

Metagenomic Information from Rumen Contents to Improve Feed Efficiency and Mitigate Methane Emissions

Professor Dr. Rainer Roehe

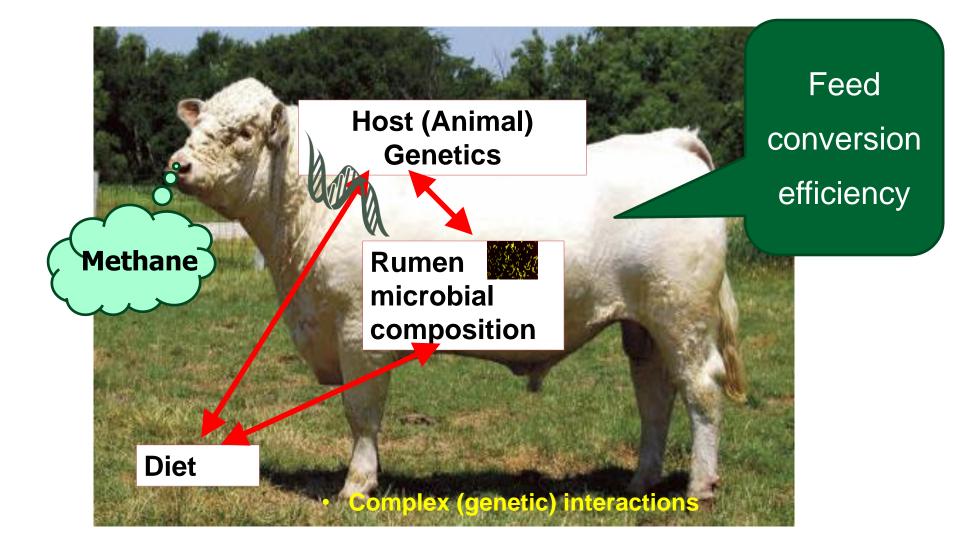
Leading the way in Agriculture and Rural Research, Education and Consulting

Host (Animal) Selection for Feed Efficiency and

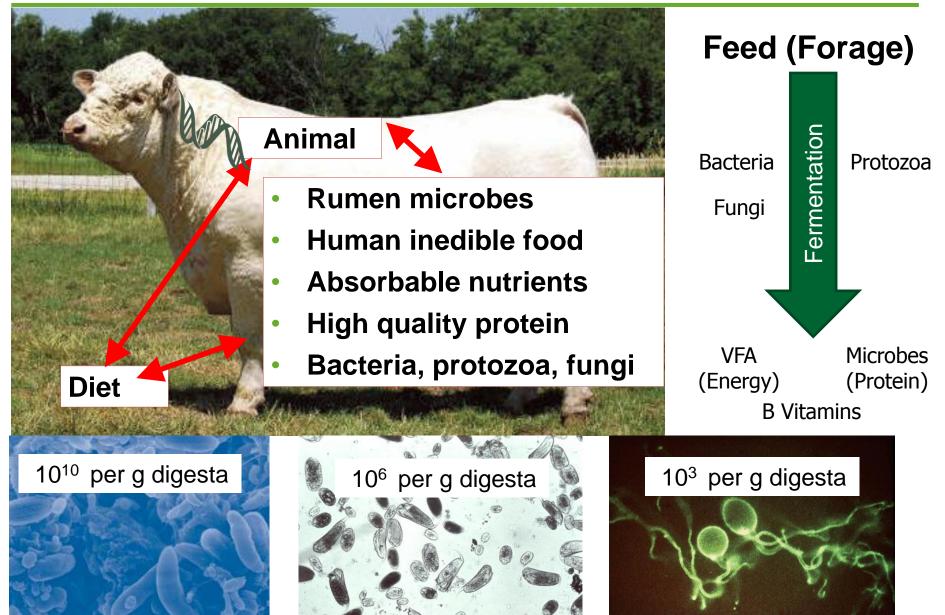
- **Methane Mitigation**
- Feed conversion efficiency (FCE) in beef cattle
 - High economic impact
 - Use of limited resources
 - Brazil second largest beef producer
- Methane
 - 7.1 billion tonnes CO_{2-eq} per annum (Gerber et al., 2013)
 - ~40% from enteric methane
- Host (Animal) Genetics
 - FCE & Methane emissions
 - Rumen microbiome information
 - Best selection criteria



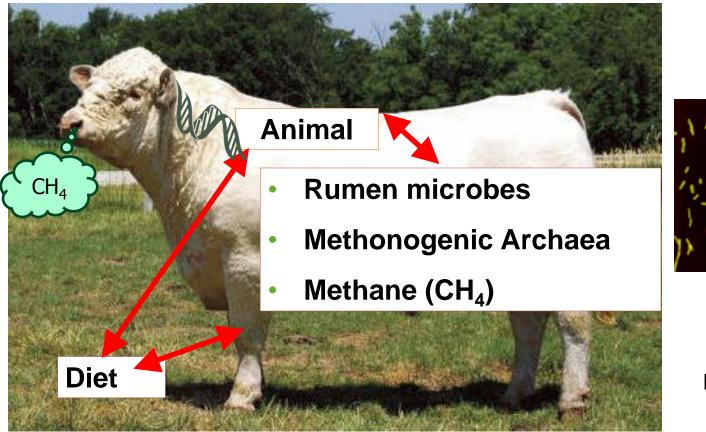
Host Genetics and Microbiome



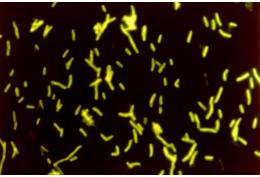
Microbes affecting Feed Efficiency (Symbiotic Relationship)

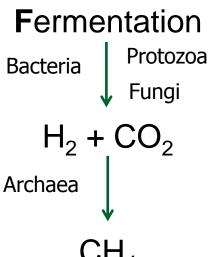


Microbes affecting Methane Emissions

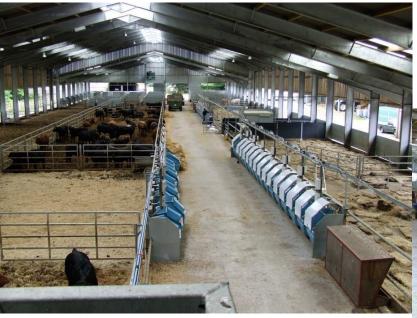


10⁸ per g digesta





Recording Feed Intake & Methane Emissions



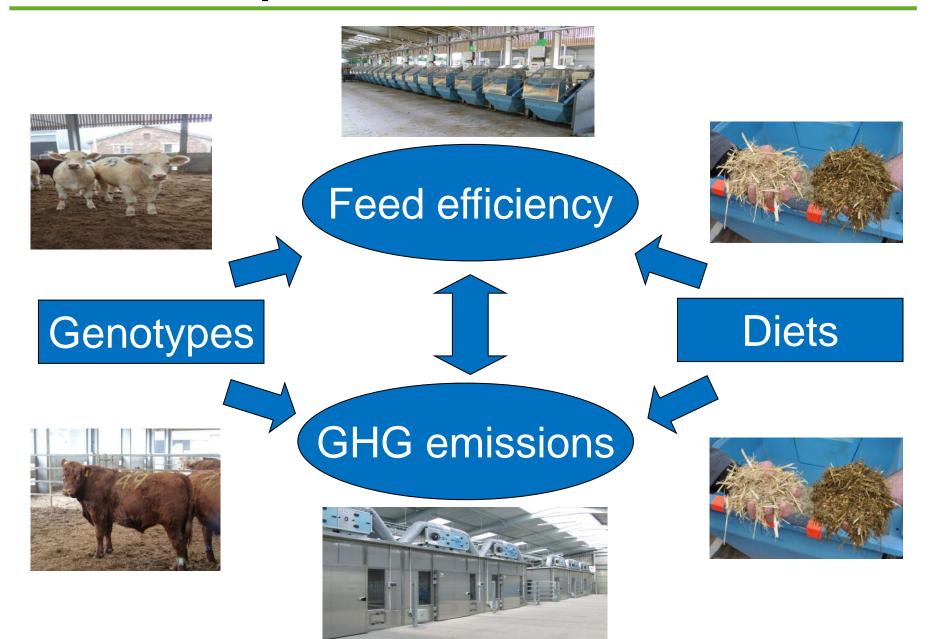
Individual feed intake



SRUC Beef Research Centre, Easter Howgate

Individual methane emissions

Experimental Beef Trials



Variation in Methane Emissions g/day between Animals

	Forage	Concentrate
A. Angus x	172–333 g/day	78–233 g/day
Limousin x	152–266 g/day	86-216 g/day
		γ/
		n methane emissions n animals
	CV =	14% – 32%

Variation in Methane Emissions (g/DMI) between Animals

	Forage	Concentrate
A. Angus x	15.9–31.4 g/DMI	7.6–18.1 g/DMI
Limousin x	14.4–30.4 g/DMI	9.3–22.8 g/DMI

Large differences in methane emissions between animals

CV = 18% - 29%

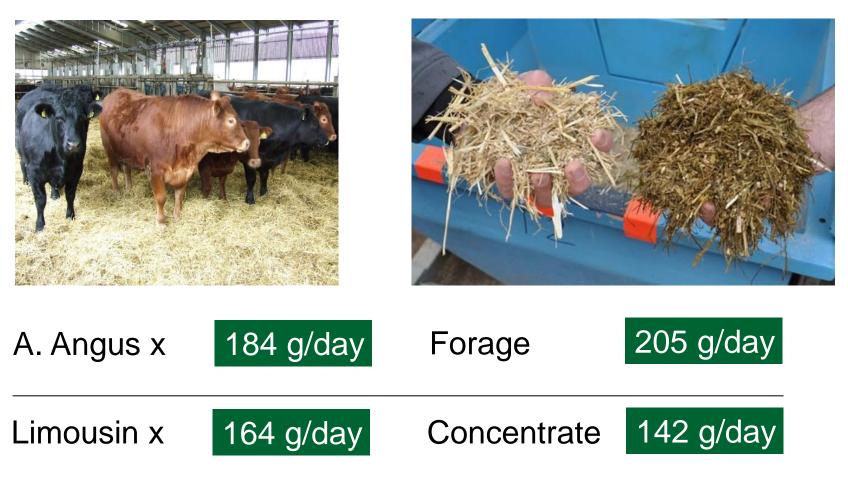
Variation in Archaea:Bacteria Ratio between Animals using Samples collected on Slaughtered Animals

	Forage	Concentrate
A. Angus x	1.5 – 11.0	0.9 – 5.8
Limousin x	2.2 – 14.0	1.4 – 4.9
	Extreme large Archaea:Bacteria rat	differences in ios between animals
	CV = 3	35% – 50%

Variation in Archaea:Bacteria Ratio between Animals using Samples collected on Live Animals

	Forage	Concentrate
A. Angus x	3.1 – 17.1	0.7 – 8.5
Limousin x	2.1 – 9.4	1.0 – 6.7
	۱	
		differences in tios between animals
	CV = 3	89% – 65%

Effect of Breed & Diet Type on Methane Emissions g/day

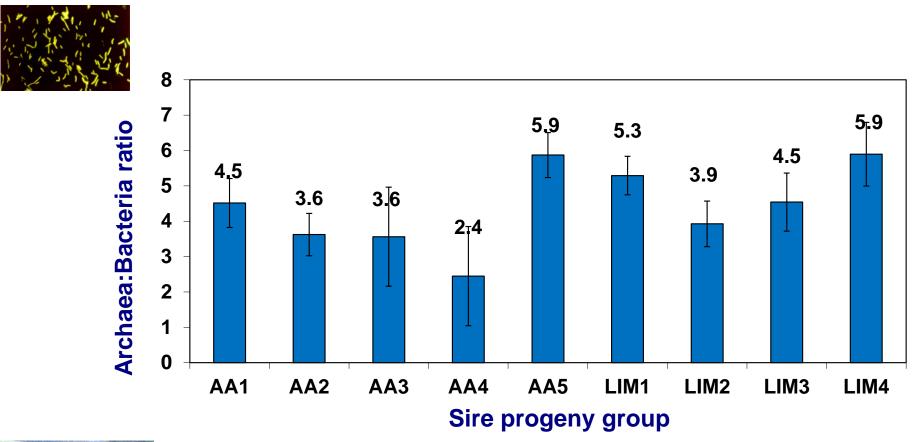


SE = 5.7

SE = 5.7

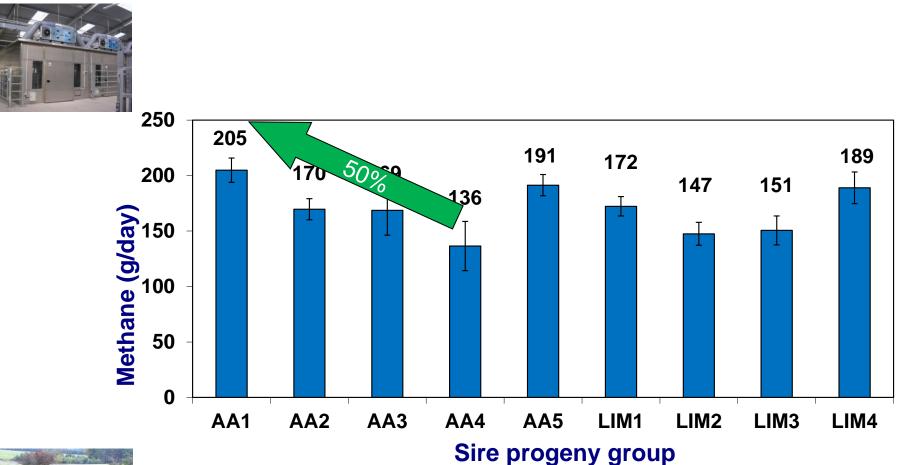
Rooke et al. (2014); Roehe et al. (2016)

Host (Animal) Genetics shapes the Microbial Community (A:B ratio)

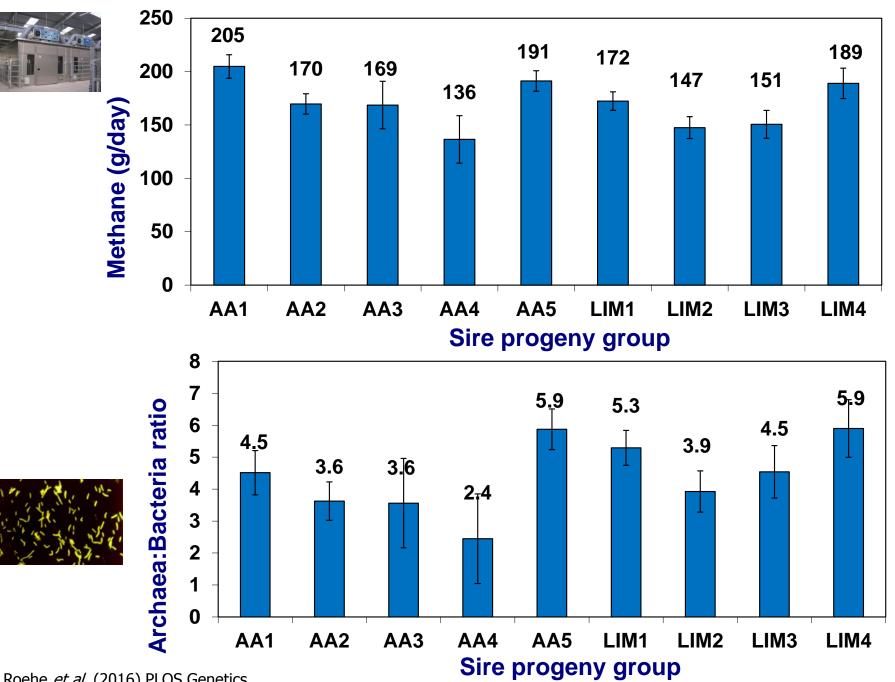




Host (Animal) Genetics affects Methane Emissions (g/day)



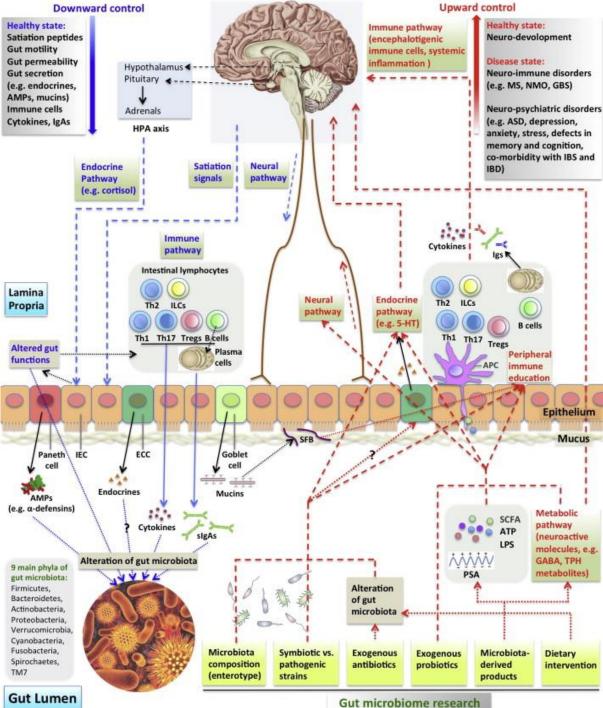




Biological Mechanisms

– Host Genetics and Microbiome Interactions –

- Rumen pH influences microbial community
 - Saliva contains bicarbonate
 - Large variation in saliva production (av. 150 l/day)
 - Differences in short chain fatty acids absorption
 - Passage rate of protons
- Variation in physical size & structure of the rumen
- Rumen contractions and passage rate of digesta
- Microbiome-gut-brain axis
 - Stress
 - Immune system
 - 'Fucose sensing', gut microbiome and host epithelia cell cross-talk



Microbiome-Gut-Brain Axis

Wang & Kasper (2014) Brain, Behavior, and Immunity

Gut Lumen

Deep Sequencing of DNA from Rumen Microbes

Metagenomic analysis

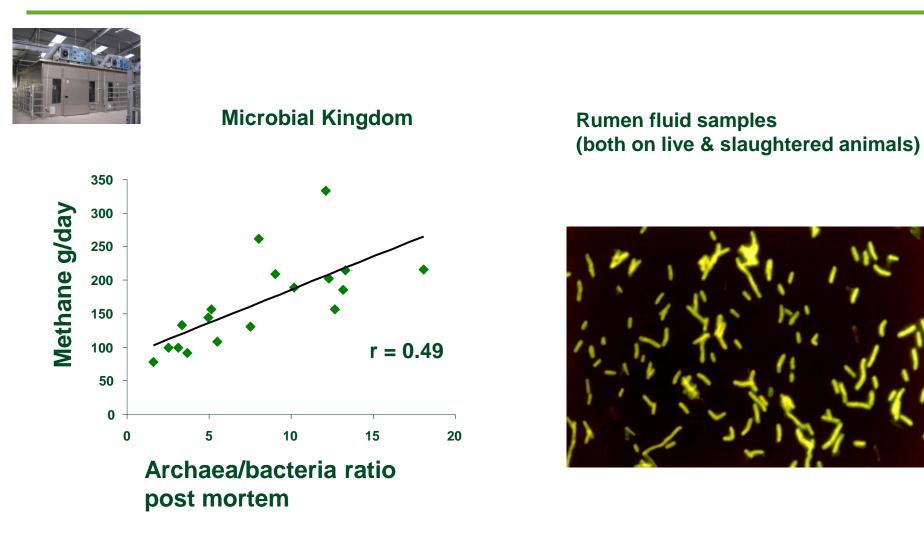
Microbial community

Domain e.g. Archaea, Bacteria Phylum e.g. Bacteroidetes, Proteobacteria Genus e.g. Methanobrevibacter, Methanosphaera Centric Microbial genes, e.g. KEGG gene orthologues

Gene-

Proteins within KEGG orthologues

Predicting Methane Emissions by Methanogenic Archaea : Bacteria Ratio



Wallace et al. (2014) Scientific Reports

Prediction of Methane by Genera

	Methane		
Genus	Estimate	VIP	R ²
Methanosphaera	0.360	1.15	0.84
VadinCA11	0.279	1.07	0.77
Methanobrevibacter	0.190	1.05	0.92
Moryella	0.098	0.98	0.77
Megasphaera	-0.092	0.90	0.83
Desulfovibrio	-0.027	0.81	0.98

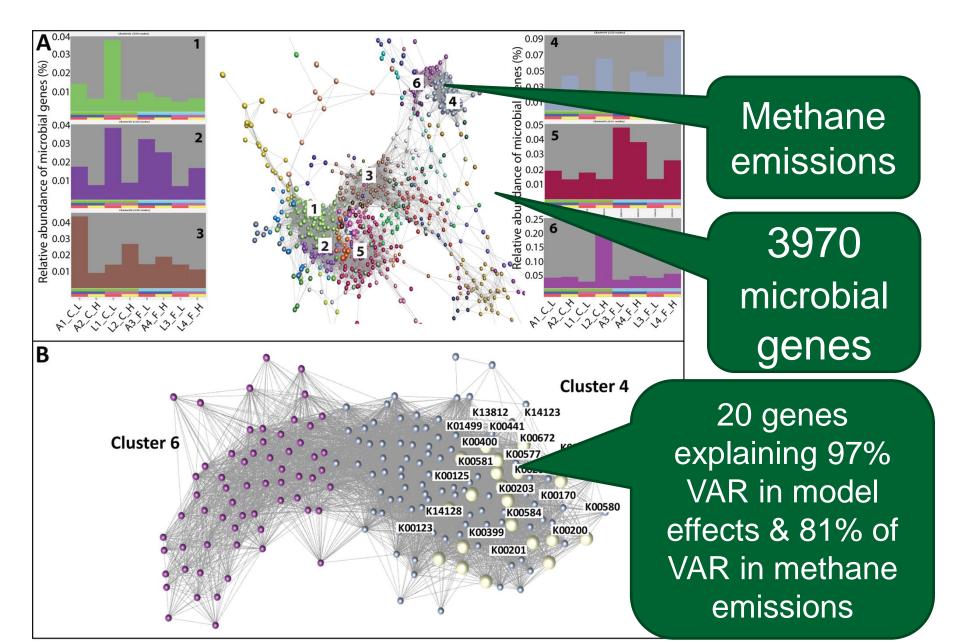
PLS model explains 89.7% of the variation in model effects and 84.5% of the variation in methane

Prediction of Feed Conversion Ratio by Genera

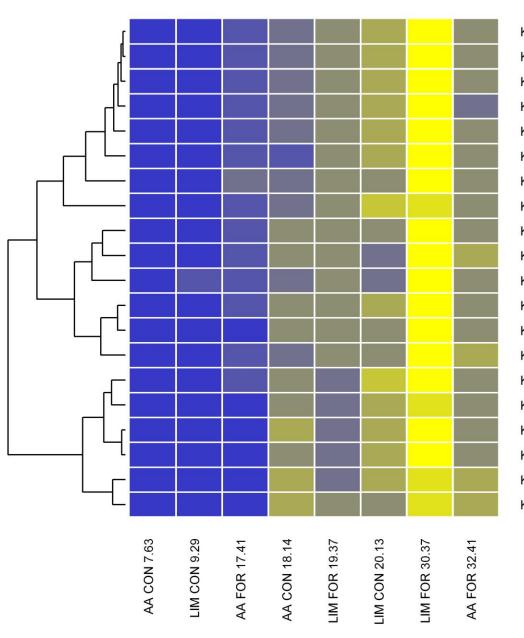
Feed conversion ratio					
Genus	Estimate	VIP	R ²		
Sphaerochaeta	0.224	1.09	0.82		
Ruminobacter	0.206	1.06	0.84		
Succiniclasticum	0.360	1.04	0.80		
Dialister	0.277	1.01	0.73		
Clostridium	0.156	0.95	0.83		
Bifidobacterium	0.074	0.83	0.66		

PLS model explains 86.9% of the variation in model effects and 73.6% of the variation in FCR

Network of Rumen Microbial Genes

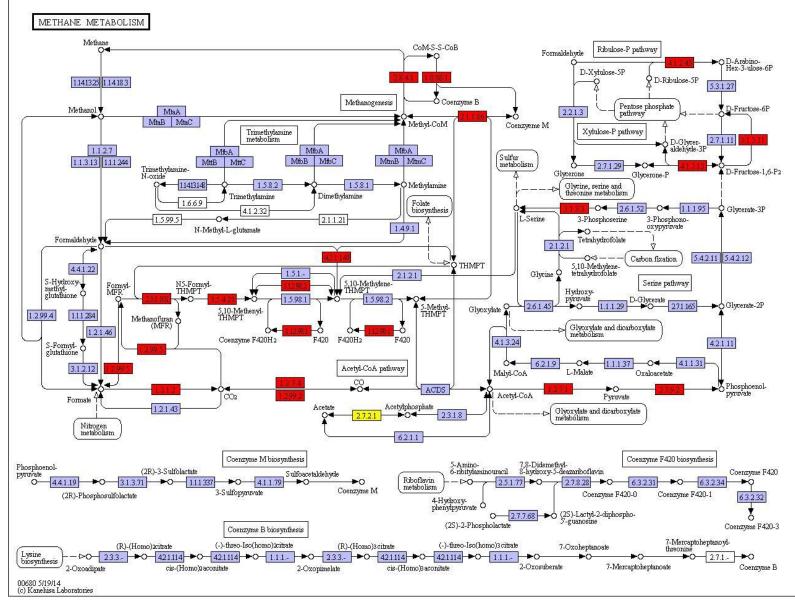


Microbial Genes associated with Methane



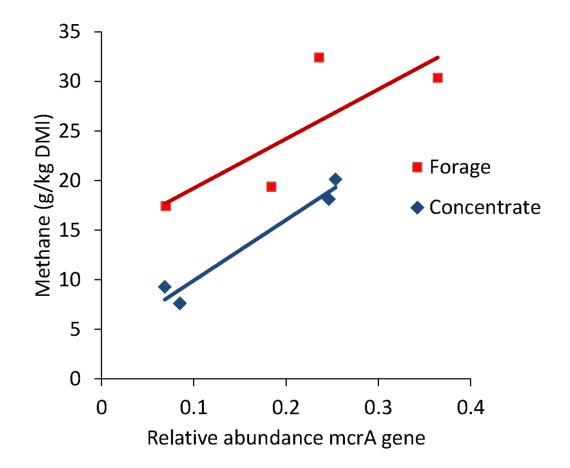
K00400 methyl coenzyme M reductase system, component A2 K00577 tetrahydromethanopterin S-methyltransferase A K13812 bifunctional enzyme Fae/Hps K00441 coenzyme F420 hydrogenase beta K00672 formylmethanofuran--tetrahydromethanopterin N-formyltransferase K14123 energy-converting hydrogenase B N K00205 formylmethanofuran dehydrogenase F K01499 methenyltetrahydromethanopterin cyclohydrolase K00170 pyruvate ferredoxin oxidoreductase, beta K00200 formylmethanofuran dehydrogenase A K00580 tetrahydromethanopterin S-methyltransferase D K00169 pyruvate ferredoxin oxidoreductase, alpha K00203 formylmethanofuran dehydrogenase D K00584 tetrahydromethanopterin S-methyltransferase H K00125 formate dehydrogenase, beta K00581 tetrahydromethanopterin S-methyltransferase E K00123 formate dehydrogenase, alpha K14128 F420-non-reducing hydrogenase G K00399 methyl-coenzyme M reductase alpha K00201 formylmethanofuran dehydrogenase B

Microbial Genes in the Methane Metabolism



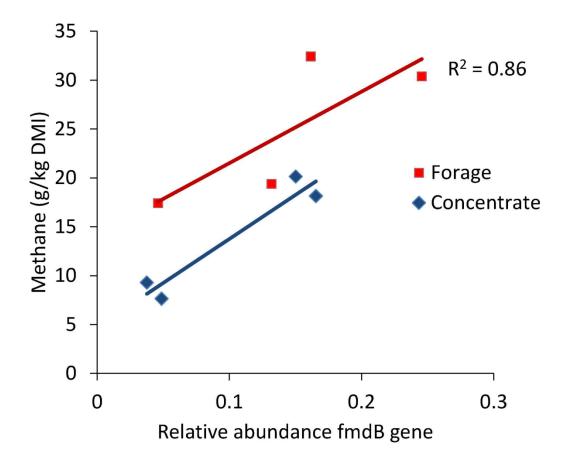
Wallace et al. (2015) BMC Genomics

Methane Emissions & mcrA Gene



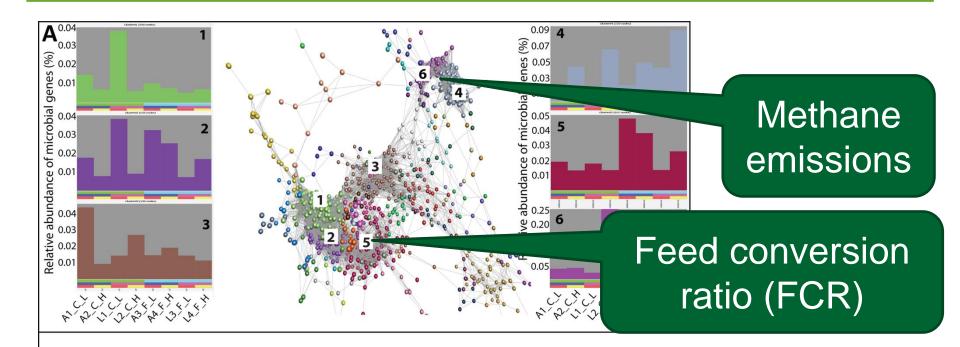
mcrA = methyl-coenzyme M reductase alpha subunit

Methane Emissions & fmdB Gene



fmdB =formylmethanofuran dehydrogenase subunit B

Microbial Genes associated with FCR



- 49 microbial genes significantly associated with feed conversion ratio explaining 81% of the variation in model effects & 88% of the variation in FCR.
- Microbial genes are related to known metabolic pathways, e.g. degradation of amino acids and proteins, protein and vitamin synthesis

									K01613 phosphatidylserine decarboxylase K03111 single-strand DNA-binding protein K03501 glucose inhibited division protein B	
									K01129 dGTPase K01104 protein-tyrosine phosphatase K02343 DNA polymerase III gamma/tau K00773 queuine tRNA-ribosyltransferase K09811 cell division transport system permease protein K07139 Putative Fe-S oxidoreductase	Microbial
									K01924 UDP-N-acetylmuramatealanine ligase K07082 UPF0755 protein K01928 UDP-N-acetylmuramoylalanyl-D-glutamate2,6-diaminopimelate ligase K01784 UDP-glucose 4-epimerase K00956 sulfate adenylyltransferase 1 K03581 exodeoxyribonuclease V alpha	genes
ſ									K00868 pyridoxine kinase K04517 prephenate dehydrogenase K01814 phosphoribosylformimino-5-aminoimidazole carboxamide ribotide isomerase K03458 nucleobase:cation symporter-2, NCS2 family K13542 uroporphyrinogen III methyltransferase / synthase	associated
									K00974 tRNA nucleotidyltransferase (CCA-adding enzyme) K08602 oligoendopeptidase F K00394 adenylylsulfate reductase, A K00375 GntR family transcriptional regulator / MocR family aminotransferase K01269 aminopeptidase K03657 DNA helicase II / ATP-dependent DNA helicase PcrA	with
									K03037 DNA heilcase in 7 A P-cependent DNA heilcase PCIA K08483 phosphotransferase system, enzyme I, Ptsl K02006 cobalt/nickel transport system ATP-binding protein K06921 Putative ATPase K00941 hydroxymethylpyrimidine/phosphomethylpyrimidine kinase K03426 NAD+ diphosphatase	feed
									K03631 DNA repair protein RecN (Recombination protein N) K01493 dCMP deaminase K00075 UDP-N-acetylmuramate dehydrogenase K02313 chromosomal replication initiator protein K11752 diaminohydroxyphosphoribosylaminopyrimidine deaminase	conversion
									K03615 electron transport complex protein RnfC K00278 L-aspartate oxidase K00766 anthranilate phosphoribosyltransferase K02008 cobalt/nickel transport system permease protein K01358 ATP-dependent Clp protease, protease K01818 L-fucose isomerase	ratio
									K01876 aspartyl-tRNA synthetase K02907 large ribosomal protein L30 K00179 indolepyruvate ferredoxin oxidoreductase, alpha K07588 LAO/AO transport system kinase K01195 beta-glucuronidase K03694 ATP-dependent Clp protease ATP-binding ClpA	
L	AA CON 6.10	AA CON 6.10	AA FOR 6.72	LIM CON 8.04	LIM FOR 8.07	LIM FOR 8.12	LIM CON 9.33	AA FOR 10.38	K02377 GDP-L-fucose synthase	016) PLOS Genetics

K01613 phosphatidylserine decarboxylase K03111 single-strand DNA-binding protein K03501 glucose inhibited division protein B K01129 dGTPase K01104 protein-tyrosine phosphatase K02343 DNA polymerase III gamma/tau K00773 queuine tRNA-ribosyltransferase K09811 cell division transport system permease protein K07139 Putative Fe-S oxidoreductase K01924 UDP-N-acetylmuramate--alanine ligase K07082 UPF0755 protein K01928 UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase K01784 UDP-glucose 4-epimerase K00956 sulfate adenylyltransferase 1

L-fucose isomerase

K00974 IKNA Hu K08602 oligoendo K00394 adenylylsi K00375 GntR fam K01269 aminopep K03657 DNA helica K08483 phosphotra K02006 cobalt/nick K06921 Putative AT K00941 hydroxymet K03426 NAD+ dipho K03631 DNA repair K01493 dCMP deam K00075 UDP-N-acet K02313 chromosoma K11752 diaminohvdrd K03615 electron trans K00278 L-aspartate of K00766 anthranilate p K02008 cobalt/nickel tr K01358 ATP-dependent Clp protease, protease K01818 L-fucose isomerase K01876 aspartyl-tRNA synthetase K02907 large ribosomal protein L30 K01195 beta-glucuronidase

lator / MocR family aminotransferase

dent DNA helicase PcrA enzyme I, Ptsl em ATP-binding protein

phosphomethylpyrimidine kinase

N (Recombination protein N)

te dehydrogenase tion initiator protein sphoribosylaminopyrimidine deaminase omplex protein RnfC

phoribosyltransferase sport system permease protein

K00179 indolepyruvate ferredoxin oxidoreductase, alpha

K07588 LAO/AO transport system kinase

Ч

CON 6.10

4A FOR 6.72 IM CON 8.04 IM FOR 8.07 -IM FOR 8.12

AA CON 6.10

IM CON 9.33 AA FOR 10.38 K03694 ATP-dependent Clp protease ATP

K02377 GDP-L-fucose synthase

GDP-L-fucose synthase

Roehe et al. (2016) PLOS Genetics

Microbial

genes

associated

with

feed

conversion

ratio

'Fucose Sensing'

Fucose

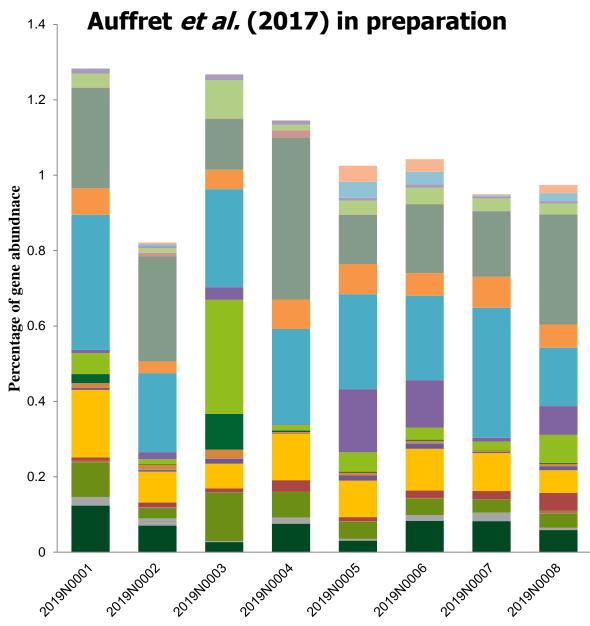
- Component of innate immunity glycoproteins (mucins)
- Intestinal mucosa
- Saliva glands
- Integrity of the mucosal barrier
- Bacterial demand for fucose
 - Degradation mucins
- FucR: L-fucose operon activator
 - Controls bacterial signalling for host mucins production
 - Controls bacterial demands for fucose with supply
- Cross-talk of microbiome & host

Conclusions Microbial Selection Criteria

Microbial information highly informative

- Relative abundance of microbial community
 - Deviation from normal distribution
 - More restricted numbers
 - No unique biological (functional) background
- Relative abundance of microbial genes
 - Most microbial genes normally distributed
 - Many thousands of microbial genes
 - Many proteins within KEGG orthologues
 - Known biological (functional) background
- Combination of taxa & microbial genes BEST!

Microbial Genes associated with Antimicrobial Resistance

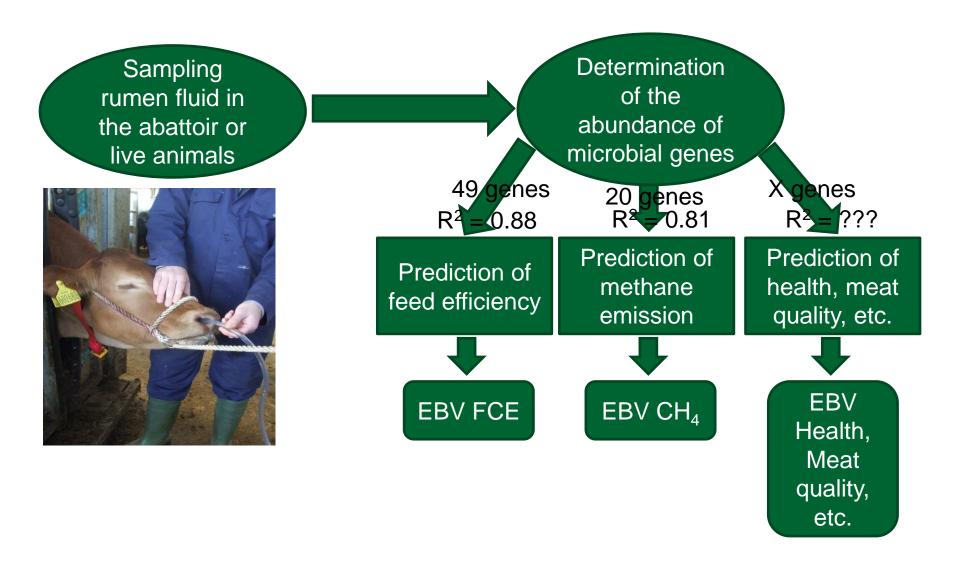


	Microcin resistance
	Heat/Cold shock protein
	Toxic resistance
	Drugs resistance
	Antibiotics production
	Antibiotics resistance
	Oxidative stress
	Biofilm formation and resistance
	Adhesion/type IV pilus
	Motility and hooking to host cells
	Other secretion system
	■ T6SS
	T4SS
	T3SS
	T2SS
	T1SS
	Iron scavenging mechanisms
	■two-component system, OmpR family, sensor histidine kinase QseC ■Quorum sensing Toxin
	Quorum sensing QS3
	■Quorum sensing QS2
-	Two-component signal transduction systems

beta-glucuronidase

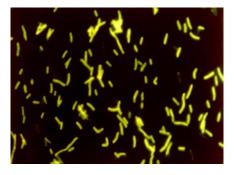
Fucose sensing

Selection using Rumen Microbial Information



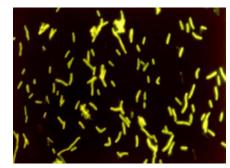
Conclusions

- Host (animal) genetic effect
 - Microbial community & microbial genes
 - Methane emissions
- Selection criterion
 - Abundance of microbial genes associated with feed conversion efficiency and methane emissions
 - Development of a microbial gene microarray
- Abundance of microbial genes
 - Health & meat quality
 - Susceptibility to heat stress
 - Biomarker for animal welfare, etc.



Conclusions

- Advantages of this selection strategy
 - Genetic improvement of difficult and costly to measure traits via abundances of microbial genes
 - Highly cost-effective
 - Microbial genes have functional background



 New era of breeding for animals (hosts) providing the best environment for efficient rumen microbes can begin!

Outlook & Recommendation

- Brazilian meat production is expected to continue its fast growth in the coming decade, according to the FAO.
- Based FAO outlook to 2024, beef production in Brazil will increase due to:
 - Increasing domestic and international demand
 - Lower projected feed costs
 - Improved animal genetics
 - Better Health and Nutrition

Recommendation

Use of Microbial Gene Information

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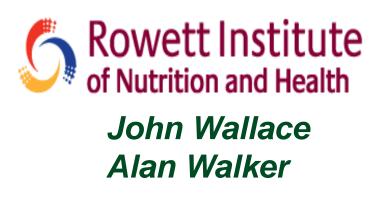




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