

REVIEW

REVIEW: The rumen microbiome: Composition, abundance, diversity, and new investigative tools

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ABSTRACT

Ruminants are able to degrade and use fibrous feed as a source of energy and nutrients because of the presence of complex anaerobic microbiota in the rumen, composed mainly of bacteria, fungi, and ciliate protozoa. Ruminal microorganisms play different roles in feed digestion and act synergistically to ferment plant structural and nonstructural carbohydrates and proteins. This review reports the latest assessment of microbiota diversity in the rumen ecosystem and summarizes the molecular techniques and the newly available “omic” technologies, based on DNA and RNA sequence analysis, which allow for new insights into the structure and functions of these complex microbial communities.

Key words: rumen, microbiota, ruminant digestion, diversity, DNA/RNA-based technique

INTRODUCTION

Herbivores, omnivores, and carnivores have in common the presence of microbial life in their gastrointestinal tract. Gut microbial communities (Figure 1), composed of bacteria, ciliate and flagellate protozoa, anaerobic fungi, and viruses, play a vital role in nutritional, physiological, immunological, and protective functions of the host. The rumen is one of the most extensively studied gut ecosystems, because of the importance of ruminants for human nutrition and the major role played by rumen microbes in nutrition and health of the ruminant animal. Indeed, ruminants, in a symbiotic relationship with the microorganisms in the rumen, degrade and use fibrous feed as a source of energy and nutrients. Ruminal microorganisms play different roles in feed digestion and act synergistically to ferment plant structural and nonstructural carbohydrates and proteins. This review provides an overview of microbial abundance and diversity in the rumen. Moreover, this paper provides an outline of the current molecular approaches used to gain a better

understanding of the structure and function of the microbial ecosystem.

RUMEN MICROBIAL ABUNDANCE AND DIVERSITY

Conventional culture-based techniques such as isolation, enumeration, and nutritional characterization have provided significant information on the diversity of the rumen microbiota. In fact, more than 200 species of bacteria and at least 100 species of protozoa and fungi inhabiting the rumen have been identified by culture-based techniques. Nevertheless, over the last 10 years the development of high-throughput sequencing techniques has allowed for a considerable increase in knowledge of the microbial diversity of the rumen ecosystem. Indeed, even if culture-based techniques are successful in isolating key representatives of rumen bacteria, archaea, and fungi, they are not well suited to characterizing the overall microbial diversity, because a vast majority of rumen species are not yet culturable. Recent studies indicate that when quantified

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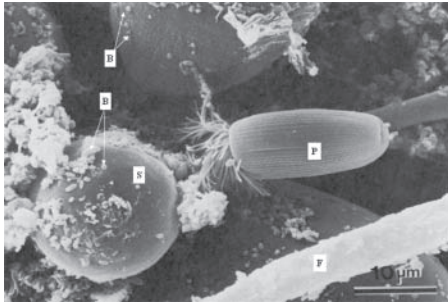


Figure 1. Rumen microbial consortium. B = bacteria; P = ciliate protozoa; S = fungal sporocyst; F = plant fiber. Scanning electron microscope photograph. B. Gaillard-Martinie, INRA UR454 Microbiology, France. Copyright INRA—not for commercial purpose.

by real-time PCR, some uncultured bacteria are as abundant as major cultured bacteria found in the rumen, suggesting that uncultured bacteria may play an important role in ruminal fermentation (Kim et al., 2011). The diversity within the rumen can be strongly affected not only by diet composition (Chaucheyras-Durand et al., 2012) but probably also by host genetics (Benson et al., 2010) and environmental factors (Uyeno et al., 2010).

Rumen Bacteria and Archaea

The diversity of bacteria and Archaea in the rumen of predominantly domesticated livestock has been studied through a metaanalysis of all curated 16S ribosomal RNA (rRNA) gene sequences deposited in the Ribosomal Database Project database as of 2010, amounting to 13,478 bacterial and 3,516 archaeal sequences (Kim et al., 2011). From this analysis, the diversity of bacterial and archaeal species in the rumen is estimated to be approximately 7,000 and 1,500 species, respectively. The bacterial sequences were assigned to 5,271 operation taxonomic units, which represented 19 existing phyla, with *Firmicutes* (~56%), *Bacteroidetes* (~31%), and *Proteobacteria* (~4%) being the predominant. More than 90% of the *Firmicutes* sequences were related to genera within the class

Clostridia. Within the *Clostridia*, *Lachnospiraceae*, *Ruminococcaceae*, and *Veillonellaceae* were the largest families. The predominant genera included *Butyrivibrio*, *Acetivibrio*, *Ruminococcus*, *Succiniclacticum*, *Pseudobutyrvibrio*, and *Mogibacterium*. Within the class *Bacilli*, *Streptococci* were dominant. In the *Bacteroidetes* phylum, the majority of sequences were assigned to class *Bacteroidia*, and *Prevotella* was the most predominant genus, the species *Prevotella ruminicola*, *Prevotella brevis*, *Prevotella bryantii*, and *Prevotella albensis* being among the most commonly found. All 5 classes of *Proteobacteria* were represented in the database of rumen sequences, with a predominance of *Proteobacteria*. It has been suggested that, as in humans, a shared core community exists between different cows because around 35 genera could be shared across 90 to 100% of the 16 cows studied in Jami and Mizrahi (2012a).

The abundance of rumen bacteria has been reported to be up to 10^{12} individuals per gram of rumen content. Taxa abundance may greatly vary across individuals. Jami and Mizrahi (2012b) used quantitative PCR (qPCR) to quantify the abundance of 13 key functional species of the rumen in 16 cows. They found that some species, such as *Eubacterium ruminantium*, a biohydrogenating species with hemicellulolytic activities, were very stable in abundance across individuals, whereas others, such as *Megasphaera elsdenii*, a lactic acid-using species, seemed to exhibit a much greater degree of variation between animals, with values spreading over 3 orders of magnitude. In the study of Kim et al. (2011), almost all the archaeal sequences were assigned to the phylum Euryarchaeota. Based on the analysis of public databases, more than 90% of sequences of rumen archaea are affiliated with methane-producing genera *Methanobrevibacter* (>60%), *Methanomicrobium* (~15%), and a group of uncultured rumen archaea commonly referred to as rumen cluster C (~16%). Within the genus *Methanobrevibacter*, 2 major groups

M. gottschalkii (contains *M. gottschalkii*, *M. thaueri*, and *M. millerae*) and *M. ruminantium* (*M. ruminantium* and *M. olleyae*) appear dominant. In terms of abundance, depending on the target gene (*rrs* or *mcrA*), qPCR data report values between 10^8 to 10^{10} gene copies per gram of rumen contents (Mosoni et al., 2011).

Rumen Protozoa

Microscopy has been the method of choice in identifying and enumerating protozoal populations in rumen samples and is still a reliable method. With this method, protozoa are generally enumerated at 10^5 to 10^6 cells per gram of rumen contents, and it has found *Entodinium* to be the dominant genus in the rumen, which can account for up to 95% of the total population in animals fed high-grain diets. Besides microscopy, protozoal diversity representing many different genera and species has been assessed through molecular techniques that are becoming more prevalent in ecological studies of ruminal protozoa (Skillman et al., 2006; Tymensen et al., 2012). For example, using a DNA fingerprinting method with various protozoa-specific PCR primers, Sylvester et al. (2004) showed the effect of diets on protozoal diversity in the rumen and duodenum and identified as major protozoal species *Epidinium caudatum*, *Entodinium caudatum*, and *Isotricha prostoma*. Other protozoal genera include *Dasytricha*, *Ostracodinium*, *Diplodinium*, *Diploplastron*, *Eudiplodinium*, *Epidinium*, *Ophryoscolex*, and *Polyplastron*.

Rumen Fungi

Anaerobic fungi have been isolated from rumen contents and feces of numerous herbivores including ruminant and monogastric animals and have been assigned recently to a new and separate phylum, Neocallimastigomycota (Griffith et al., 2010; Ligenstoffer et al., 2010). Six genera, *Neocallimastix*, *Piromyces*, *Anaeromyces*, *Caecomyces*, *Orpinomyces*, and more recently *Cyllamyces*, have been

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