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Exploring rumen methanogen genomes to identify targets for methane mitigation strategies

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ABSTRACT

Methane emissions from ruminant livestock is generated by the action of methanogenic archaea, mainly in the rumen. A variety of methanogen genera are responsible for CH_4 production, including a large group that lacks cultivated representatives. To be generally effective, technologies for reducing ruminant CH_4 emissions must target all rumen methanogens to prevent any unaffected methanogen from expanding to occupy the vacated niche. Interventions must also be specific for methanogens so that other rumen microbes can continue normal digestive functions. Thus a detailed knowledge of the diversity and physiology of rumen methanogens is required to define conserved features that can be targeted for methanogen inhibition. Genome sequencing projects are underway in New Zealand and Australia on several ruminal methanogen groups, including representatives of the genera *Methanobrevibacter*, *Methanobacterium*, *Methanosphaera*, *Methanosarcina*, and the uncultured group, Rumen Cluster C. The completed *Methanobrevibacter ruminantium* genome and draft sequences from other rumen methanogen species are beginning to allow identification of underlying cellular processes that define these organisms, and is leading to a better understanding of their lifestyles within the rumen. Although the research is mainly at the explorative stage, two types of opportunities for inhibiting methanogens are emerging, being inactivation of conserved methanogen enzymes by screening for, or designing, small inhibitors via a chemogenomics approach, and identifying surface proteins shared among rumen methanogens that can be used as antigens in an anti-methanogen vaccine.

Abbreviations: ATP, synthase (Aha); BLAST, basic Local Alignment Search Tool; CoMS-SCoB, coenzyme B-coenzyme M heterodisulphide; CRC, Beef Cooperative Research Centre; DBA, differential BLAST analyses; FGD, functional genome distribution; GHG, greenhouse gas; HSCoB, reduced coenzyme B; HSCoM, reduced coenzyme M; H_4MPT, methyl transferase (Mtr); H_4MPT, tetrahydromethanopterin; IPG, immobilized pH gradient; MALDI-TOF/TOF, matrix-assisted laser desorption ionisation-time of flight–time of flight; McrA, methyl-coenzyme M reductase; MF, methanofuran; Mtd, coenzyme F_{420}-dependent methylenetetrahydromethanopterin dehydrogenase; NZAGRC, New Zealand Agricultural GHG Research Centre; ORF, open reading frames; PGGRC, Pastoral GHG Research Consortium; RELRP, Reducing Emissions in Livestock Research Program; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SP, sinal peptides; ssrRNA, small subunit ribosomal RNA; TMH, transmembrane helices; TMHMM, trans-membrane Hidden Markov Models; 2D-DIGE, two-dimensional differential in-gel electrophoresis.

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Many of the conserved enzyme targets are involved in energy generation via the methanogenesis pathway, while the majority of the conserved surface protein targets are of unknown function. An understanding of the expression and accessibility of these targets within methanogen cells and/or microbial biofilms under ruminal conditions will be required for their development as CH_4 production mitigations.

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1. Introduction

Ruminants are vital to food production in most countries of the world and, in Australia and New Zealand, their products are important contributors to economic and social well being. In 2008, ruminant products earned ~$NZ17 billion in commodity exports (Statistics New Zealand, 2008), and the red meat industry alone contributes over 20% of total farm exports in Australia (Meat and Livestock Australia, 2009). Despite the importance of these industries to the economic vitality and food supply of New Zealand and Australia, they, like those in many other Western countries, are being confronted with public concerns and challenges to change with respect to resource use, environmental impact and public health. The biology underpinning many of these challenges is microbiological in cause and effect. A specific case is production of greenhouse gases (GHG) by ruminant livestock production systems, both directly from the animal as CH_4 emissions and indirectly via soil emissions of N_2O from urea excreted in ruminant urine.

Ruminant microbiology typically favours formation of CH_4 via methanogenesis as a fermentation end point to maintain H_2 balance. In lactating dairy cows fed a pasture based diet, emissions can be as high as 330 g of CH_4/day (Grainger et al., 2007) or ~460 L of CH_4 gas. Degradation of lignocellulose in ruminants by microbes in the reticulo-rumen results in formation of microbial fermentation products which are absorbed and used by the animal as energetic precursors and subsequent growth and productivity. However, some fermentation products, such as H_2, CO_2, formate and small methyl-containing compounds, cannot be used by the animal and are further metabolised to CH_4. Methane formation is completed by a specialised group of microbes called methanogens, and their action prevents accumulation of these end products in the rumen to keep digestive processes normal.

Methane is released from the animal via eructation and, in the atmosphere, it is slowly converted to CO_2. However, during its lifetime, CH_4 can absorb and re-emit infrared radiation, thereby contributing to global warming. Methane has 25 times the warming effect of CO_2 over a 100 year period and, when its atmospheric concentration and half life are considered, CH_4 is thought to contribute 4–9% of the global GHG effect (Forster et al., 2007).

The current global demand for food, and the predicted trend to increased meat and milk consumption, means that ruminants will remain important contributors to global GHG emissions. Countries that depend on ruminants will come under increasing pressure to have sustainable agricultural practices. There is a need to investigate the process of CH_4 formation in ruminants with a view to reducing GHG emissions. For these reasons, there are various research initiatives underway in New Zealand (*e.g.*, the Pastoral GHG Research Consortium [PGGRC]; New Zealand Agricultural GHG Research Consortium [NZAGRC]) and Australia (*e.g.*, Beef CRC for Genetic Technologies and the Reducing Emissions in Livestock Research Program [RELRP]) to address these issues and challenges.

The principal strategic context of these consortia and programs is to advance a biotechnological basis for definition and management of rumen microbiomes and, by doing so, to reduce CH_4 emissions from livestock production systems. The research approaches employed are justified by three key considerations relevant to the likelihood that the rumen microbiome, can be successfully manipulated to achieve the desired outcome. First, rumen methanogens can be inhibited under laboratory conditions and there is a reduction in CH_4 emissions as a result. Second, the rumen microbiome has been successfully manipulated before, both by the introduction and persistence of 'exotic' bacteria (*e.g.*, *Synergistes jonesii* for detoxification of the forage legume *Leucaena* spp. Jones and Megarrity, 1986), or by compromising the growth potential of 'undesirable' bacteria by use of ionophores such as monensin (Hammond et al., 1978; Bartley et al., 1983). Third, methods for measuring CH_4 output in cattle are improving and we propose that successful long term reductions of CH_4 emissions from cattle can be expedited by functional and comparative studies of the rumen microbiomes in individual animals with low CH_4 emissions. In that context, alternative pathways for H_2 utilization exist in rumen microbiomes, but an understanding of the microbial biology underpinning these pathways remains obscure.

This review focuses on the first of these three considerations. We hypothesize that ruminal methanogens possess gene product(s) that are highly conserved and are specific to their colonisation and persistence in the rumen. Identification of these gene product(s) will provide candidate targets for development of novel classes of methanogen inhibitors. We present some of our initial findings derived from application of genomic technologies with ruminant methanogens, and the new insights provided from these approaches.