Investigation of Rumen Methanogens in New Zealand Livestock

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Abstract

Methane emitted by farmed ruminants contributes 30.3% to New Zealand's anthropogenic greenhouse gas inventory. Methanogens living in the rumen produce methane from H_2 and CO_2 as a byproduct of feed fermentation. The use of vaccines and small molecule inhibitors against the methanogens are promising methods to reduce methane emissions from extensively-grazed ruminants in New Zealand. Knowledge of the methanogens present in New Zealand ruminants is an important first step for successful vaccine and inhibitor development to target all methanogens.

In this study, the methanogen diversity of farmed ruminants (sheep [*Ovis aries*], cattle [*Bos taurus*] and red deer [*Cervus elaphus*]) was investigated using molecular ecological techniques. Ruminants fed different diets had largely similar rumen methanogen communities. The major methanogen groups identified were from the *Methanobrevibacter ruminantium* clade (*Mbb. ruminantium* and closely-related species), *Methanobrevibacter gottschalkii* clade (*Mbb. gottschalkii* and closely-related species), *Methanosphaera* spp., and the putative methanogens belonging to the group designated Rumen Cluster C. A total of 37.5 - 57% of 16S rRNA genes in the rumen of a group of cows originated from members of Rumen Cluster C. Chloroform treatment of cows increased the abundance of Rumen Cluster C to 82% - 93% of archaeal 16S rRNA genes. In parallel, a total of 22% of *mcrA* genes belonged to an unassigned group of archaea, and chloroform treatment increased the unassigned group of archaea to 92% of all *mcrA* genes. This suggested that Rumen Cluster C archaea contain the gene *mcrA*.

No members of the Rumen Cluster C group have previously been cultured, and currently there is no reported rumen isolate of *Methanosphaera* spp. A strain of *Methanosphaera* sp. was isolated from a sheep rumen and initial characterization suggests that this may be a new species. Three enrichment cultures were obtained containing members of Rumen Cluster C as the only archaea. Initial studies of these enrichment cultures showed that these three isolates were from three different sub-groups of Rumen Cluster C and that they produced methane.

The investigation of methanogen diversity in New Zealand farmed ruminants and isolation of previously uncultured rumen methanogens reported here in this thesis will

significantly aid the development of methane reduction strategies for farmed ruminants in New Zealand.

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Non-standard abbreviations

BLAST	basic local alignment search tool
bp	base pairs
BSA	bovine serum albumin
C	cow
CoM	coenzyme M
D	red deer
DGGE	denaturing gradient gel electrophoresis
DM	dry matter
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
DMSO	dimethyl sulfoxide
DSMZ	Deutsche Sammlung von Mikroorganismen und Zellkulturen
EM	electron microscopy
FISH	fluorescence *in situ* hybridization
GHG	greenhouse gas
h	hour
HPLC	high-performance liquid chromatography
IR	infra-red
LB	Luria-Bertani
Ltd	Limited
M	molar
Mbb	*Methanobrevibacter*
MCR	methyl coenzyme reductase
min	minutes
MOPS	3-(N-morpholino) propanesulfonic acid
MPN	most probable number
PCR	polymerase chain reaction
qPCR	quantitative real-time polymerase chain reaction
RCC	Rumen Cluster C
RF	rumen fluid
RNA	ribonucleic acid
RODS	relative one-dimensional surface

rpm	revolutions per minute
S	sheep
SRB	sulphate reducing bacteria
subs	substrate
TGGE	temperature gradient gel electrophoresis
U	unit
UV	ultraviolet
v/v	volume per volume
VFA	volatile fatty acid
vit	vitamins
w/v	weight per volume
w/w	weight per weight
YE	yeastextract