Animal (2013), **7:s2**, pp 235–243 © The Animal Consortium 2013
doi:10.1017/S1751731113000700

animal

Genome sequencing of rumen bacteria and archaea and its application to methane mitigation strategies

S. C. Leahy[1,2*†], W. J. Kelly[1*], R. S. Ronimus[1,2], N. Wedlock[2,3], E. Altermann[1] and G. T. Attwood[1,2]

[1]*AgResearch, Grasslands Research Centre, Palmerston North 4442, New Zealand;* [2]*New Zealand Agricultural Greenhouse Gas Research Centre, Palmerston North 4442, New Zealand;* [3]*AgResearch, Hopkirk Research Institute, Palmerston North 4442, New Zealand*

(Received 14 January 2013; Accepted 19 March 2013)

Ruminant-derived methane (CH_4), a potent greenhouse gas, is a consequence of microbial fermentation in the digestive tract of livestock. Development of mitigation strategies to reduce CH_4 emissions from farmed animals is currently the subject of both scientific and environmental interest. Methanogens are the sole producers of ruminant CH_4, and therefore CH_4 abatement strategies can either target the methanogens themselves or target the other members of the rumen microbial community that produce substrates necessary for methanogenesis. Understanding the relationship that methanogens have with other rumen microbes is crucial when considering CH_4 mitigation strategies for ruminant livestock. Genome sequencing of rumen microbes is an important tool to improve our knowledge of the processes that underpin those relationships. Currently, several rumen bacterial and archaeal genome projects are either complete or underway. Genome sequencing is providing information directly applicable to CH_4 mitigation strategies based on vaccine and small molecule inhibitor approaches. In addition, genome sequencing is contributing information relevant to other CH_4 mitigation strategies. These include the selection and breeding of low CH_4-emitting animals through the interpretation of large-scale DNA and RNA sequencing studies and the modification of other microbial groups within the rumen, thereby changing the dynamics of microbial fermentation.

Keywords: genome sequencing, methane mitigation, methanogens, rumen bacteria

Implications

Development of CH_4 mitigation strategies for ruminants without disrupting normal digestive function and reducing productivity is a major challenge. Genome sequencing is an effective way of gaining information on how rumen methanogens and bacteria interact and contribute to rumen function. This knowledge will be important when considering the design and implementation of ruminant CH_4 abatement strategies. Genomic information is already contributing to vaccine and small molecule inhibitor programmes that are aimed at reducing ruminant CH_4 emissions, but it will also underpin the analysis and comprehension of metagenomic sequence data sets, allowing the generation of testable hypotheses to gain a better understanding of rumen biology.

Introduction

Ruminants are foregut fermenters and have evolved an efficient digestive system in which microbes ferment plant fibre and provide fermentation end-products and other nutrients for growth of the animal (Clauss *et al.*, 2010). Feed ingested by ruminants is fermented by a complex microbial community, which includes bacteria, ciliate protozoa, anaerobic fungi, archaea and viruses. The rumen is the first and largest foregut compartment and is the main site for microbial fermentation. The microbial ecology of the rumen has been the focus of numerous studies (reviewed by McSweeney and Mackie, 2012), but very little information is available regarding the microflora of the ruminant oral cavity and lower gastrointestinal tract. The rumen provides optimal conditions for microbes to achieve rapid breakdown of complex plant polysaccharides, with fermentation of the released sugars to produce short-chain fatty acids (SCFAs), principally acetic, propionic and butyric acids. Microbial disposal of hydrogen (H_2) generated as a fermentation end-product is essential to the optimum functioning of the ruminant digestive system (Hungate, 1967). Methanogenic archaea use H_2 derived from polysaccharide breakdown as their energy source and combine it with carbon dioxide (CO_2) to form methane (CH_4), which is belched from the animal and released to the atmosphere. Other fermentation end-products

* Contributed equally to this review.
† E-mail: Sinead.Leahy@agresearch.co.nz

including formate and methyl-containing compounds are also important substrates for methanogenesis in some organisms (Hungate *et al.*, 1970; Liu and Whitman, 2008). Ruminant-derived CH_4 accounts for about one quarter of all anthropogenic CH_4 emissions (Thorpe, 2009), and is implicated as a driver of global climate change. The development of strategies to reduce CH_4 emissions from farmed animals are currently being investigated (reviewed by Hook *et al.*, 2010; Martin *et al.*, 2010; Buddle *et al.*, 2011).

Methanogens are the sole producers of ruminant CH_4, and therefore CH_4 abatement strategies can either target the methanogens themselves or target the other members of the rumen microbial community that produce substrates necessary for methanogenesis. Consequently, exploring the relationship that methanogens have with other rumen microbes is crucial when considering CH_4 mitigation strategies for farmed ruminants, but this area remains poorly understood. Genome sequencing is an important tool to gain knowledge on both of the methanogens, and of the processes that underpin the interactions between methanogens and other rumen microbes. The ability to sequence a genome provides access to its entire gene repertoire from which its metabolic capability and mode of life can be predicted. Currently, several rumen bacterial and archaeal genome projects are either complete or underway, and the use of information from these studies in the development of methane mitigation strategies is the subject of this review.

Rumen bacterial genome projects

Following the development of techniques for cultivating strictly anaerobic organisms, a variety of bacteria were isolated from the rumen and characterized on the basis of their morphology and phenotypic properties (Bryant, 1959). Over the years >100, different bacterial species have been reported in the scientific literature. The development of cultivation-independent techniques led to a decline in isolation and cultivation studies, and results from 16S ribosomal RNA gene sequencing and metagenomic studies consistently emphasize that the majority of sequences are derived from organisms that are phylogenetically distinct from currently cultivated species (Kim *et al.*, 2011; Fouts *et al.*, 2012). In recent years, there have been few attempts to systematically bring additional organisms into cultivation; however, recent studies in Japan (Koike *et al.*, 2010; Nyonyo *et al.*, 2013) and New Zealand (Kenters *et al.*, 2011; S.J. Noel *et al.*, unpublished) have successfully increased the number of taxa of rumen bacteria that have cultured representatives. Nevertheless, the full spectrum of bacterial diversity in the rumen is only beginning to be revealed.

At present, genome sequence information is publicly available for a small number of rumen bacteria (Table 1), including several that are not regarded as major contributors to rumen function. These have been studied for a variety of reasons including improving fermentative succinate production

Table 1 *Publicly available genome sequences of rumen bacteria*

Bacteria	Strain	Genome size (bp)	Status	Family	Reference
Actinobacillus succinogenes	130Z	2 319 663	Complete	Pasteurellaceae	McKinlay *et al.* (2010)
Basfia succiniciproducens	MBEL55E	2 314 078	Complete	Pasteurellaceae	Hong *et al.* (2004)
Butyrivibrio proteoclasticus	B316	4 404 886	Complete	Lachnospiraceae	Kelly *et al.* (2010)
Desulfotomaculum ruminis	DSM 2154	3 969 014	Complete	Peptococcaceae	Spring *et al.* (2012)
Desulfovibrio desulfuricans subsp. *desulfuricans*	ATCC 27774	2 873 437	Complete	Desulfovibrionaceae	Gc00931[1]
Eubacterium cellulosolvens	6	3 384 656	Complete	Lachnospiraceae	Gi03244
Fibrobacter succinogenes subsp. *succinogenes*	S85	3 842 635	Complete	Fibrobacteraceae	Suen *et al.* (2011a)
Fibrobacter succinogenes subsp. *succinogenes*	S85	3 843 004	Complete	Fibrobacteraceae	Gc01427
Lactobacillus ruminis	ATCC 27782	2 066 657	Complete	Lactobacillaceae	Forde *et al.* (2011)
Megasphaera elsdenii	DSM 20460	2 474 718	Draft	Veillonellaceae	Marx *et al.* (2011)
Oscillibacter ruminantium	GH1	3 078 743	Draft	Ruminococcaceae	Lee *et al.* (2012)
Porphyromonas levii	DSM 23370	2 516 447	Draft	Porphyromonadaceae	Gi11783
Prevotella bryantii	$B_1$4	3 592 891	Draft	Prevotellaceae	Purushe *et al.* (2010)
Prevotella ruminicola	23	3 619 559	Complete	Prevotellaceae	Purushe *et al.* (2010)
Ruminococcus albus	7	4 482 087	Complete	Ruminococcaceae	Suen *et al.* (2011b)
Ruminococcus albus	8	4 373 730	Draft	Ruminococcaceae	Gi00517
Ruminococcus flavefaciens	FD-1	4 573 608	Draft	Ruminococcaceae	Berg Miller *et al.* (2009)
Ruminococcus flavefaciens	17	3 450 000	Draft	Ruminococcaceae	Berg Miller *et al.* (2012)
Ruminococcus flavefaciens	007C	3 713 731	Draft	Ruminococcaceae	Sanger Institute[2]
Selenomonas ruminantium subsp. *lactilytica*	TAM6421	3 003 680	Complete	Veillonellaceae	Gc02184
Slackia heliotrinireducens	DSM 20476	3 165 038	Complete	Coriobacteriaceae	Pukall *et al.* (2009)
Synergistes jonesii	ATCC 49833	2 742 000	Draft	Synergistaceae	Gi01742
Treponema saccharophilum	DSM 2985	3 449 536	Draft	Spirochaetaceae	Gi05359
Treponema sp.	JC4	3 033 760	Draft	Spirochaetaceae	Rosewarne *et al.* (2012)
Wolinella succinogenes	DSM 1740	2 110 355	Complete	Helicobacteriaceae	Baar *et al.* (2003)

Draft genomes assembled from metagenomic data (Hess *et al.*, 2011) are also available for rumen bacteria belonging to the following orders: Bacteroidales (5), Clostridiales (7), Myxococcales (1) and Spirochaetales (2). [1]Genomes online database (http://www.genomesonline.org/cgi-bin/GOLD/index.cgi).
[2]Data available at http://www.sanger.ac.uk/resources/downloads/bacteria/ruminococcus-flavefaciens.html.

(Hong *et al.*, 2004; McKinlay *et al.*, 2010), understanding their role in environmental sulphate reduction, characterization of taxonomically unusual organisms (Pukall *et al.*, 2009) and lactate utilization and potential probiotic usage (Marx *et al.*, 2011). However, most bacteria have been investigated because of their role in the processes of plant polysaccharide degradation and SCFA production, which are central to the growth and productivity of ruminant animals. Consequently, some of the first genomes to be sequenced from the rumen were those of cultivated bacteria considered to have key roles in the breakdown of the cellulose (*Fibrobacter* and *Ruminococus*) and hemicellulose (*Butyrivibrio* and *Prevotella*) components of plant cell walls. As expected, these fibrolytic bacteria have a particularly wide range of degradative abilities and produce acetate, butyrate and propionate that can be used by the animal, as well as CO_2, formate, methanol and H_2, which are substrates for methanogenesis. Other bacteria may not initiate polysaccharide breakdown, but contribute to the supporting cast that facilitates the degradation process. Genome sequence information is available for members of the genera *Eubacterium*, *Oscillobacter*, *Selenomonas* and *Treponema*, which are known to use a wide array of carbohydrates.

Although these sequenced bacteria include 21 species belonging to 14 different bacterial families, they only scratch the surface of the taxonomic and functional diversity present in the rumen. Estimates of the number of rumen microbial species based on 16S ribosomal RNA gene sequences vary from 300 to 1000 (Fouts *et al.*, 2012). Consequently, genomic studies have yet to make a sizeable impact with respect to improving our understanding of rumen microbial ecology, and how specific rumen microbes interact with the animal, the feed or each other to influence animal production or the release of greenhouse gases. In addition, there remains considerable opportunity for studies on gene expression (Dodd *et al.*, 2010), and for co-culturing studies to determine inter-species interactions (Leahy *et al.*, 2010). To develop a foundation to address some of these issues, the Hungate1000 project has been launched (www.hungate1000.org.nz). This initiative aims to generate a comprehensive reference set of rumen microbial genome sequences from cultivated rumen bacteria and methanogenic archaea, together with representative cultures of rumen anaerobic fungi and ciliate protozoa. This project will expand our knowledge of the rumen microbiome significantly.

Rumen methanogen genome projects

A variety of methanogens can be found in the rumen (Figure 1) and are estimated to comprise ~0.3% to 3% of the biomass. Surveys of archaeal 16S ribosomal RNA gene sequences from ruminants around the world fed a variety of diets show that three methanogen groups dominate. These are *Methanobrevibacter* spp., *Methanomicrobium* spp. and Rumen Cluster C (RCC), also known as Thermoplasmatales-affiliated lineage C but more recently proposed as a seventh order of methanogenic archaea, the 'Methanoplasmatales' (Paul *et al.*, 2012). Remaining groups of methanogens include representatives of the genera *Methanosphaera*, *Methanimicrococccus*, *Methanosarcina* and *Methanobacterium* (Janssen and Kirs, 2008; St-Pierre and Wright, 2013). In terms of their metabolism, methanogens typically fall into three groups, hydrogenotrophic methanogens that convert H_2 and/or formate to CH_4 and that include members of the genera *Methanobrevibacter*, *Methanomicrobium* and *Methanobacterium*, methylotrophic methanogens that metabolize H_2 plus methyl compounds to CH_4 and that include members of the genera

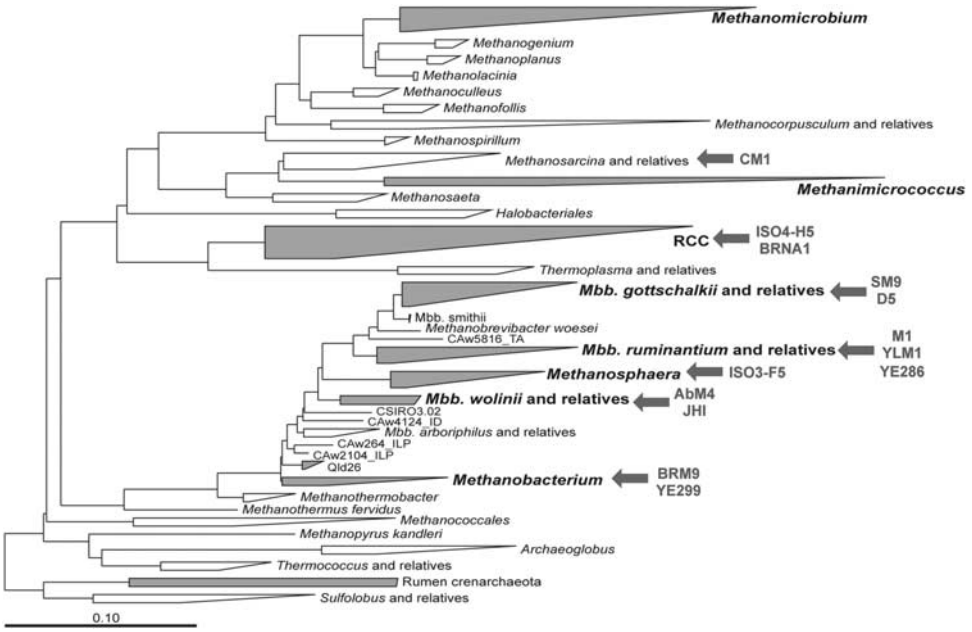

Figure 1 Phylogenetic dendrogram illustrating rumen archaea. Arrows indicate where current genome sequencing efforts are based, strains names are listed. Figure adapted from Janssen and Kirs, (2008).

Table 2 *Rumen methanogen genome sequencing projects (adapted from the study by Morgavi* et al.*, 2013)*

Genome project	Origin	Status	Country
Methanobrevibacter ruminantium M1	Bovine	Published	New Zealand
Methanobrevibacter sp. YLM1	Ovine	Draft	New Zealand
Methanobrevibacter sp. YE286	Bovine	Draft	Australia
Methanobrevibacter sp. SM9	Ovine	Complete	New Zealand
Methanobrevibacter sp. D5	Ovine	Draft	New Zealand
Methanobrevibacter sp. AbM4	Ovine	Complete	New Zealand
Methanobrevibacter sp. JHI	Bovine	Draft	Korea
Methanobacterium sp. YE299	Bovine	Draft	Australia
Methanobacterium sp. BRM9	Bovine	Complete	New Zealand
Methanosphaera sp. ISO3-F5	Ovine	Draft	New Zealand
Methanosarcina sp. CM1	Bovine	Complete	New Zealand
Rumen Cluster C sp. BRNA1	Bovine	Complete	Australia
Rumen Cluster C sp. ISO4-H5	Ovine	Complete	New Zealand

Methanosphaera, and *Methanimicrococcus*, together with the RCC organisms, and acetoclastic methanogens that utilize acetate and include members of the genus *Methanosarcina*. Obtaining representative genome sequences from each of these genera will be important to understand the metabolic capacity of these types of organisms and how they contribute to rumen fermentation processes.

Very few rumen methanogen cultures have been deposited in culture collections, and therefore the choice of well-characterized methanogen candidates for genome sequencing is limited. In recent years, new species of rumen *Methanobrevibacter* have been described (Rea *et al*., 2007), and groups in Australia and New Zealand have active programmes to isolate additional rumen methanogen cultures. Organisms within the large uncultured RCC group are of particular interest and may be targeted as enrichment cultures if they cannot be isolated in pure culture. The slow growth of rumen methanogens *in vitro*, low cell yields, difficulty in lysing cultures with pseudomurein-containing cell walls and isolate contamination are compounding issues to conduct genomic research on rumen methanogens. At the time of writing, there are two rumen methanogen genome sequences publicly available. In addition, researchers in New Zealand and Australia have several rumen methanogen genome projects that are in progress, or nearing completion (Table 2). The first rumen methanogen genome sequence was *Methanobrevibacter ruminantium* M1 (Leahy *et al*., 2010). *M. ruminantium* M1 (DSM1093) was isolated from bovine rumen contents by Bryant (1965) and is designated the neotype strain for this species. The 2.93 Mb genome of *M. ruminantium* M1 revealed an intriguing insight into the lifestyle of a rumen methanogen. A prophage genome was described and its lytic enzyme, endoisopeptidase PeiR, was shown to lyse M1 cells in pure culture. A predicted stimulation of M1 growth by alcohols was confirmed and microarray analysis indicated the upregulation of methanogenesis and formate utilization genes during co-culture with *Butyrivibrio proteoclasticus*, a formate and H_2-producing rumen bacterium. The identification of non-ribosomal peptide synthetase genes in *M. ruminantium* M1, the first reported in an archaeal species, was also an unexpected discovery. Since its publication, the M1 genome has been used to identify genes and proteins that can be targeted to reduce CH_4 production through vaccine and chemogenomic-based approaches (see following sections). The second genome sequence is that of *Methanobrevibacter* sp. JHI, isolated from the rumen of a native Korean cow (Lee *et al*., 2013). Analysis of the JHI draft genome shows it to be highly syntenous with the genome of *Methanobrevibacter* sp. AbM4 (Supplementary Figure S1), suggesting that these two organisms belong to the same species and are most similar phylogenetically to *Methanobrevibacter wolinii* (Figure 1). AbM4 was isolated from the abomasal contents of a New Zealand sheep and its genome is complete (S. C. Leahy *et al*., unpublished). A preliminary examination of the gene content of JHI and AbM4 highlights their similarity (Supplementary Figure S2). However, one striking difference is the presence of a prophage in the JHI genome that is not present in AbM4. Other rumen methanogens also contain prophage, but the role of these genetic elements in rumen microbial ecology is not understood. Comparison of the JHI and AbM4 genomes with the much larger M1 genome reveals that their gene content is largely comparable and suggests that the central metabolism and the methanogenesis pathway of the these strains is similar. All three genomes lack the coenzyme M reductase II (*mrt*) genes that are present in many other hydrogenotrophic methanogens. There are, however, some differences in cofactor biosynthesis. For example, both AbM4 and JHI contain a complete coenzyme M biosynthesis pathway in contrast to M1. With several rumen methanogen genome sequencing projects either completed or underway, comparative and pan-genomic studies will become more important, and these will help in determining the degree of conservation among genes involved in the methanogenesis pathway and the biosynthesis of methanogenic coenzymes in rumen methanogenic archaea. The genome sequences of the rumen methanogens discussed above have given us an insight into the likelihood that rumen methanogens may

occupy different niches within the rumen environment. Understanding these niche-defined roles will be important for any anti-methanogen mitigation strategy to be successful.

Methane mitigation

Advances being brought about through individual genome sequencing of rumen bacteria and archaea serve to broaden our understanding of the relationship that methanogens have with other rumen microbes. This information is helping researchers redefine and refine various approaches to CH_4 mitigation strategies.

Vaccines

Vaccines are commonly used to prevent diseases in livestock, have a proven record of prolonged efficacy, are relatively easy to administer and can be produced at a low cost. They offer green solutions to animal health problems as they are sustainable and can reduce reliance on pharmaceutical drugs and pesticides (Innes *et al.*, 2011). Therefore, vaccination of farmed ruminants against rumen methanogens offers an attractive approach to reduce their CH_4 emissions. The mode of action of a vaccine would be through the generation of antibodies to selected methanogen antigens that enter the rumen via saliva and bind to targets on the surface of rumen methanogens inhibiting their ability to produce CH_4. Given the diversity of methanogens in the rumen, a vaccine will need to contain protein antigens that have vital cellular functions and are cross-reactive for a range of ruminal methanogens. Vaccines based on whole methanogen cells have had limited success at reducing CH_4 emissions in sheep (Wright *et al.*, 2004), which may be because of a failure of this type of vaccine to produce cross-reactive antibodies. A recent strategy for vaccine development is based on identifying relevant methanogen protein antigens, which can be used for a sub-unit vaccine (Wedlock *et al.*, 2013). Such antigens need to be accessible for antibody binding, and thus vaccine candidates could include membrane associated/surface exposed proteins and adhesin-like proteins. The two main approaches to identifying such vaccine candidates have been the identification of strongly immunogenic proteins using Western blot analysis with sheep antisera raised against various methanogen fractions and the prediction of suitable vaccine antigens through bioinformatic analysis of methanogen genomes (Wedlock *et al.*, 2013). Although the former approach has identified a number of immunogenic proteins, few of these have had the attributes of a good vaccine candidate. Conversely, the availability of genomic sequences has provided guidance in the purposeful selection of potential vaccine antigens. Modern vaccine development has seen a change towards genome-based 'Reverse Vaccinology' approaches that use high-throughput *in silico* screening of an organism to identify genes that encode proteins with the attributes of a good vaccine target (Dormitzer *et al.*, 2012). This approach to identify targets for a methanogen vaccine has already proven fruitful, as sequence-based bioinformatic analysis of *M. ruminantium* M1 has led to the identification of a large number of potential vaccine targets for evaluation and, to date, two lead candidates (Leahy *et al.*, 2010; Wedlock *et al.*, 2013). The increasing number and diversity of rumen methanogen genomes available for comparative and pan-genomic studies will make this approach more powerful and will greatly assist in the search for cross-reactive and efficacious vaccine antigens.

Small molecule inhibitors

Genomic information also forms the basis of a second CH_4 mitigation strategy using small-molecule inhibitors to target enzymes essential for the growth of methanogens. An ideal inhibitor would be methanogen specific, target all methanogens in the rumen, be non-toxic to the host, not significantly affect microbes involved in fibre degradation, environment friendly, not accumulate in host tissues and cost-effective (Van Nevel and Demeyer, 1995). There are two established enzyme-based strategies to find novel inhibitors that would satisfy the above criteria. These are the *in silico* use of enzyme structure data using modelling software to screen large chemical compound libraries or the *in vitro* screening of large-scale diverse chemical compound libraries using enzyme assays. The combined use of these two strategies is a widely recognized approach used to identify and develop novel inhibitors. Enzyme-based strategies require the identification of suitable target enzymes using genome sequence data, metabolic pathway analysis and analysis of relevant literature followed by the cloning, expression and purification of target enzymes. The purified recombinant enzymes are used for identifying optimal crystal formation conditions to aid their subsequent structural determination and development of assays compatible with high-throughput screening. Any potential inhibitors need to be further tested against pure cultures of methanogens, followed by rumen fluid-based *in vitro* assays and their toxicological properties assessed before attempting animal trials. A third possible strategy is phenotypic screening, whereby methanogen cells would be directly screened against arrays of inhibitors. The advantage of this strategy is that all essential genes would be targeted simultaneously. However, this approach is technically difficult, given the cumbersome techniques required to grow methanogens, their inherently slow growth kinetics and their extreme sensitivity to oxygen.

Historically, a number of studies using various halogenated compounds have demonstrated the 'proof of principle' of small molecule inhibitors, especially in shorter-term animal experiments; however, unfortunately, the inhibitors are either unacceptable because of environmental or toxicology concerns, or tend to become less effective over time (Johnson *et al.*, 1972; Czerkawski and Breckenridge, 1975; Van Nevel and Demeyer, 1995; McCrabb *et al.*, 1997).

One consideration with a small molecule inhibitor approach is the requirement to accommodate the metabolic diversity seen in rumen methanogens. Genomic comparisons of existing rumen methanogens have already revealed enzymatic differences between the hydrogenotrophic and

methylotrophic rumen methanogens (S.C. Leahy *et al.*, unpublished). This reiterates the importance of obtaining genomic information from as wide a variety of rumen methanogens as possible. It is clear that inhibitory compounds will need to target specific phylogenetic groups, for example, the RCC, or *Methanobrevibacter* clades (Figure 1). It is envisaged that suites of inhibitors could lead to stronger and more sustained suppression of CH_4 emissions and an increased ability to counter any resistance development.

Animal breeding

Individual animals within populations of ruminant livestock show natural variation in phenotypic traits, and this variation is the basis for selection of individuals with desired traits in breeding programmes. Direct measurements of CH_4 emitted from sheep indicate that there is a natural variation between individual animals in the amount of CH_4 produced per unit of feed eaten (Pinares-Patiño *et al.*, 2003 and 2011a). Similarly, variation in efficiency of feed conversion as measured by residual feed intake (RFI) in cattle has previously been correlated with CH_4 emissions. Cattle with higher feed efficiencies are reported to produce 20% to 30% less CH_4 (Zhou *et al.*, 2009). The differences observed in ruminant CH_4 output as measured directly or as part of RFI studies, appear to be repeatable and persistent over time, and therefore an altered, but stable rumen metabolism seems to be involved, supporting the notion that a rumen microbial component contributes at least a part of the animal CH_4-emission phenotype. Logically, altered CH_4 output must involve some change in the population or activity of rumen methanogens, for example, as a result of altered substrate supply. Therefore, a change in methanogen community structure can be expected to correlate with a change in the bacterial community, which produces these substrates. Preliminary observations of rumen samples from high and low CH_4-emitting ruminants showing differences in both rumen bacteria and methanogens support such an idea. These observations open the possibility of selecting for low CH_4-emitting ruminants via animal breeding (Clark, 2013).

The exact mechanism(s) responsible for the variation in animal CH_4 emissions are currently unknown, but it is likely to have both animal and rumen microbial components. Research has indicated that the ruminal microflora contributes to host feed efficiency, but the effect is modulated by diet. Guan *et al.* (2008) showed that the microbial profiles generated from feed-efficient steers clustered together and observed higher concentrations of the SCFA fermentation end-products, butyrate and valerate, in the efficient steers. They also observed that bacterial profiles were more likely clustered within a certain breed, suggesting that host genetics may play a role in rumen microbial structure. Subsequently, Hernandez-Sanabria *et al.* (2012) examined whether specific bacterial groups were associated with cattle feed efficiency, and whether these relationships are affected by the host diet. Steers belonging to the same RFI group under both low-energy and high-energy diets were used to identify specific bacterial phylotypes related to feed-efficiency traits. Similar conclusions were drawn from a study by Carberry *et al.* (2012). Analysis of the methanogenic community by Zhou *et al.* (2009) using 16S ribosomal RNA gene sequences from feed efficient and inefficient cattle showed differences in variability and diversity. No difference was detected in total numbers of methanogens but the prevalence of organisms similar to *Methanosphaera stadtmanae* and *Methanobrevibacter* sp. strain AbM4 were two times higher in inefficient animals. In a further study, Zhou *et al.* (2010) examined ruminal methanogenic profiles in beef cattle differing in feed efficiency as well as diet (low-energy diet *v.* high-energy diet) using polymerase chain reaction denaturing gradient gel electrophoresis. For each diet, the methanogenic pattern was strongly associated with the feed efficiency of the host. Again, the total methanogen numbers did not correlate with differences in feed efficiency, diet or metabolic measurements, implying that the methanogenic community structure was more important for determining host feed efficiency under different dietary conditions.

Few studies have assessed rumen microbiomes in animals naturally divergent in CH_4 emissions, mainly because of the lack of animals with suitable CH_4 measurements. The development and use of respiration chambers for ruminants has enabled accurate and repeatable CH_4 measurements and work by researchers in New Zealand has resulted in the selection of high and low CH_4-emission lines in sheep (Pinares-Patiño *et al.*, 2011a and 2011b). The advent of high-throughput DNA sequencing technology has enabled the characterization of the rumen microbial environment to a great sequencing depth (Hess *et al.*, 2011). This approach is now being used to deep sequence the metagenome and metatranscriptome of rumen samples collected from animals with divergent CH_4 outputs. It is hoped that this work will identify rumen microbes associated with high and low CH_4 emissions in sheep and cattle, and will give a defined set of microbial genes, enzymes, metabolic pathways and genomes correlated with CH_4 production providing a large and useful public data set for targeting CH_4 reductions in ruminants. Availability of genome sequence information from individual rumen microbes will be vital to interpret large data sets of this nature.

Manipulation of the rumen microbiome

Interactions between fermentative organisms and methanogens are not well understood, but other functional groups of microbes are likely to have a strong influence on CH_4 production in the rumen, either because they produce substrates essential to methanogen survival, or because they affect the numbers of methanogens or other members of the microbiota, which produce methanogenic substrates. Modifying other microbial groups in the rumen, thereby changing the dynamics of rumen fermentation may offer the possibility of reducing CH_4 output.

Hydrogen is necessary for methanogenesis and this has led to proposals that other organisms that compete for H_2 could be used to reduce CH_4 production. In particular, bacteria capable of reductive acetogenesis (homoacetogens) use the Wood–Ljungdahl pathway to combine CO_2 and H_2 to

form acetate, which is available to the animal. The process is not energetically favoured by conditions found in the mature rumen but some homoacetogens have been reported to compete successfully with methanogens *in vitro* (Joblin, 1999; Wright and Klieve, 2011). Several homoacetogens have been isolated from the rumen, but analyses of sequences of formyltetrahydrofolate synthetase, a key enzyme of the Wood–Ljungdahl pathway, indicated that additional species remain uncultured (Henderson *et al.*, 2010). The genome sequence of a rumen acetogen *Eubacterium limosum* SA11 (W. J. Kelly *et al.*, unpublished) shows that the Wood–Ljungdahl pathway is present together with genes that enable a wide range of other substrates to be utilized including methanol and other methyl-containing compounds. Acetogenic bacteria are thought to be the dominant hydrogenotrophs in early rumen microbiota (Fonty *et al.*, 2007; Gagen *et al.*, 2012). Understanding the ecology of hydrogenotrophs in the developing digestive tract of ruminants may reveal key features that lead to the prevalence of methanogens and the restriction of homoacetogens in the adult rumen, and present new opportunities to programme microbial populations for later life through probiotic and prebiotic style approaches.

Alternatively, understanding the relationship between microbes and methanogens in the rumen could lead to approaches that reduce CH_4 production without adversely affecting normal rumen function and strategies for achieving this have been reviewed in a study by McAllister and Newbold (2008). Methanogens are dependent on the other rumen microbes to provide them with substrates essential for survival and growth. Redirecting fermentation, so that the availability of methanogenic substrates is affected, will be central to this approach. For example, studies with gnotobiotic lambs have shown that CH_4 is reduced in the rumen when the dominant fibrolytic species is a non-H_2-producer (Chaucheyras-Durnand *et al.*, 2010). Direct-fed microbials may offer a route to affecting substrate availability, and the use of bacteria involved in lactate production and utilization are hypothesized to influence CH_4 production (Seo *et al.*, 2010). *In vitro*, methanogens are known to grow better syntrophically (Sakai *et al.*, 2009). Indeed, many new rumen isolates are obtained as enrichment cultures, suggesting a co-dependence or a close association with other rumen microbes. An important area of investigation will be in elucidating which genes are involved in mediating interactions (particularly H_2, formate or methyl group transfer) between methanogens and other members of the rumen microbiome. Further characterization of the fundamental microbial biochemistry of substrate production in the rumen may provide insight for the development of effective strategies for reducing CH_4 emissions from ruminants. Homoacetogens, probiotic bacteria and methanogenic syntrophic partners are among the organisms targeted in the first phase of the Hungate1000 initiative.

Future prospects

Reducing ruminant-derived CH_4 and developing strategies that endeavour to modify microbial communities that have co-evolved with their ruminant hosts represents a considerable challenge to researchers. To be successful, a thorough understanding of microbial fermentation not just of the rumen but of the entire ruminant digestive tract will be required. Researchers have only begun to fully describe the microbial communities of the rumen and elucidate the extent of the interactions between host and microbiota. However, much remains to understand how microbes have an impact on the remainder of the ruminant digestive tract. For example, hindgut fermentation in ruminants typically provides 5% to 10% of dietary energy and is responsible for 6% to 14% of CH_4 output (Gressley *et al.*, 2011). The role hindgut microbes have to play in ruminant nutrition remains largely unstudied and may present researchers with new avenues for CH_4 reduction not previously considered. The principles and mechanisms that underlie microbial community structure and host–microbe relationships in the ruminant are complex. However, development of the host–microbe relationship begins at birth and continues throughout adult life. Incomplete information exists about the microbial diversity and colonization dynamics that occur during the developmental stage of a young ruminant. It is conceivable that early-life microbial intervention represents the ideal time point to manipulate the indigenous microbial populations of the ruminant to influence the lifelong environmental impact and productivity of the animal. As such, an appreciation of the microbes of the young ruminant and their colonization pattern will be essential to any strategy targeted for that time point.

As researchers forge ahead with the design and development of strategies that target ruminant-derived CH_4, it is clear that whole genome sequences of rumen microbes will be essential for revealing how the metabolic potential of the microbiome has an impact on the animal and the environment. Furthermore, complete genomes will assist in the taxonomic and functional assignments of data from DNA and protein-sequencing studies. Progress in rumen metagenomics has been the subject of a recent review (Morgavi *et al.*, 2013), and particular questions of interest are whether the presence of individual microbial species or particular microbial groups correlates with animal genotype, with high or low feed conversion-efficiency animals, or with high or low CH_4-producing animals. Reference genome data sets will be crucial for the analysis of any 'omics' style research.

Genome sequencing of rumen microbes through the Hungate1000 initiative will present microbiologists with new opportunities to understand rumen function, feed conversion efficiency and plant cell wall degradation with the goals of finding the balance between food production and greenhouse gas emissions. Future farming systems may have to consider farming for both the livestock and their microbes.

Acknowledgements

The authors gratefully acknowledge the financial support for the methanogen genome sequencing projects from the following New Zealand funding sources: The NZ Pastoral Greenhouse Gas Research Consortium, NZ Ministry for Agriculture and Forestry Subtainable Land Management Mitigation and Adaptation to

Climate Change and the NZ Agricultural Greenhouse Gas Research Centre. The Hungate1000 project is funded by the New Zealand Government in support of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases (http://www.globalresearchalliance.org/), and forms part of the Rumen Microbial Genomics Network (http://rmgnetwork.org.nz/) also funded by the New Zealand Government in support of the Livestock Research Group of the Global Research Alliance on Agricultural Greenhouse Gases. The Hungate1000 sequencing effort is supported by the US Department of Energy Joint Genome Institute Community Sequencing Program.

This paper was published as part of a supplement to *animal*, publication of which was supported by the Greenhouse Gases & Animal Agriculture Conference 2013. The papers included in this supplement were invited by the Guest Editors and have undergone the standard journal formal review process. They may be cited. The Guest Editors appointed to this supplement are R. J. Dewhurst, D. R. Chadwick, E. Charmley, N. M. Holden, D. A. Kenny, G. Lanigan, D. Moran, C. J. Newbold, P. O'Kiely, and T. Yan. The Guest Editors declare no conflict of interest.

Supplementary materials

For supplementary materials referred to in this article, please visit http://dx.doi.org/10.1017/S1751731113000700

References

Baar C, Eppinger M, Raddatz G, Simon J, Lanz C, Klimmek O, Nandakumar R, Gross R, Rosinus A, Keller H, Jagtap P, Linke B, Meyer F, Lederer H and Schuster SC 2003. Complete genome sequence and analysis of *Wolinella succinogenes*. Proceedings of the National Academy of Science USA 100, 11690–11695.

Berg Miller ME, Yeoman CJ, Chia N, Tringe SG, Angly FE, Edwards RA, Flint HJ, Lamed R, Bayer EA and White BA 2012. Phage–bacteria relationships and CRISPR elements revealed by a metagenomic survey of the rumen microbiome. Environmental Microbiology 14, 207–227.

Berg Miller ME, Antonopoulos DA, Rincon MT, Band M, Bari A, Akraiko T, Hernandez A, Thimmapuram J, Henrissat B, Coutinho PM, Borovok I, Jindou S, Lamed R, Flint HJ, Bayer EA and White BA 2009. Diversity and strain specificity of plant cell wall degrading enzymes revealed by the draft genome of *Ruminococcus flavefaciens* FD-1. PLoS One 4, e6650.

Buddle BM, Denis M, Attwood GT, Altermann E, Janssen PH, Ronimus RS, Pinares-Patino CS, Muetzel S and Wedlock N 2011. Strategies to reduce methane emissions from farmed ruminants grazing on pasture. The Veterinary Journal 188, 11–17.

Bryant MP 1959. Bacterial species of the rumen. Bacteriological Reviews 23, 125–153.

Bryant MP 1965. Rumen methanogenic bacteria. In Physiology of digestion of the ruminant (ed. RW Dougherty, RS Allen, W Burroughs, NL Jaskson and AD McGillard). Butterworths Publishing Inc., Washington, DC.

Carberry CA, Kenny DA, Han S, McCabe SM and Waters SM 2012. The effect of phenotypic residual feed intake and dietary forage content on the rumen microbial community of beef cattle. Applied and Environmental Microbiology 78, 4949–4958.

Chaucheyras-Durand F, Masséglia S, Fonty G and Forano E 2010. Influence of the composition of the cellulolytic flora on the development of hydrogenotrophic microorganisms, hydrogen utilization, and methane production in the rumens of gnotobiotically reared lambs. Applied and Environmental Microbiology 76, 7931–7937.

Clark H 2013. Nutrition and host effects on methanogenesis in the grazing ruminant. Animal 7 (suppl. 1), 41–48.

Clauss M, Hume ID and Hummel J 2010. Evolutionary adaptations of ruminants and their potential relevance for modern production systems. Animal 4, 979–992.

Czerkawski JW and Breckenridge G 1975. New inhibitors of methane production by rumen micro-organisms. Experiments with animals and other practical possibilities. British Journal of Nutrition 34, 447–457.

Dodd D, Moon YH, Swaminathan K, Mackie RI and Cann IK 2010. Transcriptomic analyses of xylan degradation by *Prevotella bryantii* and insights into energy acquisition by xylanolytic bacteroidetes. The Journal of Biological Chemistry 285, 30261–30273.

Dormitzer PR, Grandi G and Rappuoli R 2012. Structural vaccinology starts to deliver. Nature Reviews Microbiology 10, 807–813.

Fonty G, Joblin K, Chavarot M, Roux R, Naylor G and Michallon F 2007. Establishment and development of ruminal hydrogenotrophs in methanogen-free lambs. Applied and Environmental Microbiology 73, 6391–6403.

Forde BM, Neville BA, O'Donnell MM, Riboulet-Bisson E, Claesson MJ, Coghlan A, Ross RP and O'Toole PW 2011. Genome sequences and comparative genomics of two *Lactobacillus ruminis* strains from the bovine and human intestinal tracts. Microbial Cell Factories 10 (suppl. 1), S13.

Fouts DE, Szpakowski S, Purushe J, Torralba M, Waterman RC, Macneil MD, Alexander LJ and Nelson KE 2012. Next generation sequencing to define prokaryotic and fungal diversity in the bovine rumen. PLoS One 7 (11), e48289.

Gagen EJ, Mosoni P, Denman SE, Al Jassim R, McSweeney CS and Forano E 2012. Methanogen colonisation does not significantly alter acetogen diversity in lambs isolated 17 h after birth and raised aseptically. Microbial Ecology 64, 628–640.

Gressley TF, Hall MB and Armentano LE 2011. Productivity, digestion, and health responses to hindgut acidosis in ruminants. Journal of Animal Science 89, 1120–1130.

Guan LL, Nkrumah JD, Basarab JA and Moore SS 2008. Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency. FEMS Microbiology Letters 288, 85–91.

Hernandez-Sanabria E, Goonewardene LA, Wang Z, Durunna ON, Moore SS and Guan LL 2012. Impact of feed efficiency and diet on adaptive variations in the bacterial community in the rumen fluid of cattle. Applied and Environmental Microbiology 78, 1203–1214.

Henderson G, Naylor GE, Leahy SC and Janssen PH 2010. Presence of novel, potentially homoacetogenic bacteria in the rumen as determined by analysis of formyltetrahydrofolate synthetase sequences from ruminants. Applied and Environmental Microbiology 76, 2058–2066.

Hess M, Sczyrba A, Egan R, Kim TW, Chokhawala H, Schroth G, Luo S, Clark DS, Chen F, Zhang T, Mackie RI, Pennacchio LA, Tringe SG, Visel A, Woyke T, Wang Z and Rubin EM 2011. Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. Science 331, 463–467.

Hong SH, Kim JS, Lee SY, In YH, Choi SS, Rih JK, Kim CH, Jeong H, Hur CG and Kim JJ 2004. The genome sequence of the capnophilic rumen bacterium *Mannheimia succiniciproducens*. Nature Biotechnology 22, 1275–1281.

Hook SE, Wright AG and McBride BW 2010. Methanogens: methane producers of the rumen and mitigation strategies. Archaea 2010, 945785.

Hungate RE 1967. Hydrogen as an intermediate in the rumen fermentation. Archives of Microbiology 59, 158–164.

Hungate RE, Smith W, Bauchop T, Yu I and Rabinowitz JC 1970. Formate as an intermediate in the rumen fermentation. Journal of Bacteriology 102, 384–397.

Innes EA, Bartley PM, Rocchi M, Benavidas-Silvan J, Burrells A, Hotchkiss E, Chianini F, Canton G and Katzer F 2011. Developing vaccines to control protozoan parasites in ruminants: dead or alive? Veterinary Parasitology 180, 155–163.

Janssen PH and Kirs M 2008. Structure of the archaeal community of the rumen. Applied and Environmental Microbiology 74, 3619–3625.

Johnson DE, Wood AS, Stone JB and Moran ET 1972. Some effects of methane inhibition in ruminants (steers). Canadian Journal of Animal Science 52, 703–712.

Joblin K 1999. Ruminal acetogens and their potential to lower ruminant methane emissions. Australian Journal of Agricultural Research 50, 1307–1313.

Kelly WJ, Leahy SC, Altermann E, Yeoman J, Dunne JC, Kong Z, Pacheco DM, Li D, Noel SJ, Moon CD, Cookson AL and Attwood GT 2010. The glycobiome of the rumen bacterium *Butyrivibrio proteoclasticus* B316(T) highlights adaptation to a polysaccharide-rich environment. PLoS One 5, e11942.

Kenters N, Henderson G, Jeyanathan J, Kittelmann S and Janssen PH 2011. Isolation of previously uncultured rumen bacteria by dilution to extinction using a new liquid culture medium. Journal of Microbiological Methods 84, 52–60.

Kim M, Morrison M and Yu Z 2011. Status of the phylogenetic diversity census of ruminal microbiomes. FEMS Microbiology Ecology 76, 49–63.

Koike S, Handa Y, Goto H, Sakai K, Miyagawa E, Matsui H, Ito S and Kobayashi Y 2010. Molecular monitoring and isolation of previously uncultured bacterial strains from the sheep rumen. Applied and Environmental Microbiology 76, 1887–1894.

Leahy SC, Kelly WJ, Altermann E, Ronimus RS, Yeoman CJ, Pacheco DM, Li D, Kong Z, McTavish S, Sang C, Lambie SC, Janssen PH, Dey D and Attwood GT 2010. The genome sequence of the rumen methanogen *Methanobrevibacter ruminantium* reveals new possibilities for controlling ruminant methane emissions. PLoS One 5, e8926.

Lee GH, Kumar S, Lee JH, Chang DH, Kim DS, Choi SH, Rhee MS, Lee DW, Yoon MH and Kim BC 2012. Genome sequence of *Oscillibacter ruminantium* strain GH1, isolated from rumen of Korean native cattle. Journal of Bacteriology 194, 6362.

Lee JH, Rhee MS, Kumar S, Lee GH, Chang DH, Kim DS, Choi SH, Lee DW, Yoon MH and Kim BC 2013. Genome sequence of *Methanobrevibacter* sp. strain JH1, isolated from rumen of Korean native cattle. Genome Announcements 1, e00002-13.

Liu Y and Whitman WB 2008. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. Annals of the New York Academy of Sciences 1125, 171–189.

Martin C, Morgavi DP and Doreau M 2010. Methane mitigation in ruminants: from microbe to the farm scale. Animal 4, 351–365.

Marx H, Graf AB, Tatto NE, Thallinger GG, Mattanovich D and Sauer M 2011. Genome sequence of the ruminal bacterium *Megasphaera elsdenii*. Journal of Bacteriology 193, 5578–5579.

McAllister TA and Newbold CJ 2008. Redirecting rumen fermentation to reduce methanogenesis. Australian Journal of Experimental Agriculture 48, 7–13.

McCrabb GJ, Berger KT, Magner T, May C and Hunter RA 1997. Inhibiting methane production in Brahman cattle by dietary supplementation with a novel compound and the effects on growth. Australian Journal of Agricultural Research 48, 323–329.

McKinlay JB, Laivenieks M, Schindler BD, McKinlay AA, Siddaramappa S, Challacombe JF, Lowry SR, Clum A, Lapidus AL, Burkhart KB, Harkins V and Vieille C 2010. A genomic perspective on the potential of *Actinobacillus succinogenes* for industrial succinate production. BMC Genomics 11, 680.

McSweeney C and Mackie R 2012. Micro-organisms and ruminant digestion: state of knowledge, trends and future prospects. Commission of Genetic Resources for Food and Agriculture. Background study paper No. 61. Retrieved December 4, 2012, from http://www.fao.org/docrep/016/me992e/me992e.pdf.

Morgavi DP, Kelly WJ, Janssen PH and Attwood GT 2013. Rumen microbial (met)genomics and its application to ruminant production. Animal 7 (suppl. 1), 184–201.

Nyonyo T, Shinkai T, Tajima A and Mitsumori M 2013. Effect of media composition, including gelling agents, on isolation of previously uncultured rumen bacteria. Letters in Applied Microbiology 56, 63–70.

Paul K, Nonoh JO, Mikulski L and Brune A 2012. "Methanoplasmatales," thermoplasmatales-related archaea in termite guts and other environments, are the seventh order of methanogens. Applied and Environmental Microbiology 78, 8245–8253.

Pinares-Patiño CS, Ulyatt MJ, Lassey KR, Barry TN and Holmes CW 2003. Rumen function and digestion parameters associated with differences between sheep in methane emissions when fed chaffed lucerne hay. Journal of Agricultural Science 140, 205–214.

Pinares-Patiño CS, McEwan JC, Dodds KG, Cárdenas EA, Hegarty RS, Koolaard JP and Clark H 2011a. Repeatability of methane emissions from sheep. Animal Feed Science and Technology 166–167, 210–218.

Pinares-Patiño CS, Ebrahimi SH, McEwan JC, Dodds KG, Clark H and Luo D 2011b. Is rumen retention time implicated in sheep differences in methane emission? Proceedings of the New Zealand Society of Animal Production 71, 219–222.

Pukall R, Lapidus A, Nolan M, Copeland A, Glavina Del Rio T, Lucas S, Chen F, Tice H, Cheng JF, Chertkov O, Bruce D, Goodwin L, Kuske C, Brettin T, Detter JC, Han C, Pitluck S, Pati A, Mavrommatis K, Ivanova N, Ovchinnikova G, Chen A, Palaniappan K, Schneider S, Rohde M, Chain P, D'haeseleer P, Göker M, Bristow J, Eisen JA, Markowitz V, Kyrpides NC, Klenk HP and Hugenholtz P 2009. Complete genome sequence of *Slackia heliotrinireducens* type strain (RHS 1T). Standards in Genomic Sciences 1, 234–241.

Purushe J, Fouts DE, Morrison M, White BA, Mackie RI, Coutinho PM, Henrissat B, Nelson KE and North American Consortium for Rumen Bacteria 2010. Comparative genome analysis of *Prevotella ruminicola* and *Prevotella bryantii*: insights into their environmental niche. Microbial Ecology 60, 721–729.

Rea S, Bowman JP, Popovski S, Pimm C and Wright AD 2007. *Methanobrevibacter millerae* sp. *nov.* and *Methanobrevibacter olleyae* sp. *nov.*, methanogens from the ovine and bovine rumen that can utilize formate for growth. International Journal of Systematic and Evolutionary Microbiology 57, 450–456.

Rosewarne CP, Cheung JL, Smith WJ, Evans PN, Tomkins NW, Denman SE, Ó Cuív P and Morrison M 2012. Draft genome sequence of *Treponema* sp. strain JC4, a novel spirochete isolated from the bovine rumen. Journal of Bacteriology 194, 4130.

Sakai S, Imachi H, Sekiguchi Y, Tseng IC, Ohashi A, Harada H and Kamagata Y 2009. Cultivation of methanogens under low-hydrogen conditions by using the coculture method. Applied and Environmental Microbiology 75, 4892–4896.

Seo JK, Seon-Woo K, Kim MH, Santi D, Kam DK and Jong KH 2010. Direct-fed microbials for ruminant animals. Asian-Australasian Journal of Animal Sciences 12, 1657–1667.

Spring S, Visser M, Lu M, Copeland A, Lapidus A, Lucas S, Cheng J, Han C, Tapia R, Goodwin L, Pitluck S, Ivanova N, Land M, Hauser L, Larimer F, Rohde M, Göker M, Detter J, Kyrpides N, Woyke T, Schaap P, Plugge C, Muyzer G, Kuever J, Pereira I, Parshina S, Berner-Latmani R, Stams A and Klenk H 2012. Complete genome sequence of the sulfate-reducing firmicute *Desulfotomaculum ruminis* type strain (DL^T). Standards in Genomic Sciences 7, 304–319.

St-Pierre B and Wright ADG 2013. Diversity of gut methanogen in herbivorous animals. Animal 7 (suppl. 1), 49–56.

Suen G, Weimer PJ, Stevenson DM, Aylward FO, Boyum J, Deneke J, Drinkwater C, Ivanova NN, Mikhailova N, Chertkov O, Goodwin LA, Currie CR, Mead D and Brumm PJ 2011a. The complete genome sequence of *Fibrobacter succinogenes* S85 reveals a cellulolytic and metabolic specialist. PLoS One 6, e18814.

Suen G, Stevenson DM, Bruce DC, Chertkov O, Copeland A, Cheng JF, Detter C, Detter JC, Goodwin LA, Han CS, Hauser LJ, Ivanova NN, Kyrpides NC, Land ML, Lapidus A, Lucas S, Ovchinnikova G, Pitluck S, Tapia R, Woyke T, Boyum J, Mead D and Weimer PJ 2011b. Complete genome of the cellulolytic ruminal bacterium *Ruminococcus albus* 7. Journal of Bacteriology 193, 5574–5575.

Thorpe A 2009. Enteric fermentation and ruminant eructation: the role (and control?) of methane in the climate change debate. Climate Change 93, 407–431.

Van Nevel CJ and Demeyer D 1995. Feed additives and other interventions for decreasing methane emissions. In Biotechnology in animal feeds and animal feeding (ed. J Wallace and A Chesson), pp. 329–349. VCH Publishers, New York, NY, USA.

Wedlock DN, Janssen PH, Leahy S, Shu D and Buddle BM 2013. Progress in the development of vaccines against rumen methanogens. Animal (suppl. s2), 244–252.

Wright ADG and Klieve AV 2011. Does the complexity of the rumen microbial ecology preclude methane mitigation? Animal Feed Science and Technology 166–167, 248–253.

Wright ADG, Kennedy P, O'Neill CJ, Toovey AF, Popovski S, Rea SM, Pimm CL and Klein L 2004. Reducing methane emissions in sheep by immunization against rumen methanogens. Vaccine 22, 3976–3985.

Zhou M, Hernandez-Sanabria E and Guan LL 2009. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Applied and Environmental Microbiology 75, 6524–6533.

Zhou M, Hernandez-Sanabria E and Guan LL 2010. Characterization of variation in rumen methanogenic communities under different dietary and host feed efficiency conditions, as determined by PCR-denaturing gradient gel electrophoresis analysis. Applied and Environmental Microbiology 76, 3776–3786.