Physiology of rumen bacteria associated with low methane emitting sheep

A thesis presented in partial fulfilment of the
requirements for the degree of

Doctor of Philosophy

in

Microbiology and Genetics

at

Massey University, Palmerston North,

New Zealand

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2017

Abstract

The fermentation of feed and formation of methane (CH_4) by ruminant animals occur in the rumen, and both are microbial processes. There is a natural variation in CH_4 emissions among sheep, and this variation is heritable. Therefore, breeding for sheep that naturally produce less CH_4 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_2) or formate, which are converted to CH_4 by other rumen microbes called methanogens. It has been shown that rumen bacterial community compositions in low CH_4 emitting sheep differ to those in high CH_4 emitting sheep. This led to the hypothesis that the metabolism of dominant rumen bacteria associated with low CH_4 emitting sheep should explain the lower CH_4 yield, for example by producing less H_2 or formate than bacteria associated with high CH_4 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_4 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_2 or formate when grown in the presence of methanogens. This strengthens a previous hypothesis that sought to explain low CH_4 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_4 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_2 in the rumen. In all these possible pathways, little or no H_2 would be produced, explaining why an increased abundance of *Quinella* in the rumen would lead to lower CH_4 emissions from those sheep with high abundances of this bacterium.

Acknowledgements

If I had to make a list of the great things that happened during my PhD, then certainly getting Dr Peter Janssen as a supervisor would go on the top of this list. I sincerely thank you for your wisdom, encouragement and valuable advice throughout my PhD. You surely have made this steep learning curve easier for me. I gratefully acknowledge Dr Gemma Henderson and Dr Sandra Kittelmann for helping me to explore ARB and QIIME and thereafter, providing valuable feedback on the project. Your contributions have made a significant difference to my thesis.

I greatly appreciate Dr Mark Patchett for being supportive and providing consistent feedback. I thank Dr Graeme Attwood for invaluable suggestions and constructive criticism throughout the project. I thank Dr Sinead Leahy for providing me with much needed support in metagenomic analysis and Dr Eric Altermann for helping me with genome annotations. I thank Dr Dragana Gagic and Associate Professor Jasna Rakonjac for attending my monthly supervisor meetings and Dr Sinead Waters for being my Teagasc supervisor. I thank Dr Christina Moon, Dr Ron Ronimus, Dr Janine Kamke, Priya Soni, Faith Cox and all of the rumen microbiology team for helping and supporting me in, and outside of the lab. I thank Dr Arjan Jonker and all animal technicians for helping me with sample collection and sheep handling and Bryan Treloar for HPLC analysis. I thank Dr Ruy Jauregui for bioinformatics support and Dr Siva Ganesh and Catherine McKenzie for statistical support.

I would like to thank Teagasc, Ireland for awarding me a Walsh Fellowship, and the New Zealand Agricultural Greenhouse Gas Research Centre **(**NZAGRC**)** for providing financial support to carry out my PhD project.

I would like to thank all the fellow students in the student room for providing a great social environment. Great thanks to my diverse flatmates covering eleven countries and five continents over the years, especially Aniek, Ermanno, Stefi, Daniel, Taina, Stephen, Maarten, Judith, Savannah and Linda. Thanks are also due to my adopted, extended New Zealand family; Dr Helal Ansari, Dr Wajid Hussain, Dr Sandeep Gupta, Dr Tanu Gupta, Dr Preeti Raju, Agneta Ghose, Lovepreet Kaur, Jaspreet Singh, Debjit Dey and Shampa Dey for making this journey joyful.

And finally, to my family, mother Deo Pari Devi, father Shambhu Sharan Prasad, brother Santosh Kumar and sister Sarika Anjan for showing faith in me and providing much needed love and encouragement. Cherished memories of my grandmother Sita Devi were a source of great comfort during my present academic journey.

Dedication

To the most impressive and beautiful woman of my life, my mother, the late **Deo Pari Devi**. Like any other child, I also ran out of the words to show my gratitude and emotions for all the love and sacrifices you have made to make me a better human being. I wish you could be here to share the joy of my accomplishment, which belongs more to you than me. But I am sure that you are watching me from heaven and pouring your blessing on me as always. I dedicate not only this thesis, but all my future achievements to you.

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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_4	Methane
CO_2	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 *in situ* 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_2	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