The ruminal microbiome associated with methane emissions from ruminant livestock

Ilma Tapio¹, Timothy J. Snelling², Francesco Strozzi³ and R. John Wallace²*

Abstract

Methane emissions from ruminant livestock contribute significantly to the large environmental footprint of agriculture. The rumen is the principal source of methane, and certain features of the microbiome are associated with low/high methane phenotypes. Despite their primary role in methanogenesis, the abundance of archaea has only a weak correlation with methane emissions from individual animals. The composition of the archaeal community appears to have a stronger effect, with animals harbouring the Methanobrevibacter gottschalkii clade tending to be associated with greater methane emissions. Ciliate protozoa produce abundant H₂, the main substrate for methanogenesis in the rumen, and their removal (defaunation) results in an average 11% lower methane emissions in vivo, but the results are not consistent. Different protozoal genera seem to result in greater methane emissions, though community types (A, AB, B and O) did not differ. Within the bacteria, three different 'ruminotypes' have been identified, two of which predispose animals to have lower methane emissions. The two low-methane ruminotypes are generally characterized by less abundant H₂-producing bacteria. A lower abundance of Proteobacteria and differences in certain Bacteroidetes and anaerobic fungi seem to be associated with high methane emissions. Rumen anaerobic fungi produce abundant H₂ and formate, and their abundance generally corresponds to the level of methane emissions. Thus, microbiome analysis is consistent with known pathways for H₂ production and methanogenesis, but not yet in a predictive manner. The production and utilisation of formate by the ruminal microbiota is poorly understood and may be a source of variability between animals.

Keywords: Archaea, Methane, Microbiome, Rumen

Background

Methane is a greenhouse gas (GHG) with a global warming potential 28-fold that of carbon dioxide [1]. Agriculture makes a significant contribution to total GHG production, with estimates varying according to country and calculation method [2]. Nonetheless, a global contribution of between 7 and 18% of total anthropogenic GHG emissions is generally accepted [2]. Ruminant production accounts for about 81% of GHG from the livestock sector (calculated from Hristov et al. [2]), 90% of which results from rumen microbial methanogenesis [3]. Ruminal CH₄ production also represents a loss of energy (from 2 to 12% of gross energy intake [4]), which could in principle otherwise be available for animal growth or milk production. Lowering CH₄ emissions therefore would benefit the environment and possibly the efficiency of livestock production. More than 87% of the CH₄ produced by sheep has been estimated to be derived from the rumen [5], where a population of methanogenic archaea converts the H₂ and CO₂ produced by a complex community of ciliate protozoa, bacteria and anaerobic fungi to CH₄ [6, 7]. A massive worldwide research effort has investigated various mitigation strategies. Changes in management practices can be simple and very effective [2], while feed additives that might inhibit H₂ production, provide an alternative metabolic H sink or inhibit the archaea themselves offer opportunities beyond those straightforward management changes [6–11]. Other opportunities include chemogenomics and immunization [12–14]. One strategy that is foremost in several investigations is genetic selection of the livestock. If we can demonstrate that persistently different CH₄ emissions in different animals [14–16] can be explained by their individual
ruminal microbiomes, and that the characteristic is heritable, it should be possible to select future generations of ruminants that have intrinsically lower CH$_4$ emissions. All the strategies potentially involve changing the ruminal microbiome. The aim of this short review is to assess our current understanding of the role of different members of the microbiome in determining the extent of methanogenesis in the rumen.

The rumen microbial community
The rumen is home to a vast array of ciliate protozoa, anaerobic fungi, anaerobic bacteria and archaea. The protozoa can comprise up to half the rumen microbial biomass [17, 18], the fungi were originally estimated to be about 8% of the biomass [19] but may reach 20% in sheep [20], the archaea comprise 0.3–4% [21] and the bacteria form the remainder, typically the largest component of the microbial biomass. Our present understanding of ruminal microbiology was built initially upon a few epoch-changing advances made many years ago: Gruby & Delafond’s [22] microscopic observations of protozoa; Hungate’s [23] appreciation of the anaerobic nature of the rumen that led to, truly anaerobic culture techniques for the bacteria; Orpin’s [24] realization that some flagellate protozoa were in fact zoospores of anaerobic fungi, until then a contradiction in terms. The isolation and study of pure cultures was and remains invaluable in understanding the likely role of different species of bacteria, protozoa or fungi in the overall fermentation. Drawbacks of cultivation techniques are that only a very small number of samples can be tested, and that they suffer from bias, whereby the composition of the growth medium, generally too rich, determines which species can grow [25]. Development of molecular techniques, based mainly on ssu rRNA gene and intergenic spacer sequence (for the fungi) analyses, opened new opportunities in rumen research. Cloning and sequencing provided community analyses that were not prone to the biases imposed by cultivation techniques, although different bias was introduced by other factors, like storage conditions [26], the differential efficiency of DNA extraction from different species and amplification bias [27–29]. Related techniques for microbiome analysis quickly followed (DGGE, TGGE, T-RFLP, ARISA). Quantitative PCR and FISH enabled microbial groups or species to be quantified [30]. Now, metagenomic sequencing enables rapid community analysis to be carried out, without the cultivation bias or variation associated with primer selection or PCR amplification irregularities [25, 31]. The problem of DNA extraction remains, however, and databases are relatively weak where ruminal organisms are concerned [32]. Nevertheless, if we can use this approach to determine how the functional activity of the rumen microbial community influences methane emissions, the knowledge should enable strategies to decrease the environmental impact of livestock agriculture. Furthermore, it might be expected to improve animal production efficiency.

Ruminal community analysis relating to methane emissions

Archaea
There are two main routes for methanogenesis in the rumen, both carried out by archaea. The hydrogenotrophic pathway converts H$_2$ and CO$_2$ produced by the protozoa, bacteria and fungi to CH$_4$ [3, 6]. It is usually assumed that formate, which can be used by all the most abundant ruminal archaea, is equivalent to H$_2$ + CO$_2$, so formate is included in the hydrogenotrophic category [21, 33]. A second category of substrate for methanogenesis is methyl groups, such as those present in methylamines and methanol [34, 35]. Methylamines are derived from glycine betaine (from beet) and choline (from plant membranes), while methanol is derived from the hydrolysis of methanolic side-groups in plant polysaccharides. The most common hydrogenotrophic archaea are from the genus Methanobrevibacter, which has been divided into two subgroups, one known as the SGMT clade (Mbb. smithii, Mbb. gottschalkii, Mbb. millerae and Mbb. thaueri), the other (RO) clade comprising principally Mbb. ruminantium and Mbb. olleyae [21, 36]. Other significant hydrogenotrophic genera include Methanosphaera, Methanomicrococcus and Methanobacterium. The less abundant methylotrophs (Methanosarcinales, Methanosphaera, Methanomassiliicoccaceae) can use methylamines and methanol, and there are archaea (Methanosarcinales) that produce methane via the aceticlastic pathway (reviewed in Morgavi et al. [7]). Rumen methanogenic archaeal diversity is restricted to four orders [21] and is highly conserved across 32 ruminant species collected worldwide [32].

Intuitively, archaea should be the microbial group most closely correlated with methane emissions. However, some studies have shown no such correlation with their overall abundance while in others the correlation has been weak. Morgavi et al. [37], Zhou et al. [38], Danielsson et al. [39] and Danielsson [40] found no correspondence between the numbers of methanogens and methane emissions from dairy cows when measured using metagenomics and qPCR techniques. Kittelmann et al. [41] and Shi et al. [42] formed a similar conclusion in sheep. A weak correlation between archaeal abundance relative to bacteria was found in beef steers [43] but none was found with dairy cows in the RuminOmics project [http://www.ruminomics.eu/] when expressed as the archaea:bacteria ratio (Fig. 1). Shi et al. [42] also observed that archaeal gene expression rather than gene abundance was correlated to methane emissions from
individual sheep. It is easy to see why gene expression might be a useful proxy for methanogenesis in a static system like soil [44], but less so in a flowing system like the rumen, where for physiological reasons biomass must be directly correlated to gene abundance unless other processes, such as uncoupled CH4 production occur [45].

Given the high variability of the relationship with overall archaeal abundance, it may be that the composition of the archaeal community rather than just its size may have greater significance with regard to methane emissions. Zhou et al. [38], Danielsson et al. [39], Shi et al. [42] and Danielsson [40] all found a positive correlation between the relative abundance of Methanobrevibacter SGMT clade and methane emissions. Danielsson [40] interpreted this correlation in terms of different affinities for H2 in the two groups, with the SGMT clade possessing methyl coenzyme M reductase isozymes McrI and McrII [12], which enables the archaea to utilise H2 at higher concentrations, against the RO clade that possess only McrI [3, 12]. The dynamics of the of the archaeal community composition and thus the efficiency of H2 utilization would in turn be a consequence of differing H2 production by different bacteria [33, 41] and presumably also protozoal and fungal communities. Furthermore, the proportion of Methanosphaera spp. in total archaea was negatively associated with methane production in sheep [41], although not in beef cattle [46]. Thus, differing methane emissions are at least partly due to varying relative abundances within the community of methanogenic archaea.

Other observations regarding the archaeal community, sometimes called the archaeome, include those of Pitta et al. [47], who found that archaeal abundance increased in steers suffering frothy bloat, and Pei et al. [48], who discovered archaea associated with the rumen epithelium. In the former case, the CH4 content of the gas was not measured, so it is unclear the impact the bloat would have on methanogenesis. In the latter, the finding was surprising because the rumen wall is considered to be an aerobic/anaerobic interface, and the relative abundance of O2 might be considered to suppress the growth of the extremely O2-sensitive methanogens. In fact, one might have possibly expected CH4 oxidisers to be present, in spite of their absence from the deep ruminal digesta [49].

Ciliate protozoa

Ruminal ciliates are intimately involved in methanogenesis, partly via their abundant H2 production [50] and, taking advantage of this, their associated methanogens, which are found both as intracytoplasmic commensals and on the exterior surface of the protozoa [3, 18, 51–53]. Several studies suggested a correlation between the abundance of protozoa and methane emissions (collated in [18, 54, 55]), while others do not [37, 43]. Guyader et al. [56] conducted a meta-analysis containing 28 experiments and 91 treatments. This meta-analysis showed a linear positive relationship between log10 protozoal numbers and methane emissions expressed per unit DMI. An $r = 0.96$ showed that there is indeed a reasonably strong relationship (Fig. 2).
Defaunation (the removal of the ciliates from the rumen) has therefore been investigated in relation to methane production. Although in some cases the results of defaunation on CH₄ emissions have not been encouraging [57–60], Newbold et al. [18] carried out a meta-analysis of defaunation studies and concluded that CH₄ was decreased on average by 11%. Despite the lower CH₄ production, the total archaeal abundance was not significantly decreased in the Newbold et al. meta-analysis, suggesting that the archaeal community in defaunated animals may have a lower CH₄-emitting specific activity than that of the protozoa-associated community.

As with the archaea, the questions then revert to whether some individual protozoal genera or species, and their associated archaea, are more linked with methanogenesis than others. In general, the protozoa harbour an archaeal population that, like the general archaeal community, is dominated by *Methanobrevibacter* spp. [61–64], although differences were observed in the abundance of different archaea found in the protozoa and in the non-associated archaea [18, 61, 65] that might lead to different methanogenic specific activities in the two populations. Furthermore, archaeal colonisation abundance may differ between different protozoal species [51] and each may be associated with different predominant archaeal genera/species. Holotrichs in particular had an archaeal community that differed from entodiniomorphid protozoa [53]. Larger ciliates appear to be more heavily colonized by methanogens than smaller ciliates [53, 66], and also by bacteria, suggesting that there is not a selective colonisation by archaea [53]. The lower metabolic activity in terms of H₂ production of the larger protozoal species per unit biomass [50, 54, 58] presumably explains that smaller protozoa, and their associated archaea, will be relatively more active in methanogenesis than larger species. Indeed, in vitro studies indicated that the smaller *Entodinium* spp. were more associated with methane production than larger species like *Polyplastron multivesiculatum* [50, 58]. In vivo studies are inconsistent, however. Refaunation experiments indicated that the abundance of *Entodinium* spp. [67, 68] or holotrichs [68] correlated with higher methane emissions. A large amplicon sequencing study in sheep nevertheless found no relationship between the relative abundance of different ciliates and methane emissions [41]. Furthermore, ciliate communities fall into a small number of types (A, AB, B and O [69]) depending on interactions, principally inter-species predation. Despite the large differences in relative abundance of different protozoa types in the different community types, methane emissions could not be correlated with protozoal community structure [70]. The varying colonisation by archaea depending on the time after feeding [71] is another confounding factor in trying to evaluate the role of protozoa in methanogenesis.

**Bacteria**

Ruminal bacteria form the most diverse group within the rumen, capable of utilizing fibre, starch, protein and sugars [72]. Among numerous bacterial phyla found in different studies, Firmicutes, Bacteroidetes and Proteobacteria are the most abundant [32]. Fibrolytic bacteria, especially cellulolytic *Ruminococcus* and several *Eubacterium* spp (Firmicutes), are well studied H₂ producers. On the other hand, the prominent cellulolytic genus, *Fibrobacter*, does not produce H₂, while Bacteroidetes are net H₂ utilizers [72]. Microbiome analysis has identified three different ‘ruminotypes’ that seemed to be associated with variations in methane production by sheep [41]. The low-CH₄ production ruminotype Q was characterised by high relative abundances of the propionate-producing *Quinella ovalis*. Low-CH₄ ruminotype S had
higher abundances of lactate- and succinate-producing *Fibrobacter* spp., *Kandleria vitulina*, *Olsenella* spp., *Prevotella bryantii*, and *Sharpea azabuensis*. The high-CH₄ production rumenotype H had higher relative abundances of species belonging to *Ruminococcus*, other *Ruminococcaceae*, *Lachnospiraceae*, *Catabacteriaceae*, *Coprococcus*, other *Clostridiales*, *Prevotella*, other *Bacteroidales*, and Alphaproteobacteria. The overall interpretation would be that methane emissions depend on the abundance of the H₂-producing bacteria present; a corollary to this is the observation that chemical inhibition of methanogenesis in goats led to increases in the abundance of H₂-consuming *Prevotella* and *Selenomonas* spp. [73]. Proteobacteria were 4-fold less abundant (2.7 vs. 11.2% of bacteria) in high emitting beef cattle [46] and a similar finding was made in dairy cows [40]. The dominant family among Proteobacteria was *Succinivibrionaceae*. This finding seems to parallel the high numbers of Succinivibrionaceae in the Tammar wallaby [74], which, like the ruminant, is a herbivorous foregut fermenter. It produces only about one-fifth of the methane per unit of feed intake of ruminants, which is attributed to the large community of Succinivibrionaceae. An intriguing additional observation common to these studies [40, 41] was that within different *Prevotella* OTUs, some were correlated with a high CH₄ emissions from microbiome estimates, above, was discussed in incomplete. The relationship between bacterial abundances from microbiome estimates, above, was discussed in relation to whether bacteria form H₂, as in other anaerobes [33, 41, 43, 46], with little indication about formate production, reflected in the summary tables of Stewart et al. [72]. Although many species produce some formate, precise amounts are not known and therefore the importance of this production is difficult to estimate. Perhaps the Hungate 1000 collection (www.rmgnetwork.org/hungate1000.html) could be used as a resource to make such measurements. At present, the Hungate 1000 project has its emphasis on strengthening genetic databases [3], but much phenotypic information is being collected alongside the main thrust of the project. Assessing bacterial formate production is further complicated by the knowledge that co-culture experiments demonstrate that the metabolism of some bacteria and fungi grown in the presence of methanogens can be pulled in the direction of H₂ or formate production [82–85], so it is very difficult to be sure what the role of different species might be in the mixed rumen community. And perhaps most crucially, methanogenesis is not the sole fate of formate in the rumen. Hungate et al. [81] noted formate utilisation in the absence of

there is reason to suppose that fungal abundance might be related to methane emissions, reports are few. Kittelmann et al. [41] noted no difference in fungal community structure in relation to methane emissions from sheep. In the RuminOmics project, however, preliminary results suggest that two fungal species, *Caecomyces communis* and *Neocalimastix frontalis*, are negatively related to methanogenesis (*r* = -0.50 and -0.45, *P* < 0.001; R.J. Wallace et al., unpublished). The meta-analysis of Newbold et al. [18] noted that one of largest effects of defaunation, which leads to lower CH₄ production, was a decrease in fungal abundance. Whether this decrease is a major or direct cause of lower CH₄ production in defaunated animals is unclear.

**General considerations on variations in methanogenesis and the microbiome**

**Contribution of non-hydrogenotrophic methanogenesis**

The main substrates for methanogenesis in the rumen are known to be H₂ + CO₂, formate and compounds containing methyl groups like the methylamines and methanol [21]. In the reviews already mentioned here, formate and H₂ + CO₂ are usually considered to be equivalent as substrates for methanogenesis and formate is not treated separately. Formate feeds directly into the methanogenesis pathway at the very beginning via formate dehydrogenase [80], Hungate et al. [81] estimated that 18% of methane was formed via formate rather than H₂ + CO₂. Yet there are some important aspects of formate metabolism about which our understanding is incomplete. The relationship between bacterial abundances from microbiome estimates, above, was discussed in relation to whether bacteria form H₂ as in other analyses [33, 41, 43, 46], with little indication about formate producers. There is a large uncertainty about bacterial formate production, reflected in the summary tables of Stewart et al. [72]. Although many species produce some formate, precise amounts are not known and therefore the importance of this production is difficult to estimate. Perhaps the Hungate 1000 collection (www.rmgnetwork.org/hungate1000.html) could be used as a resource to make such measurements. At present, the Hungate 1000 project has its emphasis on strengthening genetic databases [3], but much phenotypic information is being collected alongside the main thrust of the project. Assessing bacterial formate production is further complicated by the knowledge that co-culture experiments demonstrate that the metabolism of some bacteria and fungi grown in the presence of methanogens can be pulled in the direction of H₂ or formate production [82–85], so it is very difficult to be sure what the role of different species might be in the mixed rumen community. And perhaps most crucially, methanogenesis is not the sole fate of formate in the rumen. Hungate et al. [81] noted formate utilisation in the absence of

**Anaerobic fungi**

The anaerobic fungi, like the protozoa, produce abundant amounts of H₂, along with CO₂, formate and acetate as metabolic end products [77]. Six fungal genera have been detected in the rumen but recent molecular research suggests existence of several new taxa [78], with functions still to be understood. Methanogens are found in close association with fungal hyphae [79]. Although
Fig. 3 Neighbor Joining tree of Prevotella-like OTUs that had a negative (blue dots) or positive (red dots) relation to methane (expressed in terms of g methane/kg DMI) in the 1,000-cow RuminOmics project. Multiple alignment was done using MUSCLE [111]. The Neighbor Joining tree was constructed using p-distance and pairwise-deletion parameters. The tree was resampled 1,000 times and bootstrap values are indicated. The linearized tree was computed using MEGA v5.1 [112] by using most abundant Bacteroidales OTUs to create an “outgroup”
methanogenesis, presumably by bacteria. Species like Wolinella succinogenes use formate as an energy source [72]. So, although it is usually stated that ruminal archaea utilise either H₂ + CO₂ or formate [3], it is unclear whether they are indeed equivalent for different archaea. For example, in co-cultures between rumen anaerobic fungi and three methanogens, all the methanogens used H₂ but formate was only utilised simultaneously by M. smithii [86]. The differential expression of formate dehydrogenase was one of the largest differences between high- and low-emitting sheep [42]. The formate dehydrogenase of M. ruminantium M1 was induced by co-culture with the formate-producing Butyribivibrio proteoclasticus [12]. Thus there are several reasons to conclude that thinking about formate as a substrate in vibrio proteoclasticus [12]. Thus there are several reasons to conclude that thinking about formate as a substrate in the context of microbiomes differing in their methanogenic activity might prove fruitful. Furthermore, despite the emphasis on H₂ produced by ciliate protozoa, the quantity of formate produced seems to be many times greater than H₂ [65].

The methylamines and methanol are methyl donors for methanogenesis by methylotrophic archaea, as described above. Their contribution to methanogenesis will depend to some extent on the concentration of methylamines in the diet [34, 35]. But how efficient is the process? Are methylamines converted quantitatively to CH₄ and are methylamine, dimethylamine and trimethylamine equivalent in that respect? It is possible that variation in CH₄ emissions between individual animals on some diets may be due to different efficiencies whereby methylamines are released from feed materials and converted to CH₄.

One of the more surprising findings in the Mbb. ruminantium M1 genome was the presence of three genes encoding alcohol dehydrogenase [12]. It has been demonstrated that ethanol can be used as a C source, but not as sole C source [3]. Thus, the availability of ethanol from bacterial fermentation may influence the dependence of archaea on methanogenesis for ATP production, and therefore affect the quantity of CH₄ produced.

**Influence of diet and mitigation measures**

An important principle underlying this review is that some microbiomes lead to different CH₄ emissions when other factors remain constant. Thus, key members of the microbiome leading to high or low emissions should be able to be identified. In the RuminOmics project, all dairy cows received diets that were as nutritionally similar as was possible given the different locations. Only by keeping as many other factors as possible unchanged will it be possible to dissect the role of different members of the microbial community in determining low- and high-emitting individuals. It should be noted here that we have chosen to express CH₄ production in terms of DMI, for the simple reason that it makes it easier to identify a low-CH₄ microbiome rather than a microbiome that forms less CH₄ only because the host animal eats less.

The results of microbiome analysis so far were expected in some respects, in the sense that diets high in starch content are known to lead to lower methane emissions, because starch utilising bacteria tend to produce less H₂ than others, for example [33, 72]. In a similar way, the changed fermentation stoichiometry linked with methane emissions is a very long established observation [87, 88]. New questions have been highlighted regarding different species associated with high and low CH₄ emissions under similar conditions. Unexpected correlations have been found. But many questions remain. It is also worth noting that widely different taxa may have similar metabolic activities [89], so there are several different microbiota that could lead to similar metabolic properties.

Mitigation measures have been described comprehensively elsewhere [2, 3, 6–10]. Perhaps the most promising of these is 3-nitrooxypropanol, a molecule obtained rationally by its structural similarity to methyl-CoM [90–92]. As yet we do not know the full implications of 3-nitrooxypropanol, but encouragement can be obtained that the concern that H₂ accumulation might inhibit overall fermentation does not seem to be such a problem as was suggested by some in vitro experiments [33, 93]. It is also worth noting that a 50% reduction in the growth rate of methanogens would be sufficient to cause their washout from the rumen [3, 33]. Complete inhibition of growth is therefore not necessary.

**Methane and feed efficiency**

CH₄ production and feed efficiency are linked, in the sense that a low feed efficiency, expressed as residual feed intake (RFI), is accompanied by lower CH₄ production [94–96]. The reverse does not apply, however, as has been found in dairy cows in the RuminOmics project. The findings that the abundance of certain Prevotella changes according to feed efficiency in beef cattle [97, 98] and many other taxa change in abundance [98] further emphasises our need to understand the role of Prevotella and its different biotypes on ruminal fermentation and methanogenesis. Shabat et al. [99] discovered that Megasphaera elsdenii was more abundant in low-efficiency cows, as were genes of the acrylate pathway, used by M. elsdenii in propionate formation. The explanation for lower efficiency was that M. elsdenii introduced a type of futile cycle in the production and subsequent utilisation of lactate, an energetically inefficient process.

**The influence of the host animal**

Many researchers believe, and some studies are beginning to show, that the host animal exerts a controlling
effect on its own gut microbiota [100–102]. The mechanism could conceivably be at a molecular level, perhaps via complex interactions with receptors in the rumen wall [103, 104] or antibodies in saliva [3, 105, 106]. More likely, however, is that the physical structure and dynamics of gut digesta are different in different animals. Goopy et al [15] found that lower methanogenesis in sheep was heritable and accompanied by the animals’ having smaller rumen volumes and therefore altered fluxes of nutrients through the tract. This would have the effect that less feed would be fermented in the rumen, leading to lower methanogenesis. Variations in saliva production could lead to a similar result [107]. Both would likely influence the ruminal microbiome. Therefore, caution should be exercised in interpreting microbiome analyses – the changed microbiome may be associated with, but not cause, a decrease in methanogenesis.

Ross et al. [108] found good correlations between CH₄ emissions and the broad characteristics of the microbiome. Now, metagenomics has shown that the abundance of certain groups of microbial genes can be highly predictive of CH₄ emissions [46, 109] and feed efficiency [99]. For example, 20 microbial genes explained 81% of variation in CH₄ emissions from beef cattle, while 49 genes explained 86% of variation in RFI [109]. Furthermore, the animal’s genetic background was a factor in determining these gene abundances [109]. This is the early phase of what is sure to be a fertile area in which animal-microbiome-emissions can be delineated by metagenomics profiling, and animal breeding based on these gene abundances may lead to animals with lower CH₄ emissions.

Conclusions

Recent large scale projects such as the Global Rumen Census, the Hungate 1000 and RuminOmics, from which some preliminary results are presented here, have provided new depth of insight into the composition and function of the rumen microbial community. By revealing some of the relationships between the microbiome and the animal phenotype, they have shown how understanding the role of the rumen microbiota can help in the efforts to reduce the environmental impact of livestock agriculture, in particular with the amelioration of greenhouse gas emissions. The archaea have been the main target for research, being directly associated with methane production in the rumen. However, other major microbial groups such as the ciliate protozoa, the anaerobic fungi, Succinivibrionaceae and Prevotella, among others, have been shown to be associated with both high and low methane production. The results illustrate that there are basic phenotypic characteristics, such as formate metabolism, that are insufficiently understood. When placed in the context of the many as yet uncultivated microbial species of the rumen, it becomes clear that the powerful tool of molecular analysis must be accompanied by cultural and metabolic/phenotypic analysis if we are to truly understand the relation between the ruminal microbiome and methanogenesis.

Abbreviations

ARISA: Automated ribosomal intergenic spacer analysis; DGGE: Density gradient gel electrophoresis; FISH: Fluorescence in vitro hybridization; qPCR: Quantitative polymerase chain reaction; TGGE: Temperature gradient gel electrophoresis; T-RFLP: Restriction fragment length polymorphism

Acknowledgements

The authors gratefully acknowledge the work of, and discussions with, other members of the Ruminomics consortium and thank them for their permission to use preliminary data to illustrate this article.

Funding

The Rowett Institute is funded by the Rural and Environment Science and Analytical Services Division (RESAS) of the Scottish Government. This study was financially supported by Ruminomics (project no. 289319 of EC 7th Framework Programme: Food, Agriculture, Fisheries and Biotechnology).

Availability of data and materials

The authors are bound by the Collaboration Agreement reached by the Ruminomics consortium, in which all data generated in the project become freely available in January 2018, but cannot be released without consent of the consortium before then. The original data underpinning Fig. 1 and Fig. 3 are subject to these conditions. Individual applications to release the data before 2018 should be directed to the corresponding author.

Authors’ contributions

The authors wrote the manuscript together, RJW having initiated the project. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

All authors have given their consent for submission of this article. The submission has also been approved by the publications committee of the RuminOmics consortium.

Ethics approval

Animal experimentation was conducted using protocols complying with national legislation and approved by local ethics committees.

Author details

1Green Technology, Natural Resources Institute Finland, Jokioinen, Finland.
2Rowett Institute of Nutrition and Health, University of Aberdeen, Foresterhill, Aberdeen AB10 5BD, UK. 3PTP, Via Einstein - Loc. Cascina Codazza, 26900 Lodi, Italy.

Received: 31 July 2016 Accepted: 3 January 2017
Published online: 19 January 2017

References

1. IPCC. Climate change 2014: synthesis report. In: Pachauri RK, Meyer LA, editors. Contribution of working groups I, II and III to the fifth assessment report of the Intergovernmental panel on climate change. Geneva: IPCC; 2014. p. 151.
2. Hristov AN, Oh J, Lee C, Meinen R, Montes F, Ott F, et al. Mitigation of greenhouse gas emissions in livestock production. In: Gerber PJ, Henderson B, Makkar HPS, editors. A review of options for non-CO₂ emissions. Rome: FAO; 2013. p. 226.
3. McAllister TA, Meale SJ, Vallee E, Guan LL, Zhou M, Kelly WJ, et al. Use of genomics and transcriptomics to identify strategies to lower ruminal methanogenesis. J Anim Sci. 2015;93:1431–49.
4. Johnson KA, Johnson DE. Methane emissions from cattle. J Anim Sci. 1995;73:2483–92.
13. Wright ADG, Bryant AM, Leng RA. Rates of production of methane in the rumen and large intestine of sheep. Br J Nutr. 1976;36:1–14.

14. Martin C, Morgavi DP, Dorea M. Methane mitigation in ruminants: from microbe to the farm scale. Animal. 2010;4:351–65.

15. Morgavi DP, Fonse E, Martin C, Newbold CJ. Microbial ecosystem and methanogenesis in ruminants. Animal. 2010;4:1024–36.

16. Knapp Jr, Lour GL, Vadas PA, Weiss WP, Tricicano JM. Invited review: enteric methane in dairy cattle production: quantifying the opportunities and impact of reducing emissions. J Dairy Sci. 2014;97:3231–61.

17. Kumar S, Choudhury PK, Carro MD, Griffith GW, Dagar SS, Puniya M, et al. Genetic parameters for predicted methane production and potential for reducing enteric emissions through genomic selection. J Dairy Sci. 2011;94:6122–32.

18. Hungate RE. The rumen and its microbes. New York: Academic; 1966.

19. Hungate RE. The rumen and its microbes. New York: Academic; 1966.

20. Rezaeian M, Beakes GW, Parker DS. Distribution and estimation of anaerobic fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim Feed Sci Technol. 2010;160:1–22.

21. Neill AR, Grieve DW, Dawson RN. Conversion of choline methyl groups through trimethylamine to methane in the rumen. Biochem. J. 1978;170:259–35.

22. Pitta DW, Finkach WE, Indugru N, Vecchiarelli B, Sinha R, Fulford JD. Metagenomic analysis of the rumen microbiome of steers with wheat-induced frothy bloat. Front Microbiol. 2016;7:1689.

23. Pei CX, Mao SY, Chang YF, Zhu WY. Diversity, abundance and novel 16S rRNA gene sequences of methanogens in rumen liquid, solid and epithelium fractions of Jinnan cattle. Animal. 2010;4:20–9.

24. Mitsumori M, Sun W. Control of rumen microbial fermentation for mitigating methane emissions from beef cattle. Sci Rep. 2014;4:5892.

25. Freitag TE, Toet S, Ineson P, Prosser JI. Links between methane flux and transcriptional activities of methanogens and methane oxidizers in a blanket peat bog. FEMS Microbiol Ecol. 2010;73:157–65.

26. Costa KC, Yoon SH, Pan M, Burn JA, Baliga NS, Leigh JA. Effects of H2 and formate on growth yield and regulation of methanogenesis in Methanococcus maripaludis. J Bacteriol. 2013;195:1546–62.

27. Wallace RJ, Rooke JA, McKinnon N, Duthie CA, Hyslop JJ, Ross DW, McKain N, et al. Methane yield phenotypes linked to differential gene expression in the rumen microbiome. Plos One. 2013;8:e64027.

28. Simultaneous amplicon sequencing to explore co-occurrence patterns of bacterial, archaeal and eukaryotic microorganisms in ruminant microbiota. Acta universitatis agriculturae sueciae, 2016, doctoral thesis no. 2016.

29. Methanogens: technologies, advances and prospects. Anim Feed Sci Technol. 2011;166:67–76.

30. Zhou M, McCuller TA, Guan LL. Molecular identification of ruminant methanogens: technologies, advances and prospects. Anim Feed Sci Technol. 2011;166:67–76.

31. Creevey CJ, Kelly WJ, Henderson G, Leahy SC. Determining the culturability of the rumen bacterial microbiome. Microb Biotechnol. 2014;7:467–79.

32. Newbold CJ, Cox F, Ganesh S, Jonker A, Young W, Janssen PH. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Sci Rep. 2015;5:14567.

33. Janssen PH. Influence of hydrogen on rumen methane formation and fermentation balances through microbial growth kinetics and fermentation thermodynamics. Anim Feed Sci Technol. 2010;160:1–22.

34. Daniello R, Schnurer A, Arthunov V, Bertlsson L. Methanogenic population and CH4 production in Swedish dairy cows fed different levels of forage. Appl Environ Microbiol. 2012;78:7612–9.

35. Methane in dairy cows, Impact of feed and rumen microbiota. Acta universitatis agriculturae sueciae, 2016, doctoral thesis no. 2016.

36. Simulation of rumen protozoa reveals bias in universal archaeal primers. Appl Environ Microbiol. 2012;78:4051–6.

37. Methanococcus maripaludis reveals new possibilities for controlling ruminant methane emissions. Plos One. 2013;8:e103171.

38. Orpin CG. Studies on the rumen flagellate Protomonas. J Gen Microbiol. 1975;91:249–62.

39. Orpin CG. Fungi in ruminant degradation. In: Agricultural science seminar: degradation of plant cell wall material. London: Agricultural Research Council; 1981. p. 129–50.

40. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.

41. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.

42. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.

43. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.

44. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.

45. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.

46. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.

47. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.

48. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.

49. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.

50. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.

51. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.

52. Orpin CG. Studies on the rumen flagellate Neocallimastix frontalis. J Gen Microbiol. 1975;91:249–62.
Williams AG, Coleman AG. The rumen protozoa. New York: Springer; 1992.

Guyader J, Eugene M, Noziere P, Morgavi DP, Doreau M, Martin C. Influence of rumen protozoa on methane emission in ruminants: a meta-analysis approach. Animal. 2014;8:1816–25.

Kreuzer M, Kirchgesner M, Müller H. Effect of defaunation on the loss of energy in wethers fed different quantities of cellulose and normal or steamflaked maize starch. Anim Feed Sci Technol. 1986;16:233–41.

Ranilla MJ, Jouany JP, Morgavi DP. Methane production and substrate degradation by rumen microbial communities containing single protozoal species in vitro. Lett Appl Microbiol. 2007;45:675–80.

Bird SH, Hegarty RS, Woodgate R. Persistence of defaunation effects on digestion and methane production in ewes. Aust J Exp Agric Anim Prod Sci. 2008;48:152–5.

Hegarty RS, Bird SH, Vanselow BA, Woodgate R. Effects of the absence of protozoa from birth or from weaning on the growth and methane production of lambs. Br J Nutr. 2008;100:1220–7.

Sharp R, Ziemer CJ, Stern MD, Stahl DA. Taxon-specific associations between protozoal and methanogen populations in the rumen and a model rumen system. FEMS Microbiol Ecol. 1998;26:71–82.

Iribis C, Ushida K. Detection of methanogens and proteobacteria from a single cell of rumen ciliate protozoa. J Gen Appl Microbiol. 2004;50:203–12.

Regensbogenova M, McEwan NR, Javorsky P, Kisidayova S, Michalowski T, Newbold CJ, et al. A re-appraisal of the diversity of the methanogens associated with the rumen ciliates. FEMS Microbiol Lett. 2004;238:307–13.

Tymensen LD, Beauchemin KA, McAllister TA. Structures of free-living and protozoa-associated methanogen communities in the bovine rumen differ according to comparative analysis of 16S rRNA and mcrA genes. Microbiology. 2012;158:1808–17.

Tomka U, Ushida K, Miyazaki K, Kojima Y. Methanogens associated with rumen ciliates. FEMS Microbiol Ecol. 1997;22:137–43.

Lloyd D, Williams AG, Anman R, Hayes AJ, Dunant L, Ralphs JR. Intracellular prokaryotes in rumen ciliate protozoa: detection by confocal laser scanning microscopy after in situ hybridization with fluorescent 16S rRNA probes. Eur J Protistol. 1996;32:523–31.

Jouany JP, Zaimas B, Senaud J, Grollere CA, Grain J, Thiwed P. Role of the ciliate rumen protozoa Polyplastron multivesiculatum, Entodinium sp. and Isotricha protoga in the digestion of a mixed diet in sheep. Reprod Nutr Dev. 1981;21:871–84.

Belanche A, de la Fuente G, Newbold CJ. Effect of progressive inoculation of rumen bacteria. In: Hobson PN, Stewart CS, editors. The rumen microbial ecosystem. London: Chapman & Hall; 1997. p. 467–91.

Joblin KN, Williams AG. Effect of cocultivation of ruminal chytrid fungi with Methanobrevibacter smithii on lumence stem degradation and extracellular fungal enzyme activities. Lett Appl Microbiol. 1991;12:21–4.

Joblin KN, Naylor GE, Williams AG. Effect of Methanobrevibacter smithii on xylanolytic activity of anaerobic ruminal fungi. Appl Environ Microbiol. 1990;56:2287–95.

Marvin-Sikkema FD, Richardson AJ, Stewart CS, Gottschal JC, Prins RA. Influence of hydrogen-consuming bacteria on cellulose degradation by anaerobic fungi. Appl Environ Microbiol. 1999;65:3793–7.

Demeeyer DJ, Van Nevel CJ. Methanogenesis, an integrated part of carbohydrate fermentation and its control. In: McDonald IW, Warner ACI, editors. Digestion and metabolism in the ruminant. Armidale: The University of New England Publishing Unit; 1975. p. 366–82.

Czerkawski JW. Methane production in the rumen and its significance. Wild Rev Nutr Diet. 1969;11:240–62.

Taxis TM, Wolff S, Gregg SJ, Minton NO, Zhang C, Dai J, et al. The players may change but the game remains: network analyses of ruminal microbiomes suggest taxonomic differences mask functional similarity. Nucleic Acids Res. 2015;43:9600–12.

Martinez-Fernandez G, Abecia L, Arco A, Cantalapiedra-Hijar G, Martin-Garcia AI, Molina-Maule-Alda E, et al. Effects of ethyl-3-nitrooxypropane and 3-nitrooxypropanol on ruminal fermentation, microbial abundance, and methane emissions in sheep. J Dairy Sci. 2014;97:5709–9.

Reynolds CK, Humphries DJ, Kirton P, Kindermann M, Duval S, Steinberg W. Methane production of lambs. Br J Nutr. 2008;100:1220–8.

Bauchop T, Mountfort DG. Cellulose fermentation by a rumen anaerobic fungus in both the absence and presence of rumen methanogens. Appl Environ Microbiol. 1981;42:1103–10.

Wolin MJ, Miller TL, Stewart CS. Microbe-microbe interactions. In: Hobson PN, Stewart CS, editors. The rumen microbial ecosystem. London: Chapman & Hall; 1997. p. 467–91.

Koteschkan C, Kittelmann S, Lu J, AH-Halbouni D, Jarvis GN, Muller T, et al. Internal transcribed spacer 1 secondary structure analysis reveals a common core throughout the anaerobic fungi (Neocallimastigomycota). PLoS One. 2014;9:e91928.

Bauchop T. The anaerobic fungi in rumen fibre digestion. Agric Environ. 1981;16:339–48.

Thauer RK, Kaster AK, Seedorf H, Buckel W, Hedderich R. Methanogenic archaea: ecologically relevant differences in energy conservation. Nature Rev Microbiol. 2008;6:579–91.

Hunegre RE, Smith W, Bauchop T, Yu L. Rabinovitz JC. Formate as an intermediate in the bovine rumen fermentation. J Bacteriol. 1970;102:389–97.

Bauchop T, Mountfort DG. Cellulose fermentation by a rumen anaerobic fungus in both the absence and presence of rumen methanogens. Appl Environ Microbiol. 1981;42:1103–10.

Taxis TM, Wolff S, Gregg SJ, Minton NO, Zhang C, Dai J, et al. The players may change but the game remains: network analyses of ruminal microbiomes suggest taxonomic differences mask functional similarity. Nucleic Acids Res. 2015;43:9600–12.

Martinez-Fernandez G, Abecia L, Arco A, Cantalapiedra-Hijar G, Martin-Garcia AI, Molina-Maule-Alda E, et al. Effects of ethyl-3-nitrooxypropane and 3-nitrooxypropanol on ruminal fermentation, microbial abundance, and methane emissions in sheep. J Dairy Sci. 2014;97:5709–9.

Reynolds CK, Humphries DJ, Kirton P, Kindermann M, Duval S, Steinberg W. Effects of 3-nitroxypropanol on methane emission, digestion, and energy and nitrogen balance of lactating dairy cows. J Dairy Sci. 2014;97:3777–81.

Hristov AN, Oh J, Gallifong F, Frederick TW, Harper MT, Weeks HL, et al. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proc Natl Acad Sci. 2015;112:10663–8.

Morvan B, Rieu-Lesme F, Fonty G, Gouët P. In vitro interactions between rumen H2-producing cellulolytic microorganisms and H2-utilizing acetogenic and sulfate-reducing bacteria. Anaerobe. 1996;2:175–80.

Nikrumah JD, Okine EM, Mathison GW, Schmid K, Li C, Basarab JA, et al. Moore SS Relationships of feedlot feed efficiency, performance, and feeding behavior with metabolic rate, methane production, and energy partitioning in beef cattle. J Anim Sci. 2006;84:145–53.

Hegaty RS, Goopy JP, Herd RM, McCorkell B. Cattle selected for lower residual feed intake have reduced daily methane production. J Anim Sci. 2007;85:1479–86.

Muro-Reyes A, Gutierrez-Banuelos H, Diaz-Garcia LH, Gutierrez-Pina FJ, Escarceño-Sánchez LM, Banuelos-Valenzuela R, et al. Potential environmental benefits of residual feed intake as strategy to mitigate methane emissions in sheep. J Anim Nutr Sci. 2011;15:10663–8.

Carberry CA, Kenny DA, Han S, McCabe MS, Waters SM. Effect of phenotypic residual feed intake and dietary forage content on the rumen microbial community of beef cattle. Appl Environ Microbiol. 2012;78:4949–58.

Myer PA, Smith TP, Wells JE, Kuehn LA, Freely HC. Rumen microbiome from steers differing in feed efficiency. PLoS One. 2015;10:e0121974.

Shabat SK, Sasson G, Doron-Faigenboim A, Durman T, Yaacoby S, Berg Miller ME, et al. Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants. ISME J. 2016;10:2958–72. doi:10.1038/ismej.2016.62.
100. NSW Department of Primary Industries. Genetic technologies to reduce methane emissions from Australian beef cattle. 2015. ISBN 978-1-74256-860-7.
101. Weimer PJ, Stevenson DM, Mantovani HC, Man SLC. Host specificity of the ruminal bacterial community in the dairy cow following near-total exchange of ruminal contents. J Dairy Sci. 2010;93:5902–12.
102. King EE, Smith RP, St Pierre B, Wright ADG. Differences in the rumen methanogen populations of lactating jersey and holstein dairy cows under the same diet regimen. Appl Environ Microbiol. 2011;77:5682–7.
103. Malmuthuge N, Li M, Fries P, Griebel PJ, Guan LL. Regional and age dependent changes in gene expression of Toll-like receptors and key antimicrobial defence molecules throughout the gastrointestinal tract of dairy calves. Vet Immunol Immunopathol. 2012;146:18–26.
104. Liu J, Bian G, Zhu W, Sheng-yong MS. High-grain feeding causes strong shifts in ruminal epithelial bacterial community and expression of Toll-like receptor genes in goats. Front Microbiol. 2015;6:167.
105. Williams YJ, Rea SM, Popovski S, Pimm CL, Williams AJ, Toovey AF, et al. Responses of sheep to a vaccination of entodinial or mixed rumen protozoal antigens to reduce rumen protozoal numbers. Br J Nutr. 2008;99:100–9.
106. Williams YJ, Popovski S, Rea SM, Skillman LC, Toovey AF, Northwood KS, et al. A vaccine against rumen methanogens can alter the composition of archaeanal populations. Appl Environ Microbiol. 2009;75:1860–6.
107. Appuhamy JA, Wagner-Riddle C, Casper DP, France J, Kebreab E. Quantifying body water kinetics and fecal and urinary water output from lactating Holstein dairy cows. J Dairy Sci. 2014;97:6177–95.
108. Ross EM, Moate PJ, Marett LC, Cocks BG, Hayes B. Metagenomic predictions: from microbiome to complex health and environmental phenotypes in humans and cattle. PLoS One. 2013;8:e73056.
109. Roehe R, Dewhurst RJ, Duthie CA, Rooke JA, McKain N, Ross DW, et al. Bovine host genetic variation influences rumen microbial methane production with best selection criterion for low methane emitting and efficiently feed converting hosts based on metagenomic gene abundance. PLoS Genet. 2016;12:e1005846.
110. Yu ZT, Morrison M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. Biotechniques. 2004;36:808–12.
111. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucl Acids Res. 2004;32:1792–7.
112. Tamura K, Dudley J, Nei M, Kumar S. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol. 2007;24:1596–9.