

# Importance of CAZyme Producing Microbial Flora in the Herbivore Gut

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## Abstract

Considering the complexity of the recalcitrant plant biomass which is used by the herbivores as a food source, these organisms have presence of a unique digestive chamber termed rumen. The microbial diversity associated with this organ has evolved along with their host for digestion of complex carbohydrate polymer via production of a family of enzymes termed CAZymes. Thus, the rumen acts as a potential source for discovery of novel microbes and enzymes associated with lignocellulose degradation for commercial purposes.

**Keywords:** CAZymes; Herbivores; Lignocellulose; Microbial diversity; Rumen

## Introduction

Studies of the gut microflora of various organisms have interested researchers since a while considering the dynamic relationship between these organisms and their host in terms of their bodily functions such as metabolism and defense, which have evolved over the course of host evolution and, in the case of herbivores, even play a critical role in multiple origin decisions [1]. In herbivores, an important role of these microorganisms is their ability to aid in the digestion of recalcitrant lignocellulosic biomass, which is the food source of herbivores. In addition, herbivores have evolved a specialized chamber in their digestive system, the rumen, associated with their ability to efficiently consume plant biomass as an energy source, which is also a result of symbiotic evolution [2]. This mini review focuses on the relationship between microbial diversity and the environment that the rumen of herbivores provides for the digestion of plant biomass.

## Rumen of Herbivores and Microbial Diversity

Throughout evolution, herbivores used plant biomass as their sole source of energy, but enzymes (also known as carbohydrate-active enzymes or CAZymes) required to break down cellulose and other carbohydrates associated with plant biomass were absent from their metabolism. As a result, a symbiotic association was found to be observed between different microorganisms located at different sites in the digestive tract of herbivores and the herbivorous host, which has evolved specialized chambers in its digestive system that support the growth of microbes capable of degrading lignocellulose. Based on the location of the active digestive site in the digestive system, two types of herbivores have evolved over time, pre-gastric/foregut fermenting and post-gastric/hind-gastric fermenters. The most common are foregut fermenters, whose anatomy is characterized by the presence of a four-chambered stomach separated into rumen, reticulum, omasum, and abomasum [3].

The first three chambers, i.e., rumen, reticulum, and omasum, are the fore-stomachs specifically designed to digest complex carbohydrates, followed by the abomasum, which is a true stomach. This type of digestive system is found in ruminants, which include cattle, deer, sheep, elk, goats, and most herbivores. These organisms have a unique mechanism of rumination, which means that once they ingest the plant biomass, the food does not pass

directly through the digestive tract, but is returned from the rumen to the oral cavity where it is chewed again, breaking it down into smaller particles that facilitate fermentation [4].

The rumen is a sac-like chamber and is the main part where the entire fermentation process of ruminants takes place. It is very voluminous and houses a microbial population of different microbes that have evolved over the years along with their host. The environment of the rumen can be described as low in oxygen. Anaerobic microbes perform the necessary biochemical reactions for energy production, while facultative aerobes are present to remove any oxygen that may have passed over to maintain anaerobic conditions. In addition, the absence of gastric acid, along with microbial activity, indicates that the rumen has a pH (H<sub>2</sub>O) between 6 and 7 [3].

Organisms from various life domains are present in the rumen, including bacteria, fungi, protozoa, archaea, viruses, etc. [5]. However, bacteria are considered the most important group of organisms that play an important role in the degradation of lignocellulose. These bacteria, through the production of various CAZymes, convert the complex sugar molecules into simple sugar monomers that can be used by the host for energy production [6]. Numerous studies have been conducted using both classical and modern omics-based methods to understand ruminant microbial diversity [2,7,8]. In this work, it has been reported that there are differences in microbial community composition among different animals, with differences observed even within genera and species. These differences were specifically associated with the feeding habits of organisms, with factors such as age and health status of the ruminants playing only a minor role. These studies have shown that most bacteria found belong to the phyla Bacteroidetes and Firmicutes [9].

Although fungi also produce various CAZymes for the degradation of lignocellulose, they have not been properly studied in ruminants, while archaea and protozoa are mainly important for methane production and nitrogen cycling [10].

### CAZyme Diversity in the Rumen

CAZymes comprise the entire family of enzymes that play a role in the synthesis, conversion, and metabolism of carbohydrates. Numerous CAZymes have been isolated from various sources, and a database called CAZy (<http://www.cazy.org/>) along with a dedicated encyclopedia, CAZypedia (<https://www.cazypedia.org/>), is present containing a listing of all the different CAZymes, which is updated from time to time [11]. CAZymes have been classified into six groups based on their function, namely Glycosyl Hydrolase (GH), Glycosyl Transferase (GT), Carbohydrate Esterase (CE), Polysaccharide Lyase (PL), Auxiliary Activities (AA), and Carbohydrate Binding Modules (CBM). To date, based on their function, 173 GH families, 114 GT families, 20 CE families, 42 PL families, 17 AA families, and 89 CBM families have been found with multiple subfamilies in each class (<http://www.cazy.org/>). These CAZymes are used in industry in textiles, food and beverages, pet food, and research studies [11].

One of the most important sources of these CAZymes is the rumen of herbivores. One report showed that the rumen of cattle fed Green Roughage (GR) and Dry Roughage (DR) had 28.13% and 10.93% of the total contigs for CAZyme-related genes, respectively. In the same study, the percentage of CAZyme genes for buffalo was 8.08% and 12.53% for GR and DR, respectively. Among CAZyme, dominance of GH family was observed with 39-43% of total CAZyme, followed by GT and CBM (19-24% and 20-17%, respectively). The dominant GH families were GH2, GH3, GH12, GH13, GH27, GH43, GH45, GH54, GH113, and GH128 [12]. A similar observation was made in black Bengal goat, where GH accounted for 39.73-37.88%, followed by GT (23.73-23.11%), CBM (17.65-15.61%), and CE (12.90-11.95%) [13]. In another study, GH2, GH3, GH28, GH31, GH97, GH32, GH51, GH77, GH78, and GH95 were reported to be present in the rumen microbiota of sheep, while GH2, GH3, GH31, GH97, GH77, GH32, GH51, GH109, GH28, GH78 were found in red deer and GH2, GH3, GH92, GH109, GH97, GH13, GH31, GH78, GH28, and GH77 in reindeer [9].

### Conclusion

The various studies conducted in the rumen of these herbivores suggest that they are a potential source of various CAZymes, and with advanced technologies, the chance of discovering new CAZyme classes with high efficacy has increased. Further computational design and genetic engineering approaches will be beneficial in producing novel CAZymes with high activity and stability to various environmental conditions. Therefore, a comprehensive study of microflora and CAZyme profiles, including those of fungi, may be a future prospect in this field.

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