Distinctive roles between rumen epimural and content bacterial communities on beef cattle feed efficiency: A combined analysis

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ARTICLE INFO

ABSTRACT

Rumen content-associated (RC) and epithelial tissue-attached (RT) bacterial communities are composed of different phylotypes and play distinctive roles. This study aimed to compare the composition of the RT and RC bacterial communities of steers differing in feed efficiency. The microbiota of RT and RC samples collected from sixteen beef steers with high or low residual feed intake (RFI) were analyzed through sequencing of partial 16S rRNA gene amplicons. *Bacteroidetes, Proteobacteria* and *Firmicutes* were the predominant phyla and *Prevotella* was the most abundant genus in both RC and RT bacterial communities. In total, 19 OTUs of the RC samples and 19 OTUs of the RT samples were differentially abundant (DA) between H-RFI and L-RFI steers. Among them, a common DA OTU belonged to *Prevotella* genus was identified in both RC and RT samples, making it the potential key microbial marker for indicating feed efficiency of steers. The co-occurrence of the DA OTUs among RT and RC samples suggest the importance of these two communities function as a complete system in influencing host feed efficiency.

1. Introduction

The bovine rumen consists of numerous symbiotic microorganisms including bacteria, archaea, protozoa and fungi with the bacteria being the most abundant and playing a crucial role in decomposing feed into absorbable nutrients and supplying the host with energy (Stewart et al., 1997). Rumen bacteria are usually divided into three distinct groups based on their habitats in the rumen: bacteria in the rumen fluid, bacteria associated with feed particles, and bacteria attached to the rumen epithelial wall (also defined as the epimural bacteria) (McAllister et al., 1994). Previous studies have mostly focused on exploring the ecology of fluid/content associated bacteria, and have reported that they are mainly responsible for the primary fiber digestion of the ingested feed within the rumen. Although the epimural bacterial community only constitutes 1–2% of the total rumen bacterial population, it has a distinct functional capacity from other ruminal microbial communities (Chen et al., 2011; Sadet-Bourgeteau et al., 2007, 2010) such as taking part in oxygen scavenging, tissue recycling, urea metabolism, and nutrient and energy absorption (Cheng et al., 1979; Mccowan et al., 1978). The epimural microbiota can also affect the overall ruminal microbial population as they interact with other ruminal microbial communities and

maintain rumen homeostasis (Chen et al., 2011; Petri et al., 2013). Recent evidence has shown that epimural bacteria community is diverse (Chen et al., 2011; Li et al., 2012), and similar to fluid/content associated bacteria diet can affect its diversity and it varies among individuals when they were fed the same diet (Li et al., 2012). Currently, the taxonomic composition and functional capacity of the epimural bacterial community as well as their relationship with rumen function in beef cattle have not been well defined.

In livestock production improving feed efficiency has been one of the key research areas for decades, aiming to enhance animal production while lowering feed costs. Residual feed intake (RFI), which is defined as the difference between animal's actual feed intake and the expected feed requirements for maintenance and growth, has been introduced into the beef industry as an effective measure for feed efficiency (Arthur and Herd, 2008; Nkrumah et al., 2006). Cattle with high RFI (H-RFI) are inefficient (require more feed than expected) while those with low RFI (L-RFI) are efficient (require less feed than expected) for projected body weight (Archer et al., 1999). Because rumen fluid/content-associated microbiota participate in feed fermentation, supplying the host with nutrient substrates and energy, it was proposed that they could be one of the biological factors that are attributed to host feed efficiency. Indeed,

bacterial and archaeal populations in the rumen contents (RC) were found to differ between L-RFI and H-RFI beef cattle (Hernandez-Sanabria et al., 2010; Zhou et al., 2010). Epimural bacteria, however, have not been examined in this context. We hypothesized that rumen epimural bacteria also display distinctive structure between L-RFI and H-RFI steers, and examining both RC and rumen tissue (RT) associated bacteria from the same animal may provide a more complete view of how the microbiota relates to host feed efficiency.

2. Materials and methods

2.1. Animals and sample collection

Rumen content and epithelial tissue samples were collected as part of two animal studies (Hernandez-Sanabria et al., 2010; Li et al., 2012). Briefly, animal feed intake was monitored using GrowSafe system (GrowSafe Systems Ltd., Airdrie, AB) and RFI was calculated based on the difference between the actual feed intake and the estimated feed intake as described in Hernandez-Sanabria et al. (2010) in both two studies. The raw RFI was then adjusted with backfat, and ranked according to the values. For both studies, the adjusted RFI values ≥ 0.5 were classified as H-RFI, $-0.5 < \text{RFI} < 0.5$ were classified as M-RFI, and the adjusted RFI values ≤ -0.5 were classified as L-RFI. In study one, nine animals with extreme RFI (L-RFI, lowest RFI values, $n = 4$; H-RFI, highest RFI values, $n = 5$) were selected from fifty-eight 10-month-old Hereford × Aberdeen Angus (HEAN) steers with divergent RFI phenotypes (L-RFI, $n = 20$; M-RFI, $n = 16$; H-RFI, $n = 22$) that were used in a previous study (Hernandez-Sanabria et al., 2010). Steers were fed a finishing diet consisting of 74% oats, 20% hay, and 6% feedlot supplement (32% crude protein, beef supplement containing 400 mg/kg of body weight of Rumensin, and 1.5% canola oil) and managed at the Roy Berg Kinsella Research Ranch at University of Alberta (Kinsella, Alberta, Canada). In study two, seven animals with RFI extremes (L-RFI, lowest RFI values, $n = 3$; H-RFI, highest RFI values, $n = 4$) were selected from the twenty-two 16-month-old Hereford × Aberdeen Angus steers with divergent RFI (L-RFI, $n = 11$; H-RFI, $n = 11$) from another previous study (Hernandez-Sanabria et al., 2010). Steers were housed at the Lacombe Research center and were fed a finishing diet consisting of 73.3% barley grain, 22% barley silage, 1.6% molasses, 3.1% feedlot supplement (32% crude protein, beef supplement containing Rumensin at 400 mg/kg of body weight, and 1.5% canola oil).

Rumen contents (RC) and rumen epithelial tissues (RT) were collected upon slaughter. To ensure the quality of the collected samples, both rumen content and tissue samples were collected immediately when the rumen is removed from the carcass. The RC samples were immediately preserved in RNA*later*® solution (Invitrogen, Grand Island, NY), frozen with dry ice, and then stored at -20 °C for further analysis. To remove non-adherent bacteria, the RT samples were scraped and rinsed three times with sterilized phosphate-buffered saline (PBS) solution (pH, 7.0), and then preserved with the same steps as above (Chen et al., 2011). All sample handling were finished within 10 min.

2.2. PCR amplification and amplicon sequencing

Total DNA was extracted from rumen content and tissue samples using the bead beating method as previously described (Chen et al., 2011; Li et al., 2009). Partial bacterial 16S rRNA gene sequences (V1-V3) were amplified with primer A-338 (5′- CCATCTCATCCC TGcgtgtctccgacTCAGAC-MID index- TTGCTGCCTCCCGTAGGAGT - 3′) and primer B (27F) (5′- cctatccctgtgtgccttggcagtctcagagaGTTTGA TCCTGGCTCAG- 3′) (Hamady et al., 2008). The samples from each animal were assigned a distinct MID index. The reaction solution (50 μl) included 1 μl (50 ng/μl) of template, 1 μl of 10 mM deoxynucleoside triphosphate, 2.5 U of Taq polymerase (Invitrogen, Carlsbad, CA), $1\times$ PCR buffer, 1 μl of 50 mM MgCl$_2$, 1 μl of 20 pmol of each primer, and nuclease-free water. PCR was performed using the following program:

an initial denaturation for 5 min at 95 °C; 30 cycles of 95 °C for 30 s, 53 °C for 30 s, and 72 °C for 1 min; and a final elongation for 7 min at 72 °C. The amplicons were run on a 1.2% agarose gel, and the bands with size of ~400 bp were excised for DNA purification using QIAEX II gel extraction kit (Qiagen Sciences, MD). The concentration of the eluted DNA was measured using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). Equal amounts of each amplicon (25 ng) were pooled and subjected to pyrosequencing using the Roche 454 GS-FLX+ system at the McGill University and Génome Québec Innovation center (Montreal, QC). All sequences were submitted to the NCBI SRA (Sequence Read Archive) database under BioProject ID PRJNA304183.

2.3. Sequence data analysis

Sequence reads from the 16 RT and 16 RC samples were analyzed using the quantitative insight into microbial ecology (QIIME) toolkit version 1.9 (Caporaso et al., 2010a). Briefly, sequences were assigned to samples based on their unique barcodes. Barcodes and primer sequences were then removed, and the reads with a length ≥ 200 bp and ≤ 500 bp and an average quality score of at least 20 were retained for downstream analysis. Chimeric sequences were identified for removal using the usearch81 method in the identify_chimeric_seqs.py script with the GOLD (Liolios et al., 2010) and Greengenes (August 2013 release) reference databases.

Filtered reads were then classified into operational taxonomic units (OTUs) according to the open reference-based OTU picking method using the Greengenes database with 97% similarity (MacDonald et al., 2012). Briefly, the sequences were clustered into OTUs using Usearch 8.1 (Edgar, 2010). Taxonomy was then assigned to the sequences using the RDP (ribosomal database project) classifier and PyNAST aligner, using the same Greengenes database at the default of 75% confidence (Caporaso et al., 2010b). The biom program (McDonald and Clemente, 2012) was then used to convert the biom-formated OTU file to a tabulated format suitable for further analysis.

2.4. Taxonomy and community diversity analysis

The R for amplicon-based metagenomics (RAM) package (https:// rdrr.io/cran/RAM/, accessed July 10, 2019) was used for analyzing the OTU tables obtained from QIIME. Taxonomic abundance was reported for samples based on three ranks: phylum, family and genus, for the OTUs with a relative abundance > 1% in all samples. Good's coverage was calculated using the alpha_diversity.py script in QIIME. Diversity indices including Simpson, Shannon, species richness, Chao1, inverse Simpson, and ACE for all samples were calculated using RAM package. The compare_alpha_diversity.py script in QIIME was used to compare alpha diversity indices between H-RFI and L-RFI groups as well as between RT and RC samples using default parameters. The phyloseq package in R (McMurdie and Holmes, 2013) was used to import, analyze and visualize data from the OTU biom and phylogenetic tree files. The Bray-Curtis method was selected based on a standard stated by Weiss et al. (2017) for generating a distance matrix from the abundance counts, and a phylogenetic tree. Principal coordinate analysis (PCoA) (Gower, 1967) was performed on the basis of this distance matrix using RAM scripts, and plots were subsequently generated using the ggplots package (Hadley, 2009) in R.

2.5. Differential abundance analysis

OTU and function count data from all 32 samples (16 RT and 16 RC) were converted to an edgeR (Robinson et al., 2010) DGElist for statistical analysis (McMurdie and Holmes, 2014). In edgeR, taxonomy and functions that were observed (> 2 read counts) in at least 8 out of the 16 samples for either RC or RT were used for analysis. Next, the counts data was normalized by a scaling factor Relative Log Expression (RLE)

(Anders and Huber, 2010) implemented in edgeR. The general linear model framework implemented in edgeR was used to compare the RC and RT groups with a paired design since an RT and RC sample was obtained from each animal. A multiple-testing correction was applied using the Benjamini and Hochberg (1995) method. The significance level was ascertained at α < 0.05 and the BH-corrected P-values were used to identify the differentially abundant features.

A similar approach was used to identify differentially abundant features between L-RFI and H-RFI groups using the 16 RT samples (7 L-RFI vs 9 H-RFI) obtained from both animal studies. The effect of trial one versus two was included in the testing model by using a block design. Raw P-values were used to identify significant features below the ascertained α (0.05) due to the small sample size.

2.6. Co-occurrence analysis of the DA OTUs

Co-occurrence analysis of all identified DA OTUs for both RT and RC samples were performed with co-occurrence package in R. The matrix was built to illustrate the co-occurrence pattern of the DA OTUs.

3. Results

3.1. Bacterial community analysis of RT and RC microbiota

In total, approximately 2 million reads were generated from 32 samples (16 RT and 16 RC). After quality control using QIIME, 652,666 sequences from the RT samples (40,791 ± 29,533 sequences per sample) were classified into 20,226 OTUs (2898 ± 1919 OTUs per sample). From the RC samples, 1335,687 sequences (83,480 ± 45,704 per sample) were classified into 30,916 OTUs (4051 ± 1737 per sample). Further details regarding the sequence depth and other sample-related information are provided in Supplementary Table S1. Good's coverage and Simpson index showed that the coverage and diversity of each sample varied from 0.86 to 0.97 (0.92 ± 0.03) and 0.68–0.99 (0.92 ± 0.08), respectively.

3.2. Taxonomic composition of RT and RC bacterial communities

For the RT samples, 92.40% of the OTUs were classified at the phylum level, 67.30% at the family level and 41.10% at the genus level, identifying 17 phyla, 54 families and 64 genera of bacteria, respectively. The most abundant phyla in the RT samples were *Bacteroidetes*, *Proteobacteria*, and *Firmicutes*, representing 50.16% ± 18.46%, 27.82% ± 12.78% and 19.25% ± 12.19% (mean and standard deviation) of the OTUs, respectively (Fig. 1A). The most abundant families identified

were *Prevotellaceae* (36.00% ± 18.20%), followed by *Succinivibrionaceae* (14.05% ± 20.21%) and *Campylobacteraceae* (7.16% ± 11.31%) (Fig. 1A). At the genus level, *Prevotella* was the most prominent (35.56% ± 18.11%), followed by *Campylobacter* (7.16% ± 11.30%), and *Succiniclasticum* (3.70% ± 4.70%) (Fig. 1A). For the RC samples, 94.30% of OTUs were classified at the phylum level, 73.60% were classified at family level and 51.90% were classified at the genus level. From the identified OTUs, 17 phyla, 49 family and 60 genera of bacteria were identified. The most abundant phyla in the RC samples were *Bacteroidetes* 54.63% ± 19.20%, *Firmicutes* 30.07% ± 19.73%, and *Proteobacteria* 12.78% ± 21.29% (Fig. 1B). The most abundant families included *Prevotellaceae* (39.26% ± 16.48%), *Lachnospiraceae* (11.47% ± 15.54%) and *S24–7* (9.41% ± 10.68%) (Fig. 1B). At the genus level, *Prevotella* (38.94% ± 16.35%) was the most predominant genus followed by *Campylobacter* (4.21% ± 11.57%), *Succiniclasticum* (3.71% ± 3.91%), and *Ruminococcus* (1.21% ± 0.96%) (Fig. 1B). The relative abundance (mean and standard deviation) of all identified phyla belonging to RC and RT microbiota is listed in Supplementary Table S2.

3.3. Comparison of bacterial communities differed between RC and RT samples

The principle coordinate axes (PCoA) plot using the distance matrix generated by the Bray-Curtis method in phyloseq showed a separation between the RT and RC groups (Fig. 2A). Alpha diversity indices were higher for the RT samples than the RC samples although P-value did not indicate significance (P-values: species richness, 0.766; Chao1, 0.599; Good's coverage, 0.713, Fig. 2B). At the level of individual OTUs, 57 OTUs were differentially abundant (DA) between the RT and RC groups with 6 of them having higher abundance in RT and 51 of them having higher abundance in RC (Fig. 2C).

3.4. Comparison of the RC and RT bacterial communities between H- and L- RFI steers

PCoA plots did not show RFI group-based clustering for the OTU (Bray-Curtis) distance matrices in either RC (Fig. 3A) or RT (Fig. 3B) samples. To further explore how RC and RT bacterial communities might contribute to differences in host RFI, additional analyses were conducted for individual OTUs in the RC and RT samples.

For the RC samples, 19 OTUs were found to be DA between H-RFI and L-RFI steers (Fig. 4A). Among them, 4 OTUs were more abundant in H-RFI steers and 15 were more abundant in L-RFI steers (Fig. 4B). For the RT samples, there were 19 DA OTUs between H-RFI and L-RFI group (Fig. 4C), of which 11 OTUs were more abundant in H-RFI samples and 8
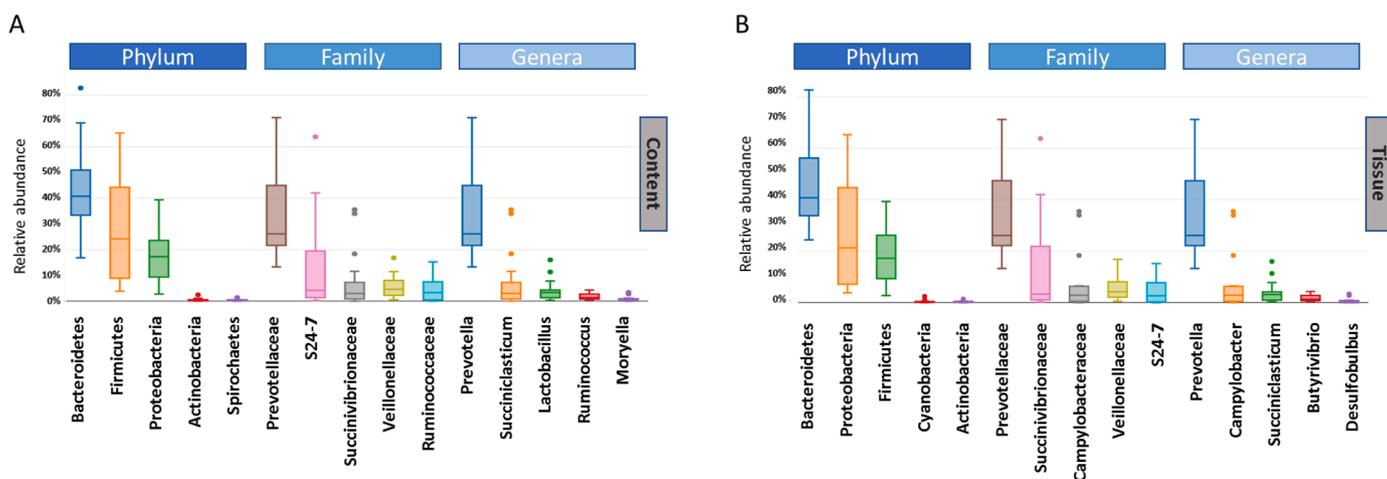
Fig. 1. Relative abundance of the top five taxon in phylum, family and genus level in both rumen content associated (A) and rumen epithelium associated (B) bacterial communities.
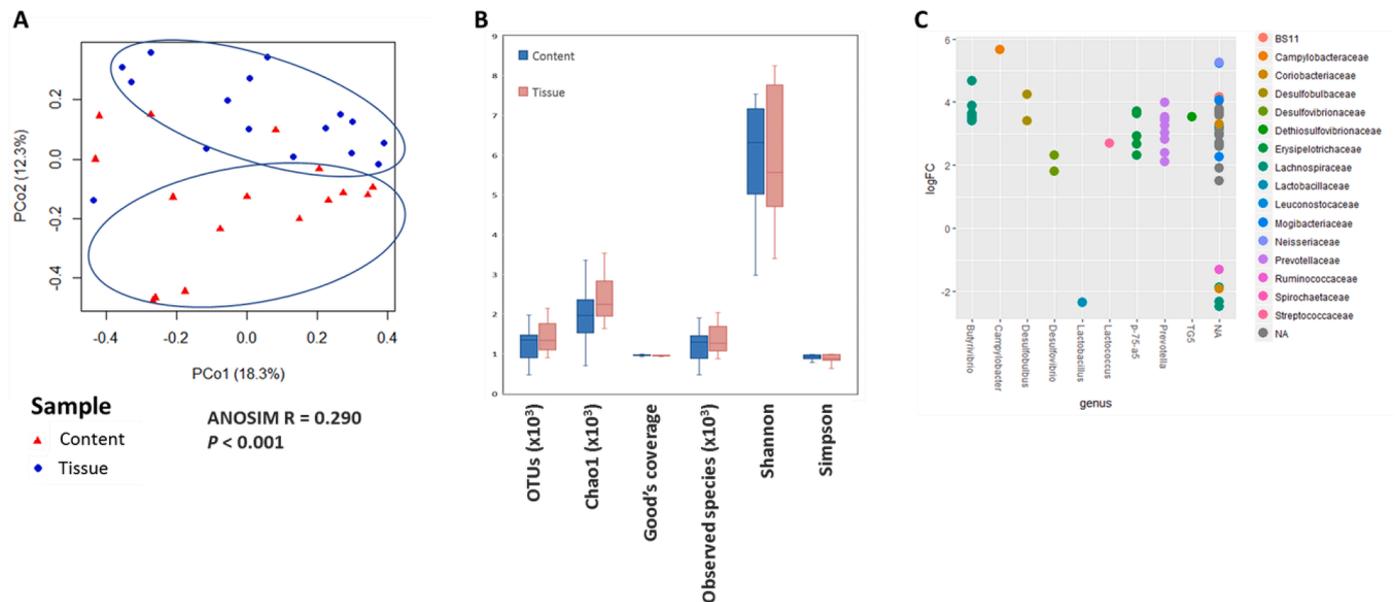
Fig. 2. Comparison of the microbial profiles between Content and Tissue samples. (A) Principal coordinate analysis showed that bacterial profiles formed two clusters based on sample type. (B) The bacterial community was more diverse in RT samples compared to RC samples. (C) Differential abundant OTUs identified between RC and RT samples.
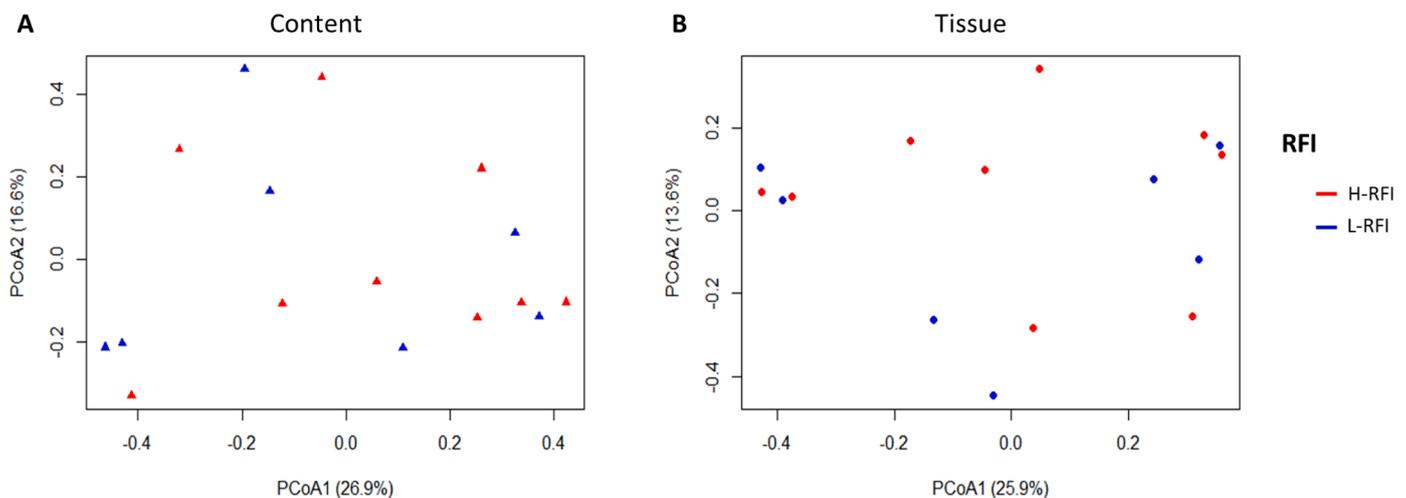
Fig. 3. Overall bacterial profiles of Content and Samples did not show clear separation between H-RFI and L-RFI steers. (A) Principal coordinate analysis plot of RC samples. (B) Principal coordinate analysis plot of RT samples.

OTUs were more abundant in L-RFI samples (Fig. 4D). Only one OTU belonging to *Prevotella* was DA in both RT (TDA11) and RC (CDA8) samples.

3.5. Co-occurrence between the DA OTUs between RT and RC samples

Co-occurrence analysis of all identified DA OTUs was then performed to illustrate the potential of microbial synergy in influencing host RFI. The OTUs DA between H-RFI and L-RFI steers for the RC and RT samples were ranked based on significance and renamed to CDA1-CDA19 and TDA1-TDA19. Taxonomic information, fold change, and *P*-value for these 38 OTUs is listed in Table S2. As shown in Fig. 5, strong correlation ($|r| > 0.80$, $P < 0.001$) was found between CDA1 (*Bulleidia*) and TDA8 (*Prevotella*), TDA13 (*Prevotella*), TDA15 (undefined genus of Ruminococcaceae), TDA18 (*Prevotella*), between CDA4 (*Prevotella*) and TDA19 (undefined genus of *Neisseriaceae*), between CDA16 (undefined genus of Clostridiales) and TDA11 (*Prevotella*), and between CDA19 (*Prevotella*) and TDA2 (undefined genus of *Paraprevotellaceae*). Moderate correlation

($0.65 < |r| \leq 0.80$, $P < 0.01$) was observed between two CDAs and two TDAs. Weak correlation ($0.5 < |r| \leq 0.65$, $P < 0.05$) was found between 5 CDAs and 3 TDAs.

4. Discussion

The impact of rumen content-associated and liquid-associated microbiota on cattle feed efficiency has been extensively examined (e. g. Lopes et al., 2021; McGovern et al., 2020), but that of the epimural microbiota has rarely been studied. It is only until recently that Tan et al. (2021) reported an epimural community with higher oxygen scavenging bacteria from more efficient steers. However, there is no study examining the content-associated (RC) and epimural community (RT) simultaneously with regard to host feed efficiency. In this study, we compared both content and epimural bacterial communities between H-RFI (less efficient) and L-RFI (more efficient) steers, aiming to identify the microbial markers for host feed efficiency and the potential mechanisms underlying.
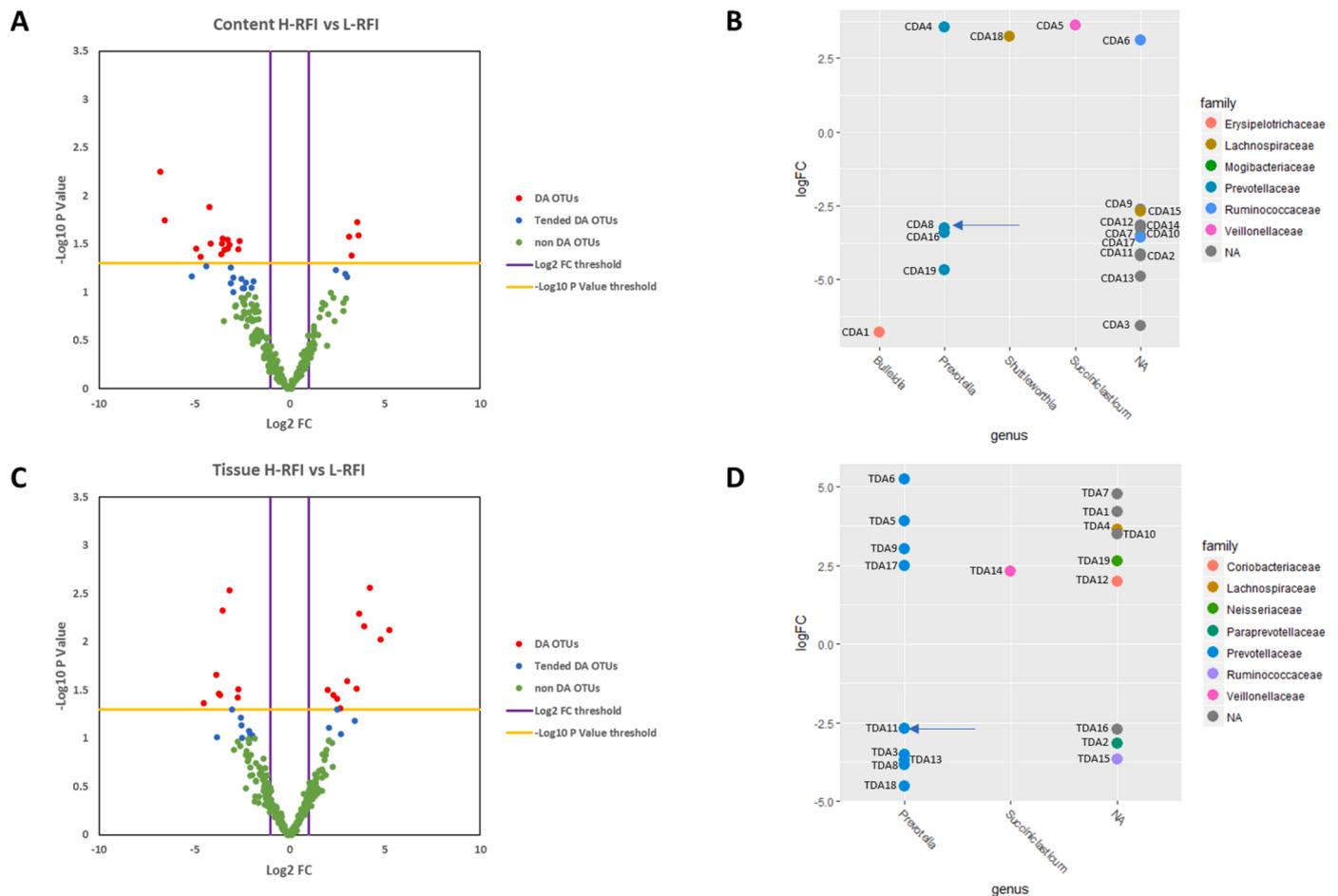
Fig. 4. Differential abundant OTUs between H-RFI and L-RFI steers. (A) Volcano plot of the OTUs identified from RC samples. DA OTUs: DA OTUs (Log2 Fold Change > 1, BH-adjusted $P < 0.05$); Tended DA OTUs: tended to be DA OTUs (Log2 Fold Change > 1, $0.05 \leq$ BH-adjusted $P < 0.1$); non DA OTUs: not DA OTUs (BH-adjusted $P \geq 0.1$). (B) Phylotypes of the differential abundant OTUs identified from RC samples. (C) Volcano plot of the OTUs identified from RT samples. DA OTUs: DA OTUs (Log2 Fold Change > 1, BH-adjusted $P < 0.05$); Tended DA OTUs: tended to be DA OTUs (Log2 Fold Change > 1, $0.05 \leq$ BH-adjusted $P < 0.1$); non DA OTUs: not DA OTUs (BH-adjusted $P \geq 0.1$). (D) Phylotypes of the differential abundant OTUs identified from RT samples. The arrow in (B) and (D) indicates the same OTU that is DA between H-RFI and L-RFI steers in both RC and RT samples.

While rumensin was added to animal feed following the regular practice on the research farms, based on our studies using the same herd of animals the supplement did not affect the (Hernandez-Sanabria et al., 2010; Li et al., 2012). The V1-V3 regions of the 16S rRNA gene was chosen for examination following the global rumen census (Henderson et al., 2015). The entire sample set was firstly compared between RC and RT. Distinctions between RC and RT bacterial communities were identified (Fig 2A), with the RT samples consisted of a more diverse community compared to the RC samples (Fig. 2B), which was in accordance with previous study (Li et al., 2012). The RC bacterial community in our study was dominated by *Bacteroidetes, Firmicutes*, and *Proteobacteria*, which is similar as reported in the previous 16S rRNA and metagenomics-based studies of dairy and beef (Brulc et al., 2009; Jami and Mizrahi, 2012). The RT bacterial community consisted of *Bacteroidetes, Proteobacteria*, and *Firmicutes*, which is different from what was reported in previous studies, namely that *Firmicutes* was the most predominant phylum, representing up to 73% of the overall RT bacterial population (Li et al., 2012; Mao et al., 2015; Petri et al., 2013). Such a discrepancy could be due to a number of factors including diet (forage-based diet (Mao et al., 2015; Petri et al., 2013) vs. grain-based diet in our study), depth of sequencing (Li et al., 2012; Mao et al., 2015), primers used (Mao et al., 2015; Petri et al., 2013) and steers used (dairy Holstein cows (Mao et al., 2015) vs. HEAN beef steers used in our study). In addition, a higher percentage of *Proteobacteria* (27.7%) was observed in RT in the current study as compared to 10–15% reported in previous

studies (Li et al., 2012; Mao et al., 2015). A high percentage of *Proteobacteria* has been reported in the epithelial tissue-attached bacteria in the gastrointestinal tract and rumen of pre-weaned calves (30.2%) (Malmuthuge et al., 2014). The phylum *Proteobacteria* consists primarily of facultative anaerobes that are insensitive towards oxygen toxicity and may play a role in oxygen scavenging that is one of the primary functions of the RT bacteria (Sadet-Bourgeteau et al., 2010). However, regardless of the relative abundance of the predominant bacterial phyla, the more diverse bacterial community in RT samples compared with RC samples observed in the current study (Fig. 2B) was in accordance with previous study (Li et al., 2012).

Considering the niches that RC and RT bacteria occupied and their varied functions in the rumen, the comparisons between H-RFI and L-RFI steers were performed for RC and RT separately. A total of 19 differential abundant OTUs were identified from the RC samples (CDAs). Among these CDAs, the functions can be speculated for the OTUs that have been classified at family level based on the known taxa being reported previously, while for the OTUs that were only classified at phylum level it is not feasible to predict their functions until their taxonomy has been refined. In previous studies, lower acetate:propionate (A:P) ratio was reported in L-RFI animals (Hernandez-Sanabria et al., 2012), suggesting that efficient animals may host a bacterial community with less acetate producers but more propionate producers. Surprisingly, in the current study acetate producers such as CDA1 (*Bulleidia*), CDA8 (*Prevotella*), CDA16 (undefined genus of Clostridiales), CDA17 (*Prevotella*), and
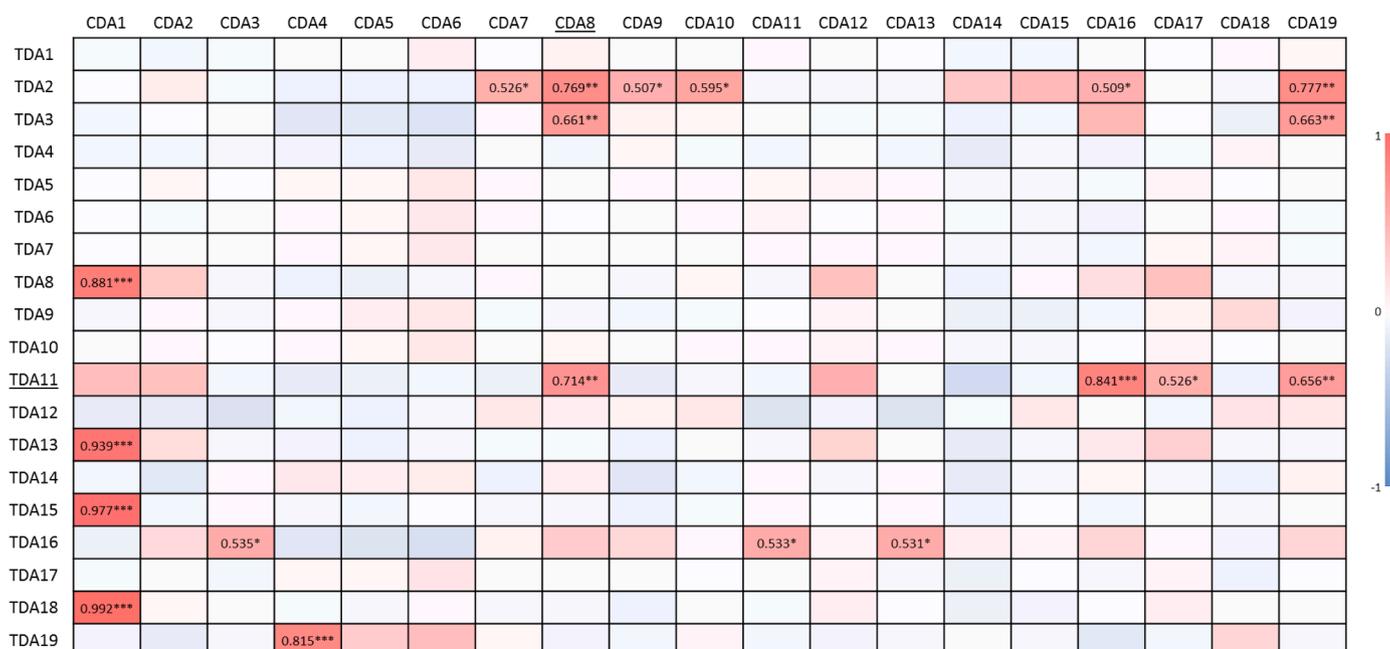
Fig. 5. The co-occurrence between the differential abundant OTUs from RC and RT samples. The OTU IDs were simplified to CDA1-CDA19 for all differential abundant OTUs identified from RC and TDA1-TDA19 for all differential abundant OTUs identified from RT samples, with the ordering followed their differential abundance significance. The phylogenic information for all differential OTUs is listed in Table S2. The two underlined differential abundant OTUs represent the same OTU.

CDA19 (*Prevotella*) were all more abundant in L-RFI steers, while CDA5 belonging to *Succiniclasticum*, which converts succinate to propionate was less abundant in L-RFI steers. These data indicated the possibility that the acetate producers although more abundant, may be less active; meanwhile the propionate producers although less abundant, may be more active in the L-RFI animals. These data warrants further validation by examining the microbial activities regarding RFI productions. *Shuttleworthia* belonging to *Lachnospiraceeae* is one of the major phylotypes involved in butyrate metabolisms (Meehan and Beiko, 2014). Butyrate concentration was previously reported to be associated with RFI in beef steers (Guan et al., 2008). CDA18 which belongs *Shuttleworthia* was more abundant in H-RFI samples, suggesting that more butyrate may be spared by the metabolic processes by CDA18 rather than being absorbed through the rumen epithelium for host utilization. A similar trend was also reported in one of our previous studies that *Lachnospiraceae* transcripts were more abundant in the rumen content of H-RFI steers (Li and Guan, 2017). It suggests that CDA18 may be more close to the phylotypes that were associated with butyrate metabolisms. Meanwhile, CDA10 and CDA 15, which also belonged to *Lachnospiraceae*, were more abundant in L-RFI steers. These two OTUs on the other hand, may be more close to the *Lachnospiraceae* phylotypes that participate in the other metabolic pathways, and may be more efficient in producing VFAs.

The differential abundant OTUs of RT samples (TDAs), may play roles in transferring molecules and/or communicate with host rather than participating in microbial fermentation. Similar as that observed for RC samples, the 9 TDAs belonging to *Prevotella* can be either associated with H-RFI or L-RFI steers. The importance of considering the functional variation in different *Prevotella* species has been raised from human studies (Ley, 2015). As *Prevotella* phylotypes have been found to be associated with RFI in steers for both RC and RT samples in multiple studies, the same consideration is recommended the rumen studies. With the phylogeny data from the current study, we were unable to define the exact functions of these TDAs of *Prevotella*. However, the rumen niche these 9 TDAs occupied (epithelium) suggest that they may involve in VFA absorption and/or microbial signaling system. TDA14 although differed from CDA5, was also classified to *Succiniclasticum*, and

was also more abundant in H-RFI steers compared to that of L-RFI steers. Currently it is unknown whether TDA14 is facilitating the propionate fusion across the rumen epithelium or not.

With the limited knowledge about the RT bacterial communities, exploring the co-occurrence between the CDAs and the TDAs may provide an alternate method in predicting the functions of the TDAs. For instance, CDA1 (*Bulleidia*) was highly co-occurred with four TDAs (TDA8, TDA13, TDA15, and TDA18) (Fig. 5). As the higher *Bulleidia* abundance was speculated to be associated with higher acetate and lactate production, the strong correlations of these four TDAs suggests the possibility that these phylotypes may participate in the pathways that transport the acetate and lactate from the rumen to the blood stream. Similarly, the other strong positive co-occurrence presented in Fig. 5 also suggest that these phylotypes are more tentatively to function cooperatively rather than competitively. With the correlations identified between CDAs and TDAs, and the proposed functions of these DA OTUs, the hypothetic mechanisms in how rumen microbial community variation contribute the variation in host RFI is proposed in Fig. S1: In L-RFI steers, the RC community may be more active in producing VFAs, while the RT community may be more active in facilitating the VFA transportations and/or better maintain the epithelium homeostasis, and more nutrients are supplied to the hosts and as such the host animals are more efficient.

One of the main limitations of the current study was that no functional genes or metagenome has been examined for the samples. As such we were not able to provide direct evidence of the functional differences between RC and RT communities. While the identified predominant microbiota was different between using 16S rRNA gene amplicon-based method (current study) and using functional gene-based method (Li and Guan, 2017; Zhou et al., 2021), and different phylotypes of the same genus may have diverse functions (e.g. a broad range of fermentation potentials reported from different *Prevotella* species) (Zhao et al., 2014; Hernandez-Sanabria et al., 2012), better sequence resolution may also help to classify the microbial phylotypes at more detailed phylogenic levels (e.g. species and strain level). Therefore, the current study has analyzed the data deep at OTU level rather than genus level, aiming to compensate the disadvantage of lacking actual measurement of the

functional genes. Future study examining the functional markers are essential to validate the phylogeny-based hypothesis on the potential mechanisms how different microbes affect host feed efficiency. It should be noted that when the samples were processed for the current study, only 454 sequencing platform allowed acquisition of longer read fragments. To obtain more comparable results of the same set of the samples, older version of analysis platform and database was used in the current study. In the future, more advanced sequencing methods and updated database will be incorporated, so that to have better interpretation of the microbial composition and functions of the samples.

5. Conclusions

This is the first study to illustrate the potential contributions of both RT and RC bacterial communities on steers' feed efficiency simultaneously. The current study has proposed a novel concept for reconsidering the microbial markers for steer feed efficiency, which a combined data from both RT and RC communities should be obtained simultaneously when developing microbial manipulation methods and microbial-marker based animal selection panel. A common differential abundant OTU belonging to *Prevotella*, CDA8/TDA11, identified in both RT and RC samples, may serve as a key indicator for steer feed efficiency. Based on the differential abundant OTUs identified in both RT and RC, a more efficient VFA producing and transportation system together with a better-maintained gut homeostasis is proposed for L-RFI steers, which ultimately leading to improved feed efficiency. The co-occurrence of the DA OTUs identified between the RT and RC have highlighted the importance in considering these two communities as a system, particularly in interpreting their impacts on host feed efficiency.

CRediT authorship contribution statement

Mi Zhou: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Bibaswan Ghoshal:** Data curation, Methodology, Writing – original draft. **Paul Stothard:** Supervision, Writing – review & editing. **Le Luo Guan:** Conceptualization, Resources, Software, Supervision, Funding acquisition, Writing – review & editing.

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.

Acknowledgement

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2021.100085.

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