José A. Jimienez-Montero, 10 Cristina Ovillo, 10 Fernando Puente-Sánohez, 10 Luis Varona, 11 Magdalena Serrano, 10 Cristina Ovillo, 10 and Oscar González-Recis¹⁷16

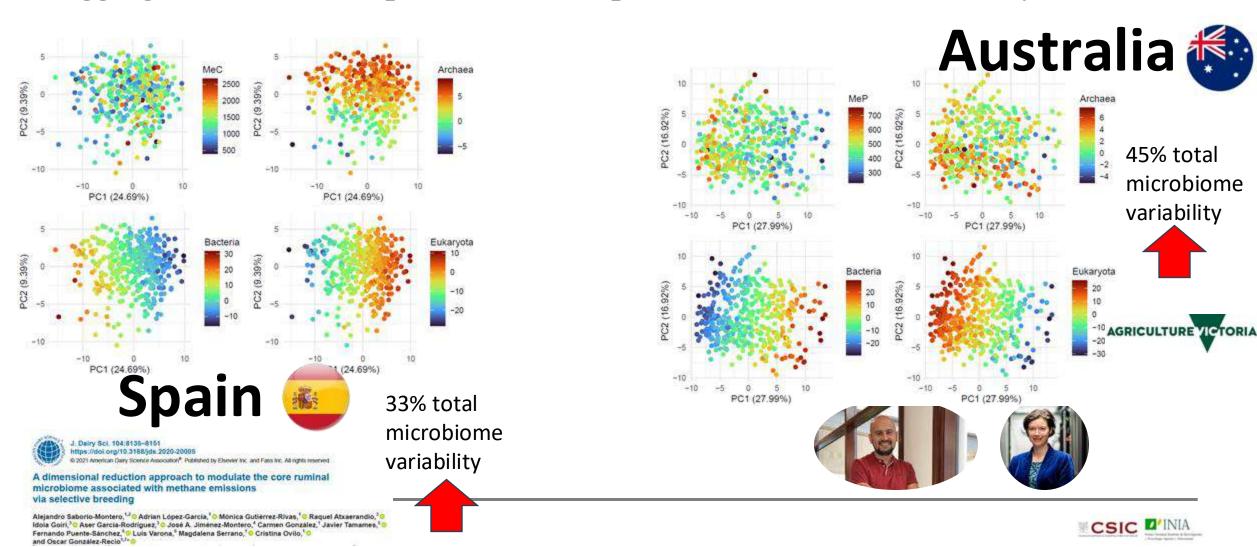
Aggregated variables with Principal Component Analysis

Heritability estimates between 0.20 and 0.30

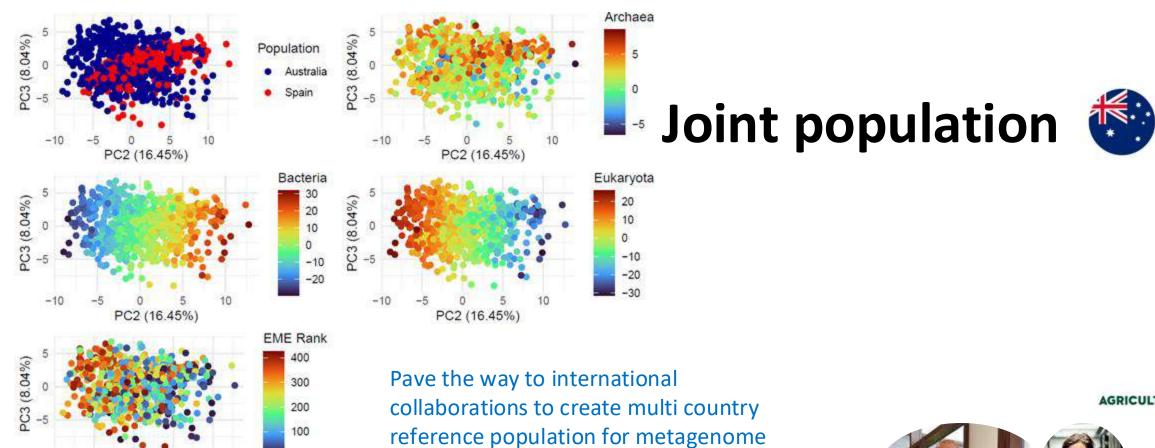


Similar microbiome composition and relationship with methane under 2 different production systems

Aggregated variables capture a relevant portion of microbial variability



Similar microbiome composition and relationship with methane under 2 different production systems



composition

PC2 (16.45%)



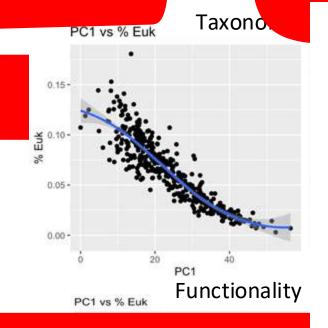


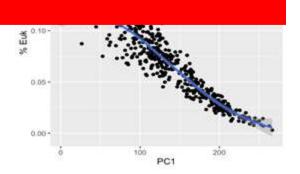
Agregated variables have a biological meaning

Eukaryotta Pelative abundance

• PC2 is related to

 $\frac{Proteobacteria}{Firmicutes + Bacteroidetes}$

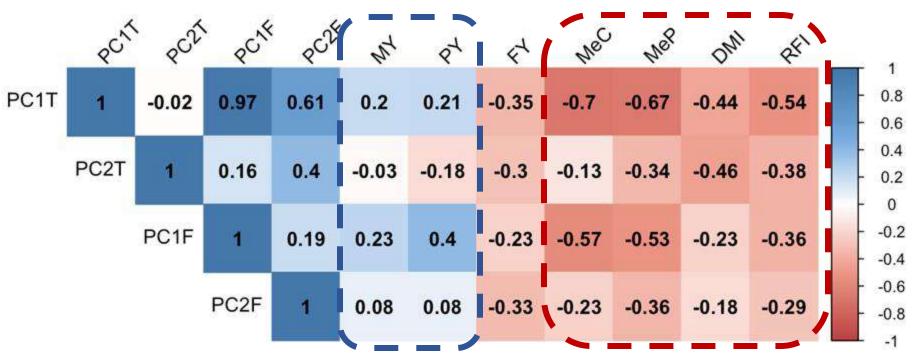


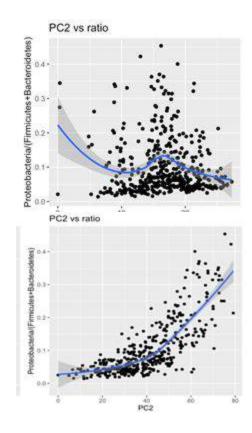




Rumen microbiome community

Genetic correlation between PC and other traits





- Aggregated microbial variables are favorably genetically correlated with milk, protein, methane emissions and feed efficiency (unfavorable with fat)
- Breeding for lower methane or feed efficiency may impact microbiota composition (dysbiosis)
- What are the expected consequences?

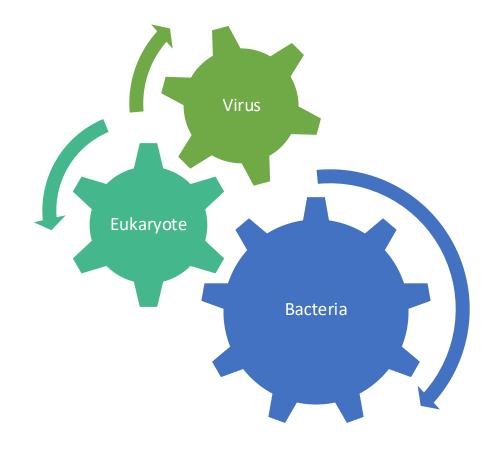


Objective

Large dimensionality, redundancy and interplay

Should the rumen microbiome composition be included as a trait of interest in dairy (and beef) cattle



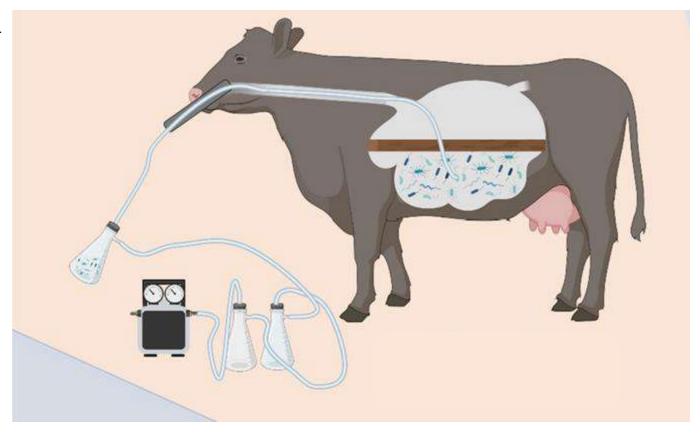




Preliminar considerations: Sample collection

- Needs proper training
- Needs to be systematic
- Sampling site may affect composition
- Test less invasive techniques
- Freeze sample immediately



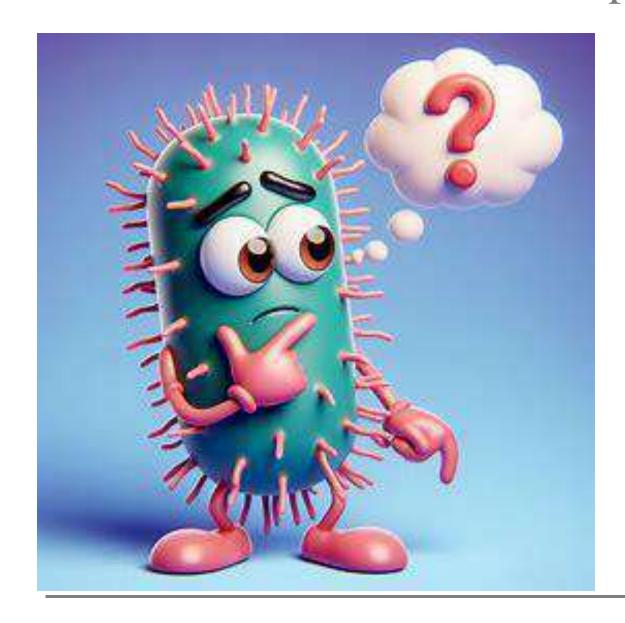




Preliminar considerations: Sample collection

- Test less invasive techniques
 - Saliva?

Preliminar considerations: Sample storage and DNA extraction





Preliminar considerations: Sample storage and DNA extraction

• Qiagen Powersoil Pro



• Zymobiomics



Preliminar considerations: Sequencing

- Whole genome sequencing
- If possible, avoid amplicon sequencing
- Long reads vs high accuracy
 - Cost
 - Resolution accuracy
 - Speed
 - Extra information



Preliminar considerations: Sequencing











Challenges at selecting for microbiome composition





UNKNOWN ECONOMIC VALUE

UNCERTAIN DIRECTION OF BREEDING (COMPLEX MICROBIAL RELATIONSHIPS)





Take home message for future metagenomic interventions in breeding programs

01

Aggregated metagenome variables can capture a large rumen microbiome variability

02

Selection for methane emissions and feed efficiency may shift rumen microbiota composition

03

International collaborations

Attractive as metagenomic traits in breeding programs for lower emissions and better efficiency.

So far, the consequences are uncertain. Need microbiome phenotyping to control changes.

Coming research efforts in this area.





