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**Physiology of rumen bacteria associated with  
low methane emitting sheep**

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## Abstract

The fermentation of feed and formation of methane (CH<sub>4</sub>) by ruminant animals occur in the rumen, and both are microbial processes. There is a natural variation in CH<sub>4</sub> emissions among sheep, and this variation is heritable. Therefore, breeding for sheep that naturally produce less CH<sub>4</sub> is a viable strategy to reduce anthropogenic greenhouse gas emissions. Rumen bacteria play a major role in feed fermentation and in the formation of hydrogen (H<sub>2</sub>) or formate, which are converted to CH<sub>4</sub> by other rumen microbes called methanogens. It has been shown that rumen bacterial community compositions in low CH<sub>4</sub> emitting sheep differ to those in high CH<sub>4</sub> emitting sheep. This led to the hypothesis that the metabolism of dominant rumen bacteria associated with low CH<sub>4</sub> emitting sheep should explain the lower CH<sub>4</sub> yield, for example by producing less H<sub>2</sub> or formate than bacteria associated with high CH<sub>4</sub> emitting sheep. In this project, the diversity and physiology of members of the bacterial genera *Quinella*, *Sharpea* and *Kandleria*, which are major bacterial groups associated with low-CH<sub>4</sub> emitting sheep, were investigated. It appeared that the genus *Quinella* is more diverse than previously suspected, and might contain at least eight potential species, although to date none have been maintained in laboratory culture. *Sharpea* and *Kandleria* contain two and one species respectively. Experiments with *Sharpea* and *Kandleria* showed that these behave like classical lactic acid bacteria that produce lactate as their major end product and did not change their fermentation pattern to produce more H<sub>2</sub> or formate when grown in the presence of methanogens. This strengthens a previous hypothesis that sought to explain low CH<sub>4</sub> emissions from sheep with *Sharpea* and *Kandleria* in their rumens, in which this invariant production of lactate was a key assumption. *Quinella* is another bacterium found in larger numbers in the rumen of some low CH<sub>4</sub> sheep. Virtually nothing is known about its metabolism. FISH probes and cell concentration methods were developed which helped in its identification and resulted in construction of four genome bins of *Quinella* that were more than 90% complete with as little as 0.20% contaminated. Bioinformatic analyses of the proteins encoded by these genomes showed that *Quinella* has the enzymes for lactate formation and for the randomising pathway of propionate formation. This indicated that lactate and propionate might be major fermentation end products of *Quinella*. Additionally, the presence of an uptake hydrogenase in the *Quinella* genomes opens up the new possibility that *Quinella* might even use free H<sub>2</sub> in the rumen. In all these possible pathways, little or no H<sub>2</sub> would be produced, explaining why an increased abundance of *Quinella* in the rumen would lead to lower CH<sub>4</sub> emissions from those sheep with high abundances of this bacterium.



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## **Dedication**

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## Abbreviations

2GenRFV	Double general substrate-Rumen Fluid-Vitamin mix
AA	Auxiliary Activities
aa	Amino acid(s)
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
BES	2-Bromoethanesulfonic acid
BLAST	Basic Local Alignment Search Tool
BLOSUM	BLOcks SUBstitution Matrix
BSA	Bovine serum albumin
CAZy	Carbohydrate-Active enZYmes
CAI	Codon adaptation index
CDS	Coding DNA sequence
cfu	Colony-forming units
CH <sub>4</sub>	Methane
CO <sub>2</sub>	Carbon dioxide
CoA	Coenzyme A
COGs	Clusters of Orthologous Groups
CRISPR	Clustered regularly interspaced short palindromic repeat
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FGD	Functional Genome Distribution
FISH	Fluorescence <i>in situ</i> hybridisation
GHG(s)	Greenhouse gas(es)
GIT	Gastrointestinal
HMM	Hidden Markov Model
IPTG	Isopropyl β-D-1-thiogalactopyranoside
KEGG	Kyoto Encyclopedia of Genes and Genomes
LB	Lysogeny broth
mRNA	Messenger RNA

NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NCBI	National Center for Biotechnology Information
NoSubRFV	Rumen fluid vitamin mix with no added growth substrates
NZ	New Zealand
O <sub>2</sub>	Oxygen
ORF	Open reading frame
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
QIIME	Quantitative Insights Into Microbial Ecology
RNA	Ribonucleic acid
TAE	Tris acetate EDTA
TE	Tris EDTA
TEM	Transmission electron micrograph/microscopy
TMH	Transmembrane helix
tRNA	Transfer RNA
UV	Ultra violet
VFA	Volatile fatty acid
v:v	Volume to volume
v:v:v	Volume to volume to volume
v/v	Volume/volume
w/v	Weight/volume
X-gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

**Measurement Units:**

°C	Degrees Celsius
μg	microgram
μL	microlitre
μm	micrometre
μM	micromolar
bp	Base pair

h	Hour
kcal	kilocalorie
kb	kilobase pairs
kDa	kilodaltons
kPa	kilopascal
kV	kilovolts
L	Litre
M	Molar
Mb	megabase pairs
mg	milligram
min	minutes
mL	millilitre
mM	millimolar
ng	nanogram
nm	nanometer
ppm	Parts per million
rpm	revolutions per minute
s	seconds

