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Impact of Feed Efficiency and Diet on Adaptive Variations in the Bacterial Community in the Rumen Fluid of Cattle

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Limited knowledge of the structure and activities of the ruminal bacterial community prevents the understanding of the effect of population dynamics on functional bacterial groups and on host productivity. This study aimed to identify particular bacteria associated with host feed efficiency in steers with differing diets and residual feed intake (RFI) using culture-independent methods: PCR-denaturing gradient gel electrophoresis (DGGE) and quantitative real-time PCR analysis. PCR-DGGE profiles were generated from the ruminal fluid of 55 steers fed a low-energy-density diet and then switched to a high-energy-density diet. Bacterial profile comparisons by multivariate statistical analysis showed a trend only for RFI-related clusters on the high-energy diet. When steers ($n = 19$) belonging to the same RFI group under both diets were used to identify specific bacterial phylotypes related to feed efficiency traits, correlations were detected between dry matter intake, average daily gain, and copy numbers of the 16S rRNA gene of *Succinivibrio* sp. in low-RFI (efficient) steers, whereas correlations between *Robinsoniella* sp. and RFI ($P < 0.05$) were observed for high-RFI (inefficient) animals. *Eubacterium* sp. differed significantly ($P < 0.05$) between RFI groups that were only on the high-energy diet. Our work provides a comprehensive framework to understand how particular bacterial phylotypes contribute to differences in feed efficiency and ultimately influence host productivity, which may either depend on or be independent from diet factors.

The complex symbiotic microbiota in the rumen are responsible for the breakdown of feed components, enabling ruminants to derive approximately 70% of their metabolic energy from the microbial fermentation of feedstuffs (9). Previous research has demonstrated that many factors influence the composition of rumen microbiota, affecting the population of certain bacterial groups (18, 22, 23, 43). Microbial diversity and activities have also been influenced by modifications to the diet (11, 51). Molecular techniques such as denaturing gradient gel electrophoresis (DGGE) demonstrated that changes in diet can affect microbial composition in the rumen (20, 32, 42). However, some studies have reported significant bacterial diversity among individuals (13, 28). Because individuals may respond differently to diet changes, identifying relationships between the differences in bacterial diversity in the rumen and host's phenotypic variations is particularly challenging, Moreover, the effect of bacterial population dynamics on host productivity characteristics, such as feed efficiency, has not been well established.

The adaptability and structural complexity of the microbial community within the ruminal ecosystem allow ruminant animals to consume a wide variety of feedstuffs (33). The type and amount of feedstuffs consumed by the host affect the nutritional supply to ruminal microbes and the end products synthesized, thereby influencing the nutrients absorbed by the host. Animals with poor feed efficiency have an increased environmental impact and cost of production (26, 39). In beef feedlots, feed accounts for up to 80% of costs (4), and up to 75% of total dietary energy consumed is used for nonproductive purposes (31). Improving feed efficiency without increasing maintenance energy expenditure may reduce the excretion of nutrients into the environment and has become economically relevant. Residual feed intake (RFI) has been used as a measurement of feed efficiency and has been defined as the difference between an animal's actual feed intake and its predicted intake, although the major biological mechanisms controlling RFI have yet to be fully elucidated (27, 38). Recent studies have suggested that bacterial structure in the rumen is associated with cattle feed efficiency (25), and PCR-DGGE band patterns have been linked to this phenotypic trait (28). Although probable associations among ruminal ecology and activities and cattle feed efficiency have been identified, there is little information on how specific bacterial groups affect whole microbial profiles and functions and whether diet affects the relationship between microbial populations and host RFI.

In this study, we hypothesized that specific bacterial groups are associated with cattle feed efficiency, and that these relationships can be affected by host diet. Culture-independent methods (PCR-DGGE and quantitative real-time PCR) were used to characterize particular bacterial groups or phylotypes associated with host feed efficiency under divergent diet and RFI conditions. Multivariate statistical analysis was used to link the diversity of bacterial communities with host productivity under two different diets (low energy density [LE] and high energy [HE] density). Three phylotypes belonging to the genera *Robinsoniella*, *Eubacterium*, and *Succinivibrio* sp., which were identified as RFI-associated bacteria, were selected for validation in the steers that remained under the same RFI category on both diets. The evaluated productive measurements were dry matter intake (DMI), average daily gain (ADG), feed conversion ratio (FCR) (feed:gain [F:G]), and residual feed intake (RFI).

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MATERIALS AND METHODS

Animals and sampling. The animal protocol was approved by the Animal Care and Use Committee for Livestock at the University of Alberta. All animals were raised at Kinsella Research Station (University of Alberta) by following the guidelines of the Canadian Council of Animal Care (14).

One hundred eighty Hereford × Aberdeen Angus steers (10 months old) were fed a mixed-ration, low-energy-density (LE) feedlot diet composed of 74% oats, 20% hay, and 6% feedlot supplement (32% crude protein [CP] beef supplement containing Rumensin [400 mg/kg of body weight] and 1.5% canola oil [metabolizable energy {ME}, 2.6 Mcal/kg]) for 90 days. After 1 week of adaptation, animals were switched to a high-energy-density (HE) feedlot diet composed of 28.3% oats, 56.7% barley, 10% alfalfa pellets, and 5% feedlot supplement (32% CP beef supplement containing Rumensin [400 mg/kg] and 1.5% canola oil [ME, 2.9 Mcal/kg]) for another 90 days. Feeding intake data were collected using the GrowSafe automated feeding system (GrowSafe Systems Ltd., Airdrie, Canada). Feed efficiency traits (DMI, ADG, FCR, and RFI) were obtained from all steers throughout both experimental periods by following procedures outlined by Basarab et al. (7). RFI was determined based on DMI and metabolic weight as described by Nkrumah et al. (39). Sixty steers were selected for their extreme RFI values (the 30 highest and 30 lowest values) on LE diet ($n = 60$). In both trials, steers were ranked and assigned to the following groups: high RFI (H-RFI; means plus 0.5 standard deviations [SD]), termed inefficient; medium RFI (M-RFI; between the means minus 0.5 SD and means plus 0.5 SD); and low RFI (L-RFI; less than the means minus 0.5 SD), termed efficient.

Rumen fluid samples (~150 to 200 ml) were collected via orogastric tubing from all steers in both LE and HE trials during the last week of the trial (days 83 to 90) before feeding using the method described by Hernandez-Sanabria et al. (28).

DNA extraction and PCR-DGGE analysis. Total DNA was extracted from rumen samples using physical disruption with the bead-beating method (25). The concentration and quality of DNA were measured, and 50 ng of total DNA was used as a template for the PCR amplification of the V2-V3 region (~200 bp) of the 16S rRNA gene of bacteria using universal bacterial primers HDA1-GC/HDA-2, as outlined by Walter et al. (52). Protocols for the purification of PCR products and the PCR-DGGE analysis followed those of previously reported studies (28).

Similarities between the PCR-DGGE band patterns of the rumen fluid samples were analyzed using Dice similarity coefficients (D_{sc}). Similarity was determined by comparing and clustering the whole profiles using BioNumerics software, v5.1 (Applied Maths, Austin, TX). Herein, hierarchical cluster comparisons were performed to group similar profiles and to generate a binary matrix of band classes. Dendrograms were generated using the unweighted pair-group method using average linkages (UPGMA) at a 1% position tolerance. The average of the D_{sc} values was calculated to compare the profiles of the H-RFI and L-RFI groups. Because RFI has been reported to be the most desirable measure of feed efficiency (3), we correlated RFI with the bacterial DGGE profiles.

Analysis of fermentation profiles: VFA and NH_3-N. Rumen fluid was subjected to volatile fatty acid (VFA) profiling using gas chromatography analysis by following standard procedures. An enzymatic assay was performed to measure NH_3-N (R-Biopharm kit; Roche Inc., South Marshall, MI) as described by Hernandez-Sanabria et al. (28).

To account for the possible dilution of rumen fluid by saliva and the time elapsed since the last meal (28), the proportion of each VFA (acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate) relative to the total VFA concentration was obtained and used as the dependent variable for the microbial metabolite analysis. Differences in VFA composition and NH_3-N within the RFI group between the two diets and within each diet in the two RFI groups were compared using the simple covariance mixed model in SAS (version 9.2; SAS Institute, Cary, NC). Statistical correlations were performed to identify interactions between the metabolites. Significance was assumed at $P < 0.05$.

Quantitative real-time qPCR. To verify the relationship between specific bacterial populations with RFI and diet, total rumen fluid DNA only from steers with the same RFI ranking under both diets (L-RFI, $n = 13$; H-RFI, $n = 6$) was subjected to quantitative PCR analysis to estimate the copy number of the 16S rRNA gene in each of the following bacterial phylotypes: *Robinsoniella peoriensis*, *Eubacterium rectale*, and *Succinivibrio dextrinosolvens*.

For the quantification of total bacterial 16S rRNA gene copy numbers, a standard curve was constructed using serial dilutions of plasmid DNA from a clone identified as *Butyrivibrio hungateii* using a method previously described by Li et al. (35). Briefly, universal bacterial primers 27F and 1492R (49) were used to amplify the full-length 16S rRNA gene from the plasmid DNA of a *Butyrivibrio hungateii* clone. The resultant PCR product was purified using the QIAquick PCR purification kit (Qiagen, Carlsbad, CA). The mass concentration of the PCR product was measured using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and converted to the molecule concentration using the following equation: DNA (number of molecules) = $(NL \times A \times 10^{-9})/(660 \times n)$, where NL is the Avogadro constant (6.02×10^{23} molecules per mol), A is the molecular weight of the molecule in the standard, and n is the length of the amplicon (in base pairs). The copy numbers of total bacteria in 50 ng of DNA were determined by relating the threshold cycle (C_T) values to the standard curves based on the following equation: $Y = -3.193 \times \log X + 35.003$ (Y, C_T value; X, copy number of 16S rRNA gene) ($r^2 = 0.996$). The copy numbers of the targeted phylotype 16S rRNA genes (per ml of rumen fluid) were calculated using the equation $(QM \times C \times DV)/(S \times V)$, where QM is the quantitative mean of the copy number, C is the DNA concentration of each sample, DV is the dilution volume of extracted DNA, S is the DNA amount (in ng) subjected to analysis, and V is the rumen fluid volume subjected to DNA extraction (56).

For the quantitative reverse transcription-PCR (qRT-PCR) analysis of the three phylotypes, Primer Express v2.0 (Applied Biosystems, Foster City, CA) was used to design primers (Table 1) targeting the sequences of the DGGE bands corresponding to bacterial groups related to the genus *Robinsoniella* that potentially are associated with RFI (see Tables 6 and 7; also see Table S4B in the supplemental material). The V2-V3 region from the existing sequences in the database and from the DGGE band clones were aligned, and the conserved sequence was used to design the primers. To check for specificity, designed primers were compared to the available sequences in the BLAST database. PCR was performed to amplify the plasmid containing the PCR-DGGE sequence insert using the following conditions: initial denaturation for 10 min at 95°C; 30 cycles at 95°C for 30 s, 58°C for 30 s, and 72°C for 60 s; and final elongation for 7 min at 72°C. qPCR conditions included 95°C for 10 min; 40 cycles of 95°C for 30 s, 58°C for 1 min, and 95°C for 15 s; and a final step of 60°C for 1 min. The *Eubacterium rectale* (5, 6) and *Succinivibrio dextrinosolvens* (37, 49) PCR assays were performed as previously described. The specificity of all primers was further verified by the amplification of amplicons of the correct size from the target products in all rumen fluid samples. Three PCR products from different samples corresponding to each of the three targeted bacterial phylotypes were selected, purified, and sequenced. When the primers amplified sequences with identities higher than 95% to the sequences of reported species in the GenBank database and to the PCR-DGGE bands, they were considered for further qRT-PCR analysis of the bacterial groups mentioned above. We used 97% similarity as the cutoff for species level and 93% similarity as the cutoff for genus level (8); because the sequences obtained from this study had only 95% identity with *Eubacterium rectale* and *Succinivibrio dextrinosolvens*, *Eubacterium* sp. and *Succinivibrio* sp. were used to represent the corresponding phylotypes. The standard curve for each phylotype was constructed using the plasmid DNA containing the inserts of the identified PCR-DGGE band from each of the three phylotypes. The copy numbers of each standard curve from the three targeted bacterial groups were calculated based on the equation used for the total bacterial population. The proportion of each phylotype was obtained after dividing the total copy number of 16S rRNA genes by

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TABLE 1 Primers used for detection of particular functional bacterial phylotypes in the present study[a]

Target bacterium	Primer sequence (5′–3′)	Annealing temp (°C)	Amplicon size (bp)	Reference(s) or source
Universal bacteria (U2)	F, ACTCCTACGGGAGGCAG R, GACTACCAGGGTATCTAATCC	50	468	49
Succinivibrio dextrinosolvens 0554	F, CGTCAGCTCGTGTCGTGAGA R, CCCGCTGGCAACAAAGG	60	80	37, 49
Eubacterium rectale ATCC 33656	F, AAGGGAAGCAACGCTGTGAA R, CGGTTAGGTCACTGGCTTC	60	200	5, 6
Robinsoniella peoriensis HGUE-09/9434	F, AAACGGATTTCTTCGGAATGAA R, TCTGTCTGTTATCCCCCTGTATGA	58	98	This study

[a] F and R indicate forward and reverse primers, respectively.

the copy numbers of the 16S rRNA gene of each targeted phylotype. The efficiencies (E) of RT-PCR were calculated from the given slopes in StepOnePlus software using the following equation: $E = [10(^{-1/\text{slope}}) - 1] \times 100\%$. qRT-PCR was performed using a StepOnePlus real-time PCR system and SYBR green (Applied Biosystems, Foster City, CA). Data generated from reactions with efficiencies between 90 and 110% were used for further analysis (55).

Analysis of variance using a mixed model in SAS was used to identify differences in total bacterial 16S rRNA gene copy numbers and in the proportion of each of the three specific bacterial phylotypes (*Succinivibrio* sp., *Eubacterium* sp., and *Robinsoniella* sp.) between RFI categories (high, $n = 6$; low, $n = 13$) within each diet (LE and HE; $n = 19$), as well as between diets within a particular RFI group. Correlations were determined among proportions of each bacterial phylotype, total copy number, ruminal metabolites, and feed efficiency traits (RFI, ADG, DMI, and FCR) using the CORR procedure in SAS. Correlations also were performed separately within diet and RFI groups. Significance was assumed when $P < 0.05$.

Statistical analysis. Data collected from only 55 steers were used for all analyses, because five animals were removed: two samples had saliva contamination, two had missing feed efficiency data for the HE diet, and one lacked sharpness in the PCR-DGGE image.

To identify the association between the PCR-DGGE profiles obtained from both HE and LE trials and RFI, bands were identified based on the positions of each band in the PCR-DGGE gels using BioNumerics software and fitted to previously reported categories (28). PROC CATMOD (SAS, version 9.2; SAS Institute, Cary, NC) was used to analyze the interaction between the recorded feed efficiency traits and the band frequency (presence or absence) on both HE and LE diets. The CATMOD procedure performs categorical data modeling that can be represented by contingency tables and calculates chi-square values for linear models of response frequencies (presence/absence of a band). To assess the relationships between PCR-DGGE bands, feed efficiency, and rumen metabolites, animals were classified under each feed efficiency/rumen fermentation variable using the criteria described above for high and low groups. The presence of particular bands representing specific bacteria for each high or low category of the described variables was analyzed only in steers that maintained H- or L-RFI rank in both diets and were classified within the high/low group of each metabolite.

To detect the RFI-associated bands within diets, the frequency of all detected bands on each RFI group was compared for the HE and LE diets using chi-square analysis. Similarly, the frequency of all bands between each diet was analyzed for the H- and L-RFI groups using the same statistical model. Two-way contingency tables of cross-classification containing the frequencies of the bands per category (high/low) and in both diets (LE/HE) were obtained using PROC FREQ in SAS. Diet-associated bands within the RFI categories and RFI-associated bands within the diets were revealed in two-way contingency tables created for each RFI group and for

TABLE 2 Variations in RFI classification of steers on two different diets[a]

Animal no.	RFI	
	LE diet	HE diet
1	H	M
11	H	H
13	L	M
23	L	L
31	L	H
33	L	L
39	H	M
65	L	M
69	L	H
75	L	L
91	L	L
95	H	H
97	H	M
101	L	L
111	L	L
119	L	L
121	L	M
129	L	L
151	H	M
159	H	M
161	H	M
163	H	M
167	H	H
173	H	H
197	L	M
205	L	L
225	H	H
249	L	M
259	L	L
271	L	L
279	H	L
287	H	H
293	L	M
311	H	M
403	H	M
407	H	M
411	H	M
423	L	L
427	H	M
439	H	M
451	L	L

[a] For illustration purposes, only the steers classified as L- or H-RFI in the LE diet were included.

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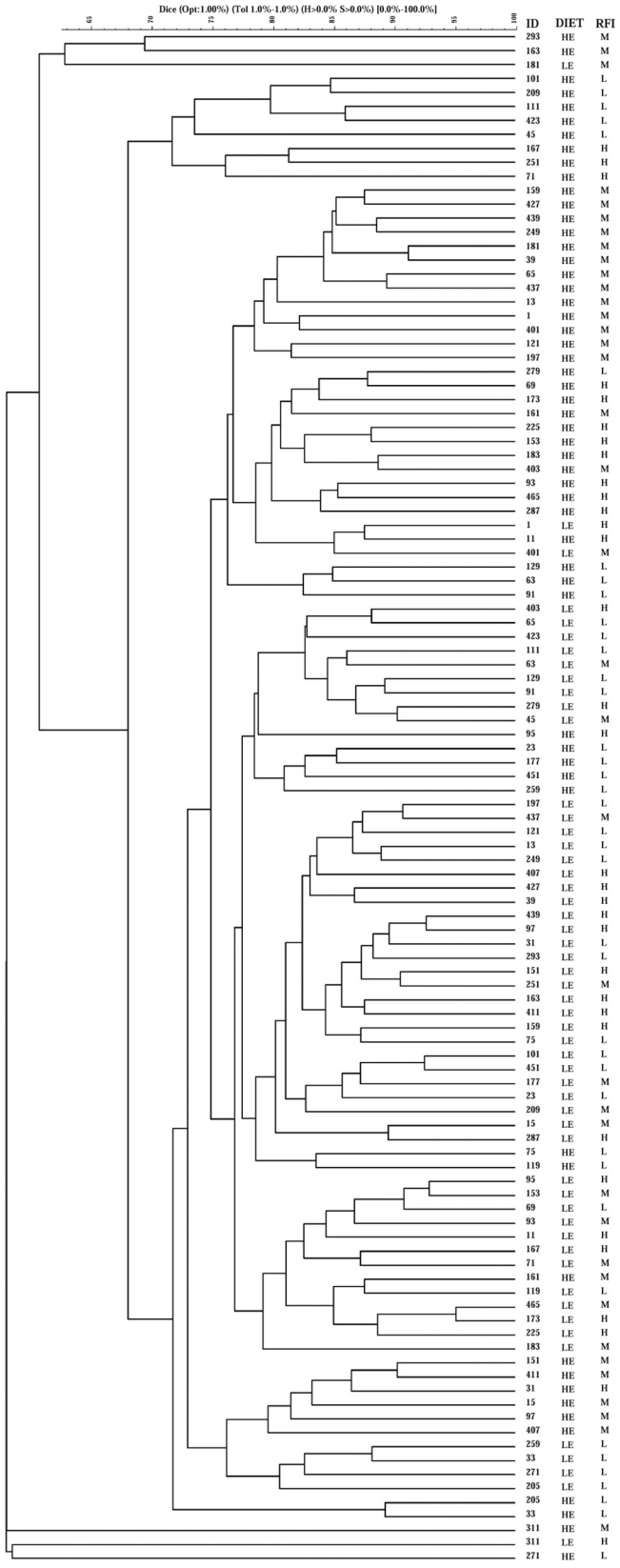

FIG 1 PCR-DGGE profiles generated from ruminal fluid DNA from 55 steers fed LE and HE diets using primers HDA1-GC and HDA2 (22 to 55% DGGE). H, M, and L represent the steers with H-RFI (means plus 0.5 SD; inefficient), M-RFI (between the means minus 0.5 SD and means plus 0.5 SD), and L-RFI (less than the means minus 0.5 SD; efficient), respectively. The comparison of the PCR-DGGE profiles was generated with the BioNumerics software package using the UPGMA method as described in the text; the first column indicates the identity (ID) of the steer, the second column corresponds to the diet, and the third column represents the RFI classification. The indices for the clustering analysis are indicated at the top of the dendrogram: Opt, optimization (setting 1.0%); Tol, position tolerance, expressed as a rounded-up value (1.0%); H and S, minimum height and minimum surface, respectively (0% used for the comparison); 0.0%–100.0%, entire length of each lane.

each diet, respectively. When the count of any of the cells was less than five, the Fisher exact test was used to calculate the probability of the table. For each of the variables, the frequency of all bands was plotted for high and low groups within the diets.

RESULTS

PCR-DGGE profiling of rumen bacterial dynamics between diets. Different RFI rankings were observed for the 55 steers under LE and HE diets. In the LE diet trial, 20 steers were allocated to H-RFI, 14 to M-RFI, and 22 to L-RFI groups. In the HE diet trial, 14 steers were allocated to H-RFI, 24 to M-RFI, and 18 to L-RFI groups. Table 2 summarized the changes in RFI ranking in the HE diet for the steers that were ranked H- or L-RFI on the LE diet ($n = 41$). Within the latter group, 19 animals (L-RFI, $n = 13$; H-RFI, $n = 6$) retained the same RFI classification during both diets and were used for the study of the association between feed efficiency variables and bacterial populations.

The evaluation of the predominant bacterial diversity in the rumen of steers with different feed efficiency and diets was compared using the UPGMA dendrograms generated. The overall comparison of bacterial profiles showed a similarity of 74.5% among all individuals in both diets, and the steers tended to group according to RFI within the HE diet cluster (Fig. 1). A separate PCR-DGGE dendrogram for the bacterial community on the HE diet alone confirmed the aforementioned grouping trend: average similarity among HE profiles was 69.8% (data not shown). A clustering tendency in LE diet animals has been reported (28).

Steers ranked as H-RFI for both diets were compared, and the similarity among them was 66.3%, whereas similarities among the L-RFI and M-RFI animals were 70.5 and 59.1%, respectively (Fig. 2). Only two individuals switched from L- to H-RFI ($D_{sc} = 46.6\%$) and one from H- to L-RFI ($D_{sc} = 81.7\%$) (data not shown). Due to the small sample size, further analyses were not performed. Because our objective was to identify the microbes associated with the H- or L-RFI animals, M-RFI steers were not selected for further analysis, as inconsistencies in their clustering trends were observed (e.g., nine M-RFI steers grouped with L- or H-RFI steers), and the M-RFI category included steers that changed their RFI dramatically when fed HE diet (from L-RFI to M-RFI or from H-RFI to M-RFI).

Characterization of fermentation metabolites in response to diet and RFI classification. Concentrations of VFAs and ammonia-nitrogen (NH_3-N) were assessed in rumen fluid samples to provide information on the associations between bacterial diversity and its functions in the rumen of animals fed LE and HE diets. The total VFA concentration was higher ($P < 0.0001$) in H-RFI steers on the HE diet, while the isobutyrate level was lower ($P < 0.05$) in this group (Table 3). Propionate, butyrate, and isovalerate remained unchanged in the L-RFI animals for both diets. DMI and ADG were significantly different between the diets in both L- and H-RFI animals; in L-RFI individuals, RFI and FCR tended to improve when the

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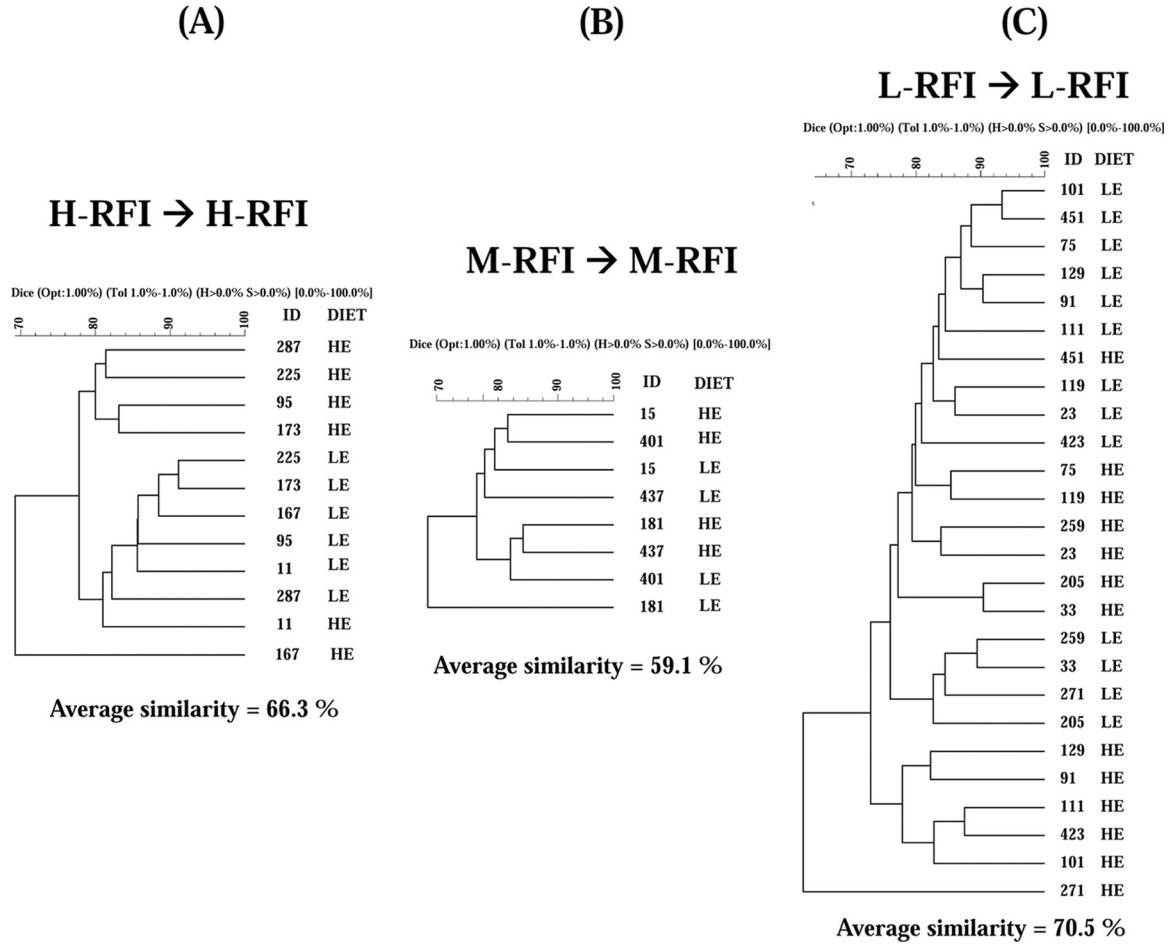

FIG 2 Comparison of PCR-DGGE bacterial profiles for different RFI groups from 19 steers (H-RFI, $n = 6$; M-RFI, $n = 4$; and L-RFI, $n = 13$) fed LE and HE diets. (A) Individuals in the H-RFI group throughout the study; (B) steers belonging to the M-RFI category; and (C) animals in L-RFI classification in both feed trials. The Dice similarity coefficient is indicated for each clustering analysis.

HE diet was fed ($P < 0.0001$). In addition, significant differences were observed in NH_3-N levels among L-RFI animals ($P < 0.05$) (Table 3). Although valerate and total VFA were significantly different in both RFI groups, only acetate was found to differ in L-RFI steers ($P < 0.05$). This difference did not have an effect on the acetate-to-propionate (A:P) ratio or the straight-to-branched (St:Br) VFA ratio of L-RFI steers; in H-RFI animals, however, the St:Br VFA ratio was significantly different. Furthermore, the correlations among fermentation metabolites and feed efficiency traits in the steers that remained either L-RFI or H-RFI on both diets were evaluated to provide information on the variations in the fermentation characteristics related to RFI. Results confirmed that for L-RFI steers, propionate, valerate, and total VFA were negatively related to RFI ($P < 0.05$) (see Table S1A in the supplemental material). In H-RFI individuals, RFI was positively correlated with NH_3-N ($P < 0.0001$), which in turn was linked with higher DMI ($P < 0.05$) (see Table S1B in the supplemental material).

Interactions between PCR-DGGE profiles, fermentation characteristics, and host feed efficiency under different diets. Using a chi-square-based procedure (PROC CATMOD in SAS), the effect of fermentation and feed efficiency measurements on the presence/absence of all bands was determined in the steers that remained in the same RFI category. The analysis of the frequencies within the steers that remained L-RFI in both diets revealed that some bands tended to be more frequent in one diet than in the other. For instance, band 63 (*Eubacterium rectale*-like, 98% identity) was the most frequent band in the LE diet, whereas band 83 (*Selenomonas ruminantium*) was not identified in any of the L-RFI steers (Table 4). Band 1 (*Prevotella* sp.) was more frequent ($P < 0.05$) in L-RFI steers than in H-RFI steers for both the LE and HE diets.

In total, 28 bands were related to RFI, but only band 1 (*Prevotella* sp.), band 5 (uncultured *Succinivibrio* sp.), band 31 (*Butyrivibrio fibrisolvens*), band 32 (*Prevotella ruminicola*), band 54 (*Succinivibrio dextrinosolvens*), band 58 (*Moryella indoligenes*), band 68 (*Succinivibrio dextrinosolvens*), band 74 (uncultured *Succinivibrio* sp.), and band 84 (*Bifidobacterium ruminantium*) were significantly different between RFI categories in the LE diet ($P < 0.05$). For the HE diet, band 1 (*Prevotella* sp.), band 3 (*Prevotella maculosa*), band 6 (*Prevotella* sp.), band 35 (uncultured *Prevotella* sp.), band 49 (uncultured *Prevotella* sp.), band 50 (*Prevotella ruminicola*), band 67 (*Succinivibrio dextrinosolvens*), band 72 (*Clostridium indolis*), band 77 (*Succinivibrio dextrinosolvens*), band 79 (*Succinomonas amylolytica*), and band 83 (*Selenomonas ruminantium*) were significantly different between RFI groups ($P < 0.05$). Thus, for the LE diet, bands 1, 5, 54, and 58 can be considered

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TABLE 3 Rumen metabolites and feed efficiency traits in steers of differing RFI and diets ($n = 19$)

Variable	Feed efficiency by group					
	H-RFI ($n = 6$)			L-RFI ($n = 13$)		
	LE (means ± SEM)	HE (means ± SEM)	*P* value	LE (means ± SEM)	HE (means ± SEM)	*P* value
Acetate[a] (%)	54.41 ± 1.34	50.16 ± 1.70	0.06	55.21 ± 1.02	52.12 ± 1.10	0.04
Propionate[a] (%)	31.57 ± 1.75	36.39 ± 2.20	0.10	33.17 ± 1.62	34.06 ± 1.72	0.71
Butyrate[a] (%)	9.56 ± 0.96	7.83 ± 1.20	0.27	7.64 ± 0.73	8.87 ± 0.77	0.25
Isobutyrate[a] (%) a	0.94 ± 0.06	0.67 ± 0.08	0.01	0.85 ± 0.06	0.72 ± 0.07	0.19
Valerate[a] (%)	1.01 ± 0.10	2.46 ± 0.12	<0.0001	1.05 ± 0.11	2.27 ± 0.11	<0.0001
Isovalerate[a] (%)	2.38 ± 0.40	2.29 ± 0.51	0.89	1.96 ± 0.21	1.81 ± 0.23	0.62
Total VFA (mM)	58.86 ± 5.53	113.19 ± 6.96	<0.0001	62.12 ± 5.59	100.29 ± 6.52	<0.0001
A:P ratio	1.86 ± 0.17	1.53 ± 0.21	0.24	1.70 ± 0.18	1.79 ± 0.19	0.74
St:Br VFA ratio	31.04 ± 4.78	53.49 ± 6.01	0.007	37.12 ± 6.73	54.84 ± 7.11	0.08
Ammonia (mM)	0.10 ± 0.16	0.20 ± 0.02	0.0002	0.11 ± 0.01	0.17 ± 0.02	0.007
DMI (kg DM)	8.65 ± 0.19	11.52 ± 0.23	<0.0001	6.96 ± 0.15	9.55 ± 0.17	<0.0001
ADG (kg)	1.26 ± 0.05	1.73 ± 0.06	<0.0001	1.22 ± 0.04	1.81 ± 0.04	<0.0001
FCR (feed:gain ratio)	6.98 ± 0.20	6.81 ± 0.25	0.60	5.75 ± 0.14	5.33 ± 0.15	0.05
RFI	0.78 ± 0.07	0.98 ± 0.09	0.09	−0.74 ± 0.08	−1.29 ± 0.09	<0.0001

[a] Values are given as a proportion of the total concentration of Volatile Fatty Acids.

L-RFI associated, and bands 31, 32, 68, 74, and 84 were H-RFI associated. In animals fed the HE diet, bands 1, 3, 50, and 77 were L-RFI associated, and bands 6, 35, 49, 67, 72, 79, and 83 were H-RFI associated (Table 4 and Fig. 3).

When analyzing bands affected by the diet switch within L-RFI steers, bands 6 (*Prevotella* sp.), 35 (uncultured *Prevotella* sp.), 49 (uncultured *Prevotella* sp.), 65 (*Robinsoniella peoriensis*), 67 (*Succinivibrio dextrinosolvens*), and 77 (*Succinivibrio dextrinosolvens*) were significantly different between diets ($P < 0.05$). Therefore, in L-RFI steers, bands 6, 35, 49, and 67 were LE diet associated,

TABLE 4 Taxonomical identification of RFI-associated PCR-DGGE bands for particular diets ($n = 19$)

PCR-DGGE band category	Result by diet						Closest related strain (GenBank accession no.)
	LE			HE			
	% L-RFI ($n = 13$)	% H-RFI ($n = 6$)	*P* value	% L-RFI ($n = 13$)	% H-RFI ($n = 6$)	*P* value	
1	46.15	0.00	0.0517	76.92	0.00	0.0021	*Prevotella* sp. (AF218619)
3	61.54	83.33	0.2846	84.62	33.33	0.0431	*Prevotella maculosa* strain W1609 (EF534315)
5	76.92	16.67	0.0270	76.92	66.67	0.3689	Uncultured *Succinivibrio* sp. clone EMP_B23 (EU794184)
6	84.62	83.33	0.4830	46.15	100.00	0.0277	*Prevotella* sp. BP1-56 (AB501155)
10	30.77	0.00	0.1483	7.69	50.00	0.0671	*Clostridium symbiosum* strain 69 (EF025909)
12	7.69	50.00	0.0671	23.08	16.67	0.4427	*Prevotella denticola* clone WWP_SS6_P23 (GU409439)
31	0.00	33.33	0.0347	0.00	0.00	0.8603	*Butyrivibrio fibrisolvens* strain Mz3 (AM039822)
32	0.00	33.33	0.0347	7.69	0.00	0.5965	*Prevotella ruminicola* (AB219152)
35	38.46	33.33	0.3831	0.00	33.33	0.0347	Uncultured *Prevotella* sp. clone JD9 (FJ268952)
41	53.85	66.67	0.3406	61.54	16.67	0.0836	*Ruminococcus* sp. ZS2-15 (FJ889653)
42	61.54	50.00	0.3406	61.54	98.36	0.0899	*Prevotella* sp. 152R-1a (DQ278861)
46	84.62	50.00	0.1342	61.54	100.00	0.0899	Uncultured *Prevotella* sp. clone JD9 (FJ268952)
48	92.31	50.00	0.0671	84.62	83.33	0.4830	*Lactobacillus* sp. DI71 (AB290831)
49	84.62	83.33	0.4830	46.15	100.00	0.0277	Uncultured *Prevotella* sp. clone Gull85-50 (FJ220908)
50	76.92	83.33	0.4427	100.00	50.00	0.0068	*Prevotella ruminicola* strain TC2-3 (AF218617)
52	92.31	50.00	0.0671	69.23	100.00	0.1483	*Succiniclasticum ruminis* strain DSM 9236 (NR_026205)
54	69.23	16.67	0.0464	92.31	100.00	0.5965	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)
58	69.23	16.67	0.0464	84.62	100.00	0.3706	*Moryella indoligenes* strain AIP 220.04 (DQ377947)
65	7.69	50.00	0.0671	46.15	83.33	0.1362	*Robinsoniella peoriensis* strain HGUE-09/9434 (GU322806)
67	84.62	50.00	0.1342	46.15	100.00	0.0277	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)
68	46.15	100.00	0.0277	69.23	50.00	0.2838	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)
70	46.15	83.33	0.1362	76.92	33.33	0.0851	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)
72	61.54	66.67	0.3831	30.77	83.33	0.0464	*Clostridium indolis* (AF028351)
74	30.77	83.33	0.0464	30.77	33.33	0.3953	Uncultured *Succinivibrio* sp. clone EMP_V30 (EU794288)
77	7.69	0.00	0.5965	76.92	16.67	0.0134	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)
79	23.08	0.00	0.2362	0.00	83.33	0.0002	*Succinimonas amylolytica* strain DSM 2873 (NR_026475)
83	0.00	0.00	0.8603	23.08	83.33	0.0227	*Selenomonas ruminantium* strain S211 (AB198441.1)
84	0.00	50.00	0.0068	15.39	0.00	0.3706	*Bifidobacterium ruminantium* strain KCTC 3425 (GU361831)

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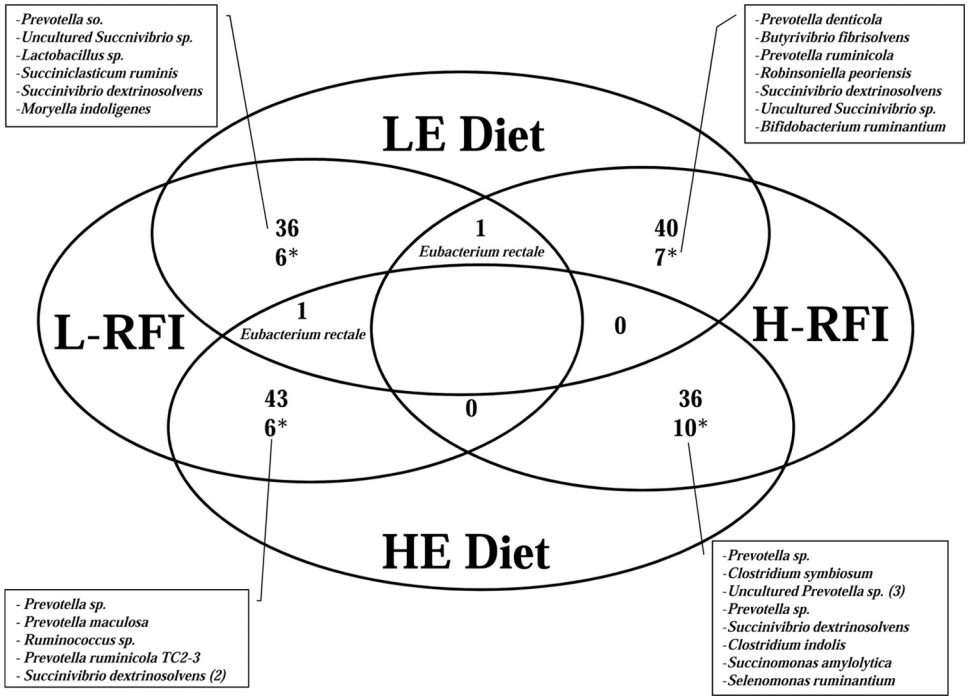

FIG 3 Specific bacterial phylotypes correlated to LE and HE diets and to L- and H-RFI groups. Numbers with asterisks indicate the number of phylotypes significantly correlated with each group; taxonomy is indicated in the squares corresponding to each quadrant.

whereas bands 65 and 77 were HE diet associated. In H-RFI animals, bands 2 (*Prevotella* sp.), 54 (*Succinivibrio dextrinosolvens*), 58 (*Moryella indoligenes*), 79 (*Succinomonas amylolytica*), and 83 (*Selenomonas ruminantium*) were significantly different between diets ($P < 0.05$). All of these bands were HE diet associated (Table 5). There were no bands exclusively related to the HE diet or to the H-RFI steers on either diet (Fig. 3).

For the animals that remained L-RFI on both diets, some trends in feed efficiency measurements were observed. For example, L-RFI steers were allocated to the low-DMI (L-DMI), high-ADG (H-ADG), and low-FCR (L-FCR) groups (see Table S2 in the supplemental material). In contrast, H-RFI individuals were in the H-DMI, L-ADG, and L-FCR groups. Therefore, frequency plots of the bands present in such groups were created to determine the particular phylotypes related to positive feed efficiency traits. Twenty bands were associated with L-DMI in the LE diet, whereas 13 were related in the HE diet. Only 5 bands were associated with H-DMI in the LE diet, while 3 were associated with H-DMI in the HE diet (Fig. 4A and B). L-RFI steers showed H-ADG, and 10 bands were associated with this trait in the LE diet group, while 6 bands were present in the HE diet group. For H-RFI individuals, three bands were associated with L-ADG in the LE diet, whereas 7 bands were present in the HE diet group. Sixteen bands were linked to L-FCR in the LE diet group, and 11 were correlated in the HE diet group. High FCR was observed for H-RFI steers: 3 bands were related in the LE diet but 6 in the HE diet (Fig. 4A and B). In the acetate proportions, L-RFI individuals were classified under the high category in the LE diet, while H-RFI animals were ranked in the low category. Four bands were associated with the L-acetate category, and 10 were prevalent in H-acetate (Fig. 4A). For the LE diet, H-isobutyrate was recorded in the L-RFI steers, and 9 bands were correlated with this characteristic; however, no clear trend could be reported for H-RFI steers. As seen in Table 3, high NH_3-N levels were observed in L-RFI steers on the LE diet, and low NH_3-N levels were detected in H-RFI individuals on the same diet; 6 bands were shown to be related to high NH_3-N and 11 to low NH_3-N (Fig. 4A and B).

Comparison of specific and total bacterial populations. To validate the identified associations revealed by the multivariate statistical analysis of PCR-DGGE profiles, three bacterial groups were selected for qRT-PCR analysis (*Succinivibrio* sp., *Eubacterium* sp., and *Robinsoniella* sp.). The results of the quantification of the total bacteria and of the specific phylotypes selected were compared for both diets and for differing RFI categories by measuring the copy numbers of total and targeted 16S rRNA genes. Only the proportion of *Succinivibrio* sp. adapted to the differences in diet ($P < 0.05$) (Table 6). In the LE diet, total bacteria tended to be increased in H-RFI animals, but the difference between RFI groups was not significant, while *Robinsoniella* sp. only showed a trend ($P < 0.10$) for an increased proportion in L-RFI steers. For the HE diet, the proportion of *Eubacterium* sp. was different between L- and H-RFI steers ($P < 0.05$), and the total bacterial population did not change between RFI groups (Table 7). Correlation analysis showed a tendency for a negative association between RFI and *Robinsoniella* sp. ($P < 0.10$) in the LE diet (see Table S3A in the supplemental material). *Eubacterium* sp. was positively associated with RFI ($P < 0.05$) and tended to be positively correlated with DMI under HE diet (see Table S3B in the supplemental material).

DISCUSSION

Feed consumed by animals is partitioned to meet host energetic requirements for production and maintenance (2). A wider understanding of the contributing factors will provide information

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TABLE 5 Taxonomical identification of diet-associated PCR-DGGE bands within particular RFI groups ($n = 19$)

PCR-DGGE band category	Result by RFI group						Closest sequence related (GenBank accession no.)
	L-RFI (%; $n = 13$)		*P* value	H-RFI (%; $n = 6$)		*P* value	
	LE diet	HE diet		LE diet	HE diet		
1	46.15	76.92	0.0924	0.00	0.00	0.5455	*Prevotella* sp. (AF218619)
2	46.15	46.15	1.0000	16.67	83.33	0.0390	*Prevotella* sp. (AF218619)
6	84.62	46.15	0.0428	83.33	100.00	0.3633	*Prevotella* sp. BP1-56 (AB501155)
9	23.08	15.39	0.3391	50.00	0.00	0.0909	*Blautia* sp. BM-C2-0 (GQ456220)
10	30.77	7.69	0.1413	0.00	50.00	0.0909	*Clostridium symbiosum* strain 69 (EF025909)
14	15.39	15.39	0.4070	50.00	0.00	0.0909	*Lachnospiraceae genomospecies* C1 (AY278618)
34	23.08	30.77	0.3109	50.00	0.00	0.0909	*Ruminococcus gauvreaui* strain CCRI 16110
35	38.46	0.00	0.0196	33.33	33.33	0.4545	Uncultured *Prevotella* sp. clone JD9 (FJ268952)
42	61.54	61.54	1.0000	50.00	100.00	0.0909	*Prevotella* sp. 152R-1a (DQ278861)
43	69.23	38.46	0.0953	66.67	66.67	0.4545	Uncultured *Roseburia* sp. clone M2-35 (EU530245)
46	84.62	61.54	0.1526	50.00	100.00	0.0909	Uncultured *Prevotella* sp. clone JD9 (FJ268952)
49	84.62	46.15	0.0428	83.33	100.00	0.5000	Uncultured *Prevotella* sp. clone Gull85-50 (FJ220908)
52	92.31	69.23	0.1413	50.00	100.00	0.0909	*Succiniclasticum ruminis* strain DSM 9236 (NR_026205)
54	69.23	92.31	0.1413	16.67	100.00	0.0076	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)
58	69.23	84.62	0.2422	16.67	100.00	0.0076	*Moryella indoligenes* strain AIP 220.04 (DQ377947)
65	7.69	46.15	0.0339	50.00	83.33	0.2424	*Robinsoniella peoriensis* strain HGUE-09/9434 (GU322806)
66	61.54	92.31	0.0727	66.67	83.33	0.4091	Uncultured *Succinivibrio* sp. clone EMP_B23 (EU794184)
67	84.62	46.15	0.0428	50.00	100.00	0.0909	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)
68	46.15	69.23	0.1588	100.00	50.00	0.0909	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)
70	46.15	76.92	0.0924	83.33	33.33	0.1136	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)
72	61.54	30.77	0.0953	66.67	83.33	0.4091	*Clostridium indolis* (AF028351)
73	61.54	23.08	0.0476	66.67	50.00	0.3788	Uncultured *Succinivibrio* sp. clone EMP_V30 (EU794288)
76	15.39	23.08	0.3391	0.00	50.00	0.0909	*Moryella indoligenes* strain AIP 220.04 (DQ377947)
77	7.69	76.92	0.0004	0.00	16.67	0.5000	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)
79	23.08	0.00	0.1100	0.00	83.33	0.0076	*Succinimonas amylolytica* strain DSM 2873 (NR_026475)
83	0.00	23.08	0.1100	0.00	83.33	0.0076	*Selenomonas ruminantium* strain S211 (AB198441.1)
84	0.00	15.39	0.2400	50.00	0.00	0.0909	*Bifidobacterium ruminantium* strain KCTC 3425 (GU361831)

on the physiological processes responsible for variations in the metabolic phenotype. During rumen fermentation, short-chain VFA and microbial proteins are formed and utilized as energy and protein sources, respectively, by the host animal (44). The efficiency of nutrient utilization is determined by the balance of such fermentation products, which ultimately can be controlled by the ruminal microbiota.

The energetic metabolism in beef cattle with different RFI has been reported to be significantly different (39). In fact, L-RFI (efficient) cattle have lower DMI and lower levels of methane emissions (26, 39). As differences in digestion contribute to approximately 10% of the differences in RFI (27), ruminal microbial fermentation must play a key role in this trait. Guan et al. (25) reported a potential association between butyrate and RFI in cattle on the HE diet. In contrast, in our study valerate was negatively related to ADG and DMI in L-RFI steers ($P < 0.05$) (see Table S1A in the supplemental material). This observation may have been the result of the decreased fermentation of amino acids in the rumen, which has been associated with inhibited methane formation (45, 46). Lower production of branched-chain fatty acids can be a consequence of the inhibition of bacterial NADH-H hydrogenases (46), indicating high concentrations of H in the ruminal medium due to the potential inhibition of methanogenic archaea or H utilization by these microorganisms (47). These findings also may relate to the lower proportion of isovalerate and a higher St:Br VFA ratio observed in L-RFI steers, suggesting that efficient animals do not divert much energy to methanogenesis (26, 55) and that more efficient N retention occurs in L-RFI steers, improving feed efficiency (16). This trend was further confirmed by the correlation between NH_3-N and RFI in H-RFI steers ($P < 0.05$) (see Table S1B in the supplemental material), suggesting the inefficient incorporation of NH_3-N into microbial protein in the rumen. Nevertheless, additional studies on the wasteful degradation of N by particular bacteria are needed.

As in our previous study linking PCR-DGGE band patterns to host RFI under the LE diet, similar associations were observed for animals on the HE diet. Due to the diet effects on rumen microbial diversity, some of these associations were altered. For instance, we observed that although some steers remained in the same RFI rank, the bacterial phylotypes associated with RFI and other phenotypic traits varied. This observation suggests that microbiota adaptation to the diet in some animals does not necessarily lead to changes in overall productive performance. In contrast, in other individuals, either a positive or negative impact of the diet on feed efficiency was evident.

Three specific phylotypes identified as RFI-associated bacteria were selected for validation in the steers that remained under the same RFI category on both diets ($n = 19$): *Succinivibrio dextrinosolvens* (band 67; H-RFI associated), *Robinsoniella peoriensis* (band 65; H-RFI associated), and *Eubacterium rectale* (band 63; L-RFI associated). *Succinivibrio dextrinosolvens* is the predominant isolate when ruminants are fed high-starch diets (40). Previous reports mention acetate and succinate (a precursor of propionate) as the major products of *Succinivibrio dextrinosolvens* (44). Because acetate proportions were higher in L-RFI steers for both diets (Table 3), *Succinivibrio* sp. might play a role in propionate

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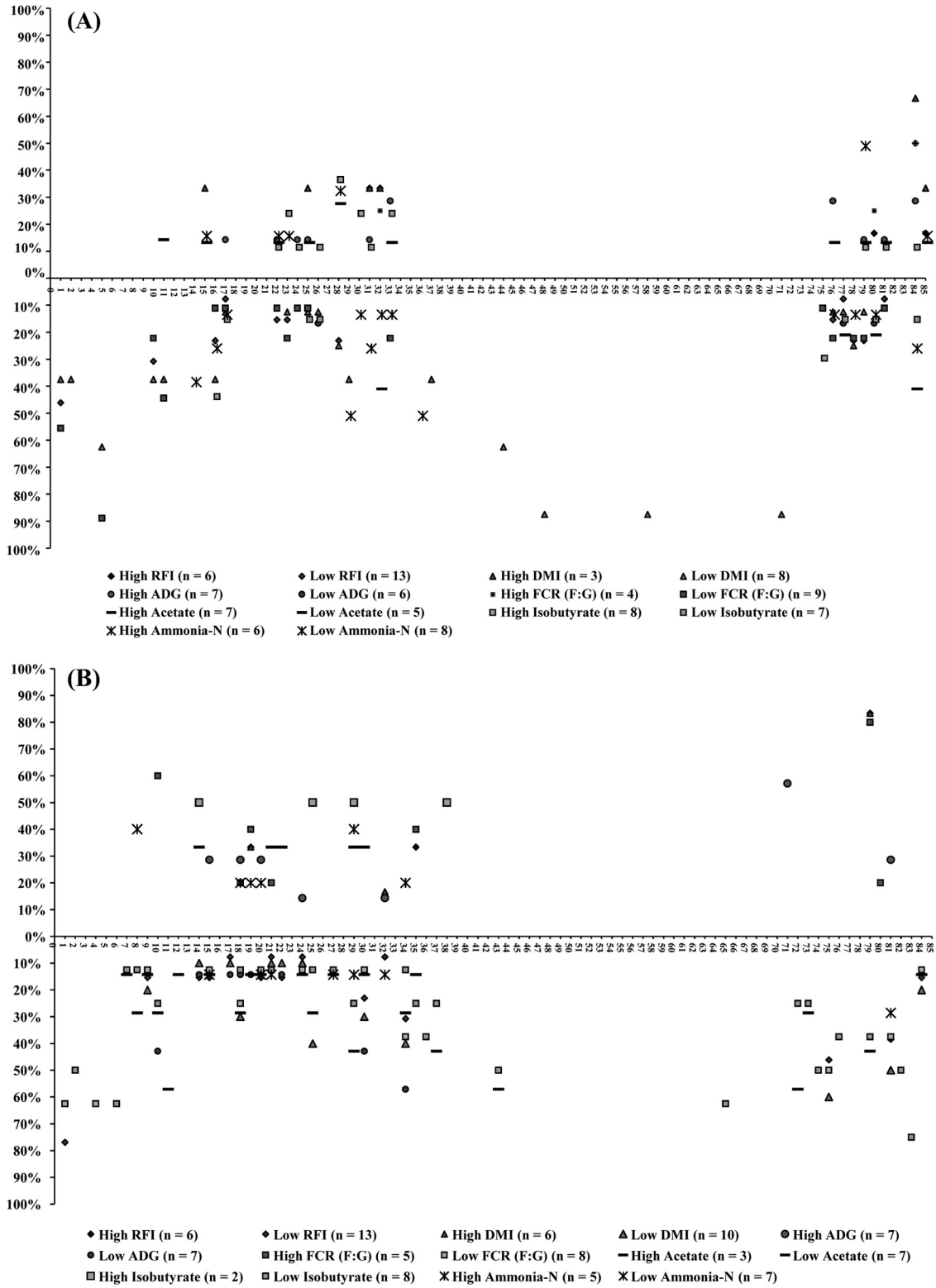

FIG 4 Frequency of PCR-DGGE bands in animals categorized on the basis of dry matter intake (DMI), average daily gain (ADG), feed conversion ratio (FCR; F:G), and acetate, isobutyrate, and NH_3-N levels on LE (A) and HE (B) diets using PROC CATMOD analysis. The *x* axis represents 85 identified bands, and the symbols plotted reflect the frequency of the bands detected in the tested population of each trait.

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TABLE 6 Differences in particular bacterial species between LE and HE diets by RFI group ($n = 19$)

Variable or species	Result by group					
	H-RFI ($n = 6$)			L-RFI ($n = 13$)		
	LE (means ± SEM)	HE (means ± SEM)	*P* value	LE (means ± SEM)	HE (means ± SEM)	*P* value
Total bacteria (copy no./ml digesta)	7.56E10 ± 2.2E10	4.44E10 ± 2.2E10	0.34	4.07E10 ± 1.52E10	6.34E10 ± 1.52E10	0.30
Succinivibrio sp. (%)	8.45 ± 1.88	0.21 ± 1.72	0.01	12.49 ± 2.34	0.09 ± 2.34	0.001
Eubacterium sp. (%)	0.13 ± 0.19	0.55 ± 0.19	0.15	0.22 ± 0.09	0.09 ± 0.09	0.28
Robinsoniella sp. (%)	0.001 ± 0.001	0.002 ± 0.001	0.62	0.005 ± 0.000	0.002 ± 0.000	0.13

synthesis, which could be readily absorbed from the rumen for hepatic gluconeogenesis, thereby improving feed efficiency in L-RFI steers. Moreover, the negative correlation of *Succinivibrio* sp. with valerate proportion (see Table S4A in the supplemental material) also may indicate the inhibition of bacterial NADH-H hydrogenases and the subsequent inhibition of methanogens (47) in the rumen of efficient steers. On the contrary, another net product from *Succinivibrio* spp. is formate, which is metabolized to $CO_2 + H_2$ and methane by rumen methanogens (36). This unfavorable effect might lead to a loss of dietary energy and subsequent decreased feed efficiency in H-RFI steers as a result of increased *Succinivibrio* sp. populations under the HE diet.

Robinsoniella peoriensis is a bacterium that is isolated from anaerobic environments (17); our previous research reported this species in the rumen for the first time (28), although its function is not yet known. Cotta et al. (17) revealed that acetate and succinate are its major metabolic end products, and it produces formate and lactate to a lesser extent. When the LE diet was fed, the proportion of *Robinsoniella* spp. tended to be decreased ($P = 0.06$) (Table 7) in H-RFI steers; however, the *Robinsoniella* sp. was positively correlated with RFI ($P < 0.05$) (see Table S4B in the supplemental material) in inefficient steers. The mechanisms underlying this relationship have yet to be explained, but they might be related to the production of formate and the consequent effects on methane production.

Eubacterium rectale has been isolated from human (6, 21) and pig (34) guts and has been identified here for the first time in bovine rumen. In our study, this was the most predominant bacterial phylotype in L-RFI steers after the diet was changed, probably due to the bacterium's tolerance of low pH (19). *E. rectale* produces butyrate and utilizes acetate (21) in the human intestine (1). For ruminants, acetate constitutes the major energy source for oxidation (24); in L-RFI steers, the acetate proportion was significantly correlated to RFI ($P < 0.05$) (see Table S1A in the supplemental material). The function of acetate in L-RFI steers might be related to potential hydrogen transfer mechanisms to reductive acetogenesis (10, 12). Indeed, Janssen (30) proposed that microorganisms that are more efficient at producing biomass will cause shifts away from H_2-producing fermentation pathways, which result in lower CH_4 formation or even lower methanogenic activity. Therefore, in L-RFI individuals, the acetate utilization characteristic of *Eubacterium* sp. may interact with the acetate-producing capacity of *Succinivibrio* sp., probably toward the consumption of excessive hydrogen, which otherwise would be directed to methanogenesis (15, 54). Moreover, increased concentrations of acetate could be associated with improved dietary energy extraction by the gut microbiota (51). Since high acetate proportions were found in the L-RFI animals on both diets in our study (Table 5), future studies regarding the role of homoacetogenic bacteria on feed efficiency as well as in the shifts of pathways that result in a smaller energy change and larger production of biomass will provide more evidence for these speculations.

Based on these observations, we speculated that cross-feeding interactions among bacterial groups with functions represented by the three phylotypes mentioned above are altered when the diet is changed; as a result, their association with RFI varied as well. Nevertheless, the three groups of bacteria studied herein do not alone account for the total variation in RFI; rather, they are representative of the phylotypes that potentially contribute to such differences. Methanogen diversity from the same group of animals has been associated with RFI (55), and the interactions described above may interfere with the CO_2-H_2 methanogenic pathway (55, 56). However, only a small number of the 85 phylotypes was validated, representing a discrete portion of the total bacterial population in the rumen; DGGE patterns only summarize the community structure rather than the actual richness of the bacterial population. Further, the identified phylotypes may represent the same species or the same strains of different species. Thus, future studies on the interactions among bacteria and other ruminal inhabitants using metagenomics techniques, such as pyrosequencing, are necessary to clarify our speculations.

Due to limited sampling access to the herd used in our study,

TABLE 7 Differences in particular bacterial species populations between L- and H-RFI groups on LE and HE diets ($n = 19$)

Variable	Results by diet					
	LE ($n = 19$)			HE ($n = 19$)		
	L-RFI ($n = 13$) (means ± SEM)	H-RFI ($n = 6$) (means ± SEM)	*P* value	L-RFI ($n = 13$) (means ± SEM)	H-RFI ($n = 6$) (means ± SEM)	*P* value
Total bacteria (copy no./ml digesta)	4.07E10 ± 1.28E10	7.56E10 ± 1.89E10	0.15	6.34E10 ± 1.71E10	4.44E10 ± 2.52E10	0.54
Succinivibrio sp. (%)	12.49 ± 3.00	8.45 ± 4.83	0.49	0.09 ± 0.05	0.21 ± 0.07	0.20
Eubacterium sp. (%)	0.22 ± 0.10	0.13 ± 0.15	0.64	0.09 ± 0.09	0.55 ± 0.14	0.02
Robinsoniella sp. (%)	0.005 ± 8.33E−6	0.001 ± 0.001	0.06	0.002 ± 0.001	0.002 ± 0.001	0.98

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rumen fluid samples used for bacterial diversity analysis were collected at a single time point before feeding, whereas feed efficiency traits were recorded for a longer period. However, previous studies suggest that prefeeding VFA concentrations are similar between diets (41, 48) and individuals (53) and are their lowest before feeding (11), representing a suitable baseline indicator (28). Moreover, longer periods of adaptation lead to more stable rumen environments and fermentation characteristics (29, 50, 53). Thus, our VFA data are valid to illustrate potential associations between specific bacterial phylotypes and fermentation profiles of individual steers and their corresponding feed efficiency traits. However, for a broader explanation of the relationships between fermentation characteristics and feed efficiency, it is essential to record intake variables and collect samples at multiple time points during the period as well as to include a defined control group.

In conclusion, we have identified probable associations between specific bacterial phylotypes and feed efficiency traits under HE and LE diets in beef cattle. Three bacterial phylotypes (*Succinivibrio* sp., *Eubacterium* sp., and *Robinsoniella* sp.) have been identified to be potentially associated with RFI based on their sequences, and they may be indicative of wider changes in the microbial population. Future efforts to isolate these bacteria from rumen and to study their metabolic pathways by whole-genome sequencing will be necessary for a full understanding of their roles in feed efficiency. Our study provides a framework to identify variations in the population of rumen bacterial groups that are influenced by diet and play a major role in the energetic metabolism in the host, thereby influencing feed efficiency. The demonstrated relationships between diet, feed efficiency, and rumen microbes also contribute to the knowledge of functional microflora and their potential for manipulation and further improvement of animal performance.

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