Landcorp Lecture

Rumen microbial community profiling as a tool to study ruminant production

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Abstract

The species composition of the rumen microbial community is shaped by the ruminant host and by characteristics of the ingested feed. The microbes present determine the ratios of volatile fatty acids, gases such as methane, and microbial protein formed from the feed. The rate of feed fermentation and the mix of products are important for animal production and for environmental impacts of ruminant farming. Differences in microbial community composition within a cohort of animals on a specific feed indicate differences in the rumen environment and presumably host animal genetics or early life history. Differences in the community when animals are on different feeds can be used to understand the impact of the feed on rumen function. Interpreting these community differences relies on understanding what might cause the selection of one community type over another. Recent research in New Zealand has developed a pipeline for studying rumen microbial community compositions. These tools have been used to help understand the basis of lower methane emissions from sheep fed forage brassica compared to ryegrass, and to understand what drives natural variations in methane emissions from sheep. This type of analysis is also starting to be used to investigate productivity differences within animal cohorts.

Keywords: bacteria; methane; microbial community; molecular ecology; rumen; ruminant

The rumen as a microbial habitat

Ruminant animals have evolved to use feed that normally has poor nutritive value for many mammals. The main feature of this adaptation is the reticulo-rumen (or rumen, for simplicity), a modification of the upper digestive tract in which feed is held while it is fermented by a community of anaerobic microbes. The animal masticates the feed when it consumes it, and then periodically regurgitates it from the rumen and re-chews it. The rumen temperature is maintained at about 39°C and, through the input of bicarbonate-containing saliva, the pH is regulated to (generally) between 5.7 and 6.7 (Hungate 1966).

The rumen microbial community is very dense, with about 3×10^{10} microbial cells per millilitre (Hungate 1966, Wolin 1979). By comparison, pasture soils contain about 2×10^{9} cells and ocean waters $<10^{6}$ cells in the same volume (Whitman et al. 1998). The microbial activity in the rumen is very high. Based on the rates of microbial end product formation, each rumen microbe is about 1100 times more active than a microbe in a temperate pasture soil (calculated from data reported by Hungate 1966; Schlesinger 1977; Whitman et al. 1998; Wolin 1979). The higher individual activity and the greater cell density mean that, per unit of volume, the rumen is 12,000 times more active than soil. This high activity is driven by the very large energy source input into the system due to the active feeding behaviour of the ruminant host. The intense microbial activity in the rumen results in very low concentrations of oxygen. Other potential electron acceptors like nitrate and sulfate are present at only low concentrations in the feed. As a consequence, anaerobic fermenting microbes establish themselves in the rumen, and dominate its microbiology.

The rumen seems to have evolved to allow grazing ruminants to survive on grass-dominated diets during droughts, when only very poor feed is available (Clauss et al. 2010). The microbial community can adapt its degradative capacity to changing feed composition through changes within the mixture of microbes, which each have different enzyme complements and metabolisms. This means that the animal's metabolic capabilities are augmented by the enormous microbial genetic diversity of its rumen microbes. Toxic compounds in the rumen can be broken down by the rumen microbes before they can be absorbed in the lower digestive tract. Most importantly, fibrous plant material that is not available as an energy source for mammals is converted to something that can be used by the ruminant host (Clauss et al. 2010).The feed fermentation results in the formation of volatile fatty acids, mainly acetate, propionate, and butyrate, which are absorbed by the animal and form a significant part of the ruminant's energy requirements (Bergman 1990). A by-product of this fermentation is hydrogen gas (H_2), which is opportunistically used by methanogens, microbes that convert the H_2 to methane (CH_4).

Nitrogen in the feed is available for use by the microbes, which may take up and incorporate nitrogenous feed components for their own biomass production, or use these as energy sources and ferment them to volatile fatty acids, releasing ammonia as a product. Other microbes may take up the ammonia and use it as a nitrogen source. Microbial cells leave the rumen into the lower digestive tract, where microbial protein can be degraded by the mammalian digestive

system and be a significant contributor to the ruminant's nitrogen demand, as well as being an additional energy source (Clauss et al. 2010).

An important feature of ruminal fermentation is the passage rate of solids and liquid, which washes microbial cells out of the rumen. Increasing the time that feed spends in the rumen allows longer for microbial attack and so increases the use of the feed components that are not digestible in the lower mammalian tract, by conversion to volatile fatty acids. The attack on fibrous plant components is slower than the fermentation of soluble components (Sutton 1971), and the passage rate of the feed has to be low enough to allow microbes to act and to multiply sufficiently to maintain their populations in the rumen. However, very low turnover would limit the amount of feed that can be eaten. In addition, very low turnover would allow the establishment of microbes that degrade volatile fatty acids to CH_4 and carbon dioxide, which would result in energy loss for the animal. The energy yields under anaerobic conditions from volatile fatty acids are very low, and support only very low microbial growth rates. Rumen passage rates are usually too high to accommodate such very low microbial growth rates. In addition, those microbes that degrade propionate and butyrate under anaerobic conditions are completely inhibited by the H_2 concentrations that are found in the rumen (Schink 1997). Therefore, such microbes are absent. Thus, rumen passage rates seem to be optimised for energy extraction by the ruminant (Clauss et al. 2010; Hungate 1966).

Rumen microbial community structure

The rumen microbial community is a growth-rate selected one. In soils, organisms can enter resting or dormant phases, or grow more slowly for long periods. In the ruminal lumen, with its continuous flow-through of solid and liquid phases, organisms present must grow continuously or they will be removed from the habitat. Each species in the rumen contents must, on average, maintain a growth rate equal to the passage rate, meaning cells must divide on average one to five times a day (Hungate 1966). Soil microbes are estimated to divide, on average, two to three times a year (Harris & Paul 1994). This constant selection at high growth rate in the rumen, combined with washout, probably has the effect of reducing the species diversity in the rumen compared to a slow-growing system like soil.

The rumen microbial community consists of members of all three domains of life: Bacteria, Archaea, and Eukarya. While more than 50 phyla of bacteria are currently recognised (McDonald et al. 2012), the rumen is dominated by only two of these phyla, Firmicutes and Bacteroidetes, and members of only three other phyla seem to be abundant, namely Fibrobacteres, Proteobacteria, and Tenericutes (de Menezes et al. 2011; G Henderson et al. unpublished data; Jami & Mizrahi 2012; Kittelmann et al. 2013; Pitta et al. 2010; Rius et al. 2012). There may be 500 to 5000 different bacterial species (Fouts et al. 2012; Hess et al. 2011; Jami & Mizrahi 2012; Kong et al. 2010; Pitta et al. 2009). The diversity of archaea is much lower, with a significant presence of only four of the 16 recognised orders of archaea. These are all methanogens, predominantly from the order Methanobacteriales, with some Methanoplasmatales, and minor representation of Methanomicrobiales and Methanosarcinales, and very low genus-level diversity within these four (Janssen & Kirs 2008; H Seedorf et al. unpublished data). Two groups of Eukarya are present: anaerobic ciliates of the orders Entodiniomorphida and Vestibuliferida, and anaerobic fungi of the order Neocallimastigales.

Many years of research have built up a detailed knowledge of a few ruminal microbes. Most species, however, remain poorly studied. Modern molecular tools and new efforts to isolate ruminal microbes suggest that a good understanding of many ruminal microbes is possible. We have increasingly good knowledge of the diversity of ruminal microbes. The major part of this new knowledge comes from the application of DNA sequencing tools. In particular, targeted sequencing of diagnostic marker genes, with continually improving technologies, has given us a good indication of the microbes present. For example, for a long time fibre degradation in the rumen was attributed to *Ruminococcus albus*, *Ruminococcus flavefaciens*, and *Fibrobacter succinogenes*. However, it appears that these probably account for 5% or less of the rumen bacteria (Mosoni et al. 2011; Pitta et al. 2010; Stevenson & Weimer 2007), and a great diversity of other bacteria are associated with the plant material (Noel 2013), including many with different and previously-unknown hydrolytic enzymes potentially involved in plant fibre hydrolysis (Brulc et al. 2009; Hess et al. 2011; Wang et al. 2013).

Rumen microbial community analyses

The rumen microbial community responds to the feed input and ruminal environment by changing its population structure over a period of hours to days. This is because of the high growth rate and subsequent selection for species that are best adapted to the inputs and conditions. This means that a survey of the rumen microbial community has the potential to tell us if there are differences in rumen function (a product of input and environment), and even what might be the significant drivers of those differences if we understand the physiology of the microbes. The simplest method of microbial community profiling is based on surveying the presence of universal marker genes, such as 16S rRNA genes for bacteria and archaea, 18S rRNA genes for ciliate protozoa, and ITS1 for anaerobic fungi (Kittelmann et al. 2013). Pipelines for microbial community analyses consist of a series of steps from rumen sampling through to microbial taxon comparisons (Fouts et al. 2012, Rius et al 2012). Comparison of data from different studies

can be affected by the methods used to obtain and analyse sequence data. In particular, the method of rumen sampling, DNA extraction, marker gene amplification, and DNA sequence analysis all influence the apparent microbial community structure. It is recommended that comparisons of data between studies be done with great caution where there are methodological differences. Standardised pipelines are recommended.

Rumen sampling

Sampling the rumen through a rumen fistula seems like the best way to obtain a good representation of the rumen contents, but the necessary surgical intervention is not practical when large numbers of animals are being compared, especially with valuable research or breeding cohorts. Oral stomach tubing is an alternative, but the exact sampling site in the rumen may vary between different attempts (Geishauer & Gitzel 1996; Shen et al. 2012). Subtle differences were noted when apparent bacterial archaeal, and ciliate protozoal communities were compared in samples taken through the fistula and using an oral stomach tube from the same animals at the same time (Henderson et al. 2013). However, differences between individual animals and ruminant species were clearly detectable using either method (Henderson et al. 2013; Lodge-Ivey et al. 2009). Nonetheless, when looking for subtle differences in structure between highly similar animals, it is recommended that caution is exercised if data obtained using different sampling methods are compared. Care must be exercised when comparing predominately liquid samples to samples containing large amounts of solid digesta. These fractions have significantly different microbial communities (Fouts et al. 2012; Henderson et al. 2013).

DNA extraction

DNA has to be extracted from the rumen contents. Because the aim is to get a representation of the different microbes present, differences in the efficiency of DNA extraction from different microbial taxa will skew the resultant data. This is not the case in DNA extractions for plant and animal typing. In a study comparing 15 different DNA extraction methods, some were found to be extremely poor in extracting microbial DNA (Henderson et al. 2013). Comparison of the ones that seemed to yield usable data showed that the apparent microbial community structures were different. Choice of DNA extraction method can therefore have a significant impact on the complement of microbes detected (Henderson et al. 2013; Ó Cuiv et al. 2010; Salonen et al. 2010). Some methods resulted in data that were highly similar, allowing cautious comparison of communities (Henderson et al. 2013).

Amplification of target DNA

DNA extracted from rumen samples consists of a mixture of genomes from the microbes and any plant DNA co-extracted with that. Only a small part of each genome consists of the marker gene of interest, and these are preferentially amplified using the polymerase chain reaction. This uses a pair of conserved oligonucleotide primers that match the DNA sequence on either side of the gene region of interest. If the primers do not match some of the target group, those species will not be in the resultant dataset. If a primer sequence binds more strongly or weakly to the DNA of a particular subgroup of the microbes being targeted, then there will be over- or under-representation of that subgroup. It is therefore clear that using well-tested primers is a necessity, and that comparisons between data obtained using different primers is unwise without understanding their limitations (Jeyanathan et al. 2011, Kittelmann et al. 2012). For example, it has been found that different primers sets give very different representations of the rumen archaeal community (Tymensen & McAllister 2012).

Data generation and analysis

The use of high-throughput, barcoded, next generation sequencing technologies such as 454 or Illumina, instead of traditional Sanger sequencing, has revolutionized microbial ecology research in the past decade. For this method, one primer of each primer pair used to amplify marker genes contains a short sequence that doesn't bind to the target DNA but instead serves as a unique identifier, or so-called barcode. A different barcode is used for each sample being analysed. This allows simultaneous DNA sequencing of many different samples (Hamady et al. 2008; Kittelmann et al. 2013). These sequencing technologies generate millions of sequencing reads in a single sequencing run and require a set of bioinformatic and statistical tools for data processing and analyses. Initially, sequencing datasets are quality filtered and each sequencing read is assigned to one of the barcodes (i.e., samples). All quality-filtered reads are then combined into a large dataset and sequencing reads are clustered by sequence similarity, e.g. all sequences with >97% similarity might be grouped into a cluster. All sequences that fall into a single cluster are considered to make up one operational taxonomic unit (OTU) and one representative sequence (usually the most abundant) for each OTU is chosen for taxonomic assignment. It is important to know that these OTUs are temporary constructs that are generated at each analysis. The taxonomic assignment is performed by comparing the representative OTU sequence against a reference database that contains sequences that each have an identity associated with them, which might be a species name or other designation. A number of pipelines for this kind of data processing and taxonomic assignment are available, such as QIIME (Caporaso et al. 2010) and mothur (Schloss et al. 2009). In this way, all OTUs are taxonomically classified and the microbial composition of the entire sample is determined. These compositions can then be compared between samples. Depending on the bioinformatic tools and parameters

used, the sequencing reads can be clustered in different ways, resulting in slight differences in identities and relative abundances of the microbes in the sample. When comparing data, the best approach would be to combine and re-analyse all sequence data again using the same quality filtering criteria, and then clustering the sequences into OTUs and taxa. That way, the sequences that fall into the same OTUs and taxa will be recognised as common. Failing that, the same reference database should be used for taxonomic assignments.

Reference databases

An important part of surveying microbial communities is the availability of accurate reference databases. By grouping the sequences at appropriate taxonomic ranks, such as the species or genus level, a more detailed description of the rumen microbial community is possible. Taxonomies from publicly-available databases are of variable quality, sometimes with poor curation of data due to inclusion of all sequences that are deposited, and some reference sequences will have incorrect taxonomic identifiers. For example, Kittelmann et al. (2012) found that >29% of ITS1 sequences from rumen anaerobic fungi in GenBank were misnamed at the genus level. In addition, the nomenclature and taxonomic separation is uneven, with large undifferentiated groups of multiple taxa decreasing the resolving power of the schemes. Because different taxonomic nomenclature is used in different databases (McDonald et al. 2012), the same microbes may be given a different name when a different reference database is used.

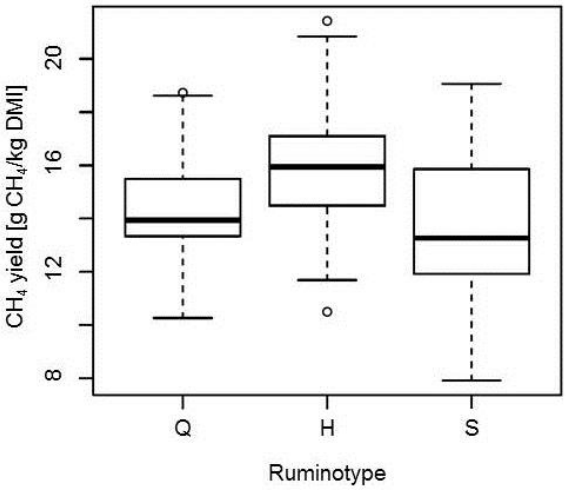
Highly curated and quality-controlled specific taxonomic schemes have been developed for rumen bacteria (G Henderson & PH Janssen unpublished data), methanogens (Janssen & Kirs 2008; H Seedorf et al., unpublished data), ciliate protozoa (Kittelmann et al. 2011; S Kittelmann et al. unpublished data), and anaerobic fungi (Kittelmann et al. 2012; Koetschan et al. 2014). In addition, progress has been made to identify homoacetogenic bacteria based on a gene coding for formyltetrahydrofolate synthetase, a diagnostic but not unique gene in this physiological type of bacteria (Henderson et al. 2010). These curated and habitat-specific reference databases allow a more accurate identification of the microbial groups present, including those from groups that contain as-yet no or few cultured or named species.

Current efforts to better understand rumen microbes

To help explain the causes or potential impacts of differences in rumen microbial community composition, attaching microbial function to the different microbial groups is very useful. This has traditionally been based on characterisation of pure culture isolates, which is limited by the availability of representatives that can be grown in the laboratory. Pioneering work by Robert Hungate and Marvin Bryant allowed cultivation and isolation of rumen microbes, and subsequent detailed study of these allowed their basic characteristics to be determined (Krause 2013). Of particular importance is the range of feed components or rumen products that a species or group of microbes breaks down or uses as an energy source, and the end-products of their metabolism. However, estimates of the total diversity of species present based on marker gene sequences suggested that, until recently, <15% of rumen microbes are available in culture (Edwards et al. 2004). There has been a recent resurgence in efforts to culture more rumen microbes in the laboratory as pure cultures that can be studied in more detail (Kenters et al. 2011; Koike et al. 2010; Noel 2013; Nyonyo et al. 2013). Isolates of rumen bacteria are being studied in more detail than ever, through sequencing of their genomes, which provides another way of assessing their metabolic capabilities (Morgavi et al. 2013). Large scale efforts to sequence genomes of a wide range of rumen microbes have been initiated recently in a New Zealand-led programme (http://www.hungate1000.org.nz, last accessed 16 May 2014). More recent advances in understanding rumen microbes come from metagenomic sequencing, in which the entire rumen DNA is treated as a single DNA pool (Brulc et al. 2009). Reassembly of microbial genomes from such metagenomic DNA (Hess et al. 2011) and selection of single microbial cells for genome sequencing (Rinke et al. 2013; Woyke et al. 2010) will allow this to be done in the future without the need to establish or maintain the source microbes in the laboratory. Of course, these methods will (probably) never be a substitute for studying the living microbe, but will allow more rapid advances to be made. These advances are allowing ever more insight into the capabilities of rumen microbes. This is particularly important when using microbial community data to infer activities. Microbes that share close phylogenetic relationships often have very similar physiologies, but not always (Achenbach & Coates 2000), which means that inferences of activity must be made cautiously when studied cultures are not available.

Not only the DNA can be sequenced to survey and compare the genetic and potential phenotypic complement of rumen microbes between samples. By sequencing the total rumen microbial RNA (the metatranscriptome), an estimate of gene expression and hence activity can be made. This has been done recently to understand differences in methanogen activity in sheep with different CH_4 emissions (W Shi et al. unpublished data). As DNA/RNA sequencing technologies and computational power improve, these approaches will become increasingly powerful. The availability of reference genomes is an important resource for these newly emerging technologies (Morgavi et al. 2013).

Figure 1 Principal coordinate analysis of Bray-Curtis dissimilarities of (A) bacterial and (B) archaeal community compositions in the rumen fluid of sheep continuously fed fresh winter forage rape or fresh perennial ryegrass. The key to the right indicates the different forages and sampling periods (P1 and P2, 8 weeks apart). The values in parentheses give the amount of variation explained by each coordinate.



Examples of rumen microbial community analyses

At present, routine analyses of microbial community composition can be made to uncover differences in gross rumen function, and estimates of functional differences can be made by comparison to the physiology of known microbial species. While there are limitations, this approach has the advantage of being able to handle hundreds of rumen samples without enormous computational complexity. Such analyses are very useful for seeing if there are potential microbial differences between rumen samples, and exploring what the drivers and consequences of such differences might be. Because the rumen is growth-rate selected, there are unlikely to be species that are abundant but inactive, and highly significant but rare species are also unlikely except for some that may be involved in transforming specific minor components in rumen contents.

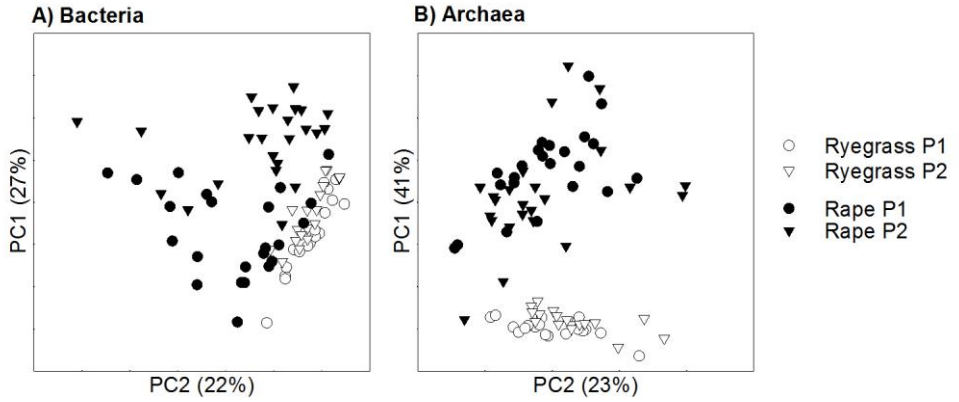
Effects of brassicas as feeds

Sheep fed brassicas consistently produce less CH_4 than do sheep fed ryegrass, expressed as CH_4 yield per unit of dry matter intake. Feeding brassicas may be a viable CH_4 emissions mitigation tool. However, understanding the basis for this lower yield has the potential to uncover general principles for CH_4 yield reduction that may extend beyond the feeding of brassicas. Sheep fed forage rape yielded 20-30% less CH_4 than sheep fed ryegrass (X Sun et al. unpublished data). The rumen microbial communities of these sheep were compared, and were shown to be fundamentally different by diet (Fig. 1). Ruminal microbial communities in the forage rape-fed sheep contained greater proportions of bacteria that probably ferment carbohydrates to lactate and then to propionate, like *Sharpea* and *Selenomonas*, while the ryegrass-fed sheep contained more H_2-producing clostridia and ruminococci. This suggests that the forage rape was fermented to less H_2 and more propionate by the feed-fermenting bacteria. The lower proportion of H_2 formed therefore accounted for the lower CH_4 yield. In parallel, more propionate and less acetate was measured in the rumens of the forage-rape-fed sheep. The results suggest a grain-like fermentation of the forage rape. Further efforts should be made to determine what the underlying mechanism might be. One possibility is that a lower ruminal pH inhibits methanogens, which results in a higher rumen H_2 concentration that selects against H_2-producing bacteria and for propionate formers (Janssen 2010). Another is that that rate of forage rape breakdown is greater than that of ryegrass, perhaps because forage rape contains more readily fermentable carbohydrates and less structural carbohydrates than ryegrass. A more rapid breakdown might result in increased ruminal H_2 concentrations, again selecting against H_2 producers (Janssen 2010). In both cases, less H_2 production results in less CH_4. Other brassicas also result in lower CH_4 yields (Sun et al. 2012), and the rumen microbial communities are similar in animals fed all these brassicas (G Henderson et al. unpublished data).

Low-methane emissions from sheep

There is a natural variation in CH_4 yield (amount of CH_4 per unit of dry matter intake) from sheep, and this is consistent over time and is heritable (Pinares-Patiño et al. 2003; Pinares-Patiño et al. 2013). Analysis of the microbial communities in sheep classed as either low CH_4 emitters or high CH_4 emitters has revealed that there is a continuum of bacterial community structures, and that this continuum is associated with the CH_4 yield (S Kittelmann et al. unpublished data). Interestingly, within the continuum are two different community types associated with low CH_4 emissions, and at the centre there is a high emission community type. One of the low-CH_4 types, designated Q-type, is characterised by a significant abundance of bacteria related to *Quinella* spp. These bacteria are thought to be propionate-producing carbohydrate fermenters that produce little or no H_2. The other low-CH_4 bacterial community, designated S-type, is characterised by bacteria related to the genera *Sharpea* and *Kandleria*. These are lactate-producing bacteria that do not produce H_2. In contrast, the high-CH_4 bacterial community, designated H-type, does not have a large abundance of these, but instead has an expanded presence of members of *Ruminococcus* and other *Ruminococcaceae*, *Coprococcus*, *Lachnospiraceae*, and other *Clostridiales*, which are known or expected to be H_2-producing bacteria. These predicted differences allow us to postulate that the basis for low CH_4 emissions in these sheep is selection for a rumen bacterial community that produces less H_2, which in

Figure 2 Average CH_4 yields (g CH_4/kg DMI) associated with 230 rumen samples from sheep classified into three ruminotypes (Q, H, and S) based on bacterial community composition. Whiskers represent the maximum and minimum values excluding outliers (circles).



turn results in less CH_4 being formed by the methanogens (Fig. 2). The animal factors that lead to selection of the different microbial communities are not yet understood. It has been observed that low CH_4 yields in sheep are associated with smaller rumen volumes (Goopy et al. 2014). This may result in a higher passage rate, which could select for such a low H_2-producing community through thermodynamic selection (Janssen 2010). However, the reason why there are two low CH_4 community types is not completely explained by this, and there may be multiple animal factors that result in one or the other low-CH_4 community type. It is also not yet clear if there are production advantages of one low-CH_4 community type over the other. Given that the fermentation pathways and products of the two low-CH_4 communities are likely to be different, there may be.

Differences in feed efficiency

Similar to the natural variation in CH_4 yield, there is also a natural variation in feed efficiency in cattle (Basarab et al. 2003; Waghorn et al. 2012). Feed efficiency, determined as residual feed intake (RFI), is a heritable trait (Berry and Crowley 2013), and some studies indicate that the rumen microbial community also differs between high and low RFI animals (Carberry et al. 2014; Zhou et al. 2009). These studies have focussed primarily on the methanogens. Zhou et al. (2009) identified *Methanobrevibacter* sp. AbM4 and Carberry at al. (2014) *Methanobrevibacter smithii* as methanogen species that differed in abundance in high and low RFI beef cattle. The reasons for the differences between RFI groups and between the studies have not yet been explored.

Community structure analysis of rumen bacteria, fungi, and protozoa in animals differing in feed efficiency has received surprisingly little attention, especially considering that these microbial groups are primarily involved in the breakdown of feed material. For the bacteria, it has been shown that there are some differences in community structure between high and low RFI animals (Hernandez-Sanabria et al. 2012), but it is not known whether and how the identified species contribute to RFI differences. The interplay between the microbial community, feeds, and animal genotype that might result in feed-specific efficiency traits is not understood. Diet-driven changes in rumen microbial community might be responsible for re-ranking of animals for RFI when feeds are changed (Berry & Crowley 2013).

Other production traits may also be associated with rumen microbial community differences, perhaps related to differences in volatile fatty acid production by different microbes. For example, the ratio of members of the bacterial phyla Firmicutes and Bacteroidetes have recently been shown to be correlated with daily milk fat yield in cows (Jami et al. 2014).

Looking into the black box

The rumen microbial community clearly changes in composition in response to changes in animal traits and feed input. The activity of these microbes is responsible for the mix of products, like volatile fatty acids, microbial protein and gases, that affect the ruminant animal's performance and environmental impact. The availability of molecular ecological methods can help in our efforts to understand rumen function and so improve animal production. As a first approach, the tools offer another way to see if an animal difference lies in rumen function. No differences in rumen microbial community, or, looking deeper, at rumen gene expression, would probably rule out rumen microbial function as a driver of the performance difference. Rumen microbial community profiling can help determine if there are multiple responses and potentially multiple drivers underlying a single animal phenotype, by looking for multiple community types. Interpreting community structure data by linking activities to microbes that are differentially present allows hypotheses to be drawn about potential causes and consequences. These tools could be (and in some cases are being) applied to environmental impacts like effects of feeds and animal genotype on CH_4 and nitrogen emissions, differences in feed efficiency, animal genetic differences in meat, milk, and wool production and qualities, and animal susceptibility to ingested toxins. As they develop

further to look at microbial activity, they could be used to help understand the impacts of different feeds on rumen function, and to understand what limits ruminal processing of ingested feed, perhaps to inform forage selection and feeding systems design.

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