Ruminal Microbiome: What is new about their contributions to ruminal fermentation and digestion and ruminant productivity?

T. G. Nagaraja
University Distinguished Professor
Department of Diagnostic Medicine/Pathobiology
College of Veterinary Medicine
Kansas State University
Manhattan, Kansas 66506-5800.
Tel: 785-532-1214
Cell: 785-341-6342
Email: tnagaraj@vet.k-state.edu

Introduction

Ruminants, particularly cattle, sheep, and goats, are important production animals for meat and milk to humans worldwide. Their importance comes from their unique digestive tract equipped with a specialized region called foregut or reticulo-rumen that carries out microbial digestion. Because of the microbial contribution to digestion, they are capable of converting fiber-based feeds, with or without grains, into high quality, protein-rich products like milk and meat. The reticulum and rumen, which are practically one compartment, are inhabited by a variety of microbes that work in concert to breakdown feeds to produce energy (volatile fatty acids; VFA), protein (microbial cells) and other nutrients like vitamins (microbial cells) to the host. The production of VFA, mainly from carbohydrates. is central to the ruminal fermentation because the process provides energy (ATP) for microbial growth, which serves as the major source of protein to the host, but also provides the animal with the precursors necessary to generate energy (mainly acetate), glucose (mainly propionate),and lipid (mainly acetate and butyrate). The fermentation of nitrogenous compounds is also an integral process because it provides the molecules (amino acids and ammonia) necessary to build microbial cell protein. In addition to the provision of nutrients, ruminal microbes are linked to host physiology, including the development of ruminal epithelium, most likely involving the modulation of host gene regulation by VFA.

Despite the global importance of ruminants and the tremendous progress that has been made to improve efficiency of milk and meat production, the rumen remains an under investigated, hence, under-characterized, microbial ecosystem. The description that 'rumen is a black box', first made several decades ago, is still applicable. At one time, rumen was the most extensively investigated anaerobic ecosystem. However, in the past 15 years, human gut microbial studies have far outpaced rumen microbiology. The human gut microbiome studies were part of the National Institute of Health-funded Human Microbiome Project, a logical extension of the Human Genome Project, to study the distribution and evolution of the constituent microorganisms in the human body (Llyod-Price et al., 2016). The impetus for the gut microbiome studies is largely because of the recognition that gut microbes have profound impact on human health and diseases (Cani et al., 2018).

Ruminal Microbes

A simple microscopic examination of ruminal fluid reveals a complex and diverse microbial

impact on human health and diseases (Cani et al., 2018).

Ruminal Microbes

A simple microscopic examination of ruminal fluid reveals a complex and diverse microbial population (Figure 1A, B, C). The population includes members of all three domains of life: Bacteria, Archaea (methanogens) and Eukarya (fungi and protozoa). The bacterial activities are absolutely essential for ruminal function and survival of the ruminant host; however, the archaeal and eukaryotic domains are not indispensable. Of the three domains, bacteria are the dominant population and most extensively investigated. Additionally, as in most microbial ecosystems, rumen also possesses acellular organisms called bacterial viruses or bacteriophages as well as fungal and protozoal phages. The structure and contribution of the viral community is the least investigated and hence not much is known about their role.

Molecular 'Omics' Methods

Initial molecular techniques were based on amplification of nucleic acids by polymerase chain reaction (PCR), both conventional and real-time, and restriction fragment length polymorphic analyses, such as ribotyping, pulsed-field gel electrophoresis, denatured gradient gel electrophoresis for identification and genetic typing. In recent years, research on rumen microbial ecology has expanded and exploded because of high-throughput and high-resolution nucleic acid sequence (DNA and RNA) and chemical separation and identification methods for protein and metabolites analyses. The advances in nucleic acid sequencing and bioinformatics analyses (whole genome sequencing, Amplicon sequencing and Metagenomics) have enabled researchers to analyze whole genome of an organism, community composition and function of an ecosystem by culture independent methods. DNA sequence information provides insight into physiologic and metabolic potential based on the whole genome, microbial community composition ('who are there?'), but does not provide a direct measure of the function ('what are they doing?'), although potential function can be deduced from the genes identified. Therefore, analysis that measure gene expressions or transcription of DNA to messenger RNA, called (meta)transcriptomics, translation of mRNA into protein, called (meta)proteomics, or ultimately production of products or metabolites, called metabolomics, are necessary to delineate functional profiling of the microbial community in the rumen.

The explosive growth in the study of gut microbes is because of the development of high-throughput and high-resolution molecular methods to unravel the community composition and functional role in the ecosystem.

Genomics of Ruminal Microbes

Genomics is the science of sequencing, mapping, and analyzing the entire complement of genetic information of an organism. Essentially, it is a genetic blueprint that provides complete information on the evolution and physiology of the organism. The process provides raw sequences that need to be assembled and annotated (read) to provide biological meaning. The process has become so inexpensive and common, the technique has become routine and often a starting point for characterizing and analyzing the metabolic potential of an organism. The

first rumen bacterial species that was genome sequenced was Fibrobacter succinogenes, a dominant fibrolytic bacterium (Jun et al., 2007). A global project on a comprehensive genomic analysis of ruminal microbes was initiated, somewhat similar human gut microbiome project. The Hungate 1000 project (www.Hunagte1000.org.nz), a global initiative launched in 2012, was designed to provide a reference set of rumen microbial genome sequences from cultivated ruminal bacteria, archaea, fungi and ciliated protozoa. The database, which are publicly available, enables researchers to analyze the physiology and metabolic potential of the organism with regard to ruminal function. At the beginning, genome sequences were 43 available for 14 bacterial species (belonging to 11 of 88 known genera in the rumen) and one methanogen. As many as 501 organisms (belonging to 73 of 88 genera) have been sequenced, referred to as Hungate genome catalog (Seshadri et al., 2018). Anaerobic fungal genomes have been difficult to sequence because of their high adenine and thymine content, repeat-sequences, complex physiology and unknown ploidy (Edwards et al., 2017). So far, whole genomes of five fungal species have been sequenced and are publicly available; however, there are no genomic sequence data on ciliated protozoa of the rumen.

The genomic sequence of an organism can provide comprehensive information on the metabolic potential. As an example, the genome of *Fibrobacter succinogenes,* a dominant fibrolytic organism, was the first ruminal bacterium to be sequenced and annotated (identification and analysis of the genes). The organism contains 3,252 genes coding for proteins and of those at least 104 genes were identified as coding for enzymes involved in plant cell wall degradation, including 33 genes for cellulose enzymes (Suen et al., 2011). Biochemical studies before genomic sequencing had only identified a dozen or so enzymes in *F. succinogenes* involved in cell wall digestion. The information gleaned from genomics of fibrolytic bacteria not only provides more information on fiber digestion in the rumen, but could potentially lead to identification of novel fibrolytic enzymes for commercial exploitations such as exogenous enzymes as feed additives or their use in biofuel production (Hess et al., 2011).

Amplicon Sequencing and Metagenomics. Sequence-based taxonomic profiling of a microbiome are carried out by amplifying 16S rRNA genes or by whole-metagenome shotgun sequencing. Amplicon sequences of 16S rRNA (reads) are commonly grouped into clusters, called as 'operational taxonomic units (OTUs)', which are then assigned to specific taxa based on sequence homology to a reference genomic sequence. In shotgun metagenomics, sequencing methods are applied to millions of random genomic fragments of DNA extracted from ruminal contents. The shotgun sequence reads are used to determine community composition, either by considering the reads individually or by first assembling them into contigs, which are then compared to a reference catalog of microbial genes or genomes. Such community analyses allow researchers to carry out taxonomic profiling of the microbial community to answer the question, 'who are present?' in the rumen. Taxonomic profiling of microbial species in the rumen have been performed on the different ruminant species (cattle, sheep, goats, and buffaloes) in relation to animal to animal variation, diet changes, ruminal disorders (acidosis, bloat, liver abscesses, low-milk fat syndrome), feed efficiency, milk production, methane production, maternal influence, feed additives, and seasonal changes, etc. (Denman et al., 2018; McCann et al., 2014). The utility and applicability of the rumen microbial profiling by molecular techniques are best evidenced by a study published by

Henderson et al. (2015). The study to assess the effects of diet, animal species and geographical location on ruminal microbial population involved 742 ruminal content samples from 32 animal species located in 35 countries. The differences in microbial communities were predominantly attributable to diet, and host factors were less influential. The protozoal communities were variable, but dominant bacteria and archaea were similar among all samples, and across animal species, diet, and geographical region a core microbiome was present (Henderson et al. (2015).

Metatranscriptomics. The metatranscriptomics, also called RNA-seq, involves sequencing all of the RNA produced by a microbial community, except ribosomal RNA, which is first depleted before sequencing. The RNA preparation is essentially messenger RNA (mRNA), which is converted to DNA, called complementary DNA (cDNA), for sequencing. A few of the studies on metatranscriptomics have focused on carbohydrate-degrading enzymes associated with microbes adherent to the fiber (Dai et al., 2015; Comtet-Marre et al., 2017). These studies have confirmed culture-base studies that major bacterial activities of fiber degradation were associated with species of the genera *Fibrobacter, Prevotella* and *Ruminococcus*, but also indicated large contribution of fungal and protozoal species.

Metaproteomics. Protein is the ultimate product of gene function, therefore, measuring protein abundance provides a more direct indicator of the functional activity of the microbes. The high-throughput method of measuring proteins and their abundance, called metaproteomics, involves mass-spectrometry-based shotgun quantification of peptide mass and abundance. The peptides are then associated with full-length proteins by sequence homology-based searches against reference databases, similar to data bases available for DNA and RNA sequences. Studies on metaproteomics of ruminal fluid are limited (Snelling and Wallace, 2017; Deusch and Seifert, 2015). The study by Deusch and Seifert (2015) identified in excess of 2,000 bacterial, 150 archaeal, and 800 fungal and protozoal proteins in the fiber adherent fraction of the ruminal digesta.

Metabolomics. The metabolomics refers to the detection, identification, and often quantification of metabolites and other small molecules in microbial communities. It is not done by predictions based on genomic information, instead, the analysis relies on techniques, such as high performance liquid chromatography, to separate chemicals, which are then identified and quantified by mass spectroscopy. Ruminal VFA analysis, a widely used technique in ruminal fermentation studies, is an example of a metabolomics. However, metabolomics, as defined now, is a more comprehensive chemical analysis that detects and quantifies all possible chemicals present in a sample. Metabolomic analysis to study the link between microbes and metabolites have been studied in several gut ecosystems. The first study on metabolomics of ruminal fluid was published by Ametaj et al (2010). The study measured ruminal metabolites of dairy cows fed diets with increasing proportions of grain. The results showed unhealthy alterations in the metabolites (increased methylamine, dimethylamine, N-nitrosodimethylamine, endotoxin, ethanol, phenylacetylglycine, etc.) in ruminal fluid of cows fed higher amounts of grains. What is not known how these alterations are linked to ruminal dysfunction.

Culture vs. Molecular methods

The understanding of the relationship between microbial community and rumen function has generally been based on culture-based analysis, particularly of bacteria and to some extent of fungi. Bacteria are the most predominant organisms in the rumen ranging from 10 to 100 billion per g and account for up to 50% of the microbial cell mass. Rumen bacterial cultivation began almost 8 decades ago with the development of anaerobic techniques, referred to as Hungate's techniques. A simple microscopic examination of ruminal contents has shown morphologically distinct bacteria, such as Lampropedia, Oscillospira, etc., which have not been cultivated yet. An advantage of microbial community analysis with nucleic acid-based techniques is that ruminal content samples need not processed immediately to maintain viability and can be archived and processed at convenience. However, with the development and application of a variety of cultivation-independent, molecular techniques, it has become clear that cultivation-based methods have only identified approximately 10 to 20% or less of the total microbial population harbored in the rumen

Ruminal Microbiome

A number of microbiome studies have attempted to relate or link community composition to rumen function and dysfunction. Jami et al (2014) reported that certain physiological parameters, such as total milk yield and milk fat yield correlated with the abundance of certain bacteria in the rumen. Xue et al (2019) reported that rumen bacterial richness and the relative abundance of several bacterial taxa were significantly different between dairy cows with high and low milk protein production. In a study that compared cows with high and low milk protein and fat percentages, concentrations of total VFA, acetate, propionate, and butyrate in high-producers were higher compared to low-producers (Wu et al., 2021). Also, the two groups displayed differences in 38 most abundant species, and genus *Prevotella* accounted for 68.8% of the species with the highest abundance in the high producers. A number of studies have addressed the link or relationship of ruminal microbiome to feed efficiency, a most important trait in the cattle production systems. Bacterial profiles in the rumens of efficient cattle (low residual feed intake) indicated differences in abundances of genera, *Butyrivibrio, Lactobacillus, Prevotella, Ruminococcus,* and *Succinivibrio* compared to inefficient cattle (high RFI; Myer et al., 2015). Li and Guan (2017) have compared cattle with high or low efficiency based on microbiome, metatranscriptomic and carbohydrate enzyme analyses. Three bacterial families (*Lachnospiraceae*, *Lactobacillaceae*, and *Veillonellaceae*) were more abundant in inefficient cattle, and they displayed greater abundance for 30 metabolic pathways and 11 carbohydrate active enzymes, whereas the efficient cattle displayed greater abundance for two metabolic pathways and one carbohydrate active enzyme. The authors suggested that rumen microbiomes of inefficient cattle are more metabolically diverse than those of efficient cattle.A detailed description of the microbiome studies in relation to hydrogen and methane production is given below.

Ruminal Microbiome: Hydrogen and Methane Production and Methane Mitigation Strategies

Hydrogen is a key product in the rumen and is produced by fermentation of both fiber and starch. The hydrogen is used in several hydrogen-sink reactions, of which, methane

production by archaeal population is the major route in the rumen (Figure 2). The utilization of H2 in an ecosystem that does not have oxygen is critical to prevent increases in the concentration of H2 and prevent disruption of the normal functioning of microbial enzymes involved in oxidation-reduction reactions. The production of H2 by one species and utilization by another species, referred to as 'inter species H2 transfer', is a major microbial interaction in the rumen (Figure 3). The interaction is thermodynamically favored to re-oxidize intracellular reduced cofactors, such as NADH FADH, FDH, etc. because of the ability of methanogens to decrease H2 concentration. Therefore, in the presence of methanogens or other H2-consuming reactions, such as succinate- or propionate producers, H2-producers shift fermentation away from formate, lactate and ethanol (products that do not yield ATP) to acetate (a product that yields ATP). The additional ATP results in higher growth, production of more enzymes, hence higher digestibility.

Although methanogens account for 1 to 4% of the total microbial population in the rumen, methanogenesis represents a major pathway to utilize hydrogen. The methanogens in the rumen are distributed free in ruminal fluid, attached to feed particles, associated with ciliated protozoa, and even attached to ruminal epithelium. Methanogens associated with protozoa and epithelium are novel phylotypes (or species), and the role of methanogens associated with ruminal epithelium has not been identified. Methanogens associated with ciliated protozoa can be intracellular, called endosymbionts, or on the surface, called ectosymbionts. Intracellular methanogens are found inside most of the common protozoal species. In contrast, the extracellular methanogens are less numerous and only 30 to 50% of the protozoan cells carry them. Protozoa produce hydrogen in large amounts in a specialized organelle called hydrogenosomes (similar to mitochondria). This hydrogen is utilized by methanogens that are inside or outside the protozoan cell, and the association represents an important microbial interaction in the rumen.

There are only a limited number of substrates that methanogens are capable of utilizing for methanogenesis. In the rumen the major substrates are CO2 and hydrogen, and formate, a product of many bacteria, particularly fiber digesters. Formate accounts for approximately up to 18% of ruminal methane. There are three major pathways of ruminal methanogenesis (Figure 4):

a. Hydrogenotrophic pathway in which H2 is used as electron donor to reduce CO2 to methane.
b. Methylotrophic pathway in which methyl group of methanol or methylamines is reduced to methane
c. Acetoclastic pathway in which the methyl group of acetate is reduced to methane.

Methane is a waste product, hence, it is expelled into the environment, which results in the loss of energy (2 to 15% of feed energy) to the animal and a anthropogenic source of greenhouse gas to the environment. Methane, as a potent greenhouse gas, is a major contributor, next only to CO2, of global warming. Methane is more potent than CO2 and estimated to account for 14% of total global greenhouse gas emissions. About 25% of the anthropogenic methane emissions are due to gut fermentations in livestock, particularly ruminants.

Although there is no relationship between methanogen abundance in the rumen to

production efficiency of the animal, the species composition of methanogenic population is different between efficient and inefficient cattle (Zhou et al. 2009). In a study that used metagenomics analysis, a significantly higher abundance of *Methanobrevibacter* was detected in the rumen of high-methane producing steers compared to low-methane producers (Wallace et al., 2015). Interestingly, a couple of studies in sheep have noted differences in rumen microbiome beyond methanogens in relation to low- or high- methane producers (Kittelmann e al., 2014; Kamke et al., 2016; Wallace et al., 2015). Two bacterial genera, *Sharpea* and *Kandleria* (Kumar et al., 2018) were associated with low methane production. A metagenomic and metatranscriptomic study conducted by Kamke et al. (2016) confirmed the relative abundance of *Sharpea* was greater in low-methane producing sheep compared to high methane producing sheep. Not much is known about these two bacterial genera, except they are anaerobic and produce predominantly D-lactic acid from sugars. Not surprisingly, another organism that is significantly enriched in low methane producers is *Megasphaera elsdenii,* a major lactic acid-fermenting bacterium in the rumen (Kamke et al., 2016; Shabat et al., 2016). Thus, methanogenesis not only is related to methanogens but also other components of the microbiome, particularly lactic acid producers and fermenters. It is possible that lactic acid pathway (production and fermentation) may be central to the production of VFA as an alternative sink to methanogenesis (Mizrahi and Jami, 2018).

Because ruminal methanogenesis results in the loss of energy, therefore, for a number of years, a major focus of researchers has been to develop an effective strategy to inhibit methane production in the rumen. The strategies that have been investigated can be broadly categorized to intervene at the following three stages of methane production (Figure 5):

1. Inhibit or reduce production of major precursors of methane production (H2 and formic acid).
2. Divert hydrogen to alternate hydrogen-sink reactions in the rumen, which include lactate, propionate and valerate production, acetate production by reduction of CO_2, and reduction of fumarate, nitrate and sulfate.
3. Eliminate or reduce methanogens in the rumen.

Because methane is the major scavenger of hydrogen in the rumen, methane inhibition results in hydrogen accumulation. It is generally assumed that hydrogen accumulation will inhibit re-oxidation of reduced cofactors like NADH and adversely affect the microbial fermentation. Therefore, strategies to mitigate methanogens should consider alternatives to sink hydrogen in the fermentation process (Wright and Klive, 2011). However, no negative effects of methane inhibition have been shown possibly because none of the methods tested inhibit 100% of methane production. Even an effective compound like bromochloromethane (BCM), which reduces methane production by about 80%, had no negative effective effects on feed intake and digestibility in goats (Mitsumori et al., 2012). Although several inhibitors of methane production were effective in in vitro studies, they were reported to be ineffective in in vivo studies.

A promising compound appears to be 3-nitroxy propanol (3-NOP), an analog of the Coenzyme M that inhibits methyl coenzyme M reductase, which is present in all methanogens and is the terminal step in methanogenesis (Ermler et al., 1993). Several studies have shown that

including 3-NOP in diets of dairy cows (Hristov et al., 2015) and beef cattle (Vyas et al., 2016) decreased methane emissions (up to 60%) with no negative effect on ruminal fermentation and animal productivity. Furthermore, inclusion of monensin in the diet had no significant interaction with the effects of 3-NOP (Vyas et al., 2018)

Researchers in New Zealand (Attwood et al., 2011; Leahy et al., 2010) have sequenced and analyzed the genome of *Methanobrevibacter ruminantium,* a major ruminal methanogen, and have identified methanogen-specific genes that code for critical enzymes for methane production, which can potentially be targeted for mitigation. The organism contains a large number of genes that encode for surface adhesion like proteins, which may be involved in mediating close association with hydrogen- producing bacterium or protozoa in the rumen. These proteins can potentially be used as antigens in a vaccine to induce antibodies to inhibit ruminal methanogens.

Conclusions

Rumen is inhabited by a dense population of microbes, which include members of all three domains of life: Bacteria, Archaea (methanogens) and Eukarya (fungi and protozoa), as well as viruses. The fermentative activities of these microbes convert complex organic feedstuffs into energy and protein, which are then used by the host for growth and production. Molecular methods to analyze bacterial community composition have identified a number of novel bacterial genera and species, which have not been cultured, therefore, nothing is known about their role in ruminal fermentation. Anaerobic fungi are the most active and effective fibrolytic organisms because of their combined mechanical (ability to penetrate plant structures) and enzymatic activities. Although ciliated protozoa contribute to digestibility of feeds and VFA production, their overall role in ruminal fermentation and contribution to the host nutrition is still an area of considerable debate and controversy. Rumen viral community analysis has identified a number of viral types and of those a small population have a significant similarity to known viruses. Viruses may be the driving factor in the evolution and stability of microbes in the rumen. Before the advent of molecular techniques, the understanding of the ruminal microbes and their contribution to the host nutrition was based on classical culture methods. In recent years, there is explosive growth on the culture-independent methods, which have provided identity and quantity of microbes and have vastly expanded our understanding of the community composition. These studies are providing answers to who is there, and how many, but provide limited information on what are they doing. Cultivation and functional characterization of species and strains of microbes identified by molecular methods remain a major challenge to rumen microbiologists. An increased functional understanding of the microbiome of the rumen as well as that of the hindgut of ruminants is essential to develop novel approaches to manipulate to improve food animal production.

References

Attwood, G. T., E. Altermann, W. J. Kelly, S. C. Leahy, L. Zhang, and M. Morrison. 2011. Exploring rumen methanogen genomes to identify targets for methane mitigation strategies. Anim. Feed Sci. Technol. 166-167:65-75.

Ametaj, B. N., Q. Zebeli, F. Saleem, N. Psychogios, M. J. Lewis, S. M. Dunn, J. Xia and D. S. Wishart. 2010. Metabolomics reveals unhealthy alterations in rumen metabolism with increased proportion of cereal grain in the diet of dairy cows. Metabolomics 6:583-594.

Cani, P. D. 2018. Human gut microbiome: Hopes, threats, and promises. Gut 67:1716-1725.

Comtet-Marre S, N. Parisot, P. Lepercq, F. Chaucheyras-Durand, P. Mosoni, E. Peyretaillade, A. R. Bayat, K. J. Shingfield, P. Peyret and E. Forano. 2017. Metatranscriptomics reveals the active bacterial and eukaryotic fibrolytic communities in the rumen of dairy cow fed a mixed diet. Front. Microbiol. 8:67.

Dai X, Y. Tian, J. Li, X. Su, X. Wang, S. Zhao, L. Liu, Y. Luo, D. Liu, H. Zheng, J. Wang, Z. Dong, S. Hu and L. Huang. 2015. Metatranscriptomic analyses of plant cell wall polysaccharide degradation by microorganisms in the cow rumen. Appl. Environ. Microbiol. 81:1375-1386.

Denman, S. E., D. P. Morgavi, and C. S. McSweeney. 2018. Review: The application of omics to rumen microbiota function. Animal. 12:233-245.

Deusch S. and J. Seifert. 2015. Catching the tip of the iceberg – evaluation of sample preparation protocols for metaproteomic studies of the rumen microbiota. Proteomics 15:3590–3595.

Edwards, J. E., R. J. Forster, T. M. Callaghan, V. Dollhofer, S. S. Dagar, Y. Cheng, J. Chang. 2017. S. Kittelmann, K. Fliegerova, A. K. Puniya, J. K. Henske, S. P. Gilmore, M. A. O'Malley, G. W. Griffith and H. Smidt. 2017. PCR and omics based techniques to study the diversity, ecology and biology of anaerobic fungi: Insights, challenges and opportunities. Front. Microbiol. 8:1657.

Ermler U, W. Grabarse, S. Shima, M. Goubeaud, and R. K. Thauer RK. 1997 Crystal structure of methyl-coenzyme M reductase: the key enzyme of biological methane formation. Science 278(5342):1457–1462

Henderson G, F. Cox, S. Ganesh, A. Jonker, W. Young and P. H. Janssen. 2015. Rumen microbial community composition varies with diet and host, but a core microbiome is found across a wide geographical range. Scientific Reports 5,14567.

Hess, M., A. Sczyrba, R. Egan, T-W. Kim, H. Chokhawala, G. Schroth, S. Luo, D. S. Clark, F. Chen, T. Zhang, R. I. Mackie, L. A. Pennacchio, S. G. Tringe, A. Visel, T. Woyke, Z. Wang and E. M. Rubin. 2011. Metagenomic discovery of biomass-degrading genes and genomes from cow rumen. Science 331:463-467.

Hristov, A. N., J. Oh, F. Giallongo, T. W. Frederick, M. T. Harper, H. L. Weeks, et al.. 2015. An inhibitor persistently decreased enteric methane emission from dairy cows with no negative effect on milk production. Proc. Natl. Acad. Sci. USA 112:10663-10668.

Li F, Guan LL. 2017. Metatranscriptomic profiling reveals linkages between the active rumen microbiome and feed efficiency in beef cattle. Appl. Environ.Microbiol. 83(9):e00061-17.

Jami, E., B. A. White, I. Mizrahi. (2014). Potential role of the bovine rumen microbiome in modulating milk composition and feed efficiency. PLoS One, 9:e85423.

Jun, H. S., M. Qi, J. K. Ha, and C. W. Forsberg. 2007. Fibrobacter succinogenes, a dominant fibrolytic ruminal bacterium" Transition to the post genomic era. Asian-Australas J. Anim. Sci. 20:802-810.

Kamke J., S. Kittelmann, P. Soni, Y. Li, M. Tavendale, S. Ganesh, P. H. Janssen, W. Shi, J. Froula and E. M. Rubin. 2016. Rumen metagenome and metatranscriptome analyses of low methane yield sheep reveals a Sharpea-enriched microbiome characterized by lactic acid formation and utilization. Microbiome 4, 56.

Kittelmann, S., C. S. Pinares-Patino, H. Seedorf, M. R. Kirk, S. Ganesh, J. C. McEwan, and P. H. Janssen. 2014. Two different bacterial community types are linked with the low-methane emission trait in sheep. PLoS One 9:e103171.

Kumar S., B. P. Treloar, K. H. Teh, C. M. McKenzie, G. Henderson, G. T. Attwood, S. M. Waters, M. L. Patchett, P. H. Janssen. 2018. Sharpea and Kandleria are lactic acid producing rumen bacteria that do not change their fermentation products when co-cultured with a methanogen. Anaerobe 54:31-35.

Leahy, S. C., W. J. Kelly, E. H. Altermann, R. S. Ronimus, C. Yeoman, D. M. Pacheco, D. Li, Z. Kong, S. McTavish, C. Sang, S. C. Lambie, P. H. Janssen, D. Dey, G. T. Attwood. 2010. The genome sequence of the rumen methanogen Methanobrevibacter ruminantium reveals new possibilities for controlling ruminant methane emissions. PLoS One 5:e8926.

Llyod-Price, J., G. Abu-Ali, and C. Huttenhower. 2016. The healthy human microbiome. Genom. Med.8:51.

McCann, J. G., T. A. Wickersham, and J. L. Loor. 2014. High-throughput methods redefine the rumen microbiome and its relationship with nutrition and metabolism. Bioinform Biol. Insights. 8:109-125.

Mitsumori, M., T. Shinkai, A. Takenaka, O. Enishi, K. Higuchi, Y. Kobayashi, Y., et al. 2012. Response in digestion, rumen fermentation and microbial populations to inhibition of methane formation by a halogenated methane analogue. Br. J. Nutr. 108: 482-491.

Myer, P. R. T. P. Smith, J. E Wells, L. A. Kuehn, and H. C. Freely (@015). Rumen microbome from steers differing in feed efficiency . PLoS One, 10:e0129174.

Seshadri R., S. C. Leahy, G. T. Attwood, K. H. Teh, S. C. Lambie, A, L. Cookson, E. A. Eloe-Fadrosh, G. A. Pavlopoulos, M. Hadjithomas, N. J. Varghese, D. Paez-Espino. Hungate project collaborators, R. Perry, G. Henderson, C. J. Creevey, N. Terrapon, P. Lapebie, E. Drula, V. Lombard, E. Rubin, N. C. Kyrpides, B. Henrissat, T. Woyke, N. N. Ivanova and W. J. Kelly. 2018. Cultivation and sequencing of rumen microbiome members from the Hungate1000 collection. Nat. Biotechnol. 36:359-367.

Shabat S. K. B., G. Sasson, A. Doron-Faigenboim, T. Durman, S. Yaacoby, M. E. B. Miller, B. A. White, N. Shterzer and I. Mizrahi. 2016. Specific microbiome-dependent mechanisms underlie the energy harvest efficiency of ruminants. ISME J. 10: 2958-2972..

Snelling, T. J., and R. J. Wallace. 2017. The rumen microbial metaproteome as revealed by SDS-PAGE. BMC Microbiol. 17:9.

Suen, G., P. J. Weimer, D. M. Stevenson, F. O. Aylward, J. Boyum, J. Deneke, C. Drinkwater, N. N. Ivanova, N. Mikhailova, O. Chertkov, L. A. Goodwin, C. R. Currie, D. Mead, P. J. Brumm. 2011. The complete genome sequence of Fibrobacter succinogenes S85 reveals a cellulolytic and metabolic specialist. PloS One 6:e18814.

Vyas D, A. W. Alemu, S. M. McGinn, S. M. Duval, M. Kindermann, and K. A. Beauchemin. 2018. The combined effects of supplementing monensin and 3-nitrooxypropanol on methane emission, growth rate, and feed conversion efficiency in beef cattle fed high-forage and high-grain diets. J. Anim. Sci. 96:2923–22938.

Vyas D, S. M. McGinn, S. Duval, M. Kindermann, and K. A. Beauchemin. 2016. Effects of sustained reduction of enteric methane emissions with dietary supplementation of 3-nitrooxypropanol on growth performance of growing and finishing beef cattle. J. Anim. Sci. 94:2024–2034.

Wallace R. J., J. A. Rooke, N. McKain, C-A. Duthie, J. J. Hyslop, D. W. Ross, A. Waterhouse, M. Watson and R. Roehe. 2015. The rumen microbial metagenome associated with high methane production in cattle. BMC Genomics 16:839.

Wright, A-D.G. and A. Klive (2010). Does the complexity of the rumen microbial ecology preclude methane mitigation? Animal Feed Sci. Technol. 166-167:248-253.

Wu, X., Huang, S., Huang, J., Peng, P., Liu, Y., Han, B., and Sun, D. (2021). Identification of the potential role of the rumen microbiome in milk protein and fat synthesis in dairy cows using metagenomic sequencing. Animals. 11:1247.

Xue, M.Y. H. Z. Sun, X. H. Wu, L. L. Guan, J. X. Liu. (2019). Assessment of rumen bacteria in dairy cows with varied milk protein yield. J. Dairy Sci., 102:5031–5041.

Zhou M. I., E. Hernandez-Sanabria, and L. L. Guan. 2009. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Appl. Environ. Microbiol. 75:6524-6533.

Figure 1A. Ruminal microbes by size

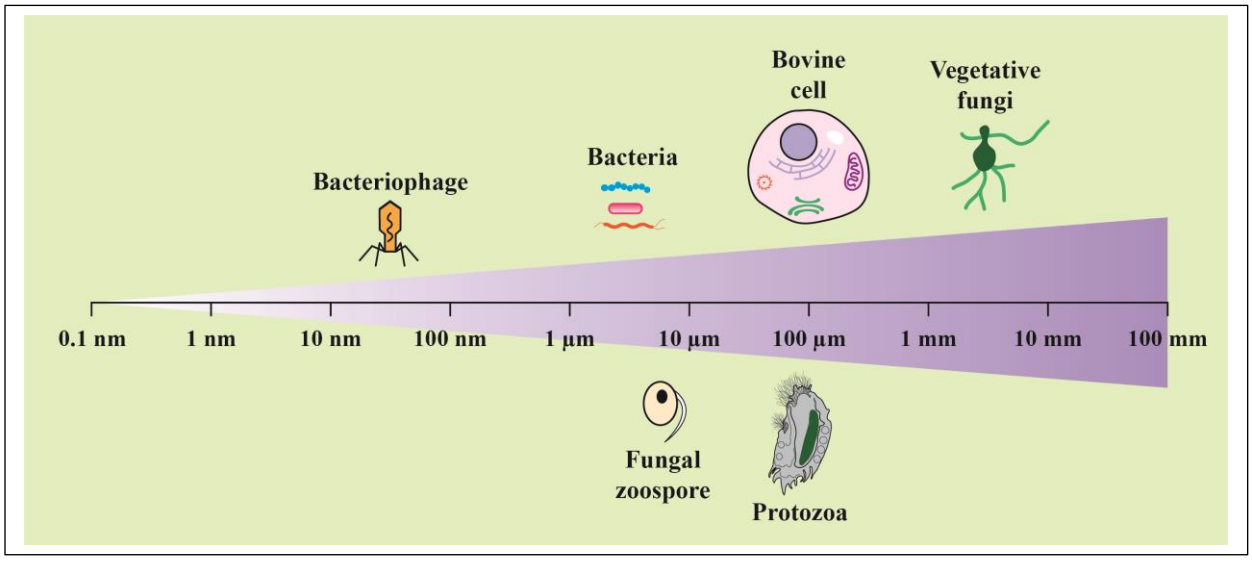

Figure 1B. Ruminal microbes by numbers

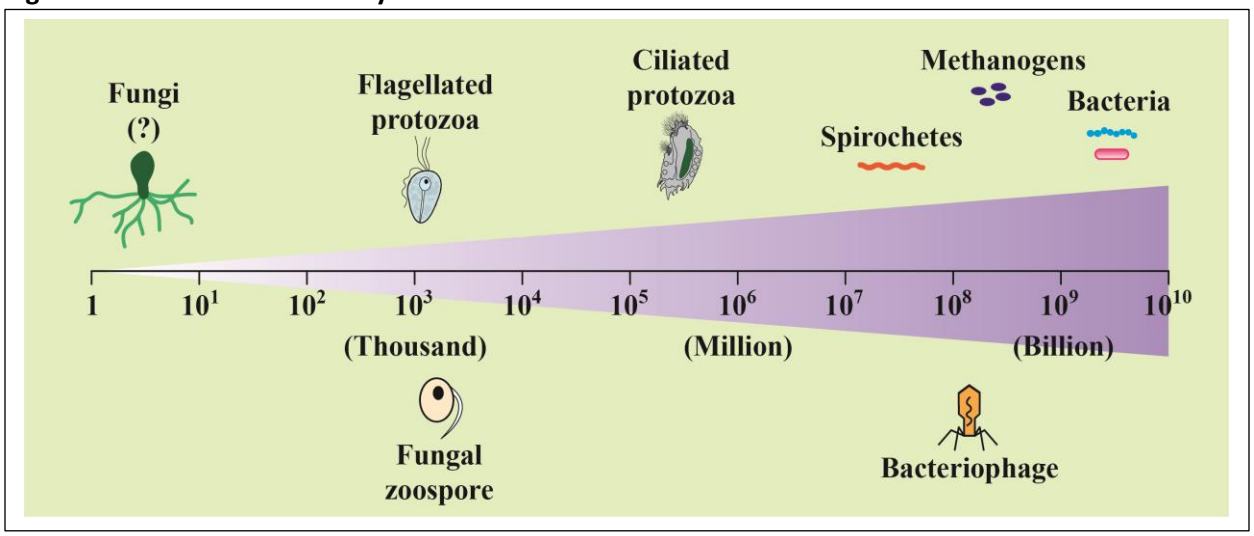

Figure 1C. Ruminal microbes by proportion

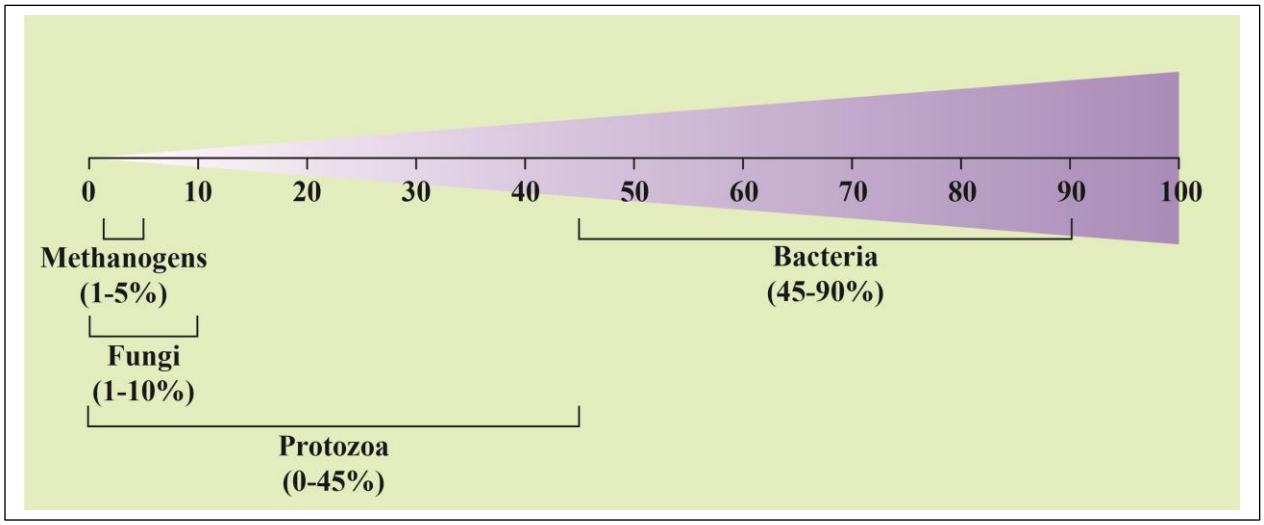

Figure 2. Hydrogen utilizing reactions in the rumen

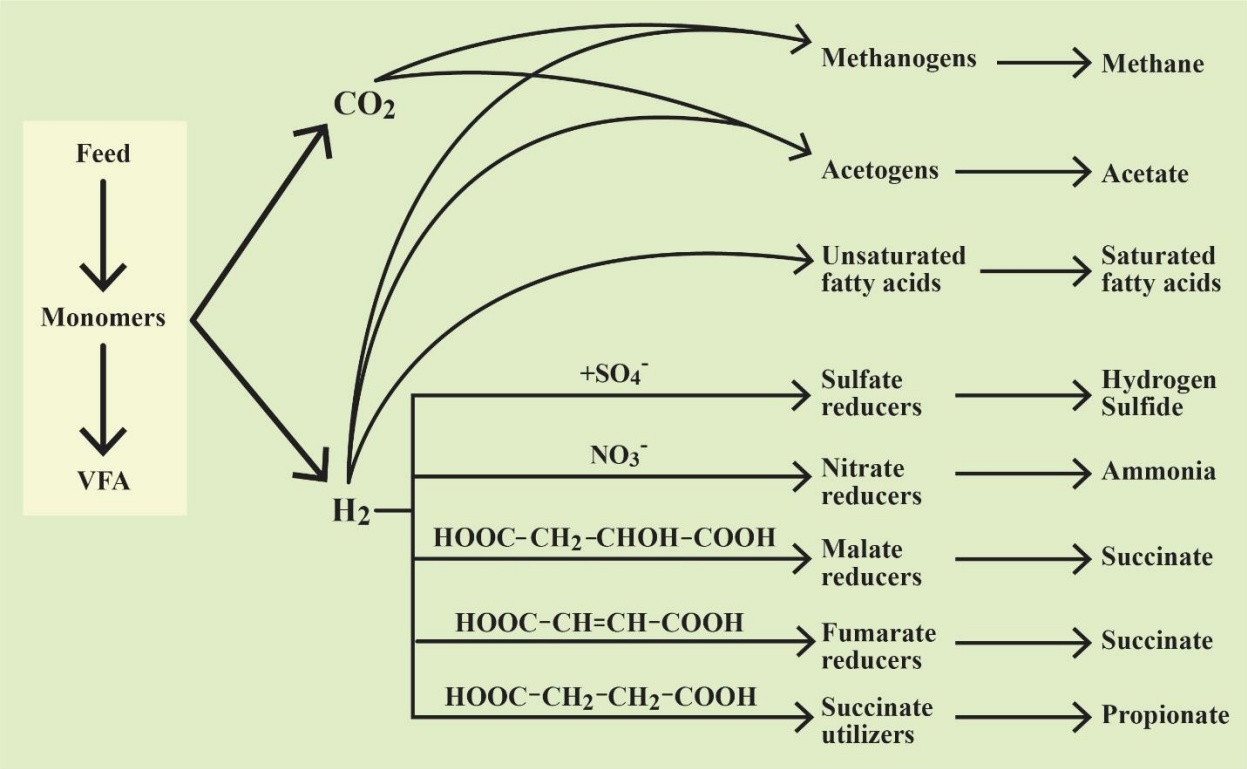

Figure 3. Interspecies hydrogen transfer in the rumen

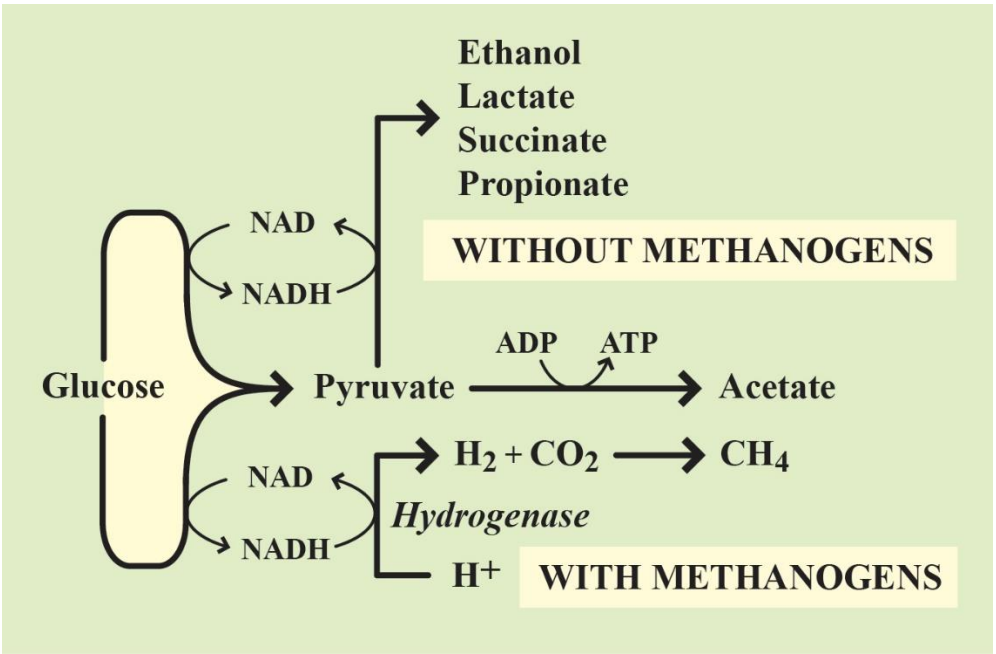

Figure 4. Major and minor pathways for methane production in the rumen

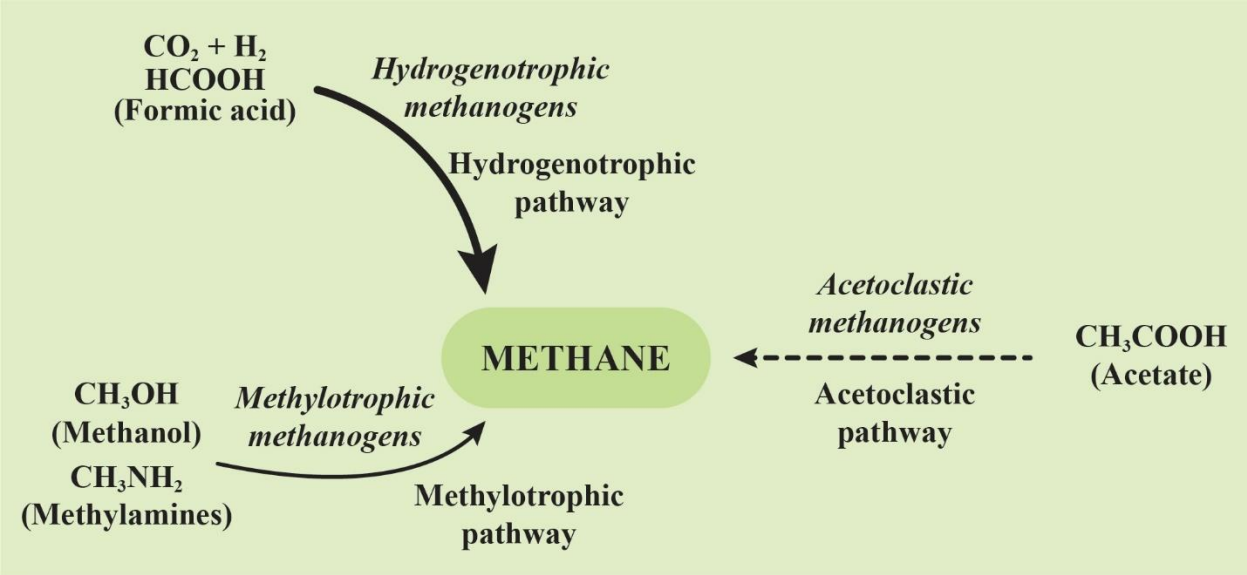

Figure 5. Stages in ruminal methanogenesis for intervention to inhibit methane production.