

# Application of Multi Omics Technologies in Ruminants Research



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**Submission:** February 07, 2017; **Published:** March 20, 2017

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## Abstract

The improving of productivity, lessening of environmental pollution and preventing nutritional metabolism diseases are crucial for ruminant livestock industry. Understanding the complicated interactions of rumen microorganisms and ruminant nutrition's is the key to achieve these goals. With the fast development of high throughput sequencing technologies and big data mining methods, multi omics technologies become to be effective tools to research rumen microbiome. We could get the deeper insight of rumen microbiome functions through metagenomics, meta-transcriptomics, meta-proteomics and metabolomics technologies.

**Keywords:** Ruminant research; Rumen microorganisms; Multi omics; Big data mining methods

## Introduction

Rumen microorganism is an enormous and complicated microbial population which contains bacteria, fungus, archaea and protozoan. The composition diversity and function of the microbiome played important roles in ruminal fermentation, A process with vital importance to ruminant nutrition. The excretion of nitrogenous wastes and the emission of methane in ruminant livestock production caused environmental pollution problems [1]. Understanding the composition and activity of microbial community in rumen is crucial for the improving of productivity and lessening of environmental pollution in ruminant livestock industry. With the development of high-throughput sequencing and omics technologies, the composition, genetic relationships, functional genes, different expressed proteins and changed metabolites of rumen microorganisms were studied systematically. We could get a deeper insight of rumen microbiome through the conjoint analysis of metagenomics, meta-transcriptomics, meta-proteomics and metabolomics.

## Metagenomics

Metagenomics, which was defined as "the genomes of the total microbiota found in nature", takes the whole genomics of environmental samples as research object, uses sequencing analysis and functional gene screening as research tools and holds the objective to find the relationships between the microbial diversity, population structure, evolutionary relationship, functional activity and the environment.

The rumen microorganism genome, regarded as the second genome of ruminants, attracted more and more attention in recent years. Traditional methods to study rumen microorganism are denatured gradient gel electrophoresis (DGGE) and 16S high-throughput sequencing. DGGE can identify the variety of rumen microorganism through PCR amplification of unique genes of microorganism [2]. The 16SrRNA genes, exist in the genomes of all bacteria, are highly conserved. Based on their conservation, the 16S high-throughput sequencing could be helpful for the finding of more microorganisms and quantity detection [3].

All methods mentioned above can screen out the varieties of rumen microorganism, however, cannot identify the gene expression changes. The metagenome, which using the next generation sequencing technology to discover the genome data in the deeper level, is beneficial for the study of rumen microorganism function. Ma Otthi as Hess (2011) identified 27,755 assumed active genes and 90 different expressed proteins, 57% of which could degrade cellulose, by metagenome technology and thus identified the rumen microorganism which is in charge of the process from cellulose to biological energy [4]. The same method was also used to reveal the influence of antibiotics on dairy cows and further identify the microorganism which is related to methane emission [5]. With the development of sequencing technologies and the methods of big data analysis, the functions of rumen microorganism could be figured out and contributed for the ruminant production.

## Meta-Transcriptomics

Metagenomics can define functional gene composition of microbial communities and analyze the genomes contained from environmental samples directly, but how natural bacterial communities responding to environmental conditions, are better answered by analysis of community mRNA rather than genomic DNA. Meta-transcriptomics is a new subject which identifies and characterizes the total RNA (including mRNA and non-coding RNA molecules) of microorganism community in specific environment of period by microarray and sequencing method mainly.

In the last two years, meta-transcriptomics begun to be used in the study of ruminants. Gullert et al. [6] compared the mixed fermentation products of animal excrement and maize straw with the rumen fluid of dairy cows by meta-transcriptomics and metagenomics, and the result showed that there were more genes encoding cellulase in rumen fluid. Kamke et al. [7] identified the rumen microorganism involving in methane yield in sheep with high methane yielding (HMY group) and low methane yielding (LMY group) with meta-transcriptomics technology. The results revealed that genes related to lactic acid, propionate and butyrate were unregulated in LMY group and the decrease of rumen superficial area and the increase of rotation rate would cause the increase of abnormal fermentation. The meta-transcriptomics study have been the popular area for the understanding of functional genes in microorganism.

## Metaproteomics

The study of metagenomics and meta transcriptomics also had limitations on the understanding of the functionality of microbial communities in ecosystems and a major challenge is to elucidate the functional role of metagenome by linking genetic structure and diversity of microbial communities with their function. Thanks to the development of high efficiency peptide ionization methods in mass spectrometry (MS), from the 1990s, proteomics developed rapidly and which allowed high sensitive protein identification. Metaproteomics was first proposed by Wilmes & Bond [8]. It was a new technology that could characterize large scale information of proteins in the environmental microbiota and further beneficial for the function analysis.

Raquet et al. [9] analyzed the lingo cellulolytic capacities of four tropical termites (*T. hospes*, *M. parvus*, *N. lujae* and *N. ephratae*) using wheat straw as sole carbon source and aimed to find the network functions of enzyme activity and lignocellulose degradation profiles by correlating the functional diversity assessed by metagenomic and metaproteomic techniques. TJ Snelling [10] explored the metaproteome of bovine ruminal digesta by feeding a high concentrate diet revealed the prokaryotic metaproteome, which was dominated by enzymes predominantly from the Firmicutes and Bacteroidetes phyla and mainly participated in glycolysis after removal of ciliate

protozoa from digesta before extraction of proteins for some highly expressed proteins of them affected the result seriously. With the development of LC-MS and sequencing technologies, metaproteomics will be more and more popular in the study of ruminant.

## Metabolomics

The metabolomics was rapidly developing in recent years by advances in the analytical methods of mass spectrometry (MS) and high-resolution nuclear magnetic resonance (NMR) spectroscopy. Metabolomics could make a quantitative analysis for metabolites by using Gas Chromatography-Mass Spectrometer (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS) or capillary electrophoresis-Mass Spectrometer (CE-MS). The developments in LC-MS and CE-MS significantly broadened the applicability of MS-based metabolomics for GC-MS was largely limited to measure volatile compounds. NMR-based metabolic profiling can be performed successfully because NMR is highly quantitative and reproducible. Microbiome could directly change its metabolic capacity and affect the organs function locally through microbial products, so that the changed products were important for us to study metabolic diseases. About 10 years ago, metabolomics began to be used to research human metabolic disorders and diseases to identify biomarkers for Type 2 Diabetes and its complications [11]. It is now widely used in studying ruminants metabolic diseases and rumen microorganism. Burim et al. [12] used eight lactating Holstein cows with cannulas in the rumen in a 4x4 Latin square arrangement of treatments to research the influence of different barley content to dairy cows with metabolomics technology, the results showed that with the increase of barley content, the content of dimethylamine, leucine, lysine and butyrate in rumen fluid were significantly increased. Saleem et al. [13] identified 246 metabolites through NMR and GC-MS analysis by using the same treatments. Metabolomics technique was also used to detect relative metabolites with subacute rumen acidosis (SARA) and made the conjoint analysis with bioinformatic tools to the cells that cultivate in vitro with different treatments [14].

## Multi Omics Conjoint Analysis

Metagenomic approaches provide new insights into genetic diversity and evolution of uncultured microorganisms. Meta-transcriptomics studies the genes' expression of microbiome, yet they could not understand what really mattered to a special treatment. Therefore, if we want to know the rumen microbiome better, multi omics conjoint analysis is essential instead of a single technology. Nowadays, metagenomics with metabolomics, metagenomics with meta-transcriptomics and metagenomics with metaproteomics analysis [15] had been addressed in some essays. The big data mining in the post-genome times is now the research hotspot, finding the suitable analysis method of multi omics data is the key to unlock the secret of rumen microorganisms.

## Conclusion

The rumen microorganism is a vast and complicated group that played important roles in ruminant health and production. Understanding the composition and activity of the rumen microbial community is crucial to improve the productivity and to lessen the environmental impact associated with ruminant livestock industry. With the development of high-throughput sequencing and multi omics technology, and the emerging of more and more data mining methods, it is not an unreachable goal to understand the rumen microorganisms' biology and function.

## Acknowledgment

Financial support for this study was provided by grants from Ministry of Science and Technology (2016YFD0700205, 2016YFD0700201) and National Natural Science Foundation of China (31572435).

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DOI: 10.19080/JDVS.2017.01.555564

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