Enumeration, Identification and Characterisation of Methanogens Colonising Pre-Ruminant Calves

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

Master of Science

at Massey University,

Palmerston North, New Zealand

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2007

Abstract

Methane-producing archaea, methanogens, in ruminant animals are a major source of anthropogenic methane. With a global warming potential 23 times greater than carbon dioxide, methane has been targeted for reduction under the Kyoto protocol. In New Zealand methane emissions from ruminant animals are major contributor to the national greenhouse gas inventory. For this reason agricultural industries are challenged with reducing methane emissions from ruminants. This investigation on methanogens in young dairy calves was carried out to obtain information on methanogen colonisation and establishment in the rumen because little is known about this process.

In this study, methanogen colonisation occurred within two days after birth in four calves that were raised in the absence of cows. Anaerobic culture techniques were used to enumerate methanogens in gut samples and showed that methanogen numbers increased over time, but dropped below detection limits in two of four calves between six and 11 days after birth. Methanogens in these two calves then reappeared at day 13. By three weeks of age methanogen densities in all four calves were approximately 10^8 cells ml^{-1}. These densities are similar to those found by other workers for 3-week old and mature ruminants. Colonies picked from anaerobic agar roll-tubes prepared from enumeration cultures yielded 31 methanogenic isolates and 28 isolates that utilised hydrogen but did not produce methane. Eleven of the 31 methanogenic isolates were selected for purification. Despite extensive efforts only four methanogens were able to be purified from the eleven isolates because of persistent non-methanogenic eubacteria also present in cultures.

A phylogenetic analysis of 16S rRNA gene sequences from purified and partially-purified methanogen isolates was carried out and dendograms constructed to identify methanogens. Some phenotypic characteristics of purified methanogens were determined. This revealed a number of methanogen species previously not found in the rumen. The results showed *Methanofollis liminatans* (three isolates), *Methanoculleus palmolei* (three isolates) or *Methanosarcina barkeri* (one isolate) were the predominant culturable methanogens colonising the rumen two days after birth. The three isolates identified as

M. liminatans were only 96.0% identical at the 16S rRNA gene level to the *M. liminatans* type strain, DSM 4140, and appear to be new a methanogen species. In gut samples collected 3-5 days after birth, *Methanobacterium bryantii* (three isolates) was found to be a predominant methanogen in some calves apparently replacing the first methanogens colonising the developing rumen. Twenty two days after birth *Methanobrevibacter thaueri* (one isolate) was identified as a predominant methanogen in one calf. These results are the first to suggest that there is a successional change in the methanogen populations as the rumen develops in young ruminants.

Consideration of the colonising species showed that *Mcl. palmolei* were obtained from only two calves (calves 10 and 12) and that *Mfl. liminatans*-like isolates were obtained only from a different cohort of calves penned separately to calves 10 and 12. These methanogens, previously found only in terrestrial or aquatic environments, are probably the primary colonising methanogens because there were no mature ruminants to provide alternative inocula. It appears that the developing rumen of young calves provides a niche suitable for opportunistic hydrogenotrophic methanogens.

A PCR investigation using targeted primers specific for seven groupings of methanogens was carried out on all rumen samples to obtain information not dependant on culturing. This analysis on DNA extracts showed methanogens belonging to the *Methanobacteriales* were present in almost every sample. Methanogens belonging to the *Methanosarcinales* and *Methanomicrobiales* were not detected in any sample. At the end of the trial (22 days), PCR analysis showed the presence of *Methanobacterium* spp. and *Methanobrevibacter* spp. in all four calves. Although there were some disagreements with results for isolates cultured, overall, PCR results confirmed the concept of successional changes in methanogen populations in pre-ruminant calves.

Acknowledgements

I would like to express my sincerest thanks to my supervisors, Dr. Keith Joblin for the original concept of this project and for challenging me at every step with his extensive knowledge of the rumen. And, Dr Mark Patchett, for all the ideas that were put forward during the writing of my thesis to give the document an overall well rounded appearance.

I am also extremely grateful to Graham Naylor for being the fountain of knowledge that he is, and for being able to describe to me some of his many intellectual and practical experiences with science. To Dr. Ron Ronimus for searching out all the small details that everybody else missed, and bringing them to my attention during editing of this thesis. A special thanks also goes to Patrice Evitt and Sam Noel for all the conversations, both intellectual and absurd, that we had.

To my family, who I did not see for long periods of time during my studies for their love, support and encouragement during all the times when I needed them to do so.

Finally, to Dr. Katia Nones for keeping me on the straight and narrow, for being there to discuss all things that are science, but being able to distract me from science when I was grumpy. It was needed.

This work was made possible due to funding from AgResearch train-up fund, the Pastoral Greenhouse Gas Research Consortium, and the Frank Sydenham memorial scholarship.

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Abbreviations

A.M.	*ante meridiem*	UV	ultraviolet
BLAST	Basic Local Alignment Search Tool	V	Volt
°C	degrees Celsius	v/v	volume per volume
cells ml^{-1}	cells per millilitre	w/v	weight per volume
DMSO	dimethylsulphoxide		
DNA	deoxyribonucleic acid		
g	gram		
kb	kilobase		
kPa	kilopascal		
L	litre		
$litre^{-1}$	per litre		
M	molar		
mg	milligram		
mg ml^{-1}	milligrams per millilitre		
ml	millilitre		
mm	millimetre		
mV	millivolt		
nm	nanometre		
pmol	picomoles		
PCR	polymerase chain reaction		
P.M.	*post meridiem*		
RNA	ribonucleic acid		
rDNA	ribosomal deoxyribonucleic acid		
rRNA	ribosomal ribonucleic acid		
rpm	revolutions per minute		
s	second		
SEM	Scanning Electron Microscope		
µg	microgram		
µg ml^{-1}	micrograms per millilitre		
µl	microlitre		

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