APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 2010, p. 6338–6350 Vol. 76, No. 19
0099-2240/10/$12.00 doi:10.1128/AEM.01052-10

Correlation of Particular Bacterial PCR-Denaturing Gradient Gel Electrophoresis Patterns with Bovine Ruminal Fermentation Parameters and Feed Efficiency Traits▽†

Emma Hernandez-Sanabria, Le Luo Guan,* Laksiri A. Goonewardene, Meiju Li, Denis F. Mujibi, Paul Stothard, Stephen S. Moore, and Monica C. Leon-Quintero

Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5

Received 30 April 2010/Accepted 30 July 2010

The influence of rumen microbial structure and functions on host physiology remains poorly understood. This study aimed to investigate the interaction between the ruminal microflora and the host by correlating bacterial diversity with fermentation measurements and feed efficiency traits, including dry matter intake, feed conversion ratio, average daily gain, and residual feed intake, using culture-independent methods. Universal bacterial partial 16S rRNA gene products were amplified from ruminal fluid collected from 58 steers raised under a low-energy diet and were subjected to PCR-denaturing gradient gel electrophoresis (DGGE) analysis. Multivariate statistical analysis was used to relate specific PCR-DGGE bands to various feed efficiency traits and metabolites. Analysis of volatile fatty acid profiles showed that butyrate was positively correlated with daily dry matter intake ($P < 0.05$) and tended to have higher concentration in inefficient animals ($P = 0.10$), while isovalerate was associated with residual feed intake ($P < 0.05$). Our results suggest that particular bacteria and their metabolism in the rumen may contribute to differences in host feed efficiency under a low-energy diet. This is the first study correlating PCR-DGGE bands representing specific bacteria to metabolites in the bovine rumen and to host feed efficiency traits.

A fundamental understanding of microbial ecology and relationships to ruminant physiology is essential for successful manipulation of ruminal microflora and subsequent improvement in animal production since rumen microflora play important roles in the nutrient and energy uptake of the host (25). Hence, principles such as niche occupancy, selective pressure, adaptation, and interactions among populations (42) as well as the kinetics of substrate utilization (18) have to be taken into account when evaluating the ruminal microflora and host interactions. Bacterial density in the rumen is high, with direct counts as high as 10 billion cells per gram of ruminal contents (19, 33). Due to the limited understanding of the complex nature of the microbial component and activities in the rumen, the mechanisms of host-microbe and microbe-microbe interactions and whether such interactions impact host biology have not been well established.

Many recent studies have employed molecularly based culture-independent techniques to investigate bacterial profiles (11, 22, 24, 39). PCR-denaturing gradient gel electrophoresis (PCR-DGGE) analysis has been applied to assess ruminal microbial diversity based upon PCR-amplified 16S rRNA fragments to study community interactions (34), monitor populations shifts (23), and screen clone libraries (10). The PCR-DGGE banding patterns are considered to be representative of the dominant bacterial groups (26) and can be applied to screen changes of dominant species in the microflora for large numbers of environmental samples. A new terminology of "microbiome" has been applied to the study of the rumen microbial community, and such studies have further confirmed the complexity of this environment (7). However, many questions remain unanswered. For example, how does the microbiome change in large numbers of animals in response to host, diet, environment, health, and other factors? Which is more important to the host, the whole microbiome or the core microbiome? What is the function of a particular microbiome? Therefore, defining the ruminal microbiome to study its functions and interactions with the host has been an immense challenge. The selection of the rumen microbiome with particular functions after screening by culture-independent methods such as PCR-DGGE, therefore, is essential for high-throughput sequence analysis.

Feed efficiency is one of the most critical factors that impact feed utilization by cattle. We hypothesized that particular bacterial populations in the rumen are associated with fermentation metabolites, which can also influence host feed efficiency. A recent study suggested that the bacterial structure may be associated with cattle's residual feed intake (14); however, the small number of animals used in this study did not provide a direct linkage between a particular microbial population and host feed efficiency traits. The rumen microbial community changes in response to the feeding time (20). Since previous studies have shown that the concentration of volatile fatty acids (VFA) at prefeeding had less variation by diet (31) or by feeding cycles (43) and because of limited access to rumen fluid sampling from the examined commercial population in this study, we centered on the characterization of prefeeding dynamics in the ruminal bacteria and in the fermentation metabolites in 58 steers to test our hypothesis. Therefore, we focused on investigating the associations between rumen bac-

* Corresponding author. Mailing address: 410 Agr/For Centre, University of Alberta, Edmonton, AB, Canada T6G 2P5. Phone: (780) 492-2480. Fax: (780) 492-4265. E-mail: lguan@ualberta.ca.

† Supplemental material for this article may be found at http://aem.asm.org/.

▽ Published ahead of print on 13 August 2010.

teria and host feed efficiency traits using PCR-DGGE analysis, aiming to identify the functional rumen microflora. The traits evaluated were daily dry matter intake (DMI), average daily gain (ADG), feed conversion ratio (FCR) (feed/gain), and residual feed intake (RFI) to measure the feed efficiency of cattle (1, 2, 28). Furthermore, we developed a multivariate statistical analysis to correlate bacterial PCR-DGGE profiles with fermentation measurements such as VFA and ammonia-nitrogen (NH_3-N) in the rumen and with feed efficiency traits, including, DMI, FCR, ADG, and RFI.

MATERIALS AND METHODS

Animals and sampling. Fifty-eight 10-month-old Hereford × Aberdeen Angus steers were raised in accordance with the guidelines of the Canadian Council on Animal Care (CCAC) (29) under feedlot conditions at the Kinsella Research Station, University of Alberta, on a finishing diet as described by Nkrumah et al. (28). The animal protocol was approved by Animal Care and Use Committee (Moore-2006-55), University of Alberta. Feeding intake data were collected using the GrowSafe automated feeding system (GrowSafe Systems Ltd., Airdrie, Alberta, Canada). Diet consisted of a total mixed finishing ration composed of approximately 74% oats, 20% hay, 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). Steers were ranked and allocated to high-RFI (H-RFI; inefficient, mean plus 0.5 standard deviation [SD], $n = 20$), medium-RFI (M-RFI; -0.5 SD $<$ mean RFI $<$ 0.5 SD, $n = 16$), and low-RFI (L-RFI; efficient, mean < -0.5 SD, $n = 22$) groups, based on calculated RFI values as described by Nkrumah et al. (28). Similarly, DMI, ADG, and FCR were obtained by following the procedures outlined by Basarab et al. (4) and Nkrumah et al. (28).

Rumen samples were collected before feeding on the same day within 1 week of completion of RFI measurement. For each animal, 50 to 100 ml of rumen fluid, including feed particles, was obtained by inserting a flexible plastic tube into the rumen. The fluid was transferred into a sterile 200-ml container, immediately frozen in dry ice, and stored at −80°C until further analysis.

DNA extraction. Total DNA was extracted from rumen samples using physical disruption with the bead beating method (14). Briefly, rumen samples were thawed, visually inspected for saliva contamination, manually homogenized, and centrifuged at 14,600 × *g* for 5 min at 4°C. The pellet was washed twice, resuspended in 1 ml of TN150 (10 mM Tris-HCl [pH 8.0], 150 mM NaCl) buffer, and transferred to a 2-ml microcentrifuge tube containing 0.3 g of zirconium beads (diameter, 0.1 mm). The cells were lysed in a BioSpec Mini Bead-Beater-8 at 4,800 rpm for 3 min, followed by phenol-chloroform-isoamyl ethanol (25:24:1) extractions. DNA was precipitated with cold ethanol and resuspended in 30 μl of TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8.0]). The amount and quality of DNA were measured using an ND 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

PCR-DGGE analysis. PCR amplifications of the V2-V3 region (~200 bp) of the 16S rRNA gene of bacteria were performed with universal bacterial primers HDA1-GC and HDA-2 using the program outlined by Walter et al. (40). All PCR products were purified with a QIAquick PCR purification kit (Qiagen, Carlsbad, CA) according to the manufacturer's instructions, and the final concentration was measured using an ND 1000 spectrophotometer (NanoDrop Technologies) before the products were subjected to DGGE analysis.

DGGE was run on 1× TAE buffer (40 mM Tris base, 20 mM glacial acetic acid, 1 mM EDTA) with a 6% polyacrylamide gel with a 22 to 55% linear denaturing gradient using the Bio-Rad DCode universal mutation detection system (Hercules, CA) and 1,294 ng of purified PCR product from each sample. The gel was run at 130 V for 4 h, stained with 0.1% (vol/vol) ethidium bromide after electrophoresis, and photographed using the FluorChem SP imaging system (Alpha Innotech, San Leandro, CA). In order to normalize for differences among different gels, a PCR product from one animal and a ladder containing purified PCR products from all animals were loaded as reference lanes on each gel.

Similarity analysis of PCR-DGGE profiles. PCR-DGGE patterns were analyzed using BioNumerics software, version 5.1 (Applied Maths, Austin, TX), with which hierarchical cluster comparisons were carried out to group similar profiles and to generate a binary matrix of band classes. All the images were normalized using the internal control samples described above, and the comparison among whole profiles was performed using the Dice similarity coefficient (D_{sc}). The dendrogram was generated using the method of unweighted pair group with mathematical averages (UPGMA) at 1% position tolerance. Furthermore, the arithmetic average of the D_{sc} (%) values was calculated. To assign categories to the fingerprint patterns, the D_{sc} was specified at 80% and an application ("script") from the BioNumerics software package was run. Accordingly, relationships between fingerprint patterns and metabolites, as well as between fingerprint patterns and RFI, were assessed.

Cloning and sequencing analysis of DGGE bands. The separated bands were excised aseptically from the gel and transferred to diffusion buffer (0.5 M ammonium acetate, 10 mM magnesium acetate, 1 mM EDTA [pH 8.0], 0.1% SDS). DNA fragments were extracted using the Qiaex II gel extraction kit (Qiagen Sciences, MD) by following the manufacturer's instructions for polyacrylamide gel extraction. Further, the extracted products were reamplified using the same HDA primer pair (without GC clamp) and the same amplification conditions mentioned above. The fresh PCR products were then cloned into the TOP10 vector (Topo TA cloning kit; Invitrogen, Carlsbad, CA) using chemical transformation. Colonies were selected on S-Gal medium (Sigma, St. Louis, MO) and randomly picked, and, from three replicates with insertions, plasmid DNA was extracted using a plasmid extraction kit (Millipore, Billerica, MA). The sequence reaction was performed in a 10-μl total volume containing 0.5 μl of BigDye, 3.2 pmol of M13 forward (CGC CAG GGT TTT CCC AGT CAC GAC) primer, 2.0 μl of 5× sequencing buffer, and 20 ng of plasmid DNA as the template with the ABI 3730 sequencing system using the ABI Prism BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA). All sequences were subjected to BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to determine the closest known taxon and were aligned using the ClustalW program (http://www.ebi.ac.uk/Tools/clustalw2). The sequence composition of each band was compared using the RDP Classifier online tool (http://rdp.cme.msu.edu/) (41).

Multivariate statistical analysis. Principal component analysis (PCA) was used to observe the structure of the data and to identify the most important response variables. Thirteen variables (Table 1) were included in the analysis (acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, total VFA, acetate/propionate ratio, branched-chain VFA/straight-chain VFA ratio, ammonia, daily dry matter intake, average daily gain, and feed conversion ratio). PCA was performed using the PRINCOMP procedure in SAS (version 9.1; SAS Institute, Cary, NC). This procedure standardizes the variables to a mean of zero and a standard deviation of one. The loadings (eigenvectors) in each principal component were retained when the loadings were greater than the absolute average eigenvalue for that component.

Each DGGE pattern from an individual steer was assigned by BioNumerics software to generate a calculated best-fit Gaussian curve for each band. All the assigned bands from each animal were then exported with the normalized relative position. According to the above PCR-DGGE profile similarity analysis, 1% tolerance was used to rectify the shifts among all the bands from all profiles. A binary matrix where all the bands were allocated into 85 new categories was created for 58 animals. Once the clustering tendency was observed, a categorical model in SAS based on maximum likelihood was fit to analyze the interaction of the phenotypic traits with the bands. All variables were categorized as high (H) and low (L) using PROC MEANS in SAS and taking 0.5 standard deviation as the cutoff point. Then they were used to define the presence of particular bands for each variable. In PROC CATMOD, the effect of all variables on the prevalence of every band was determined based on the transformation of the cell probabilities (response function). This model analyzed a data matrix containing either the averaged Gaussian position of the band or zero, indicating class. Afterwards, two-way contingency tables of cross classifications containing the frequencies of the bands per category (high/low) were obtained using PROC FREQ and results were plotted.

Analysis of fermentation parameters: VFA and NH_3-N. Rumen fluid was centrifuged, and supernatant was subjected to VFA profiling using gas chromatography (GC) analysis with a Perkin-Elmer (Waltham, MA) gas chromatographer by following standard procedures (14). An enzymatic assay was carried out to measure NH_3-N using a commercial kit (R-Biopharm Roche Inc., South Marshall, MI) by following the manufacturer's instructions, based on spectrophotometer readings at a wavelength of 340 nm (SpectraMax 190; Molecular Devices, Sunnyvale, CA).

Differences in VFA composition and ammonia were compared using the simple covariance mixed model of SAS; to find the interactions between the metabolites, statistical correlations were carried out and *P* values were recorded. Significance was assumed at *P* values of <0.05.

RESULTS

PCR-DGGE analysis of detectable bacteria in rumen samples. The predominant bacteria in the rumens of 58 steers were initially compared using PCR-DGGE analysis. The PCR-

TABLE 1. Sequence identification of the PCR-DGGE bands

PCR-DGGE band category	Taxonomy (GenBank accession no.)	Identity (%)	Trait(s) associated[a]
1	*Prevotella* sp. (AF218619)	95	
2	*Prevotella* sp. (AF218619)	97	L Acet, L Val, L A/P
3	*Prevotella maculosa* strain W1609 (EF534315)	94	
4	—[b]		
5	Uncultured *Succinivibrio* sp. clone EMP_B23 (EU794184)	100	
6	*Prevotella* sp. BP1-56 (AB501155)	95	
7	*Lactobacillus* sp. DI71 (AB290831)	100	
8	—		
9	*Blautia* sp. BM-C2-0 (GQ456220)	97	L A/P
10	*Clostridium symbiosum* strain 69 (EF025909)	98	L Prop, L But
11	*Prevotella oulorum* strain WPH 179 (NR_029147)	94	L St/Br
12	*Prevotella denticola* clone WWP_SS6_P23 (GU409439)	97	
13	*Prevotella ruminicola* isolate L16 (AY699286)	93	L RFI, L DMI, L ADG, L FCR, L Acet, L Prop, L But, L Isobut, L Val, L Isoval, L Total VFA, L A/P, L St/Br, L ammonia
14	*Lachnospiraceae* genomospecies C1 (AY278618)	99	L ammonia
15	Uncultured *Lachnospiraceae* bacterium		L FCR, L Prop, L But, L Isobut, L Val, L Isoval, L total VFA, L A/P, L St/Br
16	*Ruminococcus gauvreauii* strain CCRI 16110 (EF529620)	100	L RFI, L DMI, L ADG, L FCR, L Acet, L Prop, L But, L Isobut, L A/P, L St/Br, L ammonia
17	*Prevotella* aff. *ruminicola* Tc2-24 (AJ009933)	97	L Acet, L Prop, L But, L Total VFA, L A/P, L St/Br
18	*Prevotella ruminicola* (AB219152)	99	L DMI, H ADG, L FCR, L Prop, L But, L Isobut, L Val, L Isoval, L total VFA, L A/P, L St/Br, H ammonia
19	—		
20	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)	98	L FCR, L Acet, L But, L Val, L Isoval, L A/P
21	*Pelotomaculum thermopropionicum* SI (AP009389)	93	H ADG, L FCR, L Prop, L But, L Val, L Isoval, L Total VFA, L A/P, L St/Br
22	Uncultured *Succinivibrio* sp. clone EMP_B23 (EU794184)	100	L FCR, L Acet, L Prop, L But, L Val, L Isoval, L A/P
23	—		
24	*Clostridium populeti* strain 743A (NR_026103)	100	L FCR, L Prop, L Val, L St/Br
25	*Prevotella oulorum* (L16472.2)	94	H But, L FCR, L St/Br
26	—		
27	Uncultured *Prevotella* sp. clone T0505 (GU458954)	93	L DMI, L ADG, L Prop, L But, L Val, L St/Br
28	*Lachnospiraceae*		
29	—		
30	*Prevotella maculosa* strain GEJ21 (GU561342)	97	L ADG, L A/P
31	*Butyrivibrio fibrisolvens*, strain Mz3 (AM039822)	98	L ammonia-N
32	*Prevotella ruminicola* (AB219152)	94	
33	*Photobacterium* sp. M2 (EU046607)	87	
34	*Ruminococcus gauvreauii* strain CCRI 16110	100	L ADG, L A/P
35	Uncultured *Prevotella* sp. clone JD9 (FJ268952)	96	
36	*Butyrivibrio fibrisolvens* strain H15 (EU887842)	98	
37	Uncultured *Prevotella* sp. clone Sew1-325 (FJ219872)	95	
38	*Clostridium indolis* (AF028351)	97	
39	Prevotellaceae		
40	*Vibrio* sp. WH134 (FJ847833)	86	
41	*Ruminococcus* sp. ZS2-15 (FJ889653)	90	
42	*Prevotella* sp. 152R-1a (DQ278861)	97	
43	Uncultured *Roseburia* sp. clone M2-35 (EU530245)	100	
44	Uncultured *Prevotella* sp. clone JD9 (FJ268952)	96	
45	—		
46	Uncultured *Prevotella* sp. clone JD9 (FJ268952	96	
47	*Hespellia porcina* strain PC80 (NR_025206)	98	
48	*Lactobacillus* sp. DI71 (AB290831)	100	
49	Uncultured *Prevotella* sp. clone Gull85-50 (FJ220908)	98	
50	*Prevotella ruminicola* strain TC2-3 (AF218617)	97	
51	*Robinsoniella peoriensis* strain HGUE-09/9434 (GU322806)	98	
52	*Succiniclasticum ruminis* strain DSM 9236 (NR_026205)	97	
53	*Ruminobacter amylophilus* strain H 18 (NR_026450)	99	
54	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)	96	

Continued on following page

TABLE 1—*Continued*

PCR-DGGE band category	Taxonomy (GenBank accession no.)	Identity (%)	Trait(s) associated[a]
55	Uncultured *Succinivibrio* sp. clone EMP_J46 (EU794280	89	
56	Ruminococcaceae		
57	*Eubacterium xylanophilum* (L34628)	98	
58	*Moryella indoligenes* strain AIP 220.04 (DQ377947)	99	
59	Uncultured *Succinivibrio* sp. clone EMP_V30 (EU794288)	100	
60	*Anaerophaga thermohalophila* strain Fru22 (NR_028963)	88	
61	*Eubacterium rangiferina* (EU124830)	97	
62	*Robinsoniella peoriensis* strain HGUE-09/9434 (GU322806)	98	
63	*Eubacterium rectale* ATCC 33656 (CP001107)	98	
64	*Butyrivibrio fibrisolvens* strain H15 (EU887842)	94	
65	*Robinsoniella peoriensis* strain HGUE-09/9434 (GU322806)	98	
66	Uncultured *Succinivibrio* sp. clone EMP_B23 (EU794184)	97	
67	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)	96	
68	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)	97	
69	Uncultured *Prevotella* sp. clone 3083 (FJ976203)	93	
70	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)	95	
71	*Coprococcus eutactus* strain ATCC 27759 (EF031543)	99	
72	*Clostridium indolis* (AF028351)	97	
73	Uncultured *Succinivibrio* sp. clone EMP_V30 (EU794288)	100	
74	Uncultured *Succinivibrio* sp. clone EMP_V30 (EU794288)	100	L But
75	—		
76	*Moryella indoligenes* strain AIP 220.04 (DQ377947)	98	L Acet, L But, L A/P
77	*Succinivibrio dextrinosolvens* strain 0554 (NR_026476)	98	L Acet, L Prop, L But, L Isoval, L A/P
78	—		
79	*Succinimonas amylolytica* strain DSM 2873 (NR_026475)	94	L Isoval
80	*Ruminococcus bromii* strain YE282 (DQ882649)	93	L ADG, L Acet, L But, L Isobut, L Isoval, L total VFA, L A/P, L St/Br, L ammonia
81	—	96	L RFI, L DMI, L ADG, L FCR, L Acet, L Prop, L But, L Isobut, L Isoval, L A/P
82	—		
83	*Selenomonas ruminantium* strain: S211 (AB198441)	96	L Isoval, L St/Br, L ammonia
84	*Bifidobacterium ruminantium* strain KCTC 3425 (GU361831)	100	L Acet, L ammonia
85	—		

[a] L, low; H, high; Acet, acetate; Val, valerate; Prop, propionate; But, butyrate; St/Br, straight-chain VFA–to–branched-chain VFA ratio; Isobut, isobutyrate.
[b] —, bands could not be successfully cloned and sequenced.

DGGE profiles showed that each animal harbored an individual bacterial flora, evidenced by the presence of complex band patterns (Fig. 1), with an average Dice similarity coefficient (D_{sc}) for all PCR-DGGE profiles of 75.5%. When RFI values were included in the similarity analysis, no significant trend was observed. The RFI was chosen to be correlated with DGGE profiles because it has been described as the most desirable measure of feed efficiency (2).

Based on the positions of each band from the PCR-DGGE band patterns from all animals, 85 band categories were identified using BioNumerics software. To characterize the taxonomy of the bands, all of them were purified, cloned, and sequenced and 74 bands were identified (Table 1). The following criteria were used to determine the taxonomy of each band: a 96% or higher match between the clone sequence and the GenBank data was considered to represent identity at the species level (accession numbers are in Table 1), and a 90 to 95% match represented identity at the genus level, as given by the RDP Classifier online tool. When the accession number is not provided, the percentage corresponds to identity at the genus level, as matched by RDP Classifier. From the sequences obtained from the 74 PCR-DGGE bands, 33 of them corresponded to strains from known species of the following genera: *Prevotella*, *Clostridium*, *Ruminococcus*, *Succinivibrio*, *Butyrivibrio*, *Robinsoniella*, *Eubacterium*, *Moryella*, *Coprococcus*, *Bifidobacterium*, *Pelotomaculum*, *Succiniclasticum*, *Ruminobacter*, *Anaerophaga*, *Succinimonas*, *Selenomonas*, and *Lactobacillus*. Six sequences were identified only at the genus level (bands 1 and 2, *Prevotella*; band 24, *Clostridium*; bands 34 and 56, *Ruminococcus*; band 63, *Eubacterium*). Fifteen sequences matched uncultured clones from species of the following genera: *Prevotella*, *Succinivibrio*, and *Roseburia*. Four sequences were identified only at the family level (band 39, *Prevotellaceae*; bands 14 and 28 *Lachnospiraceae*; band 15, uncultured *Lachnospiraceae*).

Analysis of fermentation profiles. To investigate the associations between bacterial diversity and its functions in the rumen, we measured the VFA and ammonia-nitrogen (NH_3-N) concentrations of the rumen samples. To minimize the influence of the sampling method on the VFA concentrations, due to the dilution of the rumen fluid by the saliva and the time elapsed since the last meal, the proportion of each VFA to the total VFA concentration was obtained and used as the depen-

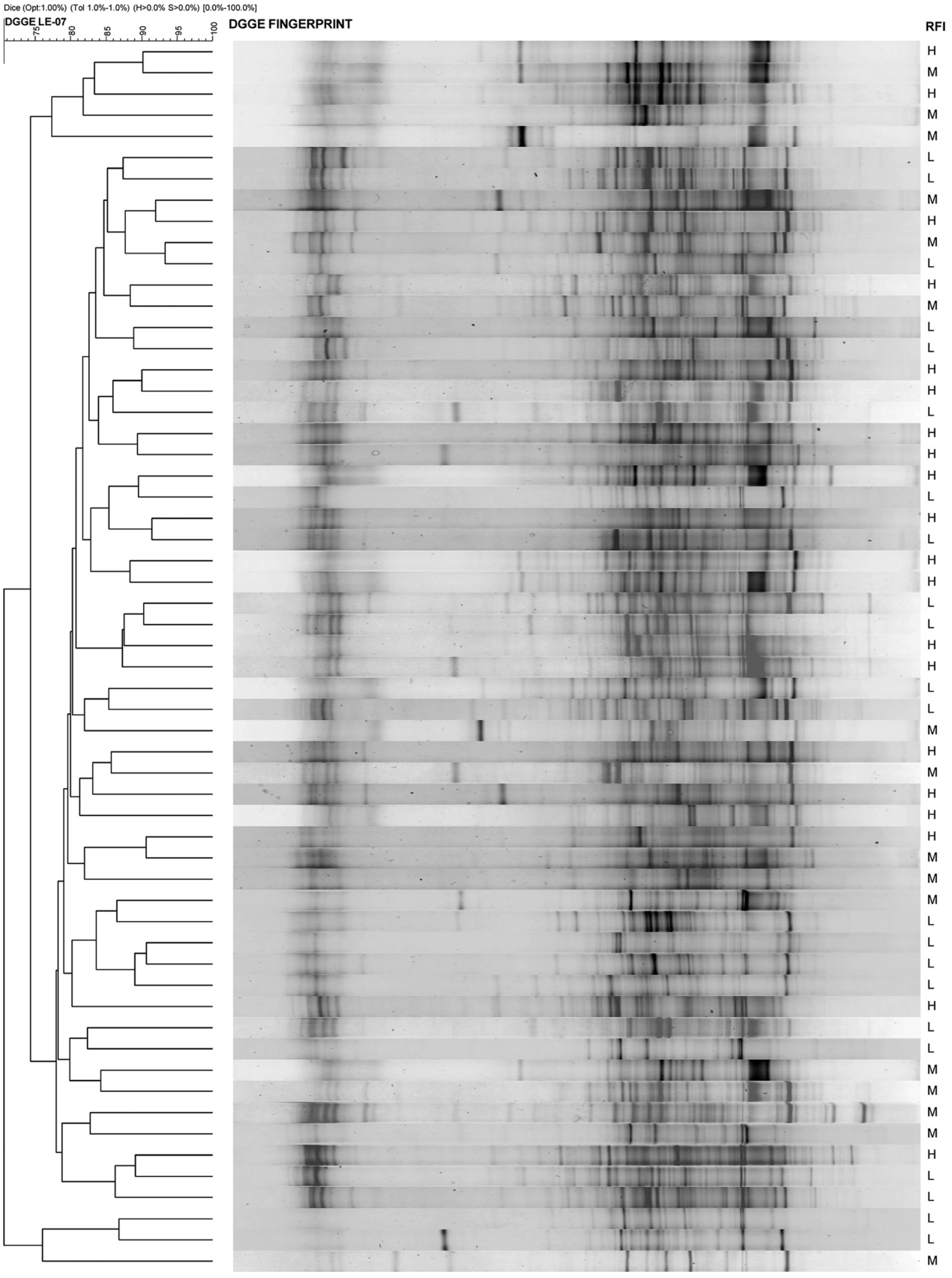

FIG. 1. PCR-DGGE profiles generated from ruminal fluid DNA from 58 steers fed with a low-energy diet using primers HDA1-GC and HDA2 (22 to 55% DGGE). H, M, and L represent the steers with high residual feed intake (RFI; a parameter to measure feed efficiency in cattle [2]) (RFI > 0.5, inefficient), medium RFI ($-0.5 <$ RFI < 0.5), and low RFI (RFI < -0.5, 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.

TABLE 2. Fermentation and feed efficiency measurements in 58 steers with differing RFIs and fed with a low-energy diet

Variable	Mean value ± SEM for:		*P*
	High RFI ($n = 20$)	Low RFI ($n = 22$)	
Acetate (%[a])	54.58 ± 1.23	54.92 ± 1.18	0.84
Propionate (%[a])	31.45 ± 1.22	33.41 ± 1.16	0.25
Butyrate (%[a])	9.51 ± 0.76	7.26 ± 0.73	0.10
Isobutyrate (%[a])	9.53 ± 0.06	8.29 ± 0.05	0.11
Valerate (%[a])	1.00 ± 0.07	1.04 ± 0.07	0.67
Isovalerate (%[a])	2.37 ± 0.15	1.92 ± 0.14	0.03
Total VFA (mM)	58.55 ± 5.49	64.17 ± 5.24	0.75
Acetate/propionate ratio	1.87 ± 0.13	1.69 ± 0.12	0.32
Straight-chain/branched-chain VFA ratio	30.95 ± 2.27	38.06 ± 2.17	0.03
Ammonia concn (mM)	0.096 ± 0.01	0.11 ± 0.01	0.43
Dry matter intake (kg)	8.65 ± 0.14	6.94 ± 0.13	<0.0001
Avg daily gain (kg)	1.26 ± 0.04	1.22 ± 0.04	0.39
Feed conversion ratio (feed/gain)	6.95 ± 0.16	5.76 ± 0.15	<0.0001

[a] Percentage of the total VFA concentration.

dent variable for the metabolite analysis. The VFA profiles detected in the rumen samples were consistent with those previously reported (13, 35). The proportion of isovalerate was significantly higher in the H-RFI animals ($P = 0.03$), while the straight-chain VFA-to-branched-chain VFA ratio was significant lower ($P = 0.03$) in the same group of animals (Table 2). Butyrate tended to be higher in the rumens of H-RFI steers ($P = 0.10$). DMI and FCR values were significantly different between L-RFI and H-RFI animals, similar to results previously reported (28). In addition, there were significant statistical correlations among all VFA, between butyrate and DMI, and between isovalerate and RFI, as well as between DMI and isovalerate (see Table S1 in the supplemental material). On the other hand, the concentrations of NH_3-N were not significantly different among different groups of animals (Table 2; see Table S1 in the supplemental material).

Correlations among PCR-DGGE profiles, fermentation characteristics, and host feed efficiency traits. To identify the correlations among rumen microbial structure, fermentation measurement, and host feed efficiency, the association among all variables showing in Table 1 was investigated using principal component analysis (PCA). Three significant principal components, describing 70% of the total variance, were extracted (data not shown). In the first principal component (PC1), the acetate/propionate ratio (A/P), isovalerate, isobutyrate, and acetate had the highest contributions and they were orthogonal to DMI and butyrate, which were described in the second principal component (PC2). Also, RFI, FCR (feed/gain), and the straight-chain VFA/branched-chain VFA ratio were in PC3, independent from the variables in PC1 and PC2. The only variable associated with RFI was FCR, as they had similar loadings in PC3. When PC1 was plotted with PC2, five variables, including butyrate, isovalerate, DMI, FCR, and RFI, were in the same quadrant (see Fig. S1 in the supplemental material).

A maximum-likelihood approach was used to reveal the direct linkage between a specific group of bands and particular fermentation/feed efficiency estimates since the above PCA analysis failed to include all the bands as variables or show the direct linkage among different variables. Using the CATMOD procedure in SAS, the effect of fermentation and feed efficiency traits on the prevalence of every band was analyzed based on 85 bands determined from all animals. Two-dimensional tables were created, and it was found that all bands were significantly different from each other by their locations on the DGGE gel ($P < 0.0001$). Based on the above analysis, the frequency plots of the bands showed the following results. Eight bands associated with DMI, 6 with high DMI and 2 with low DMI (Fig. 2A); 10 bands were correlated with ADG, 8 with high ADG and 2 with low ADG (Fig. 2B); 13 bands were associated with FCR, 5 with high FCR and 8 with low FCR (Fig. 2C); and 8 bands were exclusive to the RFI, 6 with L RFI and 2 with H RFI (Fig. 2D). Additionally, particular PCR-DGGE bands were also found to be linked to fermentation measures as follows: nine bands were linked to isovalerate, 6 with high isovalerate and 3 with low isovalerate (Fig. 2G), and 10 were linked to the straight-chain VFA/branched-chain VFA ratio, 1 with high ratio and 9 with low ratios (Fig. 2F). These two traits were significantly different between L-RFI and H-RFI animals (Table 2). Ten bands were correlated with butyrate, 5 with high butyrate and 5 with low butyrate (Fig. 2E), and four bands were associated with isobutyrate (Fig. 2G); these two traits were tentatively associated with RFI (Table 2). Similarly, associations between PCR-DGGE bands and the other 11 variables were observed (see Fig. S2A to F in the supplemental material). Some bands were commonly identified from more than one trait. For example, band 15 (uncultured *Lachnospiraceae* bacterium) was associated with low FCR, low propionate, low butyrate, low isobutyrate, low valerate, low isovalerate, low total VFA, low A/P ratio, and low straight-chain VFA/branched-chain VFA ratio and band 20 (*Succinivibrio dextrinosolvens*) was related to low FCR, low acetate, low butyrate, low valerate, low isovalerate, and low A/P ratio.

DISCUSSION

Ruminant animals derive about 70% of their metabolic energy from microbial fermentation of feed particles (5). Microbial fermentation degrades feed components and generates end products such as short-chain VFAs, carbon dioxide, methane, and ammonia. Metabolic energy is used in the synthesis of cellular components needed for microbial growth and other functions (27), whereas the VFAs are mainly absorbed and used as the main energy source by the host. To date, the relationship among the rumen microbial community, the microbial fermentation profiles, and RFI is not well studied.

We speculated that the differences in some bacterial profiles (PCR-DGGE bands) may be related to probable observed associations between the fermentation measurements and feed efficiency. In this study, attempts to link the bacterial community structure with phenotypic traits were made by correlating PCR-DGGE profiles with VFA concentrations and with various feed efficiency traits. VFA concentrations in the rumen represent the balance between microbial production and host epithelial transport and absorption. We considered that the concentration of VFA can be interpreted as one of the indicators of microbe-microbe interactions as well as host-microbe

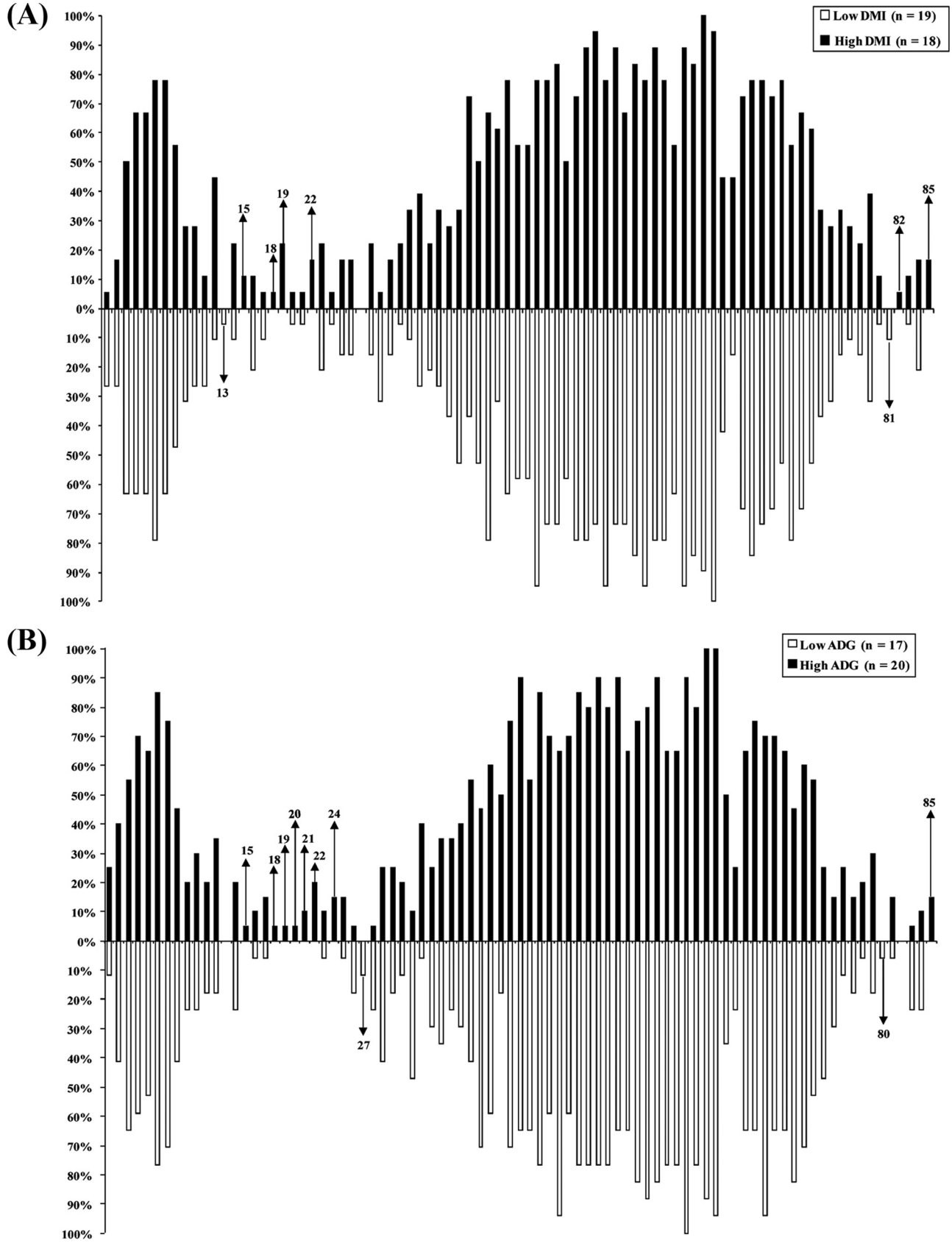

FIG. 2. Frequency of PCR-DGGE bands in animals categorized on the basis of dry matter intake (DMI) (A), average daily gain (ADG) (B), feed conversion ratio (FCR; F/G) (C), RFI (D), butyrate (E), straight-chain-to-branched-chain VFA ratio (F), isobutyrate (G), and isovalerate (H) using PROC CATMOD analysis. The *x* axis represents 85 identified bands, and the order of the bands reflects the migration locations on the PCR-DGGE gel from top to the bottom. The arrows indicate the frequency of bands detected in the tested population for each trait.

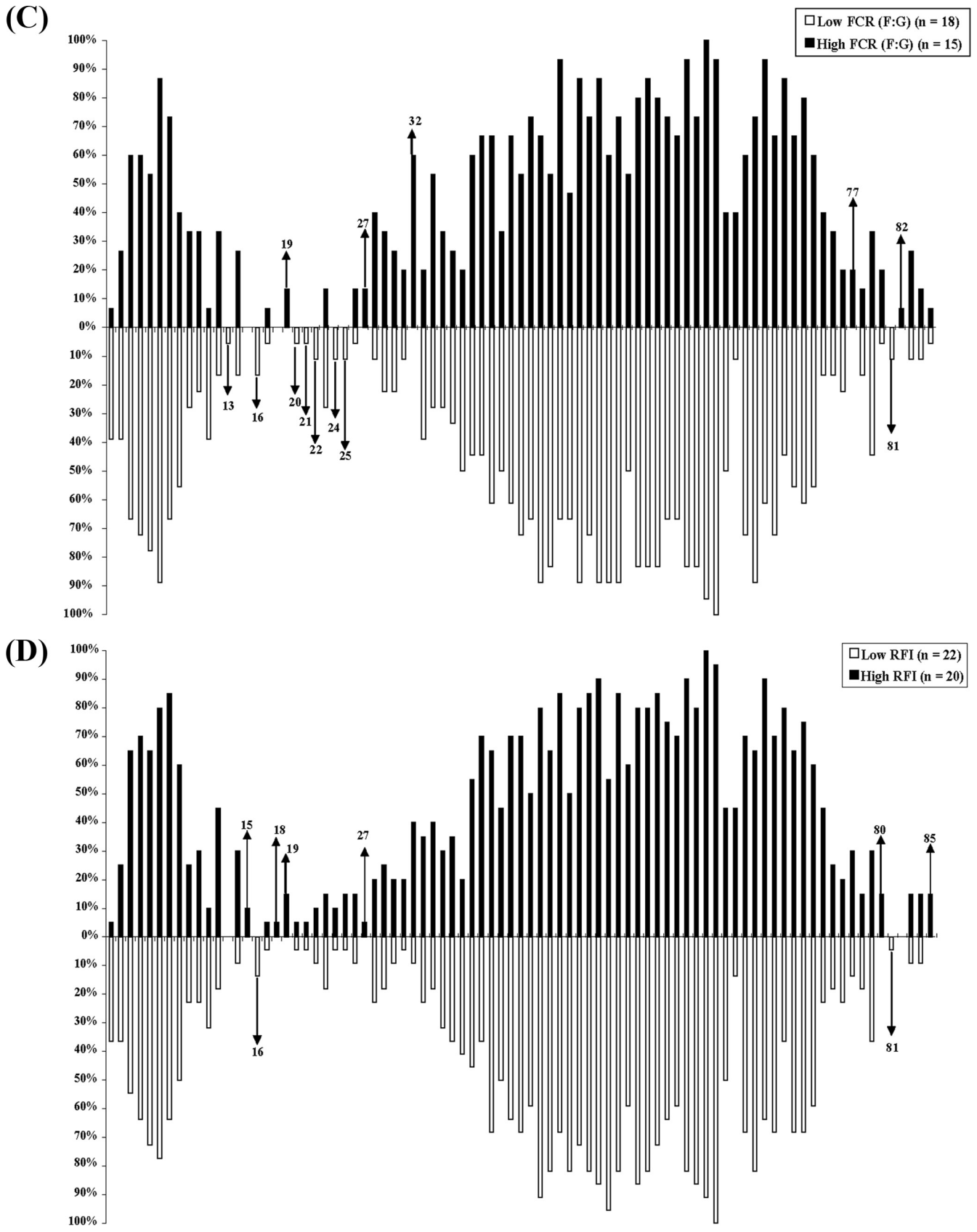

FIG. 2—*Continued.*

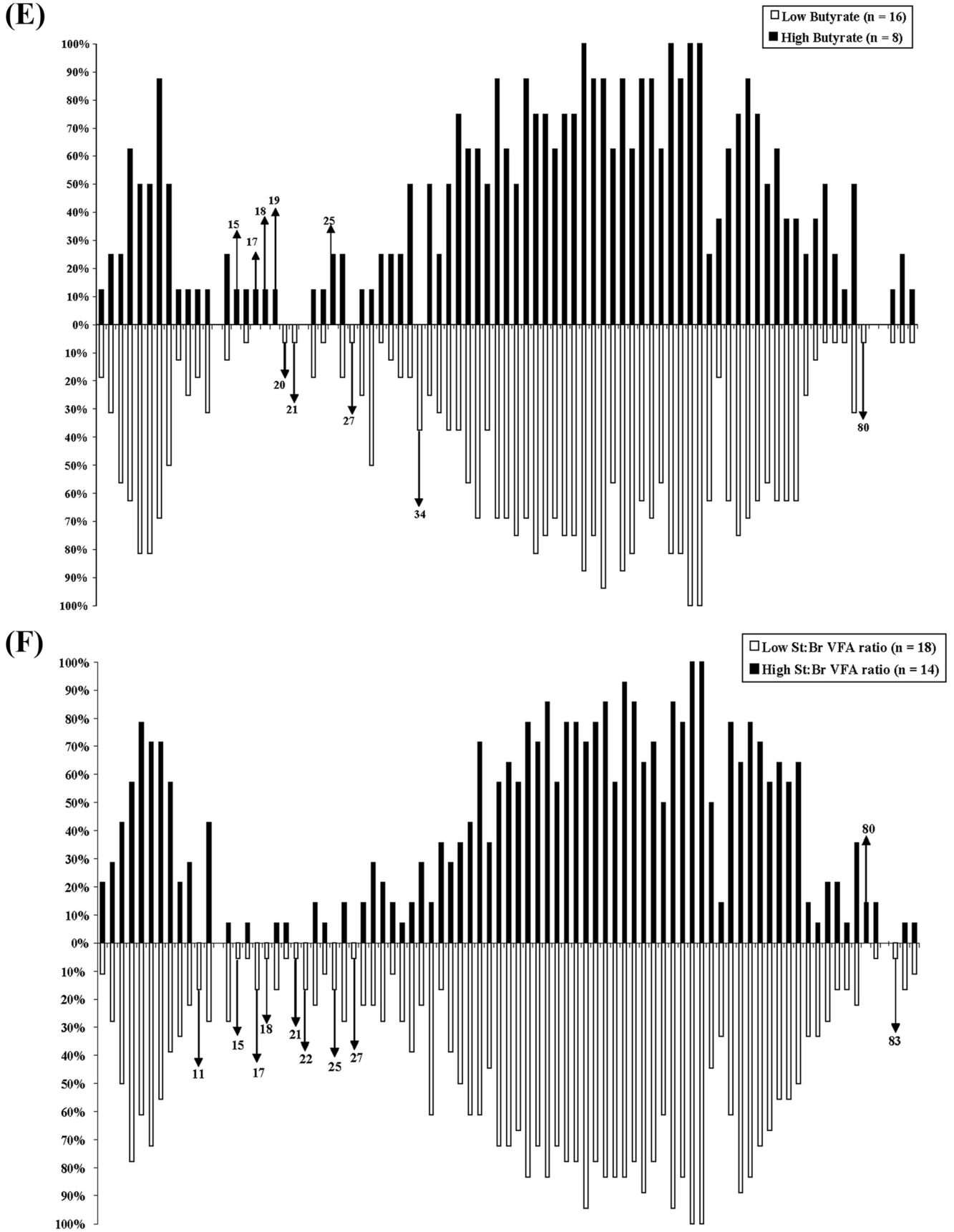

FIG. 2—*Continued.*

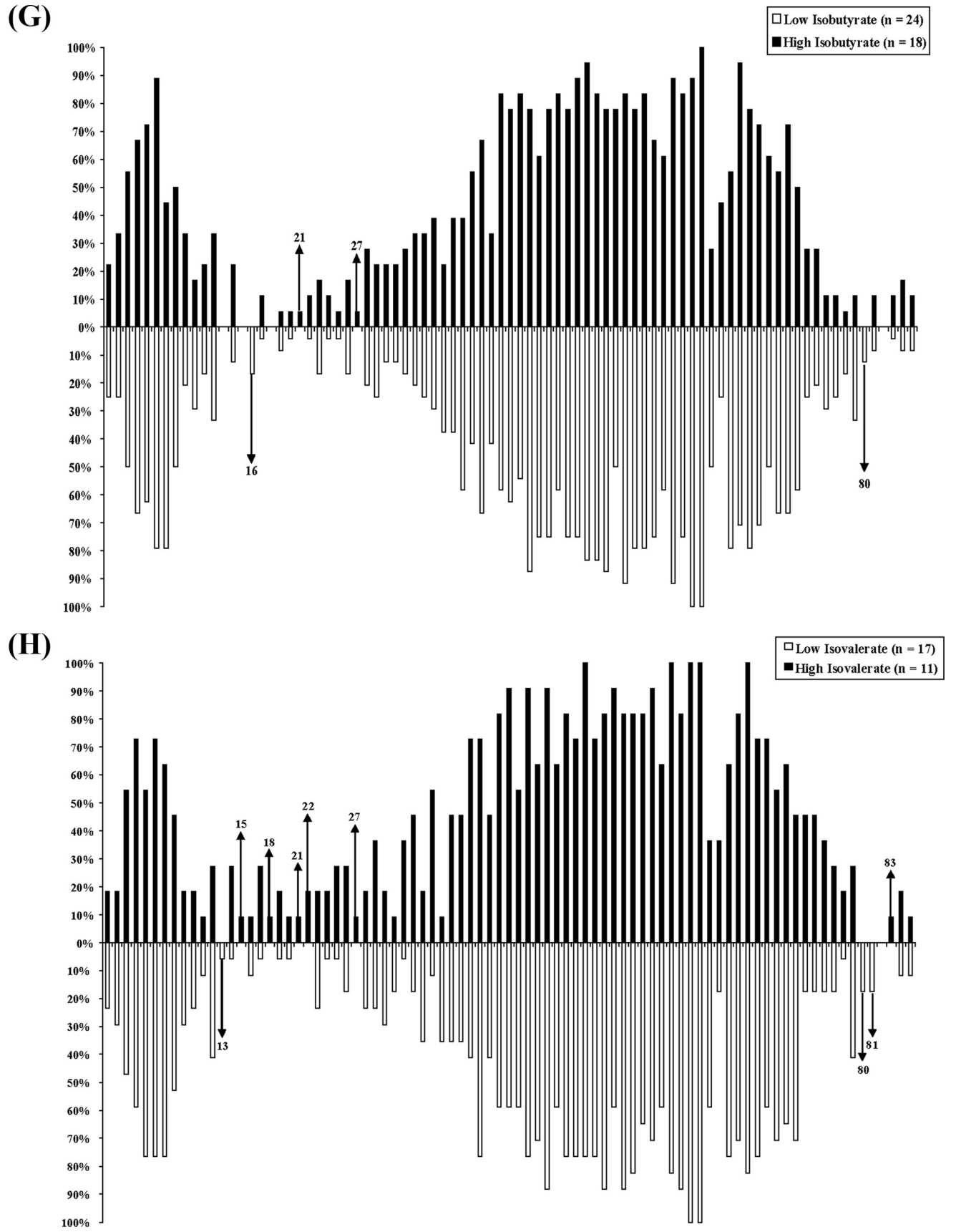

FIG. 2—*Continued.*

interactions. A direct method to categorize the whole PCR-DGGE patterns did not allow the separation of the animals with different RFIs (data not shown), suggesting that not all the dominant bacteria are relevant for this trait. From the multivariate statistical analysis, the frequencies of the bands in 58 steers revealed that all individuals have a "core" bacterial structure (bands 1 to 12, bands 37 to 75) (Fig. 2; see Fig. S2 in the supplemental material), which was not correlated with any variable examined and was present in all traits. Since all the animals shared the majority of the bands, the results agree with those observed in the PCR-DGGE dendrogram and in the pattern analysis, suggesting that some bacterial species in the rumen may be the key players influencing feed conversion in the rumen. Three sequenced bands associated with low straight-chain-to-branched-chain VFA ratio (band 11, *Prevotella* sp.), high butyrate, low FCR, and low straight-chain-to-branched-chain VFA ratio (band 25, *Prevotella oulorum*), and low ammonia-N (band 31, *Butyrivibrio fibrisolvens*) were identified. It is not surprising that, as a predominant population in the rumen (37), the *Prevotella* sp. is associated with fermentation profiles. However, *Prevotella oulorum* (band 25) was also associated with the same trait and FCR, one of the feed efficiency traits, suggesting that more than one bacterial species is associated with such a low straight-chain–to–branched-chain VFA ratio; this is also supported by the fact that the *Clostridium* sp. (band 24) is associated with low straight-chain–to–branched-chain VFA ratio and FCR. Our study has also identified potential associations of novel species with specific functions; for instance, band 21, which was associated with low FCR and low propionate, corresponds to an anaerobic propionate-oxidizing bacterium (17), *Pelotomaculum thermopropionicum.* Similarly, band 76, which was related to low acetate, low butyrate, and low A/P ratio, was identified to be *Moryella indoligenes*, an anaerobic bacterium that has acetate and butyrate as major metabolic end products (8). In addition, the band representing *Butyrivibrio fibrisolvens* is associated with ammonia-N but not with butyrate, also revealing the limitations of studying the rumen microbial community at the taxonomy level; further functional studies such as investigation of the enzymes involved in the amino acid metabolism need to be done.

The observation of significant correlation between butyrate, isovalerate, and DMI and between isovalerate and RFI in this study supplied more preliminary data to support our hypothesis of the associations between rumen microbial diversity, fermentation profiles, and host feed efficiency. The energetic metabolism has been reported to be significantly different in beef cattle with a different RFI (15, 28), suggesting that ruminal microbial fermentation plays important roles in this trait. The possible association between butyrate and DMI under a low-energy diet found in this study suggests that microbial fermentation generated substrates involved in the energetic metabolism of the host that may be associated with the differences in RFI, since DMI is a fundamental element in deriving RFI and represents the extreme end of the feed conversion axis. Although a higher concentration of butyrate has been found to be associated with L-RFI animals (14), in our study contrasting observations were recorded and lower butyrate was associated with L-RFI animals. The diet difference in the present experiment might explain the above difference, since animals tested were under a low-energy-density feedlot diet, whereas the results of Guan et al. are based on steers fed a high-energy-density feedlot diet. Previous studies showed that butyrate is mainly used as an energy source for the host (13) and that butyrate increased significantly when animals were fed a high-energy diet compared to a low-energy diet (13, 32), suggesting that this and different butyrate metabolic pathways may contribute to RFI under different diets. Therefore, the effect of the diet needs to be considered when the interaction between the ruminal microflora and host RFI is investigated. Future studies on the relationships between the differences in butyrate concentration in the same steers under the high-energy diet may supply extensive evidence which could assist in associating feed efficiency traits with this VFA.

Branched-chain VFA are derived from branched-chain amino acids such as leucine, valine, and isoleucine, and the variations in the ratio of branched-chain VFAs to straight-chain VFAs indicate altered branched-chain amino acid catabolism (30). Therefore, we considered the ratio of branched-chain to straight-chain volatile fatty acids to be an indicator of amino acid fermentation in this study. We hypothesized that the higher straight-chain–to–branched-chain VFA ratio as well as the higher concentrations of isovalerate and isobutyrate in the L-RFI animals suggests that more-efficient N flow may also be associated with improved feed efficiency when a low-energy-density diet was fed (3). Further studies on the association between microbial crude protein and RFI will supply better understanding of the relationship between microbial fermentation profiles and host feed efficiency. Although we showed possible associations between some VFAs and feed efficiency traits, our data are limited and can be biased, because the VFAs measured in this study were collected at a single time point and feed efficiency traits were recorded for a longer period. Recent studies have shown that rumen fermentation was highly associated with the time after feeding (20) and that the total VFA concentration reached the highest level at 9 h after the morning feeding but that concentrations did not differ at other sampling times (36). Although the total VFA concentrations remained above prefeeding levels throughout postfeeding, it was found that prefeeding VFA concentrations were similar for different diets (31), as well as different individual cows and feeding cycles (43). Welkie et al. (43) reported that VFA concentrations increased after feeding due to microbial fermentation, reaching a maximum value at 6 h postfeeding, and then declined to approximately the initial values. Similarly, a previous study by Bevans et al. (6) has shown that VFA concentrations were lowest before feeding, highest at 8 h after feeding, and intermediate 18 h after feeding. Hence, the VFA measured before feeding may be applied as the baseline measurement, avoiding overestimation of the rumen bacterial community, and can be representative of the variations of rumen microbial diversity for each animal. In addition, Sun et al. found that VFA concentrations peaked at 12 days after feeding goats with a diet similar to the one used in our study but returned to lower levels afterwards, indicating that longer periods of adaptation can lead to a more stable rumen environment and fermentation characteristics (38). Such a trend was supported by Hristov et al. (16), who detected a decline in VFA concentrations after 15 days of feeding a high barley diet. Thus, a sample taken after 90 days of feeding can be consid-

ered stable enough to represent the fermentation profiles. Furthermore, there is the inconvenience of cannulating 58 steers to obtain samples, and, while the variations in the VFA concentration among animals increase with oral sampling, a larger sample size leads to increased precision in estimating these variables. Lodge-Ivey et al. (21) conducted a study to determine if sampling rumen contents via a ruminal cannula or oral lavage tube would yield similar PCR-DGGE profiles of the bacterial community and fermentation metabolites. When samples were grouped according to band pattern similarity, groups were most stable according to individual animal and species rather than sampling method. These data indicate that rumen samples collected via oral lavage or rumen cannula yield similar results.

Therefore, our VFA data measured prefeeding are valid to indicate the potential associations between the rumen microbial community and fermentation profiles of the individual and its feed efficiency traits; this is a new concept that links the microbial molecular ecology to animal production. However, in order to provide a comprehensive biological relationship between VFA and feed efficiency traits, it is necessary to collect rumen samples at multiple time points during the period for recording intake variables.

In addition, the observed correlation between the butyrate and isovalerate concentrations and the feed efficiency traits might also be associated with microbial interactions such as interactions between bacteria and archaea. Another recent study revealed that the methanogenic community in L-RFI animals was more diverse than that in H-RFI animals (44), indicating that interactions between different microbial groups may also impact fermentation and feed efficiency parameters. To achieve a realistic estimate of total microbial growth as well as relative numbers of individual species within the rumen, a quantitative understanding of microbial relationships is essential.

Compared to DMI, ADG, and FCR traits, RFI has been recently considered a more desirable measurement for feed efficiency (9). Many factors, such as host genetics, diet, environment, management, and genetic-environmental interactions may directly or indirectly influence RFI. Our method of associating RFI with the identified PCR-DGGE bands (bacterial species) provides a better insight into the complexity of this trait, supporting our speculation that rumen fermentation may play a key role in this trait. Further studies of the impact of diet on RFI and on microbial diversity and fermentation parameters after switching a low-energy diet to a higher-energy diet for the same group of animals are in progress.

In conclusion, this study identified probable associations between ruminal ecology and activities and cattle feed efficiency by defining a statistical method to link the PCR-DGGE profile, microbial fermentation parameters, and RFI. This is the first attempt to categorize bacterial PCR-DGGE band patterns in the rumen and to link them to phenotypic characteristics of the host, specifically to feed efficiency. From the multiple-variable analysis, the bands representing some specific groups of bacteria may be associated with some measurable phenotypic parameters. To identify the functions of ruminal microbes, it is believed that a "consortium" may play more important roles than a single species. Our study supplies a way to identify such a consortium, which can be applied in screening the microbial community from large numbers of animals; further, the sequencing analysis of all the bands supplies information about the consortium. However, due to the limitations of the existing database, the results obtained from any sequencing analysis (including the output of the recently developed pyrosequencing technology) usually indicate many "unculturable" and "unidentified" ruminal bacteria. The statistical analysis to identify the specific bacterial PCR-DGGE profiles will identify the functions of such unculturable and unidentified ruminal species. Our work also supplies a potential method to identify functional rare (noncore) species of gut microbes contributing to host biology, which will provide fundamental knowledge for understanding the microbe-host interactions and which can also be extended to the study of functional microbes in various environmental microbial communities.

ACKNOWLEDGMENTS

This research was funded by the Alberta Livestock Industry Development Fund, the Alberta Agricultural Research Institute, and the Alberta Livestock and Meat Agency (ALIDF/AARI/ALMA 2007F041R) and by a CONACyT Scholarship.

We acknowledge Kelvin Lien, Masaaki Taniguchi, and Michael Gänzle for their kind assistance.

REFERENCES

1. **Archer, J. A., E. C. Richardson, R. M. Herd, and P. F. Arthur.** 1999. Potential for selection to improve efficiency of feed use in beef cattle: a review. Aust. J. Agric. Res. **50:**147–161.
2. **Arthur, P. F., J. A. Archer, D. J. Johnston, R. M. Herd, E. C. Richardson, and P. F. Parnell.** 2001. Genetic and phenotypic variance and covariance components for feed intake, feed efficiency, and other postweaning traits in Angus cattle. J. Anim. Sci. **79:**2805–2811.
3. **Baldwin, R. L., and J. M. Allison.** 1983. Rumen metabolism. J. Anim. Sci. **57**(Suppl. 2)**:**461–477.
4. **Basarab, J. A., M. A. Price, J. L. Aalhus, E. K. Okine, W. M. Snelling, and K. L. Lyle.** 2003. Residual feed intake and body composition in young growing cattle. Can. J. Anim. Sci. **83:**189–204.
5. **Bergman, E. N.** 1990. Energy contribution of VFA from the gastrointestinal tract in various species. Physiol. Rev. **70:**567–590.
6. **Bevans, D. W., K. A. Beauchemin, K. S. Schwartzkopf-Genswein, J. J. McKinnon, and T. A. McAllister.** 2005. Effect of rapid or gradual grain adaptation on subacute acidosis and feed intake by feedlot cattle. J. Anim. Sci. **83:**1116–1132.
7. **Brulc, J. M., D. A. Antonopoulos, M. E. Miller, M. K. Wilson, A. C. Yannarell, E. A. Dinsdale, R. E. Edwards, E. D. Frank, J. B. Emerson, P. Wacklin, P. M. Coutinho, B. Henrissat, K. E. Nelson, and B. A. White.** 2009. Gene-centric metagenomis of the fiber-adherent bovine rumen microbiome reveals forage specific glycoside hydrolases. Proc. Natl. Acad. Sci. U. S. A. **106:**1948–1953.
8. **Carlier, J. P., G. K'ouas, and X. Y. Han.** 2007. *Moryella indoligenes* gen. nov., sp. nov., an anaerobic bacterium isolated from clinical specimens. Int. J. Syst. Evol. Microbiol. **57:**725–729.
9. **Crews, D. H., Jr.** 2005. Genetics of efficient feed utilization and national cattle evaluation: a review. Genet. Mol. Res. **4:**152–165.
10. **Deng, W., D. Xi, H. Mao, and M. Wanapat.** 2008. The use of molecular techniques based on ribosomal RNA and DNA for rumen microbial ecosystem studies: a review. Mol. Biol. Rep. **35:**265–274.
11. **Edwards, J. E., B. J. Bequette, N. McKain, N. R. McEwan, and R. J. Wallace.** 2005. Influence of flavomycin on microbial numbers, microbial metabolism and gut tissue protein turnover in the digestive tract of sheep. Br. J. Nutr. **94:**64–70.
12. Reference deleted.
13. **France, J., and R. C. Siddons.** 1993. Volatile fatty acid production, p. 107–123. *In* J. M. Forbes and J. France (ed.), Quantitative aspects of ruminant digestion and metabolism. CAB International, Oxon, United Kingdom.
14. **Guan, L. L., J. D. Nkrumah, J. A. Basarab, and S. S. Moore.** 2008. Linkage of microbial ecology to phenotype: correlation of rumen microbial ecology to cattle's feed efficiency. FEMS Microbiol. Lett. **288:**85–91.
15. **Hegarty, R. S., J. P. Goopy, R. M. Herd, and B. McCorkell.** 2007. Cattle selected for lower residual feed intake have reduced daily methane production. J. Anim. Sci. **85:**1479–1486.
16. **Hristov, A. N., M. Ivan, L. M. Rode, and T. A. McAllister.** 2001. Fermentation characteristics and ruminal ciliate protozoal populations in cattle fed medium- or high-concentrate barley-based diets. J. Anim. Sci. **79:**515–524.

17. **Imachi, H., Y. Sekiguchi, Y. Kamagata, S. Hanada, A. Ohashi, and H. Harada.** 2002. Pelotomaculum thermopropionicum gen. nov., sp. nov., an anaerobic, thermophilic, syntrophic propionate-oxidizing bacterium. Int. J. Syst. Evol. Microbiol. **52:**1729–1735.
18. **Kelly, J. M., H. Park, M. Summers, and L. P. Milligan.** 1993. Interactions between protein and energy metabolism, p. 341–362. *In* J. M. Forbes and J. France (ed.), Quantitative aspects of ruminant digestion and metabolism. CAB International, Oxon, United Kingdom.
19. **Kobayashi, Y.** 2006. Inclusion of novel bacteria in rumen microbiology: need for basic and applied science. Anim. Sci. J. **77:**375–385.
20. **Li, M., G. B. Penner, E. Hernandez-Sanabria, M. Oba, and L. L. Guan.** 2009. Effects of sampling location and time, and host animal on assessment of bacterial diversity and fermentation parameters in the bovine rumen. J. Appl. Microbiol. **107:**1924–1934.
21. **Lodge-Ivey, S. L., J. Browne-Silva, and M. B. Horvath.** 2009. Technical note: bacterial diversity and fermentation end products in rumen fluid. J. Anim. Sci. **87:**2333–2337.
22. **Mackie, R. I., R. I. Aminov, W. Hu, A. C. Klieve, D. Ouwerkerk, M. A. Sundset, and Y. Kamagata.** 2003. Ecology of uncultivated *Oscillospira* species in the rumen of cattle, sheep, and reindeer as assessed by microscopy and molecular approaches. Appl. Environ. Microbiol. **69:**6808–6815.
23. **Mao, S. Y., G. Zhang, and W. Y. Zhu.** 2007. Effect of disodium fumarate on in vitro rumen fermentation of different substrates and rumen bacterial communities as revealed by denaturing gradient gel electrophoresis analysis of 16s ribosomal DNA. Anim. Feed Sci. Technol. **140:**293–306.
24. **McEwan, N. R., L. Abecia, M. Regensbogenova, C. L. Adam, P. A. Findlay, and C. J. Newbold.** 2005. Rumen microbial population dynamics in response to photoperiod. Lett. Appl. Microbiol. **41:**97–101.
25. **McSweeney, C. S., R. I Mackie, and B. A. White.** 1994. Transport and intracellular metabolism of major feed compounds by ruminal bacteria: the potential for metabolic manipulation. Aust. J. Agric. Res. **45:**731–756.
26. **Muyzer, G. E., C. de Waal, and A. G. Uitterlinden.** 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. **59:**695–700.
27. **Nagaraja, T. G., C. J. Newbold, C. J. Van Nevel, and D. I. Demeyer.** 1997. Manipulation of ruminal fermentation, p. 523–632. *In* P. M. Hobson and C. W. Stewart (ed.), The rumen microbial ecosystem, 2nd ed. Blackie Academic and Professional, London, England.
28. **Nkrumah, J. D., E. K. Okine, G. W. Mathison, K. Schmid, C. Li, J. A. Basarab, M. A. Price, Z. Wang, and S. S. Moore.** 2006. Relationships of feedlot feed efficiency, performance and feeding behaviour with digestion, energy partitioning, methane production and metabolic rate in beef cattle. J. Anim. Sci. **84:**145–153.
29. **Olfert, E. D., B. M. Cross, and A. A. McWilliams (ed.).** 1993. Guide to the care and use of experimental steers, vol. 1. Canadian Council on Animal Care, Ottawa, ON, Canada.
30. **Pátek, M.** 2007. Branched-chain amino acids, p. 129–162. *In* Amino acid biosynthesis—pathways, regulation and metabolic engineering. In V. F. Wendisch and A. Steinbuchel (ed.), Microbiology monographs, vol. 5. Springer, Berlin, Germany.
31. **Peters, J. P., J. B. Paulissen, and J. A. Robinson.** 1990. The effects of diet on water flux and volatile fatty acid concentrations in the rumen of growing beef steers fed once daily. J. Anim. Sci. **68:**1711–1718.
32. **Russell, J. B., and R. B. Hespell.** 1981. Microbial rumen fermentation. J. Dairy Sci. **64:**1153–1169.
33. **Russell, J. B., and J. L. Rychlik.** 2001. Factors that alter rumen microbial ecology. Science **292:**1119–1122.
34. **Sadet, S., C. Martin, B. Meunier, and D. P. Morgavi.** 2007. PCR-DGGE analysis reveals a distinct diversity in the bacterial population attached to the rumen epithelium. Animal **1**(7)**:**939–944. doi:10.1017/S175173110700034.
35. **Sharp, W. M., R. R. Johnson, and F. N. Owens.** 1982. Ruminal VFA production with steers fed whole or ground corn grain. J. Anim. Sci. **55:**1505–1514.
36. **Soto-Navarro, S. A., C. R. Krehbiel, G. C. Duff, M. L. Galyean, M. S. Brown, and R. L. Steiner.** 2000. Influence of feed intake fluctuation and frequency of feeding on nutrient digestion, digesta kinetics, and ruminal fermentation profiles in limit-fed steers. J. Anim. Sci. **78:**2215–2222.
37. **Stevenson, D. M., and P. J. Weimer.** 2007. Dominance of *Prevotella* and low abundance of classical ruminal bacterial species in the bovine rumen revealed by relative quantification real-time PCR. Appl. Microbiol. Biotechnol. **75:**165–174.
38. **Sun, Y. Z., S. Y. Mao, and W. Y. Zhu.** 2010. Rumen chemical and bacterial changes during stepwise adaptation to a high-concentrate diet in goats. Animal **4**(2)**:**210–217. doi:10.1017/S175173110999111x.
39. **Tajima, K., R. I. Aminov, T. Nagamine, H. Matsui, M. Nakamura, and Y. Benno.** 2001. Diet-dependent shifts in the bacterial population of the rumen revealed with real-time PCR. Appl. Environ. Microbiol. **67:**2766–2774.
40. **Walter, J., G. W. Tannock, A. Tilsala-Timisjarvi, S. Rodtong, D. M. Loach, K. Munro, and T. Alatossava.** 2000. Detection and identification of gastrointestinal *Lactobacillus* species by using denaturing gradient gel electrophoresis and species-specific PCR primers. Appl. Environ. Microbiol. **66:** 297–303.
41. **Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole.** 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl. Environ. Microbiol. **73:**5261–5267.
42. **Weimer, P. J.** 1998. Manipulating ruminal fermentation: a microbial ecological perspective. J. Anim. Sci. **76:**3114–3122.
43. **Welkie, D. G., D. M. Stevenson, and P. J. Weimer.** 2010. ARISA analysis of ruminal bacterial community dynamics in lactating dairy cows during the feeding cycle. Anaerobe **16:**94–100.
44. **Zhou, M., E. Hernandez-Sanabria, and L. L. Guan.** 2009. Assessment of the microbial ecology of ruminal methanogens in cattle with different feed efficiencies. Appl. Environ. Microbiol. **75:**6524–6533.