Rumen Microbial Community of Saanen Goats Adapted to a High-Fiber Diet in the Northeast of Iran

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ABSTRACT

Swiss Saanen goat is a widespread breed frequently found in commercial herds across the world. The present study aimed to identify the rumen microbial community of exotic Saanen goats adapted to a fibrous diet using barcoded pyrosequencing. Rumen content samples were collected from the four animals via a stomach tube after the morning graze and freeze-dried for DNA extraction. Bacterial and archaeal 16S rRNA and protozoal 18S rRNA genes were sequenced by 454 titanium pyrosequencing and analyzed using the quantitative insights into microbial ecology (QIIME) software package. Obtained results indicated that at the genus level, Prevotella (Bacteroidetes phylum) dominated the assigned sequence, with the relative abundance accounting for 29.41 ± 4.27% of the total bacteria. The second most abundant bacteria in the rumen of Saanen goats was an unclassified Bacteroidales (Bacteroidetes phylum) (11.01±0.94%). In addition, Firmicutes phylum was recorded as the second most frequent phylum and three unclassified genera, which belonged to the order Clostridiales, constituted 21.42% of the total bacteria. Entodinium was the most abundant protozoal genus, comprising 46.78 ± 9.13% of the protozoal community, followed by Epidinium and Ophryoscolex (12.37±0.06 and 11.92±7.7, respectively). Almost half the archaeal community (43.71±1.57%) was composed of Methanoplasmatales-related sequences and Methanobrevibacter gottschalkii clade (35.79±4.84%) and Methanobrevibacter ruminantium clade (13.36±6.34%) were the second and third most dominant archaea, respectively. Overall, further efforts should be made to apply culture-based methods for the identification of remarkable number of unclassified bacteria in the rumen of goats.

KEY WORDS Iran, rumen microbial community, Sannen goats.

INTRODUCTION

Water scarcity is a major challenge in the future, which adversely affects the form and functions of the animal husbandry. In harsh environments, goats are among the dominant species due to their higher adaptation capability. As such, goat population has multiplied by 2.4 times within the past 50 years, while the population of other livestock species has only been maintained or decreased (Capote, 2016). Goats have certain features to enable them to survive in continuous drought, which occasionally occurs in the dry regions of the world. Such features include skillful grazing behaviors, efficient use of water, reduced metabolism, economizing nitrogen requirements and efficient digestive system. In particular, current findings indicate that even with water restriction, body weight and production rate could be maintained in high-producing Saanen goats (Jaber et al, 2016). Therefore, goats are considered the most efficient candidates for low-water livestock farming strategies compared to sheep and cattle. The Goats differ from sheep...
and cattle in terms of feeding behaviors, level of intake, diet selection, taste discrimination, and rate of eating therefore their rumen microbial structures is incomparable (Lee et al. 2012) even with similar diet. Consequently, the knowledge obtained from other ruminant species might not be extrapolated to goats. Shi et al. (2008) reported significantly different rumen bacterial communities in three goat breeds fed on a similar diet and preserved in the same environment.

To the best of our knowledge there is lack of information about rumen microorganisms of ruminants adapted to the Iran climates. Culturing techniques can be used for random isolation of microorganisms in order to obtain isolates. This method also designates the presence of a species however; the abundance of different microbes cannot be comprehensively assessed using data from cultivation-based studies conducted in the past 50 years (Janssen and Kirs, 2008). Recent molecular-based techniques could be used to recognize the relative abundance of rumen microbes living under different nutritional regimens in various hosts (Henderson et al. 2015). Since Swiss Saanen goat is a widespread breed that is frequently found in commercial herds across the world (Capote, 2016), the present study aimed to identify the rumen microbial community of exotic Saanen goats adapted to a fibrous diet using barcoded pyrosequencing.

MATERIALS AND METHODS

Animals, feed and management

Rumen samples were collected from four late-lactating Saanen goats (body weight: 47±1.4 kg) maintained in the Livestock Research Center at the Faculty of Agriculture, Ferdowsi University of Mashhad, Iran. All experimental procedures were in accordance with the Principles of Research Ethics issued by the Ministry of Science, Research and Technology, Iran. Animals were kept in a barn and allowed to graze on an agricultural land in the vicinity, which contained residues of harvested barley grain (70%) and Alhagi maurorum (30%). Grazing occurred twice per day at 6:00-9:00 in the morning and 14:00-18:00 in the afternoon.

After the evening grazing, 500 g of ground barley grains was provided for each animal and water was provided at the intervals between grazing and staying in the barn. Rumen samples were collected from the four animals via a stomach tube two hours after the morning graze and immediately transferred to a laboratory to measure the pH.

As described in our previous study, 10 and 3 mL of rumen samples were processed for the estimation of ammonia nitrogen and volatile fatty acids (VFAs), respectively (Ebrahimi et al. 2011). Applied instruments, conditions and analytical procedures have been described by Razzaghi et al. (2016). In the current research, approximately 200 mL of the rumen contents were frozen and freeze-dried for DNA extraction.

DNA extraction, polymerase chain reaction (PCR) amplification and 454 pyrosequencing

DNA was extracted from 30 mg of freeze-dried, homogenised rumen contents using the PCQI method (Henderson et al. 2013; Rius et al. 2012). Bacterial and archaeal 16S rRNA gene regions and protozoal 18S rRNA gene regions were amplified in triplicate as described previously (Kittelmann et al. 2013; Rius et al. 2012). Primers (Integrated DNA Technologies Inc., Coralville, IA, USA) consisted of 454 titanium adapter sequences A (5’-CCA TCT CAT CCC TGC GTG TCT CCG ACT CAG-3’) or B (5’-CCT ATC CCC TGT GTG CCT TGG CAG CAG-3’), a two-base linker sequence between the barcode and the group-specific primer (Table 1), and a unique 12-base error-correcting Golay barcode attached to adapter A for sample identification followed by the specific primer sequence. Amplicons from the three microbial groups were quantified fluorometrically, normalised by sample, and pooled by microbial group. A total of 1 μg DNA of each of the three resulting pools was loaded onto an agarose gel (1%, wt:vol).

Bands were visualized and excised under blue light transillumination and amplicons were gel purified with the QIAquick Gel Extraction Kit (Qiagen). Bacterial, archaeal, and ciliate protozoal amplicons were sequenced using 454 GS FLX Titanium chemistry at Eurofins MWG Operon (Ebersberg, Germany).

Phylogenetic analysis of pyrosequencing reads

Pyrosequence data were processed and analyzed using the quantitative insights into microbial ecology (QIIME) software package version 1.5 (Caporaso et al. 2010). Sequences over 400 bp in length with an average quality score over 25 were assigned to a specific sample via 12 base error-correcting Golay barcodes. Sequence data were grouped into operational taxonomic units (OTUs) sharing over 97% (bacteria, archaea) or 100% (ciliate protozoa) sequence similarity.

Sequences were assigned to phylogenetic groups by BLAST (Altschul et al. 1990) of bacterial 16S rRNA genes against the Greengenes database (version February 2011; McDonald et al. 2012), and of archaeal 16S rRNA genes and ciliate protozoal 18S rRNA genes against in-house databases (Janssen and Kirs, 2008; Kittelmann and Janssen, 2013). Bacterial data were summarized at phylum, class, order, family and genus, and ciliate protozoal data at genus levels. Archaea were summarized using a mixed taxonomic rank scheme (Janssen and Kirs, 2008).
RESULTS AND DISCUSSION

Rumen fermentation parameters of Saanen goats used for microbial community evaluation are presented in Table 2.

Table 2: Volatile fatty acids, ammonia nitrogen and ruminal pH of Saanen goats

<table>
<thead>
<tr>
<th>Items</th>
<th>Mean±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total volatile fatty acids (VFA) (mM)</td>
<td>98.35±0.23</td>
</tr>
<tr>
<td>Molar proportion (%)</td>
<td></td>
</tr>
<tr>
<td>Acetate</td>
<td>73.75±0.12</td>
</tr>
<tr>
<td>Propionate</td>
<td>16.23±0.09</td>
</tr>
<tr>
<td>Butyrate</td>
<td>10.02±0.01</td>
</tr>
<tr>
<td>Ammonia N (mg/dL)</td>
<td>16.32±0.07</td>
</tr>
<tr>
<td>pH</td>
<td>6.68±0.08</td>
</tr>
</tbody>
</table>

In the present study, overall ruminal bacterial composition was identified and characterized using bacterial tag-encoded amplicon pyrosequencing, which was generated from the V2 and V3 regions of the 16S rRNA gene. In total, 39,535 reads were generated, with an average of 9,833 reads per each sample.

Table 3 shows percentage abundance of classified and unclassified bacterial genera identified in rumen samples. Rumen samples contained 124 bacterial genera, 39 of which had a relative abundance of ≥ 0.1%, constituting 98.41% of the bacterial population with 52.34% and 46.07% classified and unclassified bacteria, respectively.

Eighty five bacterial genera had a relative abundance of < 0.1%, accounting for only 1.59% of the total bacterial community in the rumen of Saanen goats. Overall, considering the unclassified bacteria with the relative abundance of < 0.1%, 46.73% of the total bacterial population in the rumen of goats was observed to be unclassified. Figure 1 shows relative abundances of bacterial Phylum in the rumen of Saanen goats. Bacteroidetes and Firmicutes were the first and second dominant phylum, respectively. Table 4 shows percentage occurrence of sequences identified in this study, 24 of them were taxonomically affiliated with relative abundance of more than 0.1%, including Polyplastron (8.48±2.93), Dasytricha (4.84±2.93), Eremopl-Diploplastron (4.84±2.93), Enoploplastron (4.84±2.93) and Isotricha (4.84±2.93).

In total, there were 6039 high-quality reads for the rumen archaea of the goats, with an average of 1510 reads per each sample. According to the information in Table 6, seven archaeal genera were identified in the rumen of goats, including three genera with a relative abundance of < 0.1%. Furthermore, almost half the archaeal community (43.71±1.57%) was composed of Methanoplasmatales, followed by Methanobrevibacter gottschalkii clade (35.79±4.84%) and Methanobrevibacter ruminantium clade (13.36±6.34%) as the second and third most frequent genera, respectively.

#### Table 1: Primers used to amplify bacterial and archaeal 16S rRNA genes and ciliate 18S rRNA genes

<table>
<thead>
<tr>
<th>Microbes</th>
<th>Name</th>
<th>Primer sequence (5'-3')</th>
<th>Region</th>
<th>Length (bp)</th>
<th>Annealing (˚C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Ba9F</td>
<td>GTCCCGGCGCKGCTGGCAC</td>
<td>16s RNA</td>
<td>525</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>Ba515Rmod</td>
<td>GAGTTTGATCMGGTGCTCAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Archaea</td>
<td>Ar915aF</td>
<td>GTAGGAATTTGCGGG</td>
<td>16s RNA</td>
<td>492</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Ar1386R</td>
<td>GCCGGTTGTGCCCAG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protozoa</td>
<td>RP841F</td>
<td>TCAATTGCAAGATCTATCCCC</td>
<td>18s RNA</td>
<td>511</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Reg1302R</td>
<td>GCAGTTGGATTGGARTGG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Prevotella (Bacteroidetes phylum) dominated the assigned sequence, with its relative abundance accounting for 29.41±4.27% of the total bacteria.

The second most frequent bacteria in the rumen of Saanen goats was an unclassified strain of Bacteroidales (11.01±0.94%) (Bacteroidetes phylum). The three unclassified genera belonged to the order Clostridiales, accounting for 21.42% of the total bacteria. An unclassified genus of Proteobacteria phylum and another unclassified genus of Lentisphaerae phylum had a relative abundance of 4.69±1.25 and 3.79±3.5% as the sixth and seventh most frequent bacterial genus, respectively. As can be seen in Table 4, Fibrobacter, Paludibacter, Butyrivibrio and Selenomonas were the following bacterial genera with the relative abundance of 3.29±0.70, 3.08±0.048, 2.25±0.97 and 2.22±1.10, respectively.

In the current study, 4,924 high-quality reads were available for protozoa, with an average of 1231 reads per each sample. Identified protozoal genera (n=12) found in the ruminal samples of Saanen goats and their relative abundance are presented in Table 5. According to the information in the table, Entodinium was the most abundant genus, constituting 46.78±9.13% of the protozoal community, followed by Epidinium and Ophryoscolex with the relative abundance of 12.37±0.06 and 11.92±7.7, respectively.

Moreover, there were five other dominant bacterial genera with the relative abundance of more than 0.1%, including Polyplastron (4.84±2.93), Dasytricha (4.84±2.93), Eremopl-Diploplastron (4.84±2.93), Enoploplastron (4.84±2.93) and Isotricha (4.84±2.93).

In total, there were 6039 high-quality reads for the rumen archaea of the goats, with an average of 1510 reads per each sample. According to the information in Table 6, seven archaeal genera were identified in the rumen of goats, including three genera with a relative abundance of < 0.1%. Furthermore, almost half the archaeal community (43.71±1.57%) was composed of Methanoplasmatales, followed by Methanobrevibacter gottschalkii clade (35.79±4.84%) and Methanobrevibacter ruminantium clade (13.36±6.34%) as the second and third most frequent genera, respectively.
Finally, the fourth most dominant archaeal genus was *Methanosphaera* spp. which accounted for 7.06 ± 1.42% of the total archaeal genera in the goat rumen samples.

Among the 19 bacterial phyla detected in the present study, 12 cases constituted 98.41% of the total bacteria, which might indicate that they play a pivotal role in the rumen ecosystem by occupying special ecological niches in the rumen of goats (Figure 1). As described in the results section, 46.73% of the total bacteria in the rumen of goats included unclassified bacteria. In a similar study conducted on Creole goats on a diet of alfalfa hay or alfalfa hay with corn grain (Grilli et al. 2016), the findings suggested that regardless of the diet, unclassified genera of the phylum of *Firmicutes* and *Bacteroidetes* accounted for more than 45% of the total bacterial composition.

However, total relative abundance of important fermentative cultivable genera, such as *Butyrivibrio*, *Ruminococcus* and *Selenomonas* was estimated at 10%. It has been previously published that *S. ruminantium*, which plays a pivotal role in the reduction of nitrate to nitrite, constituted 1.85% of the total quantity of bacterial 16S rRNA genes and increased its contribution to 5.94% by a feeding strategy (Asanuma et al. 2015).

Therefore, unclassified organisms in such ranges might be responsible for several biochemical pathways and must be studied using both culture and molecular techniques on rumen samples of goat origin.

In the mentioned research (Grilli et al. 2016) and recent works (Zhang et al. 2017; Liu et al. 2017), *Prevotella* genus was observed to have a relatively higher abundance compared to the other classified bacteria (greater value reported in the goats on the alfalfa hay diet alone). These findings are in line with the information presented in Table 4. In another study, Metzler-Zebeli et al. (2013) reported that when Boer, White German Noble and Toggenburg breeds were on diets with different grain proportions, the *Prevotella* spp. and *Clostridium* cluster XIVa were the most prevalent bacterial populations in the goat rumen, while exerting no effect on the rumen bacterial compositions of the breed. In another investigation, Lee et al. (2012) reported that when fattening native Korean goats were maintained on a diet containing 90% concentrate, *Prevotella* constituted 32% of the total bacterial composition. However, an unclassified genus was observed to account for approximately 55% of the bacterial population in the mentioned study.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Relative abundance (%)</th>
<th>Number of genera</th>
<th>Relative abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteroidetes</td>
<td>45.92</td>
<td>24</td>
<td>52.34</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>27.72</td>
<td>15</td>
<td>46.07</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>12.57</td>
<td>52</td>
<td>0.93</td>
</tr>
<tr>
<td>Tenericutes</td>
<td>3.60</td>
<td>33</td>
<td>0.66</td>
</tr>
<tr>
<td>Other</td>
<td>1.37</td>
<td>124</td>
<td>1.00</td>
</tr>
</tbody>
</table>
| Figure 1: Relative abundances of bacterial phylum in the rumen of Saanen goats
In this regard, Jiao et al. (2015) examined ruminal epithelial bacterial diversity of 70-day goat kids on a diet composed of 74% concentrate and 26% forage. According to their findings, Butyrivibrio (30.13%) and Campylobacter (29.99%) were the two most abundant genera in the rumen.
In the present study, unclassified genera accounted for 27.13% of the total bacteria. In another research, Cunha et al. (2011) evaluated the rumen samples of Moxotó goats grazed on the semiarid region of Brazil, discussing that the percentage of the unclassified bacteria observed in the goat rumen was higher compared to the bovine rumen due to the specific features of the species.

Table 5: Protozoal genera composition in the rumen of Saanen goats

<table>
<thead>
<tr>
<th>Genus</th>
<th>Percent±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entodinium</td>
<td>46.78±9.13</td>
</tr>
<tr>
<td>Epidinium</td>
<td>12.37±0.06</td>
</tr>
<tr>
<td>Ophryoscolex</td>
<td>11.92±7.70</td>
</tr>
<tr>
<td>Polyplostron</td>
<td>9.27±1.10</td>
</tr>
<tr>
<td>Dasytricha</td>
<td>8.31±1.25</td>
</tr>
<tr>
<td>Eremoplostron-Diploplastron</td>
<td>4.85±1.21</td>
</tr>
<tr>
<td>Enoplostron</td>
<td>3.71±4.10</td>
</tr>
<tr>
<td>Isotricha</td>
<td>2.67±1.01</td>
</tr>
<tr>
<td>Metadinium</td>
<td>0.06±0.04</td>
</tr>
<tr>
<td>Eudiplodinium</td>
<td>0.04±0.02</td>
</tr>
<tr>
<td>Anoplodinium-Diploplini</td>
<td>0.02±0.02</td>
</tr>
<tr>
<td>Ostracodinium</td>
<td>0.02±0.02</td>
</tr>
</tbody>
</table>

Analysis of the goat rumen microbiome in the current study revealed a diverse group of bacteria, several of which had not been previously identified by conventional culturing methods. The considerably high percentage of unclassified bacterial genera highlights our knowledge gap regarding the composition of goat rumen microbiota, as well as the lack of skills in the cultivation of bacterial genera, which have been detected only by culture independent methods so far.

Findings of the present study indicated that the bacterial community of goats fed high forage diet was dominated by the Firmicutes and Bacteroidetes phyla, which is in congruence with the results obtained by many workers (Cunha et al. 2011; Huo et al. 2014; Liu et al. 2015; Mao et al. 2016; Zhang et al. 2017; Liu et al. 2017), while inconsistent with the study conducted by Wetzel et al. (2017), in which Proteobacteria was reported to be the dominant phylum, appearing in 50% and 45% of the epimural microbiome of the goats on hay and hay with 30% concentrate diets, respectively.

In a recent global study, Henderson et al. (2015) collected and examined 742 samples from 32 species or subspecies of ruminants and other foregut fermenters in 35 countries and seven global regions, identifying 12 genus-equivalent protozoal groups, including Anoplodinium-Diploplini, Enoploplastron, Entodinium, Epidinium, Eremoplostron-Diploplastron, Eudiplodinium, Metadinium, Ophryoscolex, Ostracodinium, Polyplostron, Dasytricha and Isotricha.

However, the genera Entodinium, which is the smallest protozoa in the rumen, was found to be responsible for most of the bacterial protein turnover (Newbold et al. 2015), while Epidinium, a larger protozoa with great endoglucanase and xylanase activities (Newbold et al. 2015), dominated 90% of the samples.

According to the information in Table 5, only eight protozoal genera in the rumen samples of the Sannen goats had higher relative abundance than 1%. In this regard, Gürelli et al. (2016) assessed the rumen protozoal community of domestic goats on a diet of steppe shrubs in Kyrgyzstan, reporting the eight genera with genus Entodinium as the predominant protozoal genus in the rumen samples. Obtained results of the present study are in line with the aforementioned findings regarding the major ciliates living in the rumen. The Epidinium (12.37±0.06%) and Ophryoscolex genus (11.92±7.70%) were determined as the second and third most dominant protozoa in the rumen of Sannen goats in the current research. Ophryoscolex genus ferments starch with the production of acetic, butyric, and lactic acids, in addition to CO2 and H2.

Moreover, it utilizes protein sources and amino acids by producing ammonia as an end-product of nitrogenous metabolism (Williams et al. 1961). The fourth most dominant protozoal genus in the goat rumen in our research was Polyplostron, which is a large cellulolytic protozoa with higher endoglucanase and xylanase activities than the Entodinium genus. Based on their inherent characteristic to produce methane during energy metabolism, methanogens in rumen are classified into seven categories, as follows: Methanococcales, Methanopyrales, Methanobacteriales, Methanosarcinales, Methanomicrobiales, Thermoplasmatales and Methanocellales (Borrel et al. 2013). Methanogenesis is performed from H2 and CO2 by the majority of the cultured Methanococcales, Methanopyrales, Methanobacteriales, Methanomicrobiales and Methanocellales (Borrel et al. 2013).
Analysis of the global data set in this regard shows that with an average relative abundance of 61.6%, Methanobrevibacter (order Methanobacteriales) is the major genus of the rumen archaea (Janssen and Kirs, 2008).

According to the results of the present study, the three Methanobrevibacter spp. accounted for 49.2% of the total methanogens detected in the rumen samples of Sannen goats.

Similarly, Cheng et al. (2009) applied denaturing gradient gel electrophoretic (DGGE) and observed that in the rumen of goats on a diet of forage and concentrate at ratios of 100, 70:30, 50:50 and 30:70, Methanobrevibacter spp. was the most dominant methanogenic archaea.

In a previous report by Lin et al. (1997), Methanobacteriaceae was the predominant methanogen in the rumen of goats on a diet of 100% hay. Furthermore, evaluation of the ruminal archaeal sequence libraries in the Moxotó goats grazed on the semiarid region of Brazil showed similar results in terms of the liquid and solid associated fractions, reporting the higher dominance of the sequences belonging to the phylum Euryarchaeota, while the majority of the sequences were related to the genus Methanobrevibacter, accounting for 69.7% of the sequences in the rumen liquid fraction and 86.7% of the sequences in the solid-associated fraction (Cunha et al. 2011).

In this regard, Lee et al. (2012) observed that Euryarchaeota was the predominant phylum at the phylum level in the rumen of native Korean goats on a high-concentrate diet. However, all the archaeal sequences were categorized as unclassified genera.

In a longitudinal study (Wang et al. 2016) conducted on Chinese cross-bred goats aged seven days-two years, it was reported that irrespective of age, Euryarchaeota and Thaumarchaeota were the most dominant phyla, constituting 82% and 15% of the archaea, respectively. It is noteworthy that the for over 90 days, the goats were on a diet of 70% alfalfa hay and rice straw at similar proportions, as well as 30% concentrate mixture.

As described in the Results section, Methanoplasmatales was the second most dominant methanogen group in the current study. It is known as Thermoplasmatales or "rumen cluster C" (Paul et al. 2012), that is implicated in methane emissions of the rumen, possibly from methylamines (Poulsen et al. 2013) and has been reported as a major methanogen in the rumen of Australian sheep, as well as the small ruminants of Tibetan Plateau (Huang et al. 2016). Observed abundance of Methanobrevibacter gottschalkii clade within the Methanobacteriaceae family was similarly reported in the previous studies in this regard (Huang et al. 2016; Lin et al. 2015; Henderson et al. 2015).

It should be noted that the current study describes the microbial community of the goats fed on a specific diet in a particular geographical region and climate. Under such circumstances, differences with the published sources are expected. On the contrary, similarities in this regard are noteworthy, suggesting that geographical diversity has not been able to significantly influence the core of the microbial population in this species.

**CONCLUSION**

In the present study, using the 454 titanium pyrosequencing technique, relative abundance of 124 bacterial genera were found in the rumen of Saanen goats, which indicates the advantage of this method in drawing a picture of all the bacteria living in the rumen of animals on the mentioned feeding regimen. According to the results of this study, observing 46.73% of the bacterial genera, as a relatively large proportion of unclassified bacteria found in the goat rumen, could not be identified with the current knowledge from traditional culture-based techniques. Therefore, further attempts must be made to detect the remarkable unclassified bacteria using culture-based methods. Moreover it can be concluded that in comparison with protozoa and methanogens, rumen bacteria have more dark corners to be studied particularly using rumen sample from goat origin.

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