Microbial contribution to and amelioration of enteric methane emissions from domestic herbivores

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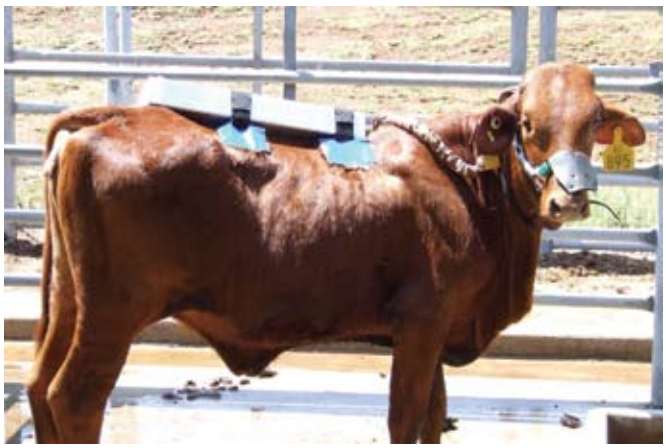
Global climate change is a major issue currently facing the international community. The primary cause of climate change arises from the human-induced increase in emission of the 'greenhouse' gases, primarily carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O). In animal agriculture, CH_4 and N_2O emissions predominate, especially CH_4. The largest source of CH_4 in the world is enteric methane from livestock (cattle and sheep), with enteric CH_4 accounting for 28% of total methane emissions. In countries reliant on agriculture for export earnings, such as Australia and New Zealand, enteric CH_4 is the major greenhouse gas from the agriculture sector and a significant contributor to total emissions (50% of New Zealand's and 14% of Australia's) [1]. Reducing CH_4 emissions is highly advantageous in delivering climate benefits in the shorter-term as CH_4 has a high radiative forcing potential (21 times that of CO_2) but a short atmospheric life (about 10 years), compared to 100 years for CO_2.



Measuring methane emissions from an individual animal using the SF6 tracer technique.

Microbial methane generation through anaerobic fermentation in the gut

Methane is generated during the digestion of plant material in the gastrointestinal tract of animals, particularly herbivores, including domestic ruminants. The generation of CH_4 is a result of the microbiological removal of hydrogen during the fermentative process.

Fermentation of plant material in the gut of herbivores takes place under anaerobic conditions [2]. Large polymeric plant components (e.g. cellulose and protein) are catabolised under reducing conditions to component monomers prior to further reduction to volatile fatty acids and organic acids. Through this process ATP is generated to satisfy the energy requirements of the microbial cell. The rumen of domestic ruminants is a gut ecosystem that has been extensively studied over the past 50 years [2] and it is known to support at least 200 distinct species of cultivable bacteria at densities up to 10^{11} mL^{-1} of contents, plus 25 genera of ciliate protozoa (10^6 mL^{-1}) and a variety of anaerobic fungi (10^5 zoospores mL^{-1}) [3]. This diversity of microbes and the differing biochemical pathways that they use to reductively capture nutrients and energy results in a great range of differing products; however, a constant feature in the generation of ATP from many of these differing pathways is the generation of hydrogen through transformation of co-factors such as NADH (to NAD+) [4, 5].

Were hydrogen to accumulate, enzymic co-factors would not be re-oxidised and further fermentation would be limited [5]. Therefore, it is important to the basic functioning of these complex microbial ecosystems that hydrogen is removed. In the gut of many herbivores, hydrogen is removed by methanogenesis. Methanogenesis is peculiar to microbes within the domain Archaea. To date, the Archaea isolated from gastrointestinal environments have all been methanogenic. Methanogenic Archaea reduce CO_2 under anaerobic conditions, generating energy for growth and maintenance of the methanogens.

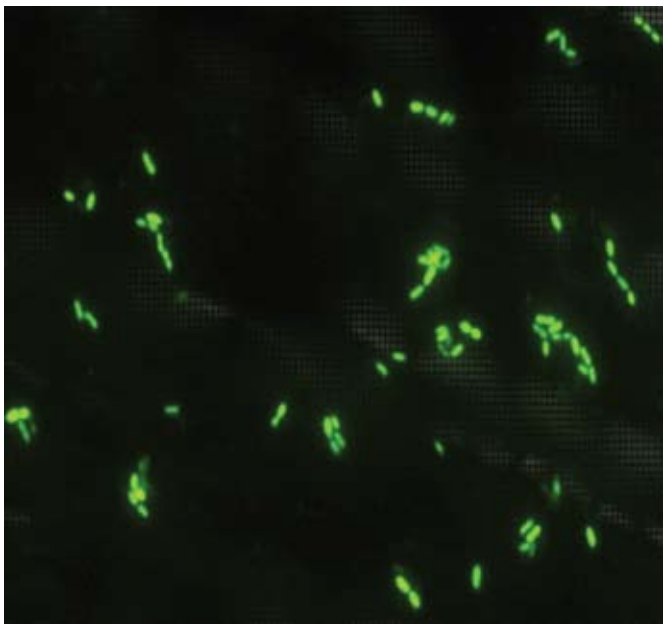
While methanogens from other environments synthesise methane from a variety of substrates (e.g. formate and acetate), rumen methanogens dominantly synthesise methane from CO_2 and

H_2 [6]. Species from three archaeal families (Methanobacteriaceae, Methanomicrobiaceae and Methanoplanaceae) have been isolated from ruminal contents [6]. However, the cultivable methanogens appear to be a small proportion of those present in the gut and many apparently novel species, genera and possibly families have been identified from 16S rRNA gene clone libraries and DNA sequence analysis [7-9]. Interestingly, the dominant methanogens in domestic livestock fall within a single genus, *Methanobrevibacter,* with the majority closely related to *Mbb. ruminantium,* at least according to 16S rRNA gene sequence similarity [7, 9].

Alternative microbial pathways that ameliorate the generation of methane in gut ecosystems

While it is necessary to remove excess hydrogen from anaerobic fermentation, it is not necessary that this occurs through the process of methanogenesis. A variety of alternative biochemical pathways do exist and in some circumstances these can be competitive with methanogenesis. Bacteria that reduce sulphates and nitrates, e.g. *Desulfovibrio* spp. and *Bacillus benzoevorans* [3, 10], consume hydrogen in the process and therefore reduce the availability of hydrogen for methanogenesis. Changing the species composition in the gut to favour those that produce propionate (which consumes hydrogen) as opposed to acetate (which yields hydrogen), as commonly occurs with feeding grain instead of pasture to ruminants, will also decrease methane emission [11]. However, the amount of hydrogen that it is possible to divert into these pathways is limited and unlikely to displace methanogenesis; however, there is another mechanism that does, in some circumstances, displace methanogenesis, namely reductive acetogenesis.

Reductive acetogenesis is mediated by bacteria and has been suggested as an alternative pathway for the disposal of hydrogen to methanogenesis [12-14]. With reductive acetogenesis, CO_2 is also reduced and hydrogen consumed, but instead of CH_4 being formed, acetate is formed and acetate can be used by the host animal as a source of energy. Reductive acetogenesis is undertaken by a broad range of genetically diverse bacteria [12, 13] and is the dominate pathway for the removal of hydrogen in a number of fermentative gut ecosystems [15-18].



Autofluorescence from *Methanobrevibacter* sp.

Wood eating termites are able to generate 33% of their total energy requirements from acetate produced by reductive acetogenesis in the hindgut [15]. Kangaroos ferment plant material in an enlarged forestomach prior to gastric digestion in a manner analogous to ruminants. Unlike ruminants, kangaroos emit very little methane [17]. Furthermore, PCR assays targeting archaea or a functional gene in the reductive acetogenesis pathway demonstrated that methanogens were below detection in eight kangaroos but all appeared to harbour populations of reductive acetogens [18]. In humans, rats and pigs [12], reductive acetogens and methanogens are present and co-exist, although reductive acetogenesis tends to dominate. Reductive acetogenesis also often out-competes methanogenesis in the ostrich hindgut (enlarged to allow for the fermentation of plant material). In the ostrich, reductive acetogenesis yields 25% of total acetate produced, which represents 7% of the bird's maintenance energy requirements [16].

Reductive acetogens are present in the rumen and the wide range of population densities reported ($<10^2$ to $>10^8$ per mL of rumen contents [12, 19]) indicates that, under some circumstances, reductive acetogenesis may be more significant in ruminants than currently accepted.

Why methanogenesis tends to dominate in the rumen and is out-competed by reductive acetogenesis in other related ecosystems is poorly understood. However, the interest in reducing greenhouse gas emissions from domestic livestock has led to considerable interest in trying to increase reductive acetogenesis at the expense of methanogenesis. Some limited success has been achieved, mainly in increasing existing ruminal acetogen populations; this has been more pronounced in studies where methanogenesis has been inhibited.

- Lopez *et al.* [20] added acetogenic bacteria to *in vitro* incubations of rumen contents with and without chemical inhibition of methanogenesis. When methanogenesis was inhibited, acetate increased and hydrogen accumulation decreased.
- With regular administration, *Ruminococcus productus* can compete with methanogens *in vitro* [21].
- Cell-free culture fluid from *Lactobacillus plantarum* 80, incubated with rumen contents, increased volatile fatty acid production (30%) and decreased CH_4 (15%). The effects were enhanced by the addition of *R. productus* and a reduction in methanogenesis by 80% was achieved *in vivo* but unfortunately this impact was short-lived [22].

Unfortunately, all current efforts to increase the role of reductive acetogens in the rumen have concentrated on boosting existing populations in ecosystems where these populations are naturally out-competed by methanogens. To date, no studies have reported whether reductive acetogens from ecosystems where they naturally out-compete methanogens could play a role in reducing emissions from cattle or sheep, nor of the mechanisms that allow these reductive acetogens to become the dominant hydrogen consumers in their natural environment. We are currently investigating reductive acetogens from the forestomach of red and grey kangaroos to understand the mechanisms that enable them to out-compete methanogens with a view to determining whether they have the attributes required to colonise the rumen of cattle and sheep and reduce methane emissions.

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From early 2009, Athol Klieve is Associate Professor in Animal Nutrition at the University of Queensland. This is a joint appointment with Queensland Primary Industries and Fisheries where he heads the Rumen Ecology Unit, as he has done over the past sixteen years.