



Study of rumen metagenome community using qPCR under different diets



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ABSTRACT

The aim of this study was to detect the major bacteria present in rumen microbiota. Here, we performed qPCR based absolute quantitation of selected rumen microbes in rumen fluid of river buffalo adapted to varying proportion of concentrate to roughage diets. Animals were adapted to roughage-to-concentrate ratio in the proportion of 100:00 (T1), 75:25 (T2), 50:50 (T3) and 25:75 (T4) respectively for 30 days. At the end of each treatment, rumen fluid was collected at 0 h and 2 h after feeding. It was found that among fibrolytic bacteria *Ruminococcus flavefaciens* (2.22×10^8 copies/ml) were highest in T2 group and followed by 1.11×10^8 copies/ml for *Fibrobacter succinogenes* (T2), 2.56×10^7 copies/ml for *Prevotella ruminicola* (T1) and 1.25×10^7 copies/ml for *Ruminococcus albus* (T4). In non-fibrolytic bacteria, the *Selenomonas ruminantium* (2.62×10^7 copies/ml) was predominant in group T3 and followed by *Treponema bryantii* (2.52×10^7 copies/ml) in group T1, *Ruminobacter amylophilus* (1.31×10^7 copies/ml) in group T1 and *Anaerovibrio lipolytica* (2.58×10^6 copies/ml) in group T4. It is most notable that *R. flavefaciens* were the highest in population in the rumen of Surti buffalo fed wheat straw as roughage source.

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Introduction

Rumen is a complex habitat in which feedstuffs are fermented primarily to a mixture of volatile fatty acids (VFAs, predominantly acetic, propionic, and butyric acids) that serve as the major nutrient source for the ruminant animal. Much of our knowledge of the ruminal metabolism of these feedstuffs was gained through in vitro study of bacterial species, most of which were isolated in the early decades of rumen microbiology (Hungate, 1996; Krause and Russell, 1996). The microbiome of the rumen is responsible for the breakdown of plant fiber which commonly occurs. The population of microbiota is highly responsive to changes in diet, age and the health of the host animal, which varies according to geographical location, season, and feeding regimen (Bryant, 1959; Hungate, 1996). Fibrolytic bacteria, protozoa, and fungi degrade fibrous material, allowing animals to utilize plant fiber for nutrition. Bacteria are the most numerous of these microorganisms and play a major role in the biological degradation of carbohydrates. *Fibrobacter succinogenes*, *Ruminococcus albus*, and *Ruminococcus flavefaciens* are presently recognized as the major cellulolytic bacterial and non-fibrolytic bacterial (*Selenomonas ruminantium*, *Succinivibrio dextrinosolvens* and *Treponema bryantii*) species found in the rumen (Forster et al., 1997; Koike et al., 2007; Shinkai and Kobayashi, 2007; Wanapat and Cherdthong, 2009). Techniques of molecular microbial ecology provide an opportunity to quantify these ruminal species with great sensitivity and precision, and several recent reports have provided a sense of which species are most abundant in the rumen under particular feeding conditions (Klieve et al., 2003; Kobayashi, 2006; Tajima et al., 2001a). Moreover, DNA-based methods offer the option of storing samples until their analysis, which could be an important advantage in field conditions (Castillo et al., 2006). The recent development of real-time polymerase chain reaction (PCR) has been successfully used for quantifying cellulolytic bacterial species (McSweeney and Denman, 2007; Tajima et al., 2001b). Real-time PCR is an approach that allows continuous monitoring of PCR product formation, and techniques vary according to the method of fluorescence generation. Real-time PCR has the ability to enumerate targeted bacteria with high sensitivity (Zimmermann and Mannhalter, 1996) and has been used to analyze various environmental samples, such as water (Layton et al., 2006) and rumen digesta (Reilly and Attwood, 1998). This technique is both reliable and simple to perform. Increased knowledge concerning the fibrolytic and non-fibrolytic bacterial population will allow insight into the fiber-digestion capabilities of ruminant animals. However, very limited research has been conducted on Indian buffalo (*Bubalus bubalis*) with regard to the ruminal bacterial population using molecular techniques. Therefore, this study was conducted to determine the ruminal bacterial (fibrolytic and non fibrolytic) population of rumen fluid in Surti buffalo fed roughage: concentrate diet" by real-time PCR techniques.

Materials and methods

Animal's diet and sample collection

The experiment was carried out on eight 2.5 year old female Surti buffaloes randomly assigned to four groups (2 in each group) reared at the Department of Animal Nutrition, College of Veterinary Science and A.H., Anand. The permission of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) was obtained prior to initiation of the study. The animals received four different total mixed rations (TMRs) of roughage to concentrate (R:C) of 100:0 (T1), 75:25 (T2), 50:50 (T3), and 25:75 (T4) in individual feeding stall. All animals received feed according to the respective R:C ratios ad lib daily, and wheat straw was used as roughage. Concentrates are high-quality, low-fiber feeds that contain a high concentration of digestible energy per unit weight and volume. Under this study, the concentrate diet consisted of 20.11% crude protein, 10.28% crude fiber, 3.8% ether extract, 52.43% nitrogen free extract, 13.38% ash, 3.38% silica, 1% phosphorus and 1.22% calcium. All animals were given free access for 2 h in the morning and evening, during which they had free access to drinking water. The feeding experiment was conducted for a period of 30 days. Sample rumen fluid was collected at 0 h and 2 h after feeding by a suction pump using a flexible stomach tube as described earlier by Khampa et al. (2006). About 100 ml rumen fluid was passed through four layers of cheese cloth to remove particulate matter. Remaining rumen fluid was stored at -80°C for further study.

DNA extraction and PCR amplification of 16S RNA genes

Total genomic DNA was isolated from rumen fluid from four groups separately by using Qiagen stool kit as per manufacturer instruction. The DNA quality and quantity were checked by 0.8% (wt/v) agarose gel electrophoresis and NanoDrop spectrophotometer (ND 1000, NanoDrop Technologies, Inc., Wilmington, DE, USA) at 260 nm.

Species specific PCR primers used for the amplification of target region of the 16S rRNA (target DNA) were chosen from the literatures (Table 1). The target DNA of all fibrolytic and non-fibrolytic bacteria were amplified from the metagenomic DNA with respective primer sets, as described previously (Muyzer et al., 1993; Tajima et al., 2001a). All quantification Real-time PCR amplification and detection were performed using ABI 7500 system software (ABI 7500, USA). The reaction was conducted in a final volume of 25 µl containing the following: 12.5 µl Qiagen DNA Master SYBR Green I, 10 pM as a forward primer, 10 pM as a reverse primer, 7.5 µl distilled water, and 2.0 µl of DNA solution of unknown concentration. The annealing temperature and amplicon size of each target are shown in Table 1. Amplicon specificity was judged based on dissociation curve of PCR end products by increasing the temperature at a rate of 1 °C every 30 s from 60 to 95 °C. All PCRs were performed in duplicate. Before starting the real time PCR assay, conventional PCRs for the validation of the specificity of the primers against target genes were performed in 25 µl using a minicycler. The PCR products were analyzed by running on 1.5% agarose gels containing ethidium bromide and visualizing for a single specific band and the absence of primer dimmer products.

Preparation of standard plasmid for real-time PCR assays

Plasmid DNA containing the respective target gene sequence, used as the standard DNA in real-time PCR, was obtained by PCR cloning using the species-specific primer sets. After the confirmation of a specific amplification of the correct size (Table 1) on an agarose gel, the PCR products were excised from the gel. The PCR products were purified using the Qiagen gel purification kit (Qiagen, CA), and ligated to PTZ571/R vector (Fermentas, UK). The ligation products were transformed to competent *Escherichia coli*

Table 1
Primers for real time PCR assay and PCR conditions.

Target	Primer sequence (5'–3')	Annealing temp. (°C)	Product size (bp)	Reference
Total bacteria	P1 – CCTACGGGAGGCAGCAG P2 – ATTACCGCGTCTGCTGG	60	194	(Muyzer et al., 1993; Mosoni et al., 2007)
<i>Fibrolytic bacteria</i>				
<i>Fibrobacter succinogenes</i>	P1 – GGTATGGGATGAGCTTGC P2 – GCCTGCCCTGAACTATC	60	446	(Tajima et al., 2001a; Wanapat and Cherdthong, 2009)
<i>Ruminococcus albus</i>	P1 – CCCTAAAAGCAGTCTTAGTTCG P2 – CCTCCTTGCGTTAGAACA	60	175	(Koike and Kobayashi, 2001; Khampa et al., 2006)
<i>Ruminococcus flavefaciens</i>	P1 – GGACGATAATGACGGTACTT P2 – GCAATC(CT)GAACTGGGACAAT	55	295	(Koike and Kobayashi, 2001; Khampa et al., 2006)
<i>Prevotella bryantii</i>	P1 – AGTCGAGCGGTAAGATTG P2 – CAAAGCGTTTCTCTCACT	68	540	(Tajima et al., 2001a; Wanapat and Cherdthong, 2009)
<i>Prevotella ruminicola</i>	P1 – GGTATCTTGAGTGAGTT' P2 – CTGATGGCACTAAAGAA	53	485	(Tajima et al., 2001a; Wanapat and Cherdthong, 2009)
<i>Non-fibrolytic bacteria</i>				
<i>Anaerovibrio lipolytica</i>	P1 – TGGGTGTITAGAAATGGATT P2 – CTCTCTGCACTCAAGAATT	57	597	Tajima et al., 2001a; Wanapat and Cherdthong, 2009)
<i>Ruminobacter amylophilus</i>	P1 – CAACCAGTCGCATTGACAG P2 – CACTACTCATGGCAACAT	57	642	(Tajima et al., 2001a; Wanapat and Cherdthong, 2009)
<i>Selenomonas ruminantium</i>	P1 – TGCTAATACCGAATGTTG P2 – TCCTGCACTCAAGAAAGA	57	513	(Tajima et al., 2001a; Wanapat and Cherdthong, 2009)
<i>Treponema bryantii</i>	P1 – ACTGCAGCGGCACTGTGAGA P2 – ACCTTACGGTGGCAGTGTCTC	57	412	(Tajima et al., 2001a; Wanapat and Cherdthong, 2009)

DH5alpha cells by heat shock. Plasmids were purified from the transformed *E. coli* using a QIAprep spin miniprep kit (Qiagen, Valencia, CA, USA), and the plasmids containing the correct insert were screened out by PCR amplification with respective primer sets. The concentration of the plasmid was determined with a NanoDrop spectrometer. Copy number of each standard plasmid was calculated using the formula; Copy No/ μ l = Concentration of plasmids (g/ μ l) \times 6.022 \times 10²³/length of recombinant plasmid (bp) \times 660, (660 = Molecular weight of nucleotide base, 6.022 \times 10²³ = Avogadro's number). Ten-fold dilution series ranging from 10 to 10⁹ copies were prepared for each target. Real-time PCR was performed with ABI system (ABI7500) using Qiagen DNA Master SYBR Green I. The optimal annealing temperature for each primer set is shown in Table 1. The 10-fold dilution series of the standard plasmid for the respective target was run along with the samples. Amplification of each sample was performed in duplicate. The copy numbers of 16S rRNA genes of all targeted per ml rumen fluid were calculated using the following equation: (QM \times C \times DV)/(S \times V), where QM was the quantitative mean of the copy number, C was the DNA concentration of each sample, DV was the dilution volume of extracted DNA, S was the DNA amount (ng) subjected to analysis and V was the rumen fluid volume subjected to DNA extraction. In the reaction, nearly perfect linear regressions ($r^2 = 0.99$ and slope $(-3.2$ to $-4)$) were obtained between threshold cycle and quantities of standard for all targets and data generated from the reaction were used for further analysis. Statistical analysis of data was performed by using software of the SPSS package (1993). The differences were determined by the method of least significant differences at the 5% level ($p < 0.05$) of data in rumen fluid at 0 h and 2 h after feeding.

Results

Real-time PCR-based quantification of representative rumen bacteria was reported in a few studies (Muyzer et al., 1993; Tajima et al., 2001a). In the present study we assess the rumen bacterial species (fibrolytic and non-fibrolytic) using real-time PCR assays. Qualitative PCR detection of fibrolytic bacteria and non-fibrolytic bacteria is shown in Fig. 1.

Figs. 2 and 3 show the population sizes of the target fibrolytic and non-fibrolytic bacteria in the rumen fluid of Surti buffalo in their responses to ratio of dietary change, as enumerated by the real-time PCR assays. Total bacteria (averaged over 4 diets) were detected to be 2.16×10^{10} copies per ml ruminal fluid (Fig. 2). Among the fibrolytic bacteria, *R. flavefaciens* was the most abundantly detected (2.22×10^8 copies/ml of ruminal fluid) (in T2) followed by, 1.11×10^8 copies/ml for *F. succinogenes* (in T2), 2.56×10^7 copies/ml for *Prevotella ruminicola* (in T1), 1.25×10^7 copies/ml for *R. albus* (in T4) and 2.56×10^4 copies/ml for *Prevotella bryantii* (in T4). In non-fibrolytic bacteria, the maximum population was observed in *S. ruminantium*, 2.62×10^7 copies/ml (in T3), followed by 2.53×10^7 copies/ml for *T. bryantii* (in T1), 1.31×10^7 copies/ml for *Ruminobacter amylophilus* (in T1) and 2.58×10^6 copies/ml for *Anaerovibrio lipolytica* (in T4). The population of

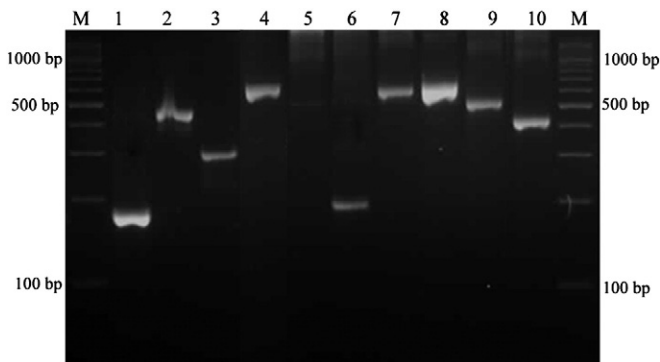


Fig. 1. Qualitative PCR detection of fibrolytic bacteria and non-fibrolytic bacteria. Lane: 1, *Ruminococcus albus*; 2, *Fibrobacter succinogenes*; 3, *Ruminococcus flavefaciens*; 4, *Prevotella bryantii*; 5, *Prevotella ruminicola*; 6, total bacteria; 7, *Ruminobacter amylophilus*; 8, *Anaerovibrio lipolytica*; 9, *Selenomonas ruminantium*; 10, *Treponema bryantii*; lane M, DNA size marker.

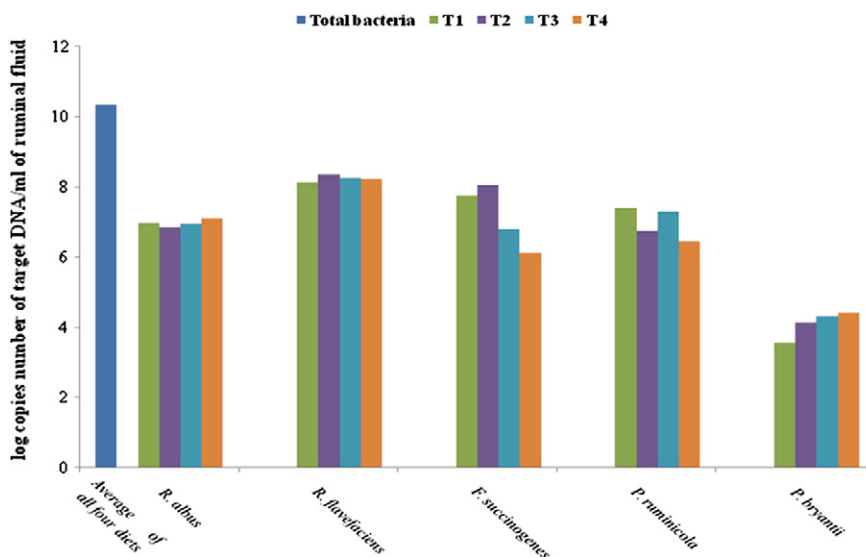


Fig. 2. Population of the fibrolytic bacterial species of Surti buffalo fed different roughage (wheat straw)-to-concentrate ratios while values were averaged from samples taken at 0 and 2 h after feeding.

P. ruminicola in T1, *P. bryantii* in T4, *F. succinogenes* in T2, *R. amylophilus* in T1, *S. ruminantium* in T3 and *A. lipolytica* in T4 group was higher among treatment groups. However, the *R. albus* and *F. succinogenes* were not significantly different between all treatment groups. The distributions of the bacteria at 0 h and 2 h are given in Tables 2 and 3.

Discussion

To the best of our knowledge, no previous study has been reported to the diet dependent changes of population size of rumen microbes of Indian buffalo. Our observations indicate that large numbers of bacteria were present in the ruminal fluid, because each bacterial species has a different copy number of 16S rRNA gene (Ozutsumi et al., 2006).

The results showed that the rumen of Surti buffalo harbored *R. flavefaciens* and *F. succinogenes* at high levels (*R. flavefaciens* > *F. succinogenes*), with both species outnumbering *R. albus*. Previous studies performed with other molecular approaches targeting either RNA (16S rRNA-targeting oligonucleotide probes) or DNA (competitive PCR) also showing that *R. albus* is the least abundant species among the three fibrolytic species in the rumen of sheep (Chaucheyras et al., 1997; Klappenbach et al., 2001; Koike and Kobayashi, 2001; Koike et al., 2003; Michalet-Doreau et al., 2002). However, the data presented here differ from those reported by Koike and Kobayashi (Chaucheyras et al., 1997), who found *F. succinogenes* to be the most abundant species in the rumen of sheep fed with alfalfa hay and concentrate. Wanapat and Cherdthong (2009) also observed that the *F. succinogenes* was most dominant in digesta and rumen fluid of swamp buffalo fed roughage and concentrate as estimated by real time PCR assay. This might be due to the primer bias which may be attributed to various host and environmental factors (diet, age and health of animal, geographical location and season) (Mosoni et al., 2007) and to the technique used (primers, real time PCR versus competitive PCR). For instance, the estimation of the number of the three cellulolytic species is much higher than that reported by competitive PCR (Chaucheyras et al., 1997), whereas it is of the same order of magnitude with reported data obtained from cattle by real-time PCR (Muyzer et al., 1993).

The dynamics of fibrolytic bacteria were in good correlation with the response to diet shift, particularly the changes of concentrate (Koike et al., 2003). In this study, changes in the feeding of wheat straw from 75% to 25% slightly decreased the population of these three cellulolytic bacterial numbers from 2.22×10^8

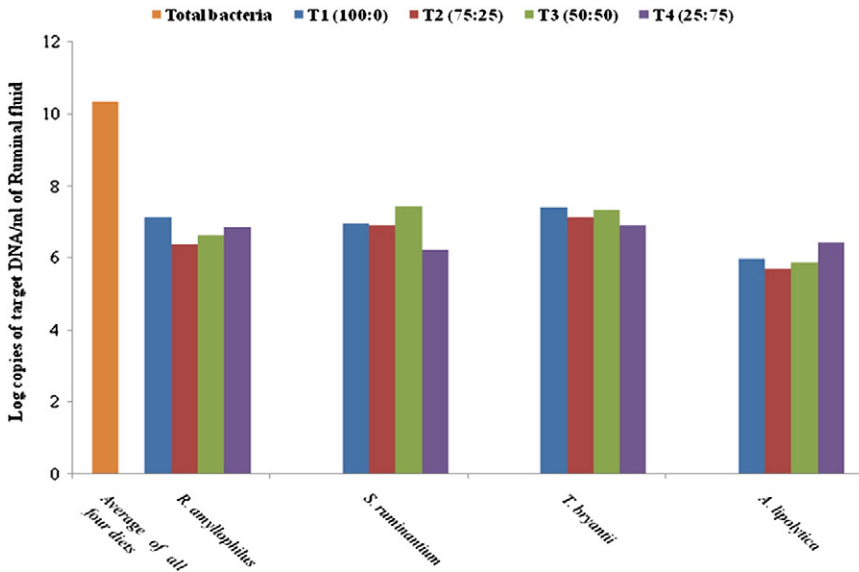


Fig. 3. Population of the non-fibrolytic bacterial species of Surti buffalo fed different roughage (wheat straw)-to-concentrate ratios while values were averaged from samples taken at 0 and 2 h after feeding.

to 1.70×10^8 copies/ml for *R. flavefaciens*, 1.11×10^8 to 1.33×10^6 copies/ml for *F. succinogenes* and 2.56×10^8 to 2.76×10^6 copies/ml for *P. ruminicola* in rumen fluid (Fig. 2) respectively. On the other hand, the population size of *R. albus* and *P. bryantii* increased from 9.42×10^6 to 1.25×10^7 and 3.5×10^3 to 2.5×10^5 respectively in response to this diet change. The proportion of roughage in the diet might influence the population size or the proportion of fibrolytic bacteria in the rumen. In addition, all the fibrolytic bacterial numbers examined in the present study were significantly different, responding to a change in proportion of wheat straw and concentrate. As the results show, the lowest numbers of the two fibrolytic bacteria (*R. albus* and *P. bryantii*) were found when increasing the level of concentrate. It is possible that dietary conditions might have influenced on reduced numbers of cellulolytic bacteria. Moreover, rumen pH together with microbial population, nature of substrates, environmental factors such as temperature, and the existence of cations and soluble carbohydrates have been suggested as factors governing bacterial attachment (Stewart and Bryant, 1998). Ruminal pH is one of the most important factors, because the fibrolytic bacteria are very sensitive to the pH change (Miron et al., 2001). When ruminants are fed fiber-deficient rations, ruminal pH declines, microbial ecology is altered, and the animals become more susceptible to metabolic disorders (Sung et al., 2007). Fibrolytic bacterial population has been shown to be decreasing on increasing concentrate proportion. As Koike et al. (2007) and Michalet-Doreau et al. (2002) quantified the cell numbers of *F. succinogenes*, *R. flavefaciens*, and *R. albus*, attached to straw and they were analyzed by competitive PCR showing that the numbers of all the three species increased gradually with increased neutral-detergent fiber disappearance. On the other hand, (Wora-anu et al., 2000; Russell and

Table 2

Comparative quantity of fibrolytic bacterial DNA from rumen fluid and sampling hours using real-time PCR techniques.

Sampling hours	Species (copies/ml ^a)				
	<i>Ruminococcus albus</i>	<i>Ruminococcus flavefaciens</i>	<i>Fibrobacter succinogenes</i>	<i>Prevotella ruminicola</i>	<i>Prevotella bryantii</i>
0 h	1.00×10^7	1.78×10^8	4.53×10^7	1.34×10^7	1.52×10^4
2 h	9.30×10^6	1.76×10^8	4.18×10^7	1.34×10^7	1.67×10^4
p-Value	0.91	0.94	0.89	1.00	0.79

^a The values were averaged on all the four diets.

Table 3

Comparative quantity of non-fibrolytic bacterial DNA from rumen fluid and sampling hours using real-time PCR techniques.

Sampling hours	Species (copies/ml ^a)			
	<i>Ruminobacter amylophilus</i>	<i>Selenomonas ruminantium</i>	<i>Treponema bryantii</i>	<i>Anaerovibrio lipolytica</i>
0 h	2.03×10^7	7.54×10^6	1.67×10^7	1.19×10^6
2 h	9.05×10^6	1.47×10^7	1.67×10^7	1.19×10^6
p-Value	0.21	0.14	1.00	1.00

^a The values were averaged on all the four diets.

Rychlik, 2001) reported that roughage- to-concentrate ratios of 100:0, 60:40, and 40:60 could decrease the cellulolytic bacterial population in swamp buffalo (5.62×10^{10} , 4.06×10^{10} , and 4.57×10^{10} CFU/ml, respectively). In addition, Tajima et al. (2001a) reported that the quantity of *F. succinogenes* DNA in animals predominantly on the hay diet fell 20-fold on the third day of the switch to a grain diet and further declined on day 28, with a 57-fold reduction in *F. succinogenes* DNA. The *R. flavefaciens* DNA concentration on day 3 declined to 10% of its initial value in animals on the hay diet and remained at this level on day 28. Therefore, in this experiment, the quantification of bacterial DNA demonstrated the decrease of the fibrolytic bacterial numbers (*R. flavefaciens* and *F. succinogenes*) as being influenced by higher concentrate feeds.

Non-fibrolytic bacteria such as *S. ruminantium* and *T. bryantii* were detected in the fiber-associated community, using comparative 16S rRNA gene analysis (Michalet-Doreau et al., 2002). The present study quantitatively confirmed the attachment of nonfibrolytic bacteria to roughage. Current understanding suggests that, they attached to the fiber at a relatively low level to that of fibrolytic bacteria (Fig. 3). In particular, *S. ruminantium* (2.62×10^7 copies/ml in T3), *T. bryantii* (2.53×10^7 copies/ml in T1) and *R. amylophilus* (1.31×10^7 copies/ml in T1) had the highest proportion among the non-fibrolytic bacterial species assessed in the present study, while the proportion of *A. lipolytica* was 2.56×10^6 copies/ml in T4 group (75% concentrate). This finding suggests the fiber-attachment ability of *S. ruminantium*. In such a relationship, fibrolytic bacteria provide the hydrolyzed product from cellulose to non-fibrolytic bacteria, while non-fibrolytic bacteria indirectly facilitate fiber degradation by preventing the accumulation of bacterial metabolites such as succinate and cellodextrins (Cheng et al., 1980).

The distribution of the fibrolytic and non-fibrolytic bacterial species in rumen fluid of the Surti buffalo is shown in Tables 2 and 3. As found among the fibrolytic bacteria in the rumen, specially two cellulolytic bacterial populations, *R. flavefaciens* and *F. succinogenes* were higher to be 1.78×10^8 and 4.53×10^7 copies/ml at 0 h than 2 h after feeding (Table 2) and accounting for 0.82% and 0.20% of total bacteria respectively (Table 4). Similarly, in non-fibrolytic bacteria, the distribution of population size of *R. amylophilus* was higher to be 2.03×10^7 copies/ml at 0 h than 2 h after feeding, while the population of *S. ruminantium* (1.47×10^7 copies/ml) was higher at 2 h than 0 h (Table 3). Indeed, the distribution of bacterial population was not significantly different between sampling hours, although the high distribution of the

Table 4

Proportions of fibrolytic and non-fibrolytic bacteria of total bacteria to ruminal fluid of Surti buffalo at 0 h.

	% Total bacteria ^a
<i>Fibrolytic bacteria^a</i>	
<i>R. albus</i>	0.045
<i>R. flavefaciens</i>	0.82
<i>F. succinogenes</i>	0.20
<i>P. ruminicola</i>	0.062
<i>P. bryantii</i>	00
<i>Non-fibrolytic bacteria^a</i>	
<i>Selenomonas ruminantium</i>	0.051
<i>Ruminobacter amylophilus</i>	0.068
<i>Treponema bryantii</i>	0.077
<i>Anaerovibrio lipolytica</i>	0.0055

^a The values were averaged on all the four diets.

fibrolytic bacteria in rumen fluid is reasonably explained by the fact that the fluid mainly consisted of plant fiber particles that were likely to have been colonized by the fibrolytic bacteria. In a study by Koike et al. (2003), the authors suggested that the increase in attached cell numbers observed could be mostly attributed to cell proliferation on the straw, at 6 h, the numbers of attached cells of the three species gradually increased and peaked at 24 h (10^9 /g dry matter (DM) for *F. succinogenes* and 10^7 /g DM for *R. flavefaciens*) or 48 h (10^6 per gram DM for *R. albus*). There are two possible explanations of the increased cell populations on the 6 h post feeding, cell proliferation after feeding and the additional attachment of new bacteria from the liquid phase or other particles. Similarly, Hungate (1996) reported that fibrolytic bacteria were more abundant in the whole digesta of rumen, presumably because many attached to the solids particles.

Although real-time PCR quantifies both viable and non-viable cells, this technique has the advantage of targeting true cellulolytic species and being a very sensitive, accurate and reproducible method, enables underlining of slight changes and allows the differentiation between the population sizes of the key cellulolytic bacteria. However, quantification of cellulolytic species numbers does not reflect the real activity of these species in the rumen. Indeed, linking community structure to activity and functionality is a central but still poorly studied issue in microbial ecology. Quantifying 16S transcripts would provide additional information as it would better reflect total bacterial activity. However, fibrolytic activity can be measured by the quantification of glycoside hydrolase transcripts. This is particularly difficult with fibrolytic species (cellulolytic), they each carry numerous genes involved in the fibrolytic function, whose relative importance is not known (Qi et al., 2005). But recently Brulc et al. (2009) observed that the glycoside hydrolase is the key enzyme in fiber-adherent bovine microbiome through pyrosequencing (Gene-centric Metagenomics).

Conclusion

In conclusion, two major representative groups of ruminal microbes covering fibrolytic and non-fibrolytic bacterial communities were demonstrated. Result revealed that *R. flavefaciens* and *F. succinogenes* were the most dominant fibrolytic among all detected fibrolytic species, which may form a multi-enzyme cellulosome complex that could play an integral role in the ability of this bacterium to degrade plant cell wall polysaccharides. Non-fibrolytic bacteria, *S. ruminantium* and *T. bryantii* were also detected with abundance in rumen fluid. The high magnitude of non-fibrolytic group on the roughage suggests the development of mutual relationships between fibrolytic and non-fibrolytic bacterial communities.

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