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Analysis of Rumen Microbial Protein Abundance of Gayals based on Metaproteomics

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ABSTRACT

Background: Presently, our understanding of the rumen of Gayals is still very shallow, which is recognized as the most effective and developed fiber degradation system in nature, with abundant microorganisms. Molecular biology technology is an effective means to study the microbial resources in the rumen.

Methods: Rumen contents of 3 Gayals (Gayals, *Bos frontalis*; G) and 3 Yellow Cattle (Yunnan Yellow Cattle, *Bos taurus*; Y) were collected in this study. Rumen microbial proteins were extracted by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS), then to analyze the bioinformatics of protein abundance was performed through the bovine rumen transcriptome database (gene.uniGeneset.fasta).

Result: The results were as follows: the differences in protein abundance of Gayals rumen bacteria in Firmicutes, *Actinobacteria*, *Ruminococcus* and *Olsenella* were significantly higher than Yellow cattle ($P<0.05$) and the difference in protein abundance of Chytridiomycota and Batrachochytrium in rumen fungus of Gayals was significantly less than that of Yellow cattle. Enrichment analysis by KEGG metabolism pathway of differentially expressed proteins in rumen microorganisms was performed, Gayals have higher abundance of β-glucosidase and 6-phosphate-β-glucosidase than Yellow Cattle.

Key words: Gayals, Metaproteomics, Microbial function, Rumen microorganism.

INTRODUCTION

Metaproteomics is a new technology that applies proteomics technology to study microbial community, which is a large-scale identification of proteins in microbial community in a specific period of time. Science protein is a direct effector for maintaining cell function, the detection of its abundance can be used to analyze the biological information of individuals in the community (Kleiner, 2012), gene expression (Hamann, 2016), community structure (Kleiner, 2017), characterization of functional enzymes (Mayers, 2017) and other biological information at the molecular level. At the same time, metabolomic analysis can provide valuable information on metabolic pathways and functional classifications and provide a deeper perspective for the in-depth study of the detailed mechanism of host microbiota interactions (Peters, 2019). It has been widely used in environmental samples, such as oral cavity (Belstrøm, 2016; Rabe, 2019), soil (Bao, 2014), seawater (Teeling, 2012), surface fresh water (Hanson, 2014), faeces (Erickson, 2012) and so on. Gayals, also known as *Bos frontalis*, mainly feeds on bamboo, *Artemisia annua*, weeds and leaves and its growth rate, especially in the early stage, is significantly higher than that of Yellow Cattle raised in agricultural areas. The purpose of this study was to understand the dominant microbial communities with high protein abundance in the rumen of Gayals and Yellow Cattle in the same habitat and to lay a foundation for the development of rumen microbial resources of Gayals.

MATERIALS AND METHODS

Animals

Rumen contents of Gayals and Yellow Cattle in the same habitat were collected from Gaoligong Mountain, Yunnan Province, then quickly frozen in liquid nitrogen tank and taken back to the laboratory for preservation at -80°C. Rumen contents of 3 Gayals (Gayals, *Bos frontalis*; G) and 3 Yellow Cattle (Yunnan Yellow Cattle, *Bos taurus*; Y) were collected in this study. The research was conducted at the Faculty of Animal Science and Technology, Yunnan Agricultural University from August 2020 to April 2021.

Extraction and quality control of microbial protein

Each rumen content sample (2g) was ground into powder in liquid nitrogen. In appropriate amounts of powder, urea cracking solution and PMSF was added; cracked on ice for 15 minutes, during the mixing once, then used ultrasonic pulverizer to ultrasonic for 5 minutes, with each ultrasonic for 9 seconds, with an interval of 4 seconds. The sample was then transferred to a centrifuge, centrifuged at 20,000g at 4°C for 5 minutes and the supernatant was extracted.

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Liquid phase tandem mass spectrometry

Inverse column information: C18 column 250 mm*75 μm*3 μm C18 (Thermofishier Scientific), chromatographic instrument: Thermo Easy nLC-1200, mass spectrometer: Q-Exactive (Thermofishier Scientific), chromatographic separation time: 155 min, phase A: 2% ACN, 0.1% formic acid, phase B: 80% ACN, 0.1% formic acid, the flow rate was 300 NL/min and the MS scanning range was (M/z): 350~1300, acquisition mode: DDA, mass spectrum resolution: 70000: MSMS scan range: (m/z): 200~2000, fragmentation mode: HCD, resolving power: 17500, Dynamic exclusion time: 20 s.

Screening of differentially expressed proteins

The screening criteria for differential proteins are: all proteins were divided into two categories: one was identified in Gayals and Yellow Cattle, the other was only identified in Gayals/ Yellow Cattle and the screening criteria for differential proteins are: P<0.05 and (FC<0.83||FC>1.20).

Bioinformatic analysis

Data analysis starts from the original mass spectrum data, firstly, a database search was performed on the spectrum (gene.uniGeneset.fasta), the qualitative and quantitative analysis of proteins was carried out and then the data were normalized. The obtained proteins were annotated and classified in terms of species and functions.

RESULTS AND DISCUSSION

Protein abundance statistics

After testing, the number of proteins identified in Gayals samples were G1(4956 pcs), G2(4972 pcs), G3(4474 pcs) and in Yellow Cattle samples were Y1 (4754 pcs), Y2 (5425 pcs), Y3 (5382 pcs).

Analysis of rumen microbial protein abundance

Rumen bacteria

At the level of rumen bacteria phyla, 20 phyla were detected in Gayals and 21 phyla in Yellow Cattle, more than 95% of the gene sets came from 5 phyla. The abundance of Firmicutes and Actinobacteria in Gayals was significantly higher than that in Yellow Cattle (P<0.05), but the abundances of Bacteroidetes, Proteobacteria and Chloroflexi were significantly lower than those of Yellow Cattle (P<0.05) (Table 1).

At the level of rumen bacteria genus, 209 and 224 genera were detected in Gayal and Yellow Cattle, respectively, among which 15 genera had protein abundance percentages more than 1%. Difference analysis showed that the abundances of *Olsenella* and *Ruminococcus* in Gayals were significantly higher than those in Yellow Cattle (P<0.05), while the abundances of *Prevotella* and *bacteroides* in Gayals were significantly lower than those in Yellow Cattle (P<0.05) (Table 2).

At the phyla level, Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria were the dominant bacteria in Gayals and Yellow Cattle. Many studies have shown that Firmicutes and Bacteroidetes play a leading role in rumen fiber degradation (Stevenson, 2007; Leng, 2011). There are a high number of fiber degrading bacteria in rumen and Firmicutes is the main phylum for fiber degradation, which contains a large number of cellulose degrading bacteria (Matsui, 2000; Krause, 2003).

Rumen fungi

At the level of rumen fungal phyla, 6 phyla were detected in Gayals and Yellow Cattle, *viz.* Ascomycota, Neocallimas tigomycota, Chytridiomycota, Basidiomycota, Glomeromycota and Cryptomycota, respectively. The abundance of Chytridiomycota in Gayals was significantly lower than that in Yellow Cattle (P<0.05) (Table 3).

At the level of rumen fungal genus, 25 genera were detected in Gayals and Yellow Cattle. Among them, 23 genera with protein abundance percentage greater than 1% were *Batrachochytrium*, *Rhizophagus*, *Piromyces*, *Absidia*, *Mortierella*, *Neocallimastix*, *Rhizopus*, *Lichtheimia*, *Chaetomium*, *Fomitiporia*, *Myceliophthora, Rozella*, *Schizosaccharomyces*, *Brettanomyces*, *Coprinopsis*, *Heterobasidion*, *Malassezia*, *Millerozyma*, *Mucor, Pestalotiopsis*, *Rhizoctonia*, *Saccharomyces*, *Tremella*, *Candida* and *Kluyveromyces*. *Batrachochytrium* and *Rhizophagus* had the highest protein abundance percentage and the abundance of *Batrachochytrium* in Gayals was significantly lower than that in Yellow Cattle (P<0.05) (Table 4).

Table 1: Significant differences of bacterial phyla.

Phylum	G (%±SD)	Y (%±SD)	G (Overall mean)	Y (Overall mean)
Firmicutes	46.79±0.92^{A}	43.32±0.73^{B}	1024.33	964.00
Bacteroidetes	34.34±0.67^{B}	38.97±1.30^{A}	752.00	867.00
Actinobacteria	7.19±0.77^{a}	5.32±0.31^{b}	157.67	118.33
Chloroflexi	0.14±0.00^{B}	0.18±0.01^{A}	3.00	4.00
Proteobacteria	2.27±0.05^{b}	2.44±0.08^{a}	49.67	54.33
Spirochaetes	3.93±0.49	4.12±0.01	86.00	91.67
Norank	2.92±0.09	3.04±0.29	64.00	67.67
Others	2.51±0.47	2.75±0.54	55.00	61.33

Note: Different lowercase letters in shoulder notes indicate significant differences compared to peers (P<0.05), with different capital letters indicating a significant difference (P<0.01), average reads: Average number of protein, the same as below.

At the phyla level, only 6 phyla were detected and the abundance of Chytridiomycota and Batrachochytrium in Gayals were significantly lower than that in Yellow Cattle ($P<0.05$). Although the content of rumen fungi is less, anaerobic fungal hyphae can penetrate the plant cell wall and dissolve lignin and produce a series of cellulose degrading enzymes with high activity, such as cellulase, hemicellulase and esterase, which can be assembled into a complex with high catalytic activity (Akin, 1990). Studies have shown that there are six major genera involved in lignocellulose degradation in the rumen anaerobic fungi: *Neocallimastix*, *Piromyces*, *Caecomyces*, *Anaeromyces*, *Orpinomyces* and *Cyllamyces*. Among them, the lignocellulose degradation efficiency of *Neocallimastix* and *Piromyces* was higher (Puniya, 2015). In this experiment, the protein abundance of *Neocallimastix* and *Piromyces* in

Table 2: Significant differences of bacterial genera.

Genus	G (%±SD)	Y (%±SD)	G (Overall mean)	Y (Overall mean)
Prevotella	16.55±0.34^{B}	18.59±0.52^{A}	362.33	413.67
Olsenella	3.47±0.38^{a}	2.37±0.17^{b}	76.00	52.67
Bacteroides	7.31±0.33^{b}	8.56±0.42^{a}	160.00	190.33
Ruminococcus	3.43±0.20^{a}	3.10±0.04^{b}	75.00	69.00
Clostridium	4.14±0.23	3.99±0.26	90.67	88.67
Treponema	3.41±0.38	3.67±0.00	74.67	81.67
Alistipes	2.98±0.48	3.47±0.03	65.33	77.33
Faecalibacterium	3.17±0.01	3.00±0.13	69.33	66.67
Nnorank	16.71±0.24	16.33±0.30	366.00	363.33
Butyrivibrio	2.84±0.29	2.77±0.23	62.00	61.67
Eubacterium	2.45±0.19	2.34±0.11	53.67	52.00
Blautia	2.13±0.05	1.99±0.08	46.67	44.33
Selenomonas	1.80±0.57	1.45±0.16	39.67	32.33
Others	29.61±0.56	28.37±0.94	648.33	631.67

Table 3: Significant differences of fungi phyla.

Phylum	G (%±SD)	Y (%±SD)	G (Overall mean)	Y (Overall mean)
Chytridiomycota	14.6±0.27^{b}	16.87±1.01^{a}	8.67	10.67
Norank	27.56±1.20	25.27±0.85	16.33	16.00
Neocallimastigomycota	15.74±0.87	15.72±2.34	9.33	10.00
Ascomycota	15.24±2.41	13.65±0.92	9.00	8.67
Glomeromycota	14.00±2.09	14.25±0.88	8.33	9.00
Basidiomycota	11.27±1.34	11.08±0.68	6.67	7.00
Cryptomycota	3.38±0.17	3.17±0.19	2.00	2.00

Table 4: Significant differences of fungi genera.

Genus	G (%±SD)	Y (%±SD)	G (Overall mean)	Y (Overall mean)
Batrachochytrium	14.36±0.69^{b}	16.87±1.01^{a}	8.67	10.67
Rhizophagus	13.79±2.34	14.25±0.88	8.33	9.00
Piromyces	8.84±0.88	8.90±2.04	5.33	5.67
Rhizopus	7.18±0.89	6.88±1.19	4.33	4.33
Neocallimastix	6.63±0.13	6.82±0.46	4.00	4.33
Mortierella	6.63±0.13	6.33±0.39	4.00	4.00
Absidia	5.54±1.07	4.15±1.50	3.33	2.67
Lichtheimia	4.97±0.10	4.75±0.29	3.00	3.00
Chaetomium	3.32±0.06	3.17±0.19	2.00	2.00
Myceliophthora	3.32±0.06	3.17±0.19	2.00	2.00
Rozella	3.32±0.06	3.17±0.19	2.00	2.00
Schizosaccharomyces	3.32±0.06	3.17±0.19	2.00	2.00
Fomitiporia	2.77±0.98	3.17±0.19	1.67	2.00
Mucor	2.75±0.91	3.17±0.19	1.67	2.00
Others	13.28±1.89	12.06±1.02	8.00	7.67

Gayals was more than 1%, which belonged to the main genus detected by rumen fungi and which was of great significance to the high cellulose degradation activity of Gayals. Among them, *Ruminococcus flavus* and *Ruminococcus alba* are the important fibrous decomposing bacteria in the rumen (Henderson, 2015). *Ruminococcus* was the dominant genus of rumen bacteria in Gayals and Yellow Cattle, which was consistent with the previous research results and the protein abundance of *Ruminococcus* in Gayals was significantly higher than that in Yellow Cattle ($P<0.05$), which may be related to the strong fiber degradation ability of Gayals.

Differentially expressed proteins

The results showed that there were 561 up-regulated proteins (the protein expression of Yellow Cattle was higher than that of Gayals) and 172 down-regulated proteins (the protein expression of Gayals was higher than that of Yellow Cattle) in the 733 differentially expressed proteins, only 27 proteins were identified in Gayals and 33 proteins were identified in Yellow Cattle (Table 5). Firmicutes and Bacteroidetes were the most abundant among up-regulated proteins and down-regulated proteins. Among the up-regulated proteins, 6.42% were more than 5-fold difference. There were three proteins with more than 10-fold difference, which were from norank (the differential protein was 23.23-fold), *Bacteroides sp.4_1_36* in Bacteroides and *Tannerella Forsythia ExBB* protein (K03561) (the differential protein was 11.36-fold). Among the down regulated proteins, there were three proteins with a differential multiple of more than 10 fold and the biggest differential multiple was *Bacteroides sp CAG:770*-the differential protein PYG (k00688) (16.26 fold), followed by two norank differential proteins (16.21 and 11.13 fold, respectively). Fig 1 showed the difference of rumen microbial proteins between Gayals and Yellow Cattle. Each point in the figure represents a specific protein, it can be seen from the figure that the number of up-regulated proteins is more than that of down-regulated proteins.

Function of rumen microbial protein

Functional annotation of differentially expressed protein KEGG

Total 733 differentially expressed proteins were annotated by KEGG and 142 metabolic pathways were found. The most active metabolic pathway was Glycolysis/Gluconeogenesis pathway. There were 14 metabolic pathways with the number of differential proteins more than 1% and 128 metabolic pathways with less than 1%, more than 5% of the metabolic pathways were metabolic pathways (11.78%), of which 11.42% were down-regulated (protein expression in Gayals was higher than that of Yellow Cattle), metabolism of microorganisms in different environments (8.42%), down-regulated protein accounted for 18.05%, carbon metabolism effect (7.10%), expression of down-regulated protein accounted for 15.29%, antibiotic biosynthesis (7.27%) and down-regulated protein was 16.38%, the metabolic synthesis

Table 5: Differential expression proteins between G and Y.

Designation	Total protein	Differential protein	Up regulated- protein	Down regulated- protein	Only in G	Only in Y
Quentities	4535	733	561	172	27	33

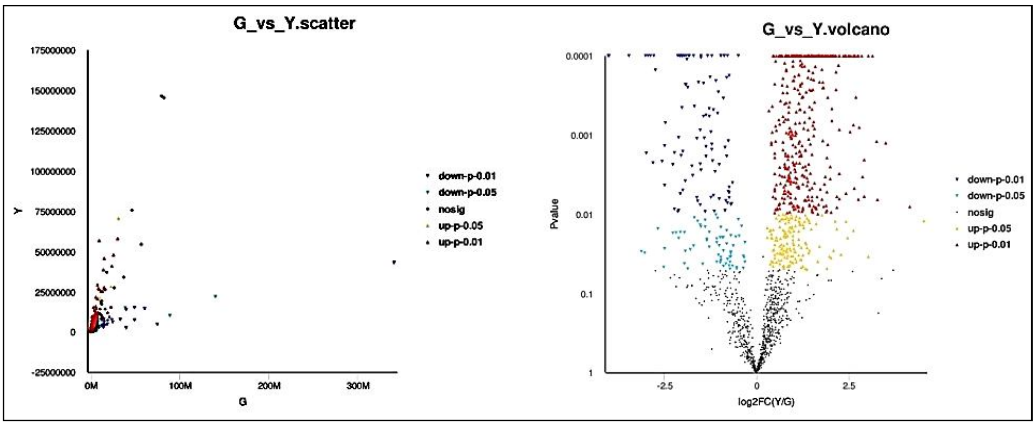

Fig 1: Volcanic map of differential protein between Gayals and Yellow Cattle.

The up value means that the protein expression of Gayals is lower than that of Yellow Cattle, the down value means that the protein expression of Gayals is higher than that of Yellow Cattle and the density of dots represents the number of differential proteins. The left figure is the volcano map of differential protein; the abscissa is the multiple change value of protein difference between the two samples, that is, the value obtained by dividing the expression of sample Y by the expression of sample D, which is logarithmized, the ordinate is the statistical T-test P value of protein expression difference and the smaller the P value is, the more significant the expression difference is. The right figure is the scatter plot of differential proteins; the abscissa and ordinate in the figure represent the protein expression in the two samples and the values are logarithmically processedÿeach point represents a specific protein, the abscissa value corresponding to a specific point is the protein expression in sample 1 and the ordinate value is the protein expression in sample 2.

of secondary organisms (6.44%), down-regulated protein expression accounted for 20.38%, Glycolysis/gluconeogenesis (5.58%), down-regulated protein accounted for 16.91%. In the KEGG functional annotation, a large number of sequences were involved in carbohydrate metabolism and the main metabolic pathways include starch and sucrose metabolism, glycolysis/gluconeogenesis metabolism and pyruvate metabolism, *etc.* Among the identified differentiated protein metabolism pathways, most of the differentiated proteins came from the dominant phyla of rumen bacteria-Firmicutes, Bacteroidetes, Actinobacteria and Proteobacteria. The abundance of β-glucosidase of Clostridium in Gayals is higher than that in Yellow Cattle, as one of the important enzymes of fiber decomposition, β-glucosidase can completely hydrolyze cellobiose to glucose, rumen ferments glucose to maintain its own growth and finally ferments into volatile fatty acids, which can be absorbed by rumen wall to provide energy for ruminants.

Carbohydrate metabolism

Starch and sucrose metabolic pathway

In the pathway of starch sucrose metabolism (Fig 2), the difference protein quantity of Firmicutes was more than Bacteroides in the rumen bacteria of Gayals and Yellow Cattle and the most detected enzymes were phospho glucomutase (K01835, 5.4.2.2) and glucose-1-phosphate acyltransferase (K00975, 2.7.7.27). Glucoso phosphate protease was mainly from Firmicutes and partly from Actinobacteria and Cyanobacteria, the enzyme species with significant difference (P<0.01) in the down-regulation of protein expression (the protein expression of Gayals was higher than that of Yellow Cattle) were *Atopobium sp. oral taxon 199*, *Atopobium rimae*, *Atopobium sp. BS2*, *Selenomonas ruminantium*, *Olsenella sp. oral taxon 809*, *Atopobium parvulum*, *Olsenella uli*, *Bacteroides sp. CAG:714*, the differences were significant (P<0.05) of enzymatic species were *Clostridium sp. KNHs209*, *Fischerella sp. PCC 9431*, *Clostridium sp. CAG:307*, *Collinsella tanakaei*, *Olsenella uli.* Among them, the abundance of β-glucosidase (K05349, 3.2.1.21) produced by *Clostridium sp.KNHS209* from Firmicutes of Gayals was significantly higher than that of Yellow Cattle (P<0.05). In the diagram of Starch and sucrose metabolic pathway, *sucrose-specific IIB component,* β-glucosidase (3.2.1.21), glucose-1-phosphate acyltransferase (2.7.7.27), glycogen phosphorylase (2.4.1.1), phosphoglucomutase (5.4.2.2) and glucose-6-phosphate isomerase (5.3.1.9) were down-regulated.

Glycolytic/gluconeogenic pathway

In the glycolysis/gluconeogenesis pathway (Fig 3), the differential proteins mainly come from Bacteroides and Firmicutes. A variety of enzymes were noted in the rumen glycolysis/gluconeogenesis process of Gayals and Yellow Cattle and it was found that glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12) was a relatively abundant protein involved in this biological process in the rumen of Gayals. This enzyme was involved in the first step of the glycolytic pathway, catalyzing the reversible oxidative phosphorylation of D-glyceraldehyde-3-phosphate to 1, 3-bisphosphate-D-glycerol in the presence of NAD+ and phosphate and the process was most commonly facilitated by Firmicutes. Pyruvate feroxidase and phosphopyruvate kinase (4.1.1.32) were also detected, mainly from Bacteroides and Firmicutes,

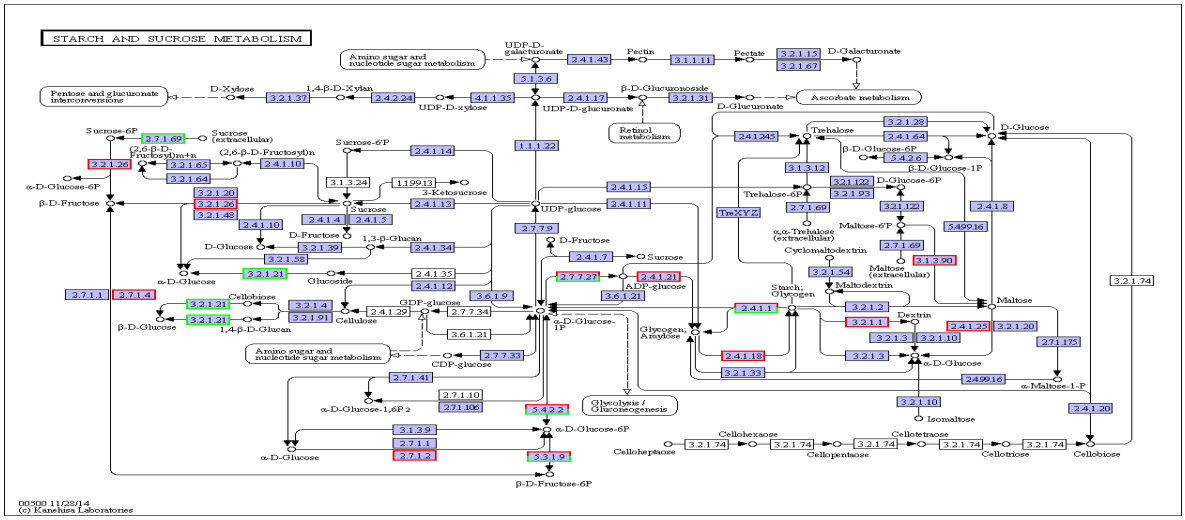

Fig 2: KEGG notes on starch and sucrose metabolism.

The gene products with red/green border in the figure belong to the differential protein detected in this study, in which green represents the down regulated protein (the protein expression of Gayals is higher than that of Yellow Cattle), red represents the up regulated protein (the protein expression of Yellow Cattle is higher than that of Gayals), all the gene products with blue background box in the figure belong to the background protein and the gene products with white background box do not belong to the KO score class system, the same below.

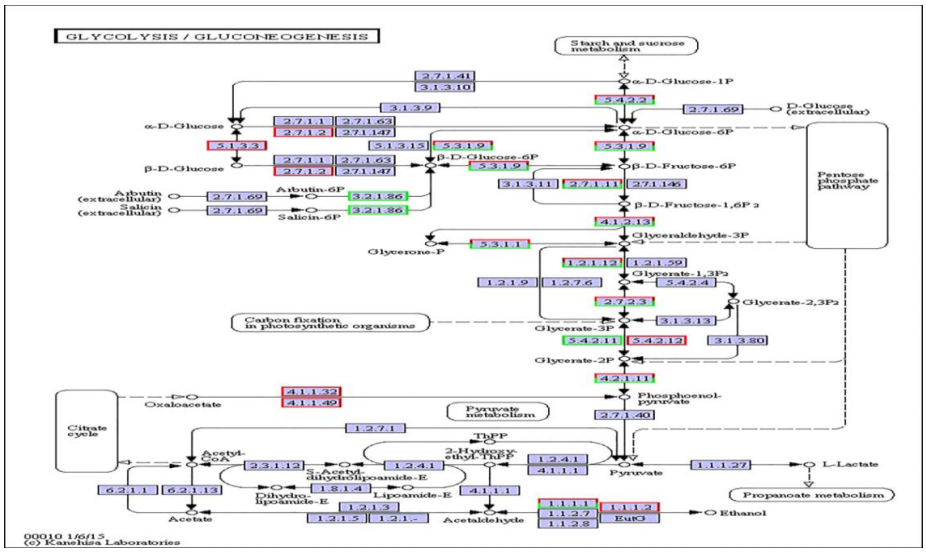

Fig 3: Species distribution at glycolysis/gluconeogenesis pathway of phyla level.

while the rest were from Fibrobacteria, Chlorobacteria, Spirochetes, Ignavibacteriae and Actinobacteria. Phosphofructokinase (PFK) was the rate limiting enzyme in the third step of glycolysis, which came from Firmicutes, the expression of down-regulated in *Selenomonas* bovis and up-regulated in Firmicutes *bacterium CAG:227*, *Lachnospiraceae bacterium AC2028* and *Blautia sp. CAG:257, Ruminococcus sp. CAG: 17*. In down-regulated expression, alcohol dehydrogenase (1.1.1.1) produced by Bautia sp. Cag: 237, *Atopobium sp. BS2*, *Eggerthia catenaformis*, phosphoglucomutase (5.4.2.2) produced by *Clostridium sp. Cag: 307* and triosephosphate isomerase (5.3.1.1) produced by *Atopobium rimae*, *Collinsella Tanakaei*, *Oscillibacter sp.1-3*, glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12) produced by *Eggerthia Catenaformis.* Phosphofructokinase (PFK) is the third rate limiting enzyme of glycolysis which comes from lunamonas ruminant and the expression of PFK in Gayals rumen is higher than that in Yellow Cattle. Compared with Yellow Cattle, the abundance and species sources of glyceraldehyde-3-phosphate dehydrogenase and phosphofructokinase in the rumen of Gayals were less, the abundance of *Olsenella* profusa 6-phosphate-β-glucosidase in Gayals was significantly higher than that in Yellow Cattle (P<0.05) in the abundance analysis, it is speculated that 6-phosphate-β-glucosidase is one of the key enzymes in the degradation of crude fiber in Gayals, 6-phosphate-β-glucosidase can catalyze 6-phosphate-β-glucoside compounds such as 6-phosphate-cellobiose and 6-phosphate-cellulose oligosaccharide to generate glucose-6-phosphate and decompose cellulose (Thompson, 1999; Desai, 2010). In the metabolic pathway, 6-phospho-β-glucosidase decomposes Arbutin-6P and Salicin-6P into β-D-glucose for subsequent catabolism. (2-3)-diphosphoglyceride phosphoglycerate mutase can catalyze the conversion of Glycerate-3P to Glycerate-2P, which is an important enzyme in gluconeogenesis and glycolysis. Previous studies detected the digestive protein products in rumen fluid of dairy cows fed high concentrate dairy cows by two-dimensional polyacrylamide gel electrophoresis (2DSDS-PAGE), indicating that many prokaryotic proteomes contained enzymes involved in glycolysis, such as glyceraldehyde-3-phosphate dehydrogenase, phosphoenolpyruvate carboxylic kinase(PEPCK), phosphoglycerate kinase and triosephosphate isomerase (Snelling, 2017).

Butanoate metabolic pathway

In the butanoate metabolic pathway (Fig 4), the number of differential proteins Firmicutes was more than Bacteroides between Gayals and Yellow Cattle, the most identified protein was Pyruvate feroxidase (K03737), the down-regulated enzymes were acetolactate synthase (2.2.1.6) and butyryl CoA dehydrogenase (1.3.8.1) and the acetolactate synthase was from Fusobacteria, acyl CoA dehydrogenase was mainly from Firmicutes and partly from Bacteroidetes. There are two production pathways of butyric acid, namely the direct conversion of butyric acid to acetic acid and the malonyl-CoA pathway, butyric acid can promote the development of rumen epithelium (Sakata, 1978).

Pyruvate metabolic pathway

In the pathway of pyruvate metabolism (Fig 5), the number of differential proteins in Firmicutes was more than Bacteroides between Gayals and Yellow Cattle and the most identified protein was pyruvate feroxidase (K03737), mainly from Firmicutes and Bacteroides, *Kandleria vitulina*, *Acidaminococcus sp.CAG:917*, *Coprococcus catus and [Ruminococcus] Obeum of Firmicutes*, *Coriobacteriaceae bacterium BV3Ac1* and *Atobicium fossor* of Actinomycetes, then *Prevotella sp. CAG: 755* of Bacteroidetes in the rumen of Gayals were higher than those of Yellow Cattle. The down-regulated enzymes in metabolic pathway were pyruvate

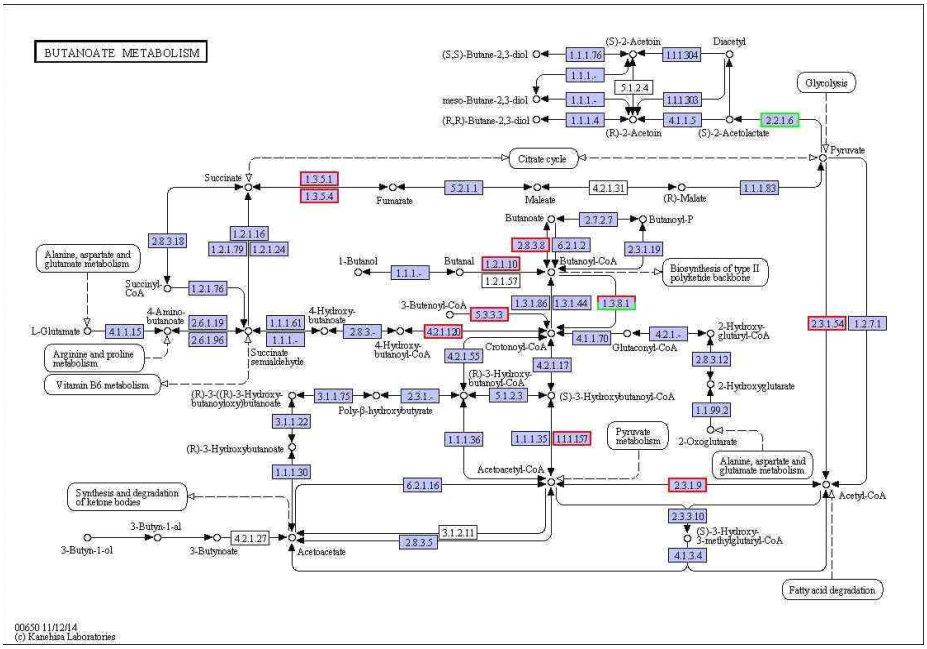

Fig 4: Species distribution at butanoate metabolic pathway of phylum level.

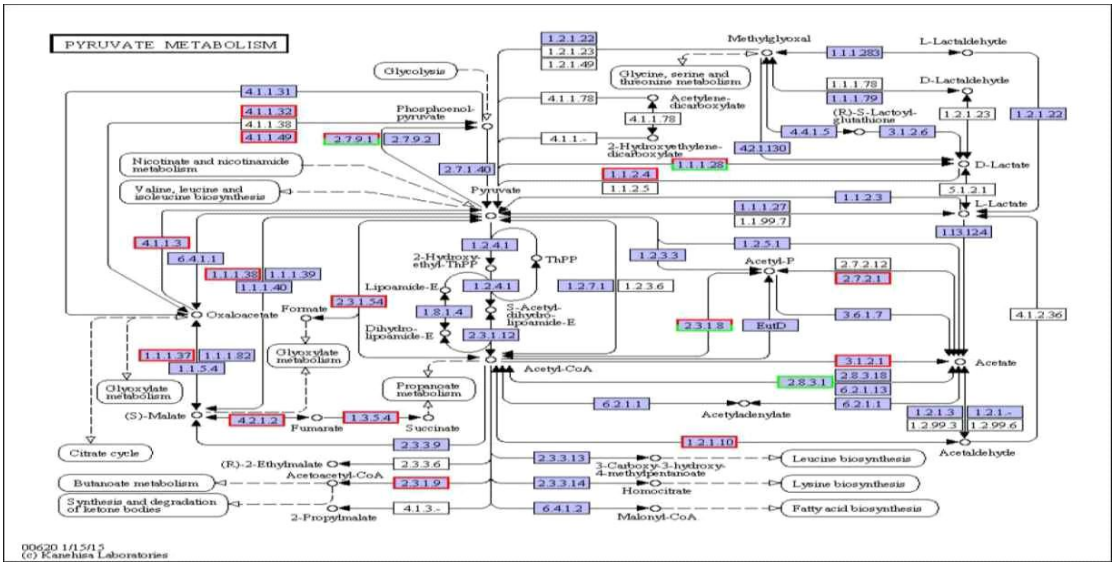

Fig 5: Species distribution at pyruvate metabolism pathway of phylum level.

phosphate dikinase (4.1.1.49), D-lactate dehydrogenase (1.1.1.28), phosphotransacetylase (2.3.1.8) and pyruvate CoA transferase (2.8.3.1). There are three pathways for acetic acid formation, namely, direct conversion of pyruvate to acetic acid, the acetyl-CoA pathway and acetyl-phosphate pathway (Söllinger, 2018). According to the pyruvate metabolic pathway diagram, the acetyl-CoA pathway and acetyl phosphate pathway are annotated in Gayals and Yellow Cattle, the acetyl-CoA synthesis pathway was reversible when the protein expression of Yellow Cattle was higher than that of Gayals, but the pathway was unidirectional when the protein expression of Gayals was higher than that of Yellow Cattle and the acetyl-CoA hydrolase was from *Phascolarctobacterium succinatutens*. It was previously found that *Phascolarctobacterium wakonense sp. nov* could produce both pyruvic acid and acetic acid when supplemented with pyruvate indicating that *Phascolarctobacterium* plays an important role in rumen microbial acetic acid synthesis and acetylphosphate pathway is distributed in both up-regulation and down-regulation of protein expression (Shigeno, 2019). Studies have shown that when feeding a large number of roughages, ruminants mainly obtain propionic acid through succinic acid decarboxylation pathway (Oba, 2003), but there are few

enzymes annotated by succinic acid pathway. D-lactate dehydrogenase and propionate-CoA transferase were annotated in the lactic acid-acrylic acid pathway and the expression of propionate-CoA transferase was down-regulated, D-lactate dehydrogenase came from Firmicutes and was only quantified in Gayals.

CONCLUSION

The rumen microbes of Gayals and Yellow Cattle were mainly bacteria and the rumen bacterial community structure had some differences, in the phylum Firmicutes and Actinomycetes, the protein abundance of rumen bacteria in Gayals was significantly higher than that in Yellow Cattle. In the functional annotation of differential proteins, the abundance of Bacteroides differential proteins in Yellow Cattle was higher than that in Gayals and Firmicutes played an important role in rumen degradation of Gayals. In the different protein metabolism pathways, the overall expression of β-glucosidase and 6-phospho-β-glucosidase in Gayals was higher than that in Yellow Cattle, which were the key enzymes for rumen crude fiber degradation in Gayals.

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Conflict of interest

There was no conflict of interests regarding the publication of this article.

REFERENCES

Akin, D.E. and Borneman, W.S. (1990). Role of rumen fungi in fiber degradation. Journal of Dairy Science. 73: 3023-3032.

Puniya, A.K., Singh, R. and Kamra, D.N. (2015). Rumen Microbiology: From Evolution to Revolution(1st Edn). Berlin: Springer (India) Private Limited, New Delhi.

Bao, Z., Okubo, T., Kubota, K., Kasahara, Y., Tsurumaru, H. anda, M., Ikeda, S. and Minamisawa, K. (2014). Metaproteomic identification of diazotrophic methanotrophs and their localization in root tissues of field-grown rice plants. Applied and Environmental Microbiology. 80: 5043-5052.

Belstrøm, D., Jersie-Christensen, R.R., Lyon, D., Damgaard, C., Jensen, L.J., Holmstrup, P. and Olsen, J.V. (2016). Metaproteomics of saliva identifies human protein markers specific for individuals with periodontitis and dental caries compared to orally healthy controls. Peer J. 4: e2433.

Desai, S.K., Nandimath, K. and Mahadevan, S. (2010). Diverse pathways for salicin utilization in Shigella sonnei and *Escherichia coli* carrying an impaired bgl operon. Archives of Microbiology. 192: 821-833.

Erickson, A.R., Cantarel, B.L., Lamendella, R., Darzi, Y., Mongodin, E.F., Pan, C., Shah, M., Halfvarson, J., Tysk, C., Henrissat, B., Raes, J., Verberkmoes, N.C., Fraser, C.M., Hettich, R.L. and Jansson, J.K. (2012). Integrated metagenomics/ metaproteomics reveals human host-microbiota signatures of Crohn's disease. PloS One. 7: e49138.

Hamann, E., Gruber-Vodicka, H., Kleiner, M., Tegetmeyer, H.E., Riedel, D., Littmann, S., Chen, J., Milucka, J., Viehweger, B., Becker, K.W., Dong, X., Stairs, C.W., Hinrichs, K. U., Brown, M.W., Roger, A.J. and Strous, M. (2016). Environmental Breviatea harbour mutualistic Arcobacter epibionts. Nature. 534: 254-258.

Hanson, B.T., Hewson, I. and Madsen, E.L. (2014). Metaproteomic survey of six aquatic habitats: discovering the identities of microbial populations active in biogeochemical cycling. Microbial. Ecology. 67: 520-39.

Henderson, G., Cox, F., Ganesh, S., Jonker, A., Young, W., Global Rumen Census Collaborators. and Janssen, P. H. (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.

Kleiner, M., Thorson, E., Sharp, C.E., Dong, X., Liu, D., Li, C. and Strous, M. (2017). Assessing species biomass contributions in microbial communities *via* metaproteomics. Nature Communications. 8: 1558.

Kleiner, M., Wentrup, C., Lott, C., Teeling, H., Wetzel, S., Young, J., Chang, Y.J., *et al.* (2012). Metaproteomics of a gutless marine worm and its symbiotic microbial community reveal unusual pathways for carbon and energy use. Proceedings of the National Academy of Sciences of the United States of America. 109: E1173-E1182.

Krause, D.O., Denman, S.E., Mackie, R.I., Morrison, M., Rae, A.L., Attwood, G.T. and McSweeney, C.S. (2003). Opportunities to improve fiber degradation in the rumen: Microbiology, ecology and genomics. FEMS Microbiology Reviews. 27: 663-693.

Leng, J., Xie, L., Zhu, R., Yang, S., Gou, X., Li, S. and Mao, H. (2011). Dominant bacterial communities in the rumen of Gayals (*Bos frontalis*), Yaks (*Bos grunniens*) and Yunnan Yellow Cattle (*Bos taurs*) revealed by denaturing gradient gel electrophoresis. Molecular Biology Reports. 38: 4863-4872.

Matsui, H., Ogata, K., Tajima, K., Nakamura, M., Nagamine, T., Aminov, R.I. and Benno, Y. (2000). Phenotypic characterization of polysaccharidases produced by four Prevotella type strains. Current Microbiology. 41: 45-49.

Mayers, M.D., Moon, C., Stupp, G.S., Su, A.I. and Wolan, D.W. (2017). Quantitative metaproteomics and activity-based probe enrichment reveals significant alterations in protein expression from a mouse model of inflammatory bowel disease. Journal of Proteome Research. 16: 1014-1026.

Oba, M. and Allen, M.S. (2003). Dose-response effects of intrauminal infusion of propionate on feeding behavior of lactating cows in early or midlactation. Journal of Dairy Science. 86: 2922-2931.

Peters, D.L., Wang, W., Zhang, X., Ning, Z., Mayne, J. and Figeys, D. (2019). Metaproteomic and metabolomic approaches for characterizing the gut microbiome. Proteomics. 19: e1800363.

Rabe, A., Gesell Salazar, M., Michalik, S., Fuchs, S., Welk, A., Kocher, T. and Völker, U. (2019). Metaproteomics analysis of microbial diversity of human saliva and tongue dorsum in young healthy individuals. Journal of Oral Microbiology. 11: 1654786.

Sakata, T. and Tamate, H. (1978). Rumen epithelial cell proliferation accelerated by rapid increase in intraruminal butyrate. Journal of Dairy Science. 61: 1109-1113.

Shigeno, Y., Kitahara, M., Shime, M. and Benno, Y. (2019). Phascolarcto bacterium wakonense sp. nov., isolated from common marmoset (*Callithrix jacchus*) faeces. International Journal of Systematic and Evolutionary Microbiology. 69: 1941-1946.

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

Söllinger, A., Tveit, A.T., Poulsen, M., Noel, S.J., Bengtsson, M., Bernhardt, J., Frydendahl Hellwing, A.L., Lund, P., Riedel, K., Schleper, C., Højberg, O. and Urich, T. (2018). Holistic Assessment of Rumen Microbiome Dynamics through Quantitative Metatranscriptomics Reveals Multifunctional Redundancy during Key Steps of Anaerobic Feed Degradation. mSystems. 3: e00038-18.

Stevenson, D.M. and Weimer, P.J. (2007). Dominance of Prevotella and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. Applied Microbiology and Biotechnology. 75: 165-174.

Teeling, H., Fuchs, B.M., Becher, D., Klockow, C., Gardebrecht, A., Bennke, C.M., Kassabgy, M., *et al.* (2012). Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science. 336: 608-611.

Thompson, J., Ruvinov, S.B., Freedberg, D.I., Hall, B.G. (1999). Cellobiose-6-phosphate hydrolase (CelF) of *Escherichia coli*: Characterization and assignment to the unusual family 4 of glycosylhydrolases. Journal of Bacteriology. 181: 7339-7345.