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Does the complexity of the rumen microbial ecology preclude methane mitigation?

André-Denis G. Wright[a,*], Athol V. Klieve[b,c]

[a] Department of Animal Science, College of Agriculture and Life Sciences, University of Vermont, Burlington, VT 05405, USA
[b] Schools of Animal Studies and Veterinary Science, University of Queensland, Gatton, Queensland 4343, Australia
[c] Agri-Science Queensland, Department of Employment Economic Development and Innovation, Yeerongpilly, Queensland 4105, Australia

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ABSTRACT

Ruminant livestock are responsible for production of a portion of greenhouse gases, particularly methane (61 Tg/yr) which is believed to contribute to global warming and climate change. Methane is an end product of fermentation of plant material by the microbial ecosystem in the rumen. Methanogenesis is undertaken by methanogenic archaea and is a mechanism by which H_2 is removed from fermentation in order to regenerate biochemical co-factors such as NAD+. The microbial ecosystem is very complex and involves thousands of species of bacteria (10^{10}–10^{11} cells/ml), archaea (10^{7}–10^{9} cells/ml), protozoa (10^{4}–10^{6} cells/ml), fungi (10^{3}–10^{6} cells/ml), and viruses (10^{9}–10^{10} cells/ml), which interact with the feed, their host and each other. This ecosystem is relatively poorly understood, particularly inter-species interactions and interactions with the host. Less than 15% of the microbial species in the gastrointestinal tract have been cultured and characterised. However, knowledge of this ecosystem is accumulating, particularly with the advent of molecular biology and culture independent technologies. New high throughput sequencing methodologies, such as pyrosequencing, will greatly improve the rate of knowledge acquisition and techniques such as Stable Isotope Probing will enhance our ability to understand species inter-relationships. While we can expect an increase in our knowledge of this complex ecosystem, and an improved ability to predictably lower CH_4 emissions, examples of successful reductions already exist, including use of feeds (*e.g.*, cereal grains) and chemical additives (*e.g.*, 2-bromo-ethane sulfonate, bromochloromethane). Achieving meaningful reductions in CH_4 emissions may be possible with advances in our knowledge of the intricacies of this complex ecosystem.

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1. Introduction

In addition to industrial processes, current agricultural practices contribute to the global issue of greenhouse gas emissions. In many countries, ruminant livestock are the largest source of CH_4 emissions from the agricultural sector. There are

Abbreviations: VFA, volatile fatty acids.

* Corresponding author. Tel.: +1 802 656 0155; fax: +1 802 656 8196.
E-mail address: agwright@uvm.edu (A.-D.G. Wright).

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~1.1 billion large ruminants in the world (FAO, 2000), and during ruminal digestion (*i.e.*, enteric fermentation), ruminant animals, such as cattle, water buffalo, sheep and goats, convert fibrous plant materials into volatile fatty acids (VFA) and microbial protein, and thereby produce CH_4 as an end product. Enteric CH_4 is eructated by ruminants, with the amount emitted dependent on the animal's digestive system, particularly its resident microbiome, and the amount and type of diet consumed.

The Intergovernmental Panel on Climate Change Fourth Assessment Report revised the global warming potential of CH_4 from 23 to 25 times that of CO_2 (Forster et al., 2007). In Australia and America, enteric CH_4 accounts for ~70 and 73%, respectively, of agricultural CH_4 emissions (National Greenhouse Gas Inventory, 2010; Environmental Protection Agency, 2010). This equates to nearly 55.6 million tonnes CO_2 equivalents of CH_4 from Australian livestock and nearly 141 million tonnes of CO_2 equivalents of CH_4 from American livestock (National Greenhouse Gas Inventory, 2010; Environmental Protection Agency, 2010). Methane's potency as a greenhouse gas and its short atmospheric life compared to other greenhouse gases such as N_2O (~12 *versus* 114 years; Environmental Protection Agency, 2010) make it worthy of mitigation. In addition, CH_4 also represents a loss of feed energy from animal agriculture which, if captured, would result in greater efficiency, productivity and profitability in animal agriculture.

2. Rumen microbiota

The rumen is a unique environment that houses thousands of species of bacteria, methanogenic archaea, protozoa, fungi and viruses. These microbes form a dense (*e.g.*, bacterial cells are above 10^{11}/ml of contents), complex community of organisms that interact to play an important role in digestion of feed and the supply of energy and protein to the host in the form of VFA and microbial protein (Hungate, 1966). Not only is the ecosystem complex, it is also relatively poorly understood, particularly inter-species interactions and interactions with the host. It is estimated that less than 15% of the gastro-intestinal tract microorganisms have been cultured and characterised (Mackie et al., 2002).

However, knowledge of this ecosystem is rapidly accumulating, particularly with the advent of molecular biology and culture independent technologies. DNA 'fingerprinting' techniques such as Temperature Gradient Gel Electrophoresis and Denaturing Gradient Gel Electrophoresis are now commonly used to follow changes in microbial diversity and species composition in ruminal communities when ruminants are fed different diets or when physiological and other factors could be expected to exert an influence on these communities (Kocherginskaya et al., 2005; Klieve et al., 2007; Ouwerkerk et al., 2008). Denaturing Gradient Gel Electrophoresis has also been used to profile archaeal (*i.e.*, methanogen) populations and, combined with DNA cloning and sequencing, to identify methanogen species (Ouwerkerk et al., 2008; Wright et al., 2009). Real time PCR is enabling enumeration of taxonomic groupings from a single species to an entire domain of microbes (Klieve et al., 2003). Thus, the fate of introduced species and changes to specific populations of microbes can now be accurately quantified. Fluorescent *in situ* hybridisation (FISH) can enumerate specific populations as well as be used in conjunction with Denaturing Gradient Gel Electrophoresis and real time PCR to increase the robustness of population compositions, as well as the additional benefit of providing insight into spatial relationships between microbes and feed material (Milinovich et al., 2008).

New technologies are rapidly emerging that promise to enhance the rate at which knowledge of the ecosystem and its interrelationships will advance. Two of these are high throughput DNA sequencing methodologies, such as 454 pyrosequencing (Dowd et al., 2008; Pope et al., 2010), and Stable Isotope Probing (SIP; Dumont and Murrell, 2005). Pyrosequencing is a next generation sequencing platform initially used for whole genome sequencing which generates hundreds of thousands of short sequences (~100–500 bp in length) per sample. Development of barcoding (*i.e.*, attaching short pieces of DNA of known sequences) has allowed simultaneous sequencing of multiple samples and enabled the technology to become a major new tool for use in molecular ecology (Meyer et al., 2007; Parameswaran et al., 2007). Stable Isotope Probing will complement FISH and other technologies by determining the functionally active members of a community and the specific roles they play within the ecosystem. Stable Isotope Probing is achieved by introducing a substrate containing a stable isotope, usually C or N, into the ecosystem, wherein microbes which can use the substrate incorporate the label into their DNA. The heavier DNA can be physically separated from lighter DNA and sequenced using standard techniques to reveal the identity of the functionally active microbes (Date et al., 2010). This technique has not, as yet, been widely applied to gut ecosystems, but appears to be a tool that could help to understand functional relationships between microbes and dietary components.

2.1. Methanogens

In the past decade, there has been an increasing interest in the rumen microbiome, especially the methanogenic archaea, also called methanogens (*i.e.*, of Euryarcheota). This has primarily resulted from the role of these organisms in production of CH_4 by domesticated livestock. Enteric CH_4 is produced in the rumen of livestock when H_2, released by other microbes (*e.g.*, rumen protozoa, fungi) during fermentation of substrates, is used by methanogens to reduce CO_2 (Stewart et al., 1997).

Unlike bacteria, methanogenic archaea lack peptidoglycan in the cell wall, but they contain pseudomurein (*e.g.*, *Methanobrevibacter*, *Methanobacterium*), heteropolysaccharide (*e.g.*, *Methanosarcina)*, or protein (*e.g.*, *Methanomicrobium)* in their cell walls (Hobson and Stewart, 1997). Methanogen cell shapes vary, from rod shape with variable motility (*e.g.*, *Methanobrevibacter ruminantium*, *Methanomicrobium mobile*), or without motility (*e.g.*, *Methanobacterium formicicum*), to coccoid shaped (*e.g.*, *Methanosarcina barkeri*, *Methanococcus* spp.). Most methanogens (*e.g.*, *Methanobrevibacter, Methanobac-*