



## Research article

# The characteristics of intestinal flora of Tibetan sheep in different regions at high altitude were revealed based on metagenomic technique

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## ABSTRACT

Tibetan sheep play a vital role in the livelihoods of herders and are an important part of the ecosystem of the Tibetan Plateau. In order to study the characteristics of the gut microorganisms of Tibetan sheep at high altitude, this study employed macrogenomic techniques to analyse the diversity and differences in the gut flora of Tibetan sheep in different regions of high altitude and high cold. The results demonstrated that at the phylum level, the dominant phylum in the ileocecum segment of Tibetan sheep in Qilian, Henan and Gonghe counties was identical, namely *Euryarchaeota*, *Firmicutes* and *Ascomycota*. At the level of the archaeobacterial genus, the dominant bacteria of the ileocecal segment of Tibetan sheep in Qilian County, Henan County and Gonghe County were *Methanobrevibacter*. At the level of bacterial genus, the dominant bacteria of Tibetan sheep ileocecal in Qilian County and Henan County were *Bacteroides*, while in Gonghe County, the dominant bacteria were *Bifidobacterium*. At the level of fungal genus, there were notable differences in the abundance of Tibetan sheep ileocecal genus across different regions. However, the abundance of cecum genus exhibited a more consistent trend across regions. From the perspective of functional prediction, the metabolic pathways enriched in the intestinal segments of Tibetan sheep in different regions were found to be identical, with the relative abundance of each functional gene also being essentially uniform. This result will provide a foundation for further research on the mechanism of action of gut microbes in ruminants at high altitude and alpine regions.

## 1. Introduction

Tibetan sheep represent a valuable genetic resource of the Qinghai-Tibet Plateau in China. They are primarily distributed in Qinghai, Tibet, Gansu, and other alpine areas with an altitude of approximately 4000 m, and they provide indispensable elementary resources to local pastoralists [1]. The Qinghai-Tibet Plateau and its surrounding areas exhibit considerable regional variation, which has led to the formation of distinct Tibetan sheep populations. These include the plateau type Tibetan sheep, Euler type Tibetan sheep, valley type Tibetan sheep and black Tibetan sheep. Plateau type Tibetan sheep in Qinghai Province can be further subdivided into the Qilian white Tibetan sheep and the Guide black fur sheep, among others [2,3]. Qilian County is situated in the northwestern region of

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Qinghai Province, with an average elevation of 3500 m. The region is renowned for its vast population of white Tibetan sheep, which retain various characteristics of plateau Tibetan sheep. Because of its good gloss, pure color and strong elasticity of wool, it is not only known as the crown of carpet wool, but also known as “Qilian big white wool” [4,5]. Henan County is located in the southeastern region of Qinghai Province, with an average elevation of 3600 m. Its principal livestock products are Euler type Tibetan sheep, or Euler sheep for short. As a valuable breed resource of meat sheep on the Qinghai-Tibet Plateau, this breed of Tibetan sheep provides a high-quality variety source for the development of an organic meat sheep industry with plateau characteristics. This is due to its outstanding meat production performance and unique meat quality characteristics [6]. Gonghe County, located in the northeast of Qinghai Province, has an average altitude of 3200 m. The dominant livestock breed is plateau Tibetan sheep, which accounts for more than 90 % of Tibetan sheep in Qinghai Province. Gonghe County is the primary source of local meat due to its extensive breeding and stable resources [7].

A significant number of microorganisms inhabit the intestinal tract of animals, which are essential for the healthy growth and development of the host. Bi [8] et al. observed that the fetal intestine of prenatal lambs was predominantly composed of *Firmicutes*, *actinomycetes*, and other bacteria. Dias [9] et al. studied the changes in the gastrointestinal flora of calves at four stages before weaning were examined. The results indicated that the richness of the rumen flora increased with age, while that of the large intestine flora decreased. In a study of the gastrointestinal flora of goats of different days of age, Li [10] et al. observed that the diversity of the flora increased with age. They noted that *Proteobacteria* were replaced by *Firmicutes* in the large intestine, and that the abundance of *Barnella* and *Trichospira* increased. The gut microbiota of ruminants interacts with other microorganisms to facilitate the efficient absorption of nutrients from the diet. The interaction between the intestinal microecology and the immune regulation of ruminants is influenced by a number of factors, including the animal variety, the breeding environment and other variables. The disruption of intestinal flora homeostasis, resulting from the invasion of pathogens or other factors, can lead to metabolic disorders in the host, which in turn can give rise to a range of intestinal diseases [11–13]. Therefore, the experiment employed Tibetan sheep at high altitude and high cold as the research object, to analyse the variability of intestinal flora of Tibetan sheep in different regions of Qinghai Province using macrogenomes. This will provide a scientific basis for further relevant studies on intestinal flora of plateau ruminants.

## 2. Materials and methods

### 2.1. Sample collection

A total of nine Tibetan sheep intestinal contents were collected from the following locations: Qilian County, Haibei Tibetan Autonomous Prefecture, Qinghai Province; Henan Mongolian Autonomous County, Qinghai Province; and Republican County, Hainan Tibetan Autonomous Prefecture, Qinghai Province. These samples were divided into three groups (Table 1), The Qilian group (QL), Henan group (Henan, HN), and Gonghe group (Gonghe, GH) each comprise three replicates. The ileum (Huichang, HC) and cecum (Mangchang, MC) contents were selected as representative intestinal segments of the small intestine and large intestine, respectively. The intestinal tracts of different Tibetan goats in different regions were labelled and transferred to  $-80^{\circ}\text{C}$  for preservation.

### 2.2. Genomic DNA extraction and sequencing

The original sample were transported to Ovicon Company at a low temperature (below  $0^{\circ}\text{C}$ ). DNA samples were analyzed by agarose gel electrophoresis and Qubit quantitative analysis, and qualified DNA samples were constructed and tested. Qualified DNA libraries were sequenced by Illumina HiSeq high-throughput sequencing platform, and the raw data obtained from sequencing was used for bioinformatic analysis.

### 2.3. Bioinformatics and statistical analysis

Quality control, assembly, gene prediction and functional annotation of sequencing data were performed. By comparing and analysing the sequencing data with the database of archaea, bacteria and fungi, the species, functional annotation and taxonomic information among the samples were obtained, and the species with differential abundance were identified.

### 2.4. $\beta$ diversity analysis

The two-dimensional or three-dimensional data obtained by Principal Component Analysis (PCA) and Partial Least Squares Discrimination Analysis (PLS-DA) were mapped so as to know the spatial distribution characteristics based on macrogenomic composition, quantify the magnitude of differences between samples, and visualize the Bray-Curtis distance matrix obtained for

**Table 1**  
Sources of experimental animals and related information.

Area/Group	Altitude(m)	Sex	Age (year)
Qilian (QL)	3500	Male	2
Henan (HN)	3600	Male	2
Gonghe (GH)	3200	Male	2

clustering analysis.

### 2.5. Species annotation analysis

The Megan 6 software was employed to generate tables of composition and abundance distribution for each sample at the taxonomic levels of phylum, order and genus. These tables were obtained for the dominant species in each sample at different taxonomic levels.

### 2.6. Function annotation analysis

The results of the CAZyme, eggNOG, KOG, and KEGG annotations were used to obtain the corresponding abundance of each protein. The number of pathways annotated to different classes and classifications was counted according to the classification to which each CAZyme, eggNOG, KOG, and KEGG belonged.

## 3. Results and analysis

### 3.1. $\beta$ diversity analysis

The results of the PCA (Fig. 1A) analyses indicate that HN-HC and QL-HC are more closely related to QL-MC and HN-MC. Conversely, GH-HC and GH-MC are more distantly related to QL-HC, QL-MC, HN-HC, and HN-MC. This suggests that the intestinal microbial abundance compositions of Tibetan sheep in the QL and HN regions are similar, with relatively minor differences. The results of the PLS-DA (Fig. 1B) analyses indicate that the proximity of HN-HC and QL-HC suggests that the microbial abundance compositions of the ileum of QL and HN were similar, with only minor differences. The proximity of QL-MC, HN-MC and HN-MC indicates that the species abundance composition of the cecum was similar in the three groups of Tibetan sheep. This indicates that there are differences in the intestinal flora of Tibetan sheep in QL, HN and GH.

### 3.2. Results of annotation of intestinal archaea levels in Tibetan sheep from different regions

In this study, the intestinal microbiota was observed to contain relatively large proportions of *Euryarchaeota*, *Methanobacteriales*, *Methanobrevibacter* and *Methanosphaera*. *Euryarchaeota* was the predominant phylum in the cecum and ileum of Tibetan sheep across all three groups (Fig. 2A–a). At the genus level (Fig. 2B–b), *Methanobrevibacter* was the dominant bacterium in HC in the three groups, and it was more than 96 % in GH group, followed by *Methanosphaera* in QL group and HN group (5.28 % and 4.73 %, respectively), and only 1.65 % in GH group. *Methanobrevibacter* was the dominant bacterium in the three groups, followed by *Methanosphaera*. However, the relative abundance of *Methanocorpusculum* was higher in the QL and HN groups (10.70 % and 5.10 %, respectively), and lower than 0.5 % in GH group.

### 3.3. Annotated results of intestinal bacterial levels in Tibetan sheep from different regions

The results of this study indicate that *Firmicutes*, *Clostridiales*, *Erysipelotrichales*, *Bacteroides* and *Clostridium* represent a significant proportion of the intestinal microbiota. At the phylum level (Fig. 3A–a), *Firmicutes* were the predominant bacteria in the HC and MC of Tibetan sheep in the three groups. Secondly, the relative abundance of *Actinobacteria* in the HC of the GH group was found to be higher than in the other groups. Additionally, the relative abundance of *Bacteroidetes* in the HC of the QL and HN groups was also found to be

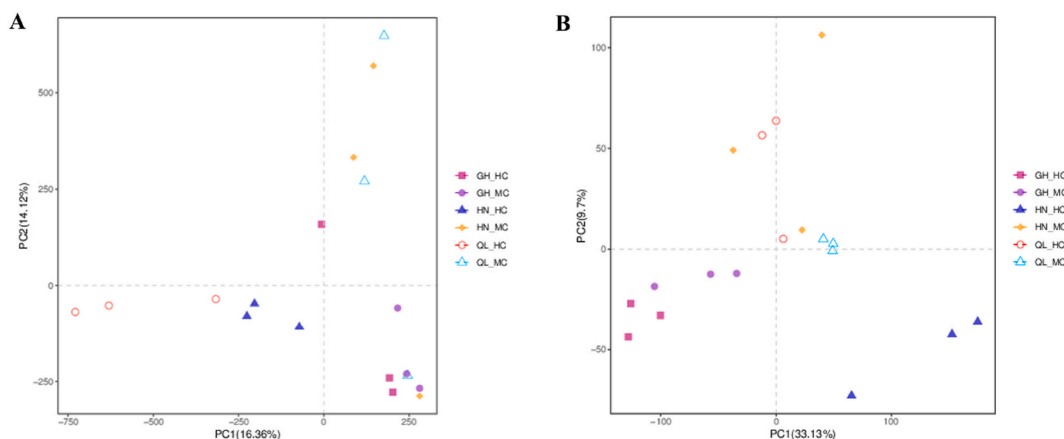


Fig. 1. Analysis of  $\beta$ -diversity of intestinal microorganisms in Tibetan sheep from different regions (A. PCA analysis, B. PLS-DA analysis).

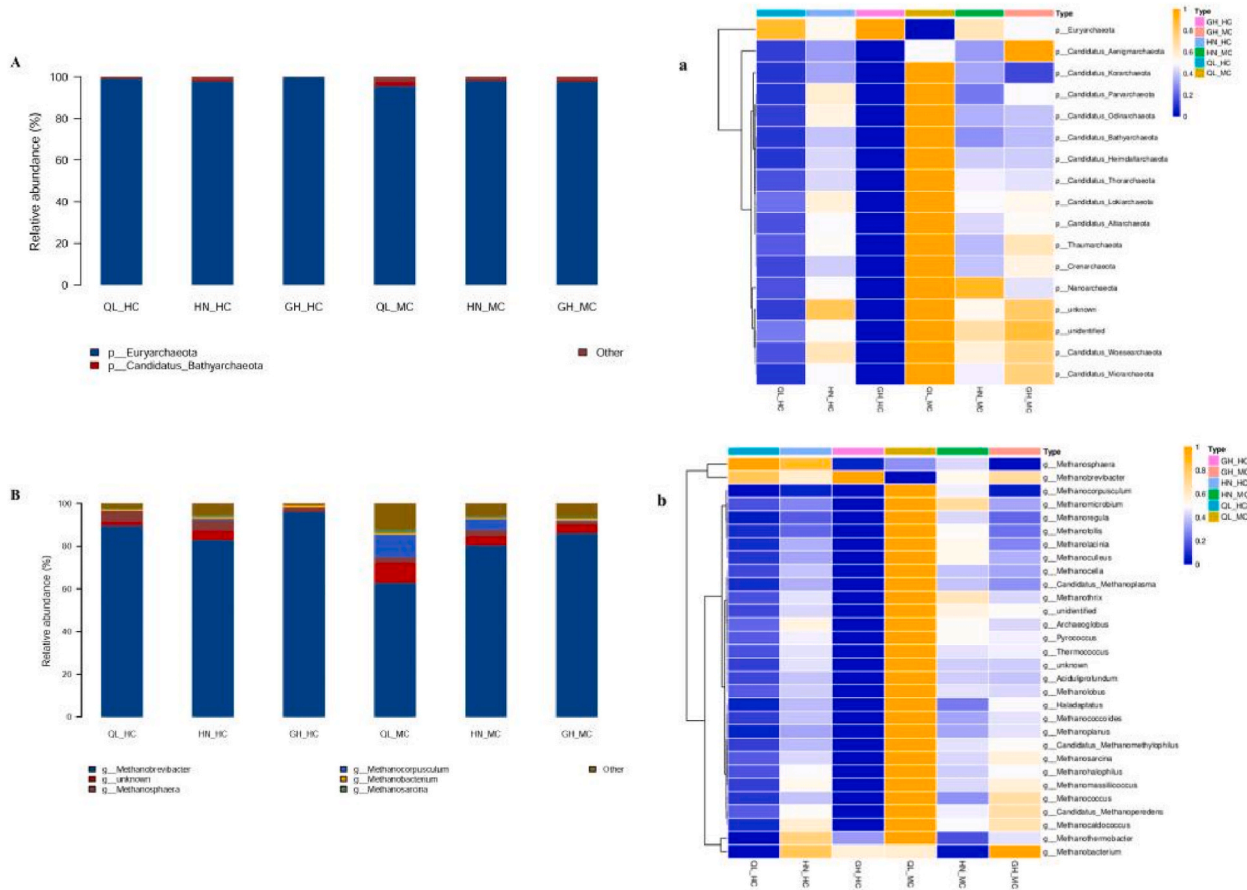


Fig. 2. Map of relative abundance of intestinal archaea in Tibetan sheep from different regions (A-a. phylum level, B-b. genus level).

higher than in the other groups. The relative abundance of *Bacteroidetes* in the three groups of Tibetan sheep MC was higher. At the genus level (Fig. 3B–b), the relative abundance of *Bacteroides* (4.88 % and 10.82 %, respectively), *Clostridium* (8.32 % and 7.40 %, respectively), *Alistipes* (1.56 % and 4.54 %, respectively) and *Prevotella* (1.53 % and 3.33 %, respectively) in the HC of the QL and HN groups was high, with *Bacteroides* (0.13 %), *Clostridium* (5.07 %), *Alistipes* (less than 0.1 %), *Prevotella* (0.14 %), and relative abundance of *Bifidobacterium* (25.22 %), and *Olsenella* (9.14 %) being found to be higher in HC of GH group. The relative abundance of *Clostridium*, *Ruminococcus*, *Eubacterium*, *Bacteroides*, *Alistipes*, and *Prevotella* was higher in the three groups of Tibetan sheep MC.

### 3.4. Annotated results of intestinal fungal levels in Tibetan sheep from different regions

The results of this study indicate that *Ascomycota*, *Pleosporales*, *Saccharomycetales*, *Mucorales*, *Paraphaeosphaeria*, *Saccharomyces* and *Colletotrichum* represent a significant proportion of the intestinal microbiota. However, the abundance of each fungi varied in different intestinal segments. There were differences, and the differences in relative abundance of HC microflora composition in Tibetan sheep from different regions were relatively obvious compared to MC. At the phylum level (Fig. 4A–a), the predominant fungi in HC and MC were *Ascomycota*, followed by *Mucoromycota* and *Basidiomycota* in descending order of abundance. At the genus level (Fig. 4B–b), *Paraphaeosphaeria*, *Ascochyta* had higher relative abundance in HC of QL group (7.22 %, 5.23 %, respectively); *Rhizopagus\_<glomeromycetes>*, *Mucor* had higher relative abundance in HC of HN group (3.65 %, 3.07 %); *Saccharomyces*, *Mucor*, *Nakaseomyces*, *Aspergillus* were higher in relative abundance in HC of GH group (24.50 %, 15.27 %, 9.18 %, 3.31 %, respectively). The relative abundance of *Colletotrichum*, *Mucor*, *Aspergillus*, *Fusarium*, and *Pseudogymnoascus* was found to be higher in the three groups of Tibetan sheep MC.

### 3.5. Species level analysis of intestinal microorganisms in Tibetan sheep from different regions

At the bacteria level (Fig. 5A), the differential species are *Bacteroidales* at the order level in GH-MC; the primary differential species are *Butyrivibrio* at the genus level in HN-MC; the important differential species are *Akkermansia*, *Glycaniphila* at the species level in QL-MC; the primary differential species are *Coriobacteriaceae* at the family level in GH-HC; *Bacteroidales* at the order level in HN-HC; and

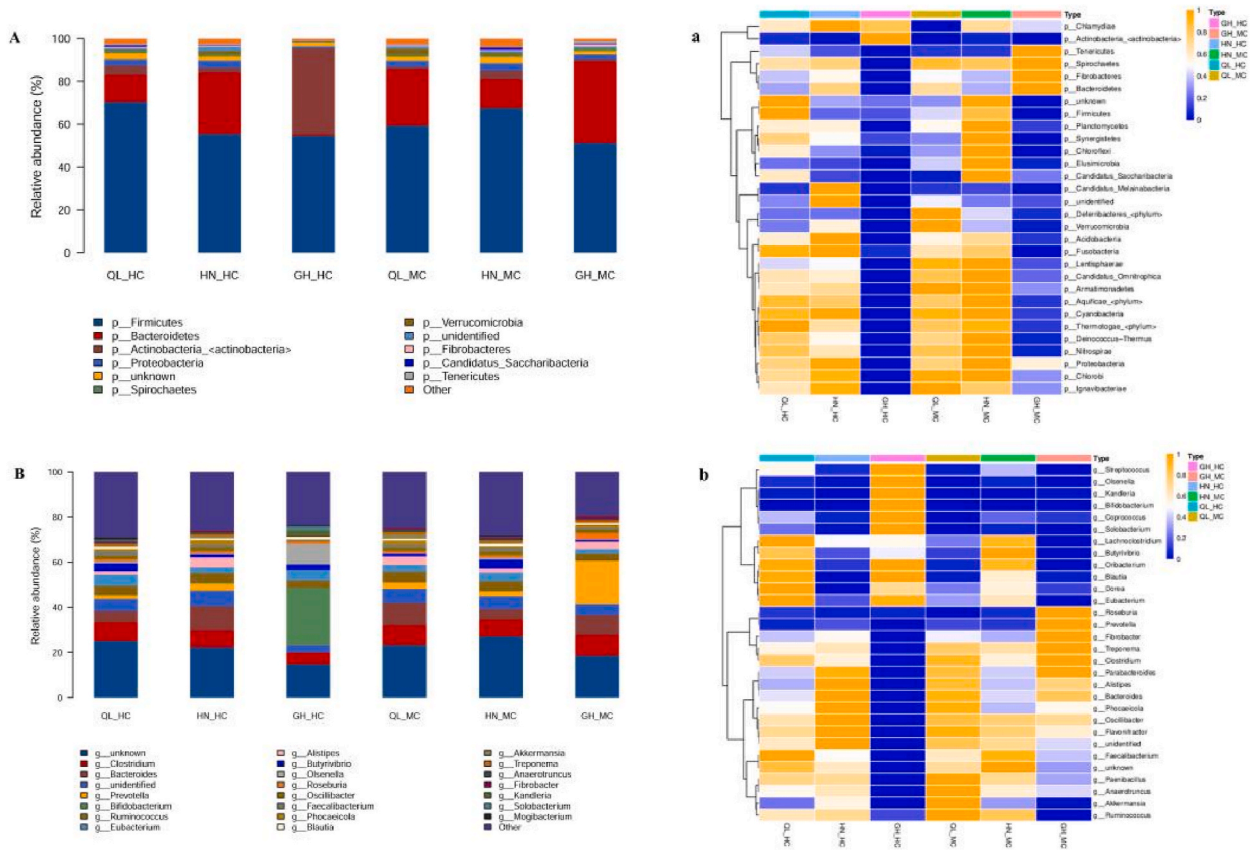


Fig. 3. Map of relative abundance of intestinal bacteria in Tibetan sheep from different regions (A-a. phylum level, B-b. genus level).

*Peptococcaceae* at the family level in QL-HC. At the fungal level (Fig. 5B), the important differential species were *Paxillaceae* at the family level in GH-MC; the main differential species were *Ustilaginales* at the order level in HN-MC; the primary differential species were *Atheliales* at the order level in QL-MC; the primary differential species in GH-HC were *Mucor\_ambiguus* at the species level; *Microbotryaceae* at the family level in HN-HC; and *Dothideomycetes* at the phylum level in QL-HC.

### 3.6. Annotation analysis of intestinal microbial functions in different regions

Based on CAZyme database statistics (Fig. 6A): The intestinal microorganisms of Tibetan sheep in the QL, HN and GH groups all have the highest number of genes annotated in the Glycoside Hydrolases (GH), followed by the Glycosyl Transferases (GT), Carbohydrate Binding Modules (CBMs) and Carbohydrate Esterases (CE) carbohydrate esterases showed similar gene abundance in intestinal microorganisms CAZyme annotation in Tibetan sheep in GH, HN and QL regions. KEGG database statistics (Fig. 6B): The number of genes annotated by Metabolism in the QL, HN and GH groups were the highest in the Level 1 classification. Among them, Carbohydrate metabolism, Global and overview maps, Amino acid metabolism and Nucleotide metabolism were the most abundant genes in Level 2. However, The Environmental Information Processing domain encompasses three distinct Level 2 classifications, with Membrane transport and Signal transduction having the highest number of genes. KOG database statistics (Fig. 6C): The number of genes annotated in Translation ribosomal structure and biogenesis of intestinal microorganisms in QL, HN and GH groups were the highest, followed by Amino acid transport and metabolism. General function prediction only, Carbohydrate transport and metabolism, Post-translational modification protein turnover chaperones, Energy production and conversion, Posttranslational modification protein turnover chaperones, energy production and conversion. eggNOG database statistics (Fig. 6D): The number of genes annotated in Carbohydrate transport and metabolism of intestinal microorganisms in QL, HN and GH groups were the highest, and followed by Amino acid transport and metabolism. Replication, recombination and repair, Translation, ribosomal structure and biogenesis, The Cell wall/membrane/envelope biogenesis.

## 4. Discussion

The gut of animals is a vital digestive site, and the intestinal microbes that inhabit it play an important role in the growth of the animal, forming a symbiotic relationship with the host. The intestinal flora is also regarded as the “external organ” of the host, playing

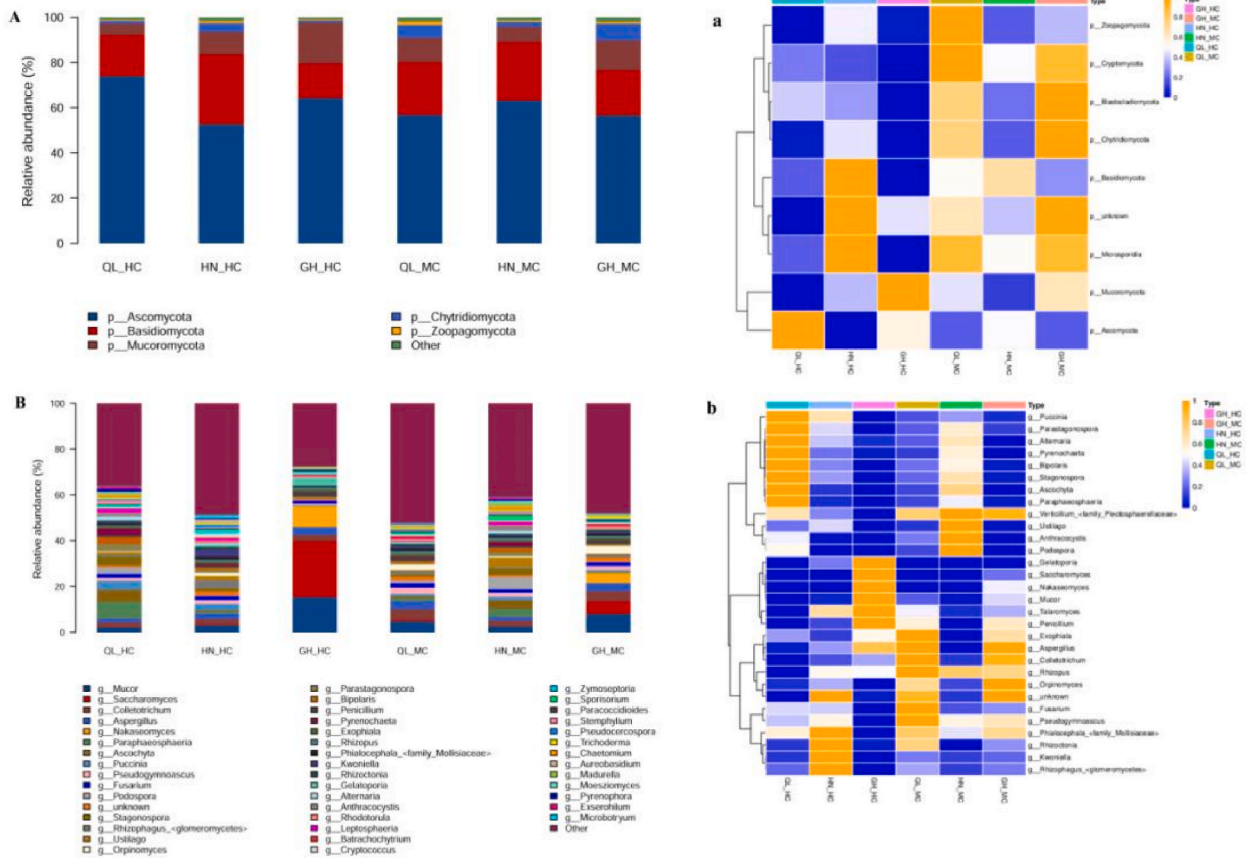


Fig. 4. Map of relative abundance of intestinal fungi in Tibetan sheep from different regions (A-a. phylum level, B-b. genus level).

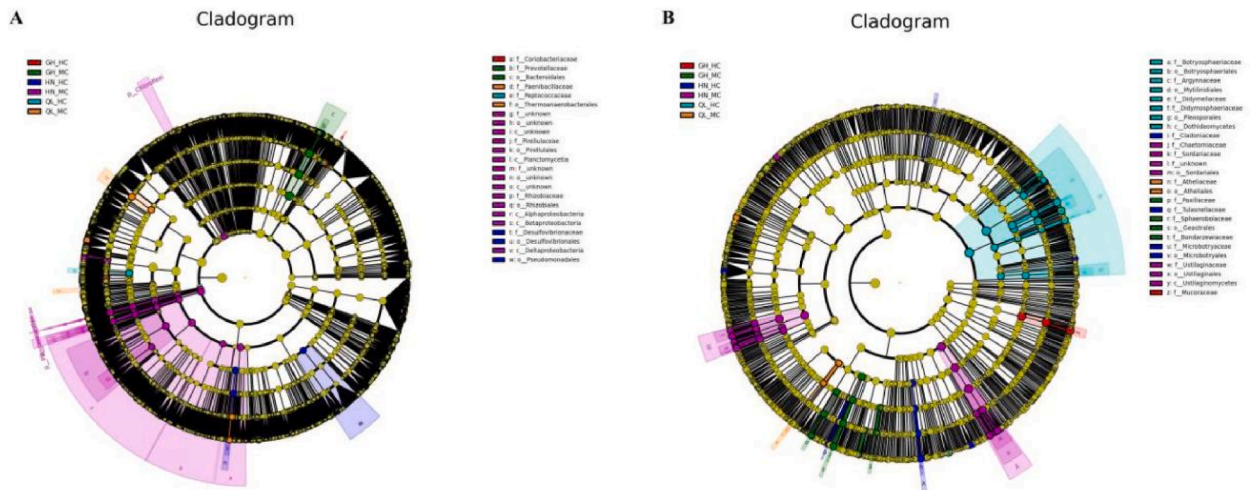
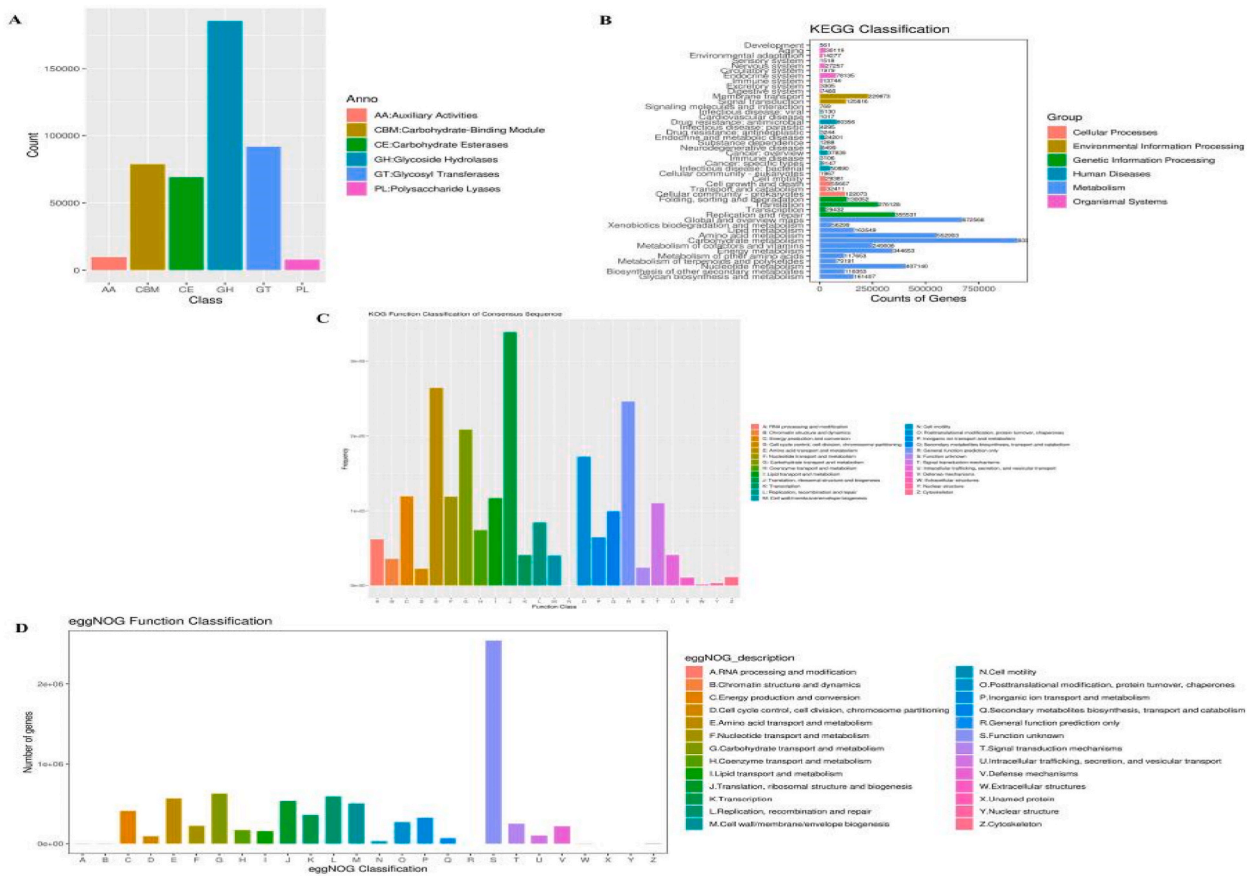


Fig. 5. Lefse analysis of intestinal microorganisms in Tibetan sheep from different regions (A. bacteria level, B. fungi level).

a role in assisting the host's immune regulation, nutrient absorption and other functions [14–16]. The analysis of intestinal flora composition based on traditional culture technology is the most commonly used research method in the field of microbial research. However many microorganisms in the gut are not easily isolated and cultured, and the current metagenomic sequencing technology not only eliminates the step of separating microorganisms in culture samples, but also does not require amplification of specific genes, which represents a new research method [17–20]. In this study, we used metagenome technology to reveal the composition and



**Fig. 6.** Functional prediction of intestinal microorganisms in Tibetan sheep from different regions based on database analysis (A. CAZyme; B. KEGG; C. KOG; D. eggNOG).

function of the intestinal flora of Tibetan sheep in different regions of the alpine, to further elaborate the important role of intestinal microorganisms on digestion and absorption, growth and development, or disease prevention and control of Tibetan sheep, with a view to providing references for the healthy development of the animal husbandry industry.

This study analyzed from the perspectives of bacteria, archaea and fungi: The dominant intestinal microorganisms of Tibetan sheep in different regions of QL, HN and GH were *Euryarchaeota* at the phylum level of archaea; *Methanobrevibacter* at the level of genus. This is in agreement with the results of diversity analysis of rumen bacteria and methanogenic archaea in yaks studied by Xue [21] et al. *Methanogenic* archaea are a kind of anaerobic bacteria that can produce methane gas by using simple compounds, and are highly conservative among ruminants worldwide. They can convert hydrogen, formic acid and acetic acid produced in rumen fermentation into methane and CO<sub>2</sub>, from which energy can be obtained [22–25], In addition, these methanogenic bacteria elevate short-chain fatty acid levels by metabolising nutrients and metabolites from other bacteria [26]. The dominant bacterial at the phylum level in the intestinal tract of Tibetan sheep in different regions was *Firmicutes*. The dominant bacterium at the genus level in the ileum of the QL and HN groups was *Bacteroides*, while in the ileum of the GH group it was *Bifidobacterium*. The relative abundance of *Bifidobacterium*, *Clostridium*, *Ruminococcus*, *Eubacterium*, and *Bacteroides* was higher in the cecum of Tibetan sheep in different regions. The relative abundance of *Prevotella* in the cecum of the GH group was significant. The *Firmicutes* and *Bacteroidetes* are effective in the degradation of fibrous material, absorption or storage of energy, and promotion of fat deposition; *Clostridium* and *Prevotella* can help hosts decompose cellulose and degrade structural carbohydrates in food, and play a crucial role in amino acid metabolism of ruminants [27–32]. Other studies have shown that when the plant fiber content in the diet is high, the intestinal flora is dominated by *Bacteroides*; when the protein content in the diet is high, *Firmicutes* account for a larger proportion; *Clostridium* can be involved in regulating the immune response mediated by intestinal T cells [33–35], *Bifidobacterium* is capable of regulating homeostasis and anti-inflammatory immunity within the body [36], and these bacteria play a pivotal role in maintaining intestinal barrier function and promoting intestinal health. Different from previous studies [37], in this study, the dominant bacterial phylum in the intestinal tract of plateau Tibetan sheep at the portal level was *Firmicutes*, which was consistent with the dominant intestinal microbial phylum of monogastric animals. Furthermore, the dominant bacterial genera at the genus level were *Bacteroides*, *Bifidobacterium*. The observed difference may be attributed to the breeds of the selected animals, their age, and their feeding style. It is also probable that this is a consequence of the Tibetan sheep’s attempts to adapt to the high altitude environment in the high plateau region. At the fungal level, the dominant phyla

were *Ascomycota*. The dominant ileal bacteria in QL, HN and GH group were *Paraphaeosphaeria*, *Rhizophagus* <*glomeromycetes*> and *Saccharomyces* respectively at the genus level. The relative abundance of *Colletotrichum* and *Mucor* in cecum of Tibetan sheep from different regions was higher. Many studies have shown that fungi are an important source of lead compounds for drugs, and the expression of fungal silent gene clusters can be regulated through chemical epigenetic modification and molecular biological manipulation, so as to discover more secondary metabolites of fungi [38–43], providing references for the research of antibacterial drugs. However, the mechanism of fungi in the gut of ruminants remains unclear, and further studies are needed. Furthermore, a considerable number of unidentified or unclassified genera were identified in the intestinal microbiota of Tibetan sheep. It was postulated that plateau Tibetan sheep may exhibit a distinctive intestinal microbiome. In conclusion, The results of this study demonstrated some degree of overlap in the relative abundance of gut species composition in the QL, HN and GH regions, this was evidenced by differences in the ileal microbiota and essentially similar cecum microbiota. However, the abundance of gut species composition was found to be more similar in the HN and QL regions.

The results of this experiment indicate that there are differences in the genera enriched in the intestinal flora of Tibetan sheep in different regions. To further explore the role of the microbiota in the gut, the functional prediction of the intestinal microorganisms of Tibetan sheep in different regions was carried out through the CAZyme, eggNOG, KOG, and KEGG databases. The results of this analysis revealed that the metabolic pathways enriched in the intestinal tracts of Tibetan sheep have a commonality. The highest number of genes annotated to these pathways was found in the following categories: Glycoside Hydrolases (GH), Carbohydrate transport and metabolism, General function prediction only, Biological Process, Translation ribosomal structure and biogenesis, and Metabolism. These pathways are inextricably linked to the dominant bacterial species in the host intestine [44–46], gene enrichment also reflects host digestive metabolism, indicating the importance of the intestinal microbiota in gut health in Tibetan sheep. Studies have reported that nutrients such as carbohydrates in food are fermented in the colon and cecum, that is, short-chain fatty acids SCFAs can be generated under the action of anaerobic microorganisms in the large intestine, thus regulating the development of rumen epithelium and intestine of goats [47]. In addition, more than 35 % of the enzymes required for animal digestion and metabolism are produced in intestinal flora, and 25 % of them are involved in carbohydrate metabolism [48,49]. These results suggest that carbohydrate transport and metabolism play an important role in the maintenance of body growth and development. The metabolism of intestinal microorganisms is closely related to the feeding habits of the host. In the long-term evolution process, intestinal flora will respond to changes in food types [50]. The gene function enrichment pathways annotated by the intestinal microorganisms of Tibetan sheep in the QL, HN and GH regions were found to be largely consistent. This phenomenon may be attributed to the general similarity of grazing pathways, feeding practices, and living environments among Tibetan sheep.

### Ethical approval statement

All experimental animal tests in this study were conducted in accordance with the Animal Ethics Committee of Qinghai University (SL-2023022).

### Data availability statement

Has data associated with your study been deposited into a publicly available repository?

Answer: NO.

### CRedit authorship contribution statement

**Zifeng Gong:** Writing – original draft. **Guisheng Ye:** Writing – review & editing. **Shuqin Xu:** Investigation. **Xi He:** Investigation.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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### References

- [1] Y. Ren, R. Ciwang, J. Wang, et al., Effect of different feeds on the fungi microbiome of suffolk crossed with Tibetan sheep, *Life* 13 (11) (2023) 2210, <https://doi.org/10.3390/life13112210>.
- [2] Z.Z. Guo, Z.N. Guo, W.C. Guo, Comparison of the determination of muscle nutrients of valley type Tibetan sheep, Qinghai semi-fine wool sheep and Qinghai Tibetan sheep, *Journal of Animal Science and Veterinary Medicine* 43 (2) (2024) 5–7. [https://kns.cnki.net/kcms2/article/abstract?v=8WLnD7pOpNE8GLmtGJ-XL\\_VgZPzYNi3aekt2UiaJuTk\\_4fptbjX22Ud-GcO7gLVi3gvvHnGATDyDZEyS\\_J7ZRDS11DY1kAmm2-Udpg6nbrQlvhQf0gdNlkkcKkU8tLVmp3CU4vNFBPeXYf\\_29fDNaQ==&uniplatform=NZKPT&language=CHS](https://kns.cnki.net/kcms2/article/abstract?v=8WLnD7pOpNE8GLmtGJ-XL_VgZPzYNi3aekt2UiaJuTk_4fptbjX22Ud-GcO7gLVi3gvvHnGATDyDZEyS_J7ZRDS11DY1kAmm2-Udpg6nbrQlvhQf0gdNlkkcKkU8tLVmp3CU4vNFBPeXYf_29fDNaQ==&uniplatform=NZKPT&language=CHS).
- [3] T. Guo, X. Wang, Q. Zhang, et al., Comparative analysis of the composition of fatty acids and metabolites between black Tibetan and chaka sheep on the Qinghai-Tibet Plateau, *Animals (Basel)* 12 (20) (2022) 2745, <https://doi.org/10.3390/ani12202745>.



- [4] G. Qiao, P. Xu, T. Guo, et al., Genome-wide detection of structural variation in some sheep breeds using whole-genome long-read sequencing data, *J. Anim. Breed. Genet.* (2024), <https://doi.org/10.1111/jbg.12846>.
- [5] Mao Li Cuo, Performance measurement and path analysis of White Tibetan sheep in Qilian County, Heilongjiang Anim. Sci. Vet. Med. (7) (2012) 79–80, <https://doi.org/10.13881/j.cnki.hljxmsy.2012.07.057>.
- [6] Yan Ming Yi, Integration and demonstration of characteristic ecological animal husbandry technology in Qinghai and Henan communities on Qinghai-Tibet Plateau, *Chinese Qinghai Journal of Animal and Veterinary Sciences* 48 (5) (2018) 55–56. [https://kns.cnki.net/kcms2/article/abstract?v=8WLnD7pOpNHVdM0Av3G57tEP5e5H0F77AQn0wMqEFyF65O\\_XKsuPAztzVCAY-i0I-zozzwXgzln66jm00brSVhjHqKLi5Nar770jObLz6NxcYNURN0anqU6Ai2nu-cwJzb55AsrZoAclj\\_G1lQdA=&uniplatform=NZKPT&language=CHS](https://kns.cnki.net/kcms2/article/abstract?v=8WLnD7pOpNHVdM0Av3G57tEP5e5H0F77AQn0wMqEFyF65O_XKsuPAztzVCAY-i0I-zozzwXgzln66jm00brSVhjHqKLi5Nar770jObLz6NxcYNURN0anqU6Ai2nu-cwJzb55AsrZoAclj_G1lQdA=&uniplatform=NZKPT&language=CHS).
- [7] Duo He Ming, Fei Meng, Xu Hai Tao, et al., Analysis on superovulation effect of Qinghai Tibetan sheep with plateau type, *China Animal Industry* (10) (2019) 60–62. [https://kns.cnki.net/kcms2/article/abstract?v=8WLnD7pOpNFpCuiNem00JJ79Fns25d\\_644s8MCnoY46exgkLzGiqe-GTOVPOpvcqgJmmOZEzKvQf93-bBohzByMrcnGXuzX2LJkR-aK3PKXQ6EtLwG0ibbZzW1MPqRsLtscoYe4WETi1xwstAQutQ=&uniplatform=NZKPT&language=CHS](https://kns.cnki.net/kcms2/article/abstract?v=8WLnD7pOpNFpCuiNem00JJ79Fns25d_644s8MCnoY46exgkLzGiqe-GTOVPOpvcqgJmmOZEzKvQf93-bBohzByMrcnGXuzX2LJkR-aK3PKXQ6EtLwG0ibbZzW1MPqRsLtscoYe4WETi1xwstAQutQ=&uniplatform=NZKPT&language=CHS).
- [8] Y.L. Bi, Y. Tu, N. Zhang, et al., Multiomics analysis reveals the presence of a microbiome in the gut of fetal lambs, *Gut* 70 (5) (2021) 853–864, <https://doi.org/10.1136/gutjnl-2020-320951>.
- [9] J. Dias, M.I. Marcondes, S. Motta de Souza, et al., Bacterial community dynamics across the gastrointestinal tracts of dairy calves during preweaning development, *Appl. Environ. Microbiol.* 84 (9) (2018) e02675, <https://doi.org/10.1128/AEM.02675-17>.
- [10] B. Li, K. Zhang, C. Li, et al., Characterization and comparison of microbiota in the gastrointestinal tracts of the goat (*Capra hircus*) during preweaning development, *Front. Microbiol.* 10 (2019) 2125, <https://doi.org/10.3389/fmicb>.
- [11] P. Cholewińska, K. Czyż, P. Nowakowski, et al., The microbiome of the digestive system of ruminants—a review, *Anim. Health Res. Rev.* 21 (1) (2020) 3–14, <https://doi.org/10.1017/S1466252319000069>.
- [12] M.G. Rooks, W. Garrett, Gut microbiota, metabolites and host immunity, *Nat. Rev. Immunol.* 16 (6) (2016) 341–352, <https://doi.org/10.1038/nri.2016.42>.
- [13] H. Tilg, N. Zmora, T.E. Adolph, et al., The intestinal microbiota fuelling metabolic inflammation, *Nat. Rev. Immunol.* 20 (1) (2020) 40–54, <https://doi.org/10.1038/s41577-019-0198-4>.
- [14] P.P. Lyons, J.F. Turnbull, K.A. Dawson, et al., Effects of low-level dietary microalgae supplementation on the distal intestinal microbiome of farmed rainbow trout *Oncorhynchus mykiss* (Walbaum), *Aquacult. Res.* 48 (5) (2017) 2438–2452, <https://doi.org/10.1111/are.13080>.
- [15] Xu C. Li, X. Wang, et al., Gut microbiota and its modulation for healthy farming of Pacific white shrimp *Litopenaeus vannamei*, *Reviews in Fisheries Science & Aquaculture* 26 (3) (2018) 381–399, <https://doi.org/10.1080/23308249.2018.1440530>.
- [16] B. Zhou, Y. Yuan, S. Zhang, et al., Intestinal flora and disease mutually shape the regional immune system in the intestinal tract, *Front. Immunol.* 11 (2020) 575, <https://doi.org/10.3389/fimmu.2020.00575>.
- [17] V. Torsvik, L. Øvreås, Microbial diversity and function in soil: from genes to ecosystems, *Curr. Opin. Microbiol.* 5 (3) (2002) 240–245, [https://doi.org/10.1016/S1369-5274\(02\)00324-7](https://doi.org/10.1016/S1369-5274(02)00324-7).
- [18] C.X. Wang, D.J. Meek, P. Panchal, et al., Isolation of poly-3-hydroxybutyrate metabolism genes from complex microbial communities by phenotypic complementation of bacterial mutants, *Appl. Environ. Microbiol.* 72 (1) (2006) 384–391, <https://doi.org/10.1128/AEM.72.1.384-391.2006>.
- [19] J. Zhou, Microarrays for bacterial detection and microbial community analysis, *Curr. Opin. Microbiol.* 6 (3) (2003) 288–294, [https://doi.org/10.1016/S1369-5274\(03\)00052-3](https://doi.org/10.1016/S1369-5274(03)00052-3).
- [20] Z.L. He, J. Nostrand, L.Y. Wu, et al., Development and application of functional gene arrays for microbial community analysis, *Trans. Nonferrous Metals Soc. China* 18 (6) (2008) 1319–1327, [https://doi.org/10.1016/S1003-6326\(09\)60004-2](https://doi.org/10.1016/S1003-6326(09)60004-2).
- [21] D. Xue, H. Chen, F. Chen, et al., Analysis of the rumen bacteria and methanogenic archaea of yak (*Bos grunniens*) steers grazing on the Qinghai-Tibetan plateau, *Livest. Sci.* 188 (2016) 61–71, <https://doi.org/10.1016/j.livsci.2016.04.009>.
- [22] W. Lan, C. Yang, Ruminant methane production: associated microorganisms and the potential of applying hydrogen-utilizing bacteria for mitigation, *Sci. Total Environ.* 654 (2019) 1270–1283, <https://doi.org/10.1016/j.scitotenv.2018.11.180>.
- [23] R. Seshadri, S.C. Leahy, G.T. Attwood, et al., Cultivation and sequencing of rumen microbiome members from the Hungate 1000 collection, *Nat. Biotechnol.* 36 (4) (2018) 359–367, <https://doi.org/10.1038/nbt.4110>.
- [24] S. Kittelmann, H. Seedorf, W.A. Walters, et al., Simultaneous amplicon sequencing to explore cooccurrence patterns of bacterial, archaeal and eukaryotic microorganisms in rumen microbial communities, *PLoS One* 8 (2) (2013) e47879, <https://doi.org/10.1371/journal.pone.0047879>.
- [25] X. Ding, R. Long, Q. Zhang, et al., Reducing methane emissions and the methanogen population in the rumen of Tibetan sheep by dietary supplementation with coconut oil, *Trop Anim. Health Prod* 44 (7) (2012) 1541–1545, <https://doi.org/10.1007/s11250-012-0103-7>.
- [26] M. Primec, M. Klemenak, D. Di Gioia, et al., Clinical intervention using *Bifidobacterium* strains in celiac disease children reveals novel microbial modulators of TNF- $\alpha$  and short-chain fatty acids, *Clin. Nutr.* 38 (3) (2019) 1373–1381, <https://doi.org/10.1016/j.clnu.2018.06.931>.
- [27] K.R. Amato, S.R. Leigh, A. Kent, et al., The role of gut microbes in satisfying the nutritional demands of adult and juvenile wild, black howler monkeys (*Alouatta pigra*), *Am. J. Phys. Anthropol.* 155 (4) (2014) 652–664, <https://doi.org/10.1002/ajpa.22621>.
- [28] K.R. Amato, S.R. Leigh, A. Kent, et al., The gut microbiota appears to compensate for seasonal diet variation in the wild black howler monkey (*Alouatta pigra*), *Microb. Ecol.* 69 (2) (2015) 434–443, <https://doi.org/10.1007/s00248-014-0554-7>.
- [29] L. Zhu, Q. Wu, J. Dai, et al., Evidence of cellulose metabolism by the giant panda gut microbiome, *Proc. Natl. Acad. Sci. U. S. A.* 108 (43) (2011) 17714–17719, <https://doi.org/10.1073/pnas.1017956108>.
- [30] H. Zhang, M. Shao, H. Huang, et al., The dynamic distribution of small-tail han sheep microbiota across different intestinal segments, *Front. Microbiol.* 9 (2018) 32, <https://doi.org/10.3389/fmicb.2018.00032>.
- [31] G. Gong, S. Zhou, R. Luo, et al., Metagenomic insights into the diversity of carbohydrate-degrading enzymes in the yak fecal microbial community, *BMC Microbiol.* 20 (1) (2020) 302, <https://doi.org/10.1186/s12866-020-01993-3>.
- [32] M.Y. Xue, H.Z. Sun, X.H. Wu, et al., Multi-omics reveals that the rumen microbiome and its metabolome together with the host metabolome contribute to individualized dairy cow performance, *Microbiome* 8 (1) (2020) 64, <https://doi.org/10.1186/s40168-020-00819-8>.
- [33] E.F. Murphy, P.D. Cotter, S. Healy, et al., Composition and energy harvesting capacity of the gut microbiota: relationship to diet, obesity and time in mouse models, *Gut* 59 (12) (2010) 1635–1642, <https://doi.org/10.1136/gut.2010.215665>.
- [34] M.J. Slifker, R.M. Friendship, J.S. Weese, Longitudinal study of the early-life fecal and nasal microbiotas of the domestic pig, *BMC Microbiol.* 15 (1) (2015) 184, <https://doi.org/10.1186/s12866-015-0512-7>.
- [35] G. Cao, F. Tao, Y. Hu, et al., Positive effects of a *Clostridium butyricum*-based compound probiotic on growth performance, immune responses, intestinal morphology, hypothalamic neurotransmitters, and colonic microbiota in weaned piglets, *Food Funct.* 10 (5) (2019) 2926–2934, <https://doi.org/10.1039/c8fo02370k>.
- [36] S.J. Gavzy, A. Kensiski, Z.L. Lee, et al., *Bifidobacterium* mechanisms of immune modulation and tolerance, *Gut Microb.* 15 (2) (2023 Dec) 2291164, <https://doi.org/10.1080/19490976.2023.2291164>.
- [37] F.F. Domínguez, M.E.V. Crisanto, R.L.S. Castro, et al., Metagenomic analysis of the intestinal microbiome in goats on cactus and *Salicornia*-based diets, *Open Vet. J.* 12 (1) (2022) 61–68, <https://doi.org/10.5455/OVJ.2022.v12.i1.7>.
- [38] A. Schueffler, T. Anke, Fungal natural products in research and development, *Nat. Prod. Rep.* 31 (10) (2014) 1425–1448, <https://doi.org/10.1039/c4np00060a>.
- [39] W.G. Wang, H. Wang, L.Q. Du, et al., Molecular basis for the biosynthesis of an unusual chain-fused polyketide, gregatin A, *J. Am. Chem. Soc.* 142 (18) (2020) 8464–8472, <https://doi.org/10.1021/jacs.0c02337>.
- [40] M.A. Witek, E. Tenconi, S. Rigali, et al., Functional analysis of the *N*-Acetylglucosamine metabolic genes of *Streptomyces coelicolor* and role in control of development and antibiotic production, *J. Bacteriol.* 194 (5) (2012) 1136–1144, <https://doi.org/10.1128/JB.06370-11>.
- [41] L. Li, L.W. MacIntyre, S.F. Brady, Refactoring biosynthetic gene clusters for heterologous production of microbial natural products, *Curr. Opin. Biotechnol.* 69 (2021) 145–152, <https://doi.org/10.1016/j.copbio.2020.12.011>.

- [42] P. Bharatiya, P. Rathod, A. Hiray, et al., Multifarious elicitors: invoking biosynthesis of various bioactive secondary metabolite in fungi, *Appl. Biochem. Biotechnol.* 193 (3) (2021) 668–686, <https://doi.org/10.1007/s12010-020-03423-6>.
- [43] L.C. Pillay, L. Nekati, P.J. Makhwitine, et al., Epigenetic activation of silent biosynthetic gene clusters in endophytic fungi using small molecular modifiers, *Front. Microbiol.* 13 (2022) 815008, <https://doi.org/10.3389/fmicb.2022.815008>.
- [44] J.M. Brulc, D.A. Antonopoulos, M.E. Miller, et al., Gene-centric metagenomics of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases, *Proc. Natl. Acad. Sci. U.S.A.* 106 (6) (2009) 1948–1953, <https://doi.org/10.1073/pnas.0806191105>.
- [45] W. Huo, W. Zhu, S. Mao, Impact of subacute ruminal acidosis on the diversity of liquid and solid-associated bacteria in the rumen of goats, *World J. Microbiol. Biotechnol.* 30 (2) (2014) 669–680, <https://doi.org/10.1007/s11274-013-1489-8>.
- [46] L. Wang, K. Liu, Z. Wang, et al., Bacterial community diversity associated with different utilization efficiencies of nitrogen in the gastrointestinal tract of goats, *Front. Microbiol.* 10 (2019) 239, <https://doi.org/10.3389/fmicb.2019.00239>.
- [47] J.M. Wong, R. de Souza, C.W. Kendall, et al., Colonic health: fermentation and short chain fatty acids, *J. Clin. Gastroenterol.* 40 (3) (2006) 235–243, <https://doi.org/10.1097/00004836-200603000-00015>.
- [48] A. Kala, D.N. Kamra, A. Kumar, et al., Impact of levels of total digestible nutrients on microbiome, enzyme profile and degradation of feeds in buffalo rumen, *PLoS One* 12 (2) (2017) e0172051, <https://doi.org/10.1371/journal.pone.0172051>.
- [49] S.R. Gill, M. Pop, R.T. Deboy, et al., Metagenomic analysis of the human distal gut microbiome, *Science* 312 (5778) (2006) 1355–1359, <https://doi.org/10.1126/science.1124234>.
- [50] R.E. Ley, M. Hamady, C. Lozupone, et al., Evolution of mammals and their gut microbes, *Science* 320 (5883) (2008) 1647–1651, <https://doi.org/10.1126/science.1155725>.