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Identification of the core rumen bacterial taxa and their population dynamics during the fattening period in Japanese Black cattle

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Abstract

The rumen microbiota comprises a vast range of bacterial taxa, which may affect the production of high-quality meat in Japanese Black cattle. The aim of this study was to identify core rumen microbiota in rumen fluid samples collected from 74 Japanese Black cattle raised under different dietary conditions using 16S rRNA gene amplicon sequencing. In the rumen of fattening Japanese Black cattle, 10 bacterial taxa, showing >1% average relative abundance and >95% prevalence, irrespective of the dietary conditions and the fattening periods, were identified as the core rumen bacterial taxa, which accounted for approximately 80% of the rumen microbiota in Japanese Black cattle. Additionally, population dynamics of the core rumen bacterial taxa revealed two distinct patterns: *Prevotella* spp. and unclassified Bacteroidales decreased in the mid-fattening period, whereas unclassified Clostridiales, unclassified Ruminococcaceae, *Ruminococcus* spp., and unclassified Christensenellaceae increased during the same period. Therefore, the present study reports the wide distribution of the core rumen bacterial taxa in Japanese Black cattle, and the complementary nature of the population dynamics of these core taxa, which may ensure stable rumen fermentation during the fattening period.

KEYWORDS
beef cattle, Japanese Black cattle, rumen microbiota

1 | INTRODUCTION

Ruminant animals harbor dense and diverse microbial populations in their rumen. The rumen microbes ferment feed and produce volatile fatty acids (VFAs), the main energy source for the host. Of the ruminal microbes, including bacteria, protozoa, and fungi, bacteria are considered to play vital roles in nutrient acquisition for the host animals; they constitute the largest biomass pool among the rumen microbes (Allison, 1993), and a majority (>90%) of the microbial genes expressed in the rumen are bacterial in origin (Brulc et al., 2009). Furthermore, comprehensive analysis of 16S rRNA genes has demonstrated the presence of more than 5,000 bacterial species in the rumen (Kim et al., 2011). A study from the Global Rumen Census project (Henderson et al., 2015) identified abundant bacterial groups, including Prevotella spp., unclassified Clostridiales, unclassified Bacteroidales, unclassified Ruminococcaceae, unclassified Lachnospiraceae, Ruminococcus spp., and Butyrivibrio spp., in the rumen as the core members of the rumen microbiota, irrespective of the ruminant species.

It is well known that the population sizes of particular bacterial species or groups in the rumen (the composition of the rumen microbiota) are affected by several factors such as host species (Henderson et al., 2015; Iqbal et al., 2017), dietary conditions (Tajima et al., 2001), and even cattle breeds. Paz et al. (2016) compared the compositions of rumen microbiota of Holstein and Jersey dairy cows under the same feeding management and demonstrated that the relative abundances of Prevotellaceae and Lachnospiraceae were significantly different between the breeds. Although members of the core rumen microbiota have been reported in a previous study, their distribution in different cattle breeds needs further investigation to extend our understanding of the key players in stable rumen fermentation.

Japanese Black cattle are the most popular beef breed, accounting for 67.2% of the beef cattle in Japan (MAFF, 2019). This breed is known to produce high-quality meat in terms of taste, tenderness, and marbling (Gotoh et al., 2009). To produce highly marbled meat, Japanese Black cattle are fed approximately five metric tons of concentrate diet during the fattening period, which is generally about 20 months long starting from the age of 10 months, and the proportion of concentrate diet is gradually increased up to approximately 90% (as fed) of the total diet. A high-concentrate diet can cause a drop in the ruminal pH, increasing the risk of digestive disorders such as subacute ruminal acidosis (Khafipour et al., 2009) and leading to loss of appetite, thereby resulting in low meat quality (Abdela, 2016). Consequently, stable rumen fermentation during the fattening period, wherein animals are given a high-concentrate diet, is one of the most important aspects of high-quality meat production in Japanese Black cattle. Therefore, it is necessary to identify the core members of the rumen microbiota in this breed and their relation to fermentation to achieve stable rumen fermentation through appropriate feeding management.

Recently, Ogata et al. (2019, 2020) studied the rumen microbiota of Japanese Black cattle using nine animals and showed high relative abundances of unclassified Ruminococcaceae, unclassified Lachnospiraceae, and Prevotella spp. during the fattening period. The abundance of these bacterial groups supported a previous report (Henderson et al., 2015) in which the core members of the rumen microbiota were determined using 742 samples from 32 animal species of 35 countries. However, further investigation with a larger sample size is necessary to identify the core members of the rumen microbiota in Japanese Black cattle. Therefore, in the present study, the findings of the previous studies on the rumen microbiota of Japanese Black cattle were validated using a larger sample size ($n = 74$). In addition, the rumen fluid samples of Japanese Black cattle that were fed different diets on different farms were subjected to rumen microbiota analysis. We hypothesized that if bacterial groups showed similar distribution and population dynamics under different conditions (locations and diets), it could be assumed that those bacterial groups were widely distributed in the rumen of Japanese Black cattle. Therefore, the objective of the present study was to generalize the core rumen microbiota that contributes to stable rumen fermentation in Japanese Black cattle.

2 | MATERIALS AND METHODS

2.1 | Animals and sampling

The animal experimental procedures used in the present study were approved by the Animal Care Committee National Livestock Breeding Center (Authorized Number: H20-53) and performed in accordance with the principles and guidelines for animal use set by the National Livestock Breeding Center.

In the present study, 74 Japanese Black cattle that were fed one of the four diets on five farms were sampled (Table 1). Thirty-seven animals were raised under the standard dietary conditions for Japanese Black fattening cattle following the conventional feeding program in the respective farms and were assigned to the Standard group (Table 1). Twenty-one animals in the Rice group were fed the same diet as the Standard group, except that the concentrate was partially replaced by steamed unhulled rice. The cattle in the corn silage (CS) group ($n = 8$) were offered CS-based total mixed ration (TMR), whereas 40% of the concentrate in the TMR was replaced with rice for the corn silage–rice (CSR) group ($n = 8$) (Table 1).

In the Standard group, cattle were fed roughage {rice straw (5.4% crude protein [CP] and 63.1% neutral detergent fiber [NDF] on a dry matter [DM] basis), orchardgrass hay (7.3% CP and 69.8% NDF on a DM basis), or wheat straw (2.4% CP and 81.1% NDF on a DM basis)} and concentrate (15.8% CP and 43.3% starch on a DM basis) in the ratio ranging from 37:63 to 8:92 depending on the fattening period (Table 1). In the Rice group, feeding regimens were the same as the Standard group except that 10%–15% of the concentrate was replaced by steamed unhulled rice (7.5% CP and 76.2% starch on a DM basis). In the CS group, cattle were fed TMR in which corn silage (8.4% CP, 25.7% NDF, and 25.7% starch on a DM basis) and concentrate (15.8% CP and 43.3% starch on a DM basis) were mixed in a ratio of 50:50 (DM basis) throughout the fattening period. In the CSR

TABLE 1 Animals and dietary conditions used in the present study

Farm location (prefectures)	Dietary group	Number of cattle	Early[a] Roughage (kg)	Early[a] Concentrate (kg)	Mid[b] Roughage (kg)	Mid[b] Concentrate (kg)	Late[c] Roughage (kg)	Late[c] Concentrate (kg)
Akita	Standard[d]	3	2.5	8.0	2.5	9.0	2.0	8.0
	Rice[e]	3	2.5	8.0	2.5	9.0	2.0	8.0
Fukushima	Standard[d]	6	3.0	7.0	1.5	10.0	1.5	10.0
	Rice[e]	6	3.0	7.0	1.5	10.0	1.5	10.0
Iwate	Standard[d]	6	4.0	7.0	2.0	10.0	2.0	10.0
	Rice[e]	6	4.0	7.0	2.0	10.0	2.0	10.0
Miyagi	Standard[d]	6	3.0	7.0	1.5	9.0	1.0	7.0
	Rice[e]	6	3.0	7.0	1.5	9.0	1.0	7.0
Hokkaido	Standard[d]	16	1.5	10.5	1.0	10.4	0.9	10.1
	CS[f]	8	24.9		22.8		19.6	
	CSR[g]	8	25.9		23.6		19.8	

[a]Dietary composition for cattle at 14 months of age in the farms in Akita, Fukushima, Iwate, and Miyagi and for cattle at 17 months of age in the farm in Hokkaido.

[b]Dietary composition for cattle at 22 months of age in the farms in Akita, Fukushima, Iwate, and Miyagi and for cattle at 21 months of age in the farm in Hokkaido.

[c]Dietary composition for cattle at 26 months of age in the farms in Akita, Fukushima, Iwate, Miyagi, and Hokkaido.

[d]Cattle were fed roughage {rice straw (5.4% crude protein [CP] and 63.1% neutral detergent fiber [NDF]), orchardgrass hay (7.3% CP and 69.8% NDF), or wheat straw (2.4% CP and 81.1% NDF)} and concentrate (15.8% CP and 43.3% starch) in the ratio given in the table.

[e]Feeding regimens were the same as the Standard group except that 10%–15% of the concentrate was replaced by steamed unhulled rice (7.5% CP and 76.2% starch).

[f]Cattle were fed total mixed ration in which corn silage (8.4% CP, 25.7% NDF, and 25.7% starch) and concentrate (15.8% CP and 43.3% starch) were mixed in the ratio of 50:50 (dry matter basis) throughout the fattening period.

[g]Feeding regimens were the same as the CS group except that 40% of concentrate was replaced by unhulled rice (7.5% CP and 76.2% starch).

group, feeding regimens were the same as the CS group except that 40% of the concentrate was replaced by unhulled rice (7.5% CP and 76.2% starch on a DM basis). The animals were fed twice daily at 08:30 and 16:00.

Rumen fluid was collected from individual cattle using a stomach tube, 4 h after feeding in the morning. The sampling time points were as follows: 14, 22, and 26 months of age (considering as early, mid-, and late fattening periods, respectively) for the farms in Akita, Fukushima, Iwate, and Miyagi, and 17, 21, and 26 months of age (considering as early, mid-, and late fattening periods, respectively) for the farm in Hokkaido. Collected rumen fluid samples were stored at $-80°C$ without separating the solid and liquid fractions until use. Rumen fluid collection from one animal in the Standard group raised at the farm in Iwate was not conducted during the mid- and late fattening periods due to a metabolic disorder.

2.2 | Measurement of fermentation parameters

The rumen fluid samples were subjected to pH measurement using a pH electrode (LAQUAtwin B-712; Horiba, Kyoto, Japan) and VFA measurement using a gas chromatograph (GC-14B; Shimadzu, Kyoto, Japan), as described previously (Oh et al., 2017). NH_3–N levels in

the rumen samples were measured using the phenol–hypochlorite method (Weatherburn, 1967).

2.3 | 16S rRNA gene amplicon sequencing for the analysis of bacterial community

Microbial DNA was extracted and purified from 250 μl of rumen fluid using the repeated bead-beating plus column method (Yu & Morrison, 2004) with a commercial kit (QIAmp DNA Stool Mini Kit; Qiagen, Hilden, Germany). The DNA concentration was quantified using a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). Microbial DNA was diluted to a final concentration of 5 ng/μl for all the samples and subjected to amplification of the V3–V4 region of the 16S rRNA gene using the primer set S-D-Bact-0341-bS-17 (5′-CCTACGGGNGGCWGCAG-3′) and S-D-Bact-0785-aA-21 (5′-GACTACHVGGGTATCTAATCC-3′) (Herlemann et al., 2011). The PCR mixture consisted of 12.5 μl of $2 \times$ KAPA HiFi HotStart ReadyMix (Roche Sequencing, Basel, Switzerland), 5.0 μl of each primer (1.0 μM), and 2.5 μl of DNA. The PCR steps were performed according to the following program: initial denaturation at 95°C for 3 min, followed by 25 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s, and a final extension step at

72°C for 5 min. Amplicons were purified using AMPure XP beads (Beckman-Coulter, Brea, CA, USA), followed by 2 × 300 bp paired-end sequencing on the Illumina MiSeq platform (Illumina, San Diego, CA, USA) with MiSeq Reagent Kit v3. Raw sequences were deposited in the NCBI Sequence Read Archive under the accession no. PRJNA701844.

2.4 | Bioinformatics analysis

Data obtained from amplicon sequencing using the MiSeq platform were analyzed using the QIIME2 version 2019.4 (Bolyen et al., 2019). Paired-end reads were filtered, dereplicated, merged, and chimera-filtered using q2-dada2 plugin (Callahan et al., 2016) to generate amplicon sequence variants (ASVs). The taxonomic classification of ASVs was performed at the genus level using the recently updated Greengenes database (v.13_8). ASVs classified as archaea, mitochondria, and chloroplasts were removed from the analysis. Three samples (one from the Standard group in the mid-fattening period and two from the Rice group in the early fattening period) with less than 1,500 reads remaining after this step were excluded from further analysis. Filtered ASV count table was exported to the R program version 3.6.2 (R Core Team, 2019) for downstream analyses. Alpha diversity indices, including Chao1 and Shannon, were calculated based on rarefied ASV count table using the Phyloseq Bioconductor packages (McMurdie & Holmes, 2013) in the R program. The relative abundance of each bacterial taxon was calculated by dividing the number of reads assigned to each bacterial taxon by the total number of reads. Only taxa with average relative abundance >0.1% and with a prevalence >20% in at least one fattening period were considered and used for the analysis. Particularly, the bacterial taxa, which showed >1% average relative abundance and >95% prevalence, irrespective of the dietary groups and the fattening periods, were identified as the core rumen bacterial members and subjected to further analysis.

2.5 | Statistical analyses

All statistical comparisons were performed using the R program. Differences in the fermentation parameters within the respective dietary groups were evaluated among the fattening periods using a repeated measures model with fixed effects of the fattening period and random effects of the cattle. If a significant effect of the fattening period was observed, Tukey's honest significant differences (HSD) test was performed for multiple comparisons. Alpha diversity indices within each dietary group were compared among the fattening periods, and statistical significance was determined using analysis of variance (ANOVA) and Tukey's HSD test. The Pearson correlation among bacterial abundances was analyzed at the genus level and visualized by corrplot package version 0.84 (Wei & Viliam, 2017) in the R program. p values of <0.05 and <0.10 were considered statistically significant and trend, respectively.

3 | RESULTS

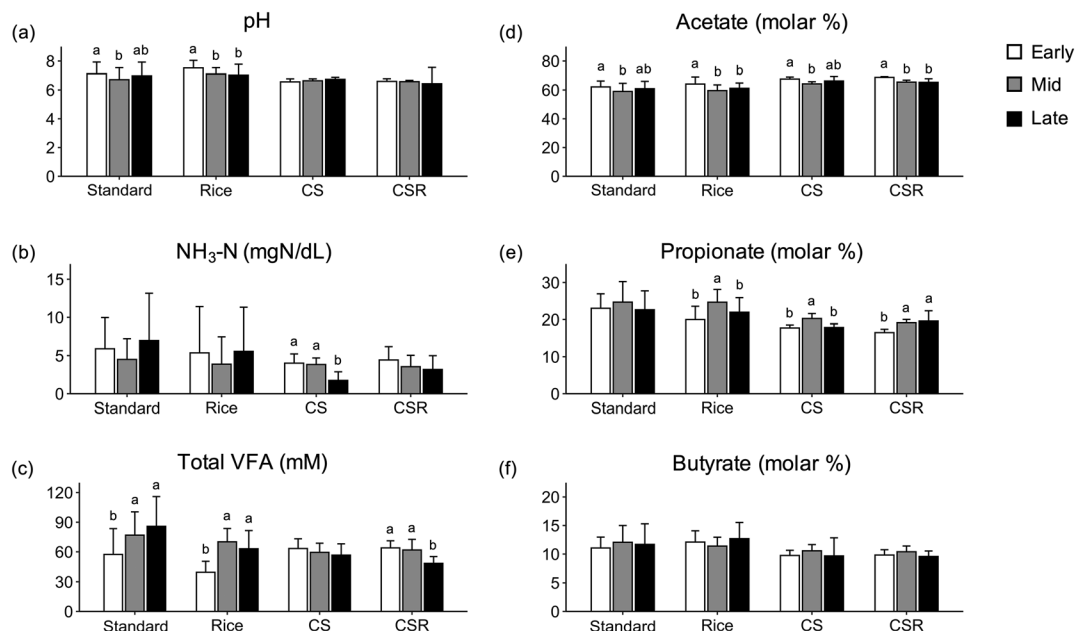### 3.1 | Changes in rumen fermentation during fattening periods

Changes in the rumen fermentation parameters during the fattening periods are shown in Figure 1. The total VFA concentrations and proportions of acetate and propionate fluctuated in all dietary groups, whereas ruminal pH, NH_3–N concentrations, and butyrate proportions did not exhibit apparent change during the fattening periods. The total VFA concentration increased ($p < 0.05$) from the early to mid-fattening period in the Standard and Rice groups, whereas it decreased ($p < 0.05$) from the early and mid-fattening periods to the late fattening period in the CSR group. Acetate proportion decreased ($p < 0.05$) from the early to mid-fattening period, irrespective of the dietary groups (Figure 1d). Conversely, the proportion of propionate increased ($p < 0.05$) from the early to mid-fattening period except for the Standard group (Figure 1e).

3.2 | Identification of the core rumen bacterial taxa by amplicon sequencing

Amplicon sequencing of the rumen fluid samples yielded a total of 3,601,426 high quality reads, with an average of 16,908 ± 7,603 reads per sample. Table 2 shows the results of alpha diversity indices (Chao1 and Shannon) of rumen microbiota in Japanese Black cattle at the early, mid-, and late fattening periods. Both indices differed significantly ($p < 0.05$) among the fattening periods, except for the CSR group, and were lower in the mid-fattening period. After filtering out taxa with an average relative abundance >0.1% and with a prevalence >20% in at least one fattening period, a total of 51 taxa at the genus-level were screened (Table S1). Based on the cut-off criteria (>1% average relative abundance and >95% prevalence across all samples), 10 bacterial taxa, namely, *Prevotella* spp. (22.89 ± 13.49%), unclassified Clostridiales (13.46 ± 6.73%), unclassified Ruminococcaceae (10.14 ± 4.43%), unclassified Bacteroidales (10.12 ± 5.83%), *Ruminococcus* spp. (7.59 ± 4.34%), unclassified F16 (4.10 ± 4.23%), unclassified Pirellulaceae (3.44 ± 4.21%), unclassified Lachnospiraceae (3.20 ± 1.67%), *Butyrivibrio* spp. (2.20 ± 2.41%), and unclassified Christensenellaceae (2.04 ± 1.48%), were identified as the core rumen bacterial taxa in Japanese Black cattle (Table S1).

3.3 | Population dynamics of the core rumen bacterial taxa during the fattening periods

The distribution of the core rumen bacterial taxa in the respective dietary groups is shown in Figure 2. The abundance of core rumen bacterial taxa accounted for approximately 80% of the total reads from amplicon sequencing, regardless of the dietary groups or the fattening periods. However, the composition of the rumen microbiota varied among the fattening periods in respective dietary groups. To verify

FIGURE 1 Changes in rumen fermentation parameters in Japanese Black cattle during the fattening period. Levels of ruminal pH (a), concentrations of NH_3–N (b), total volatile fatty acid (VFA) (c), and molar proportions of acetate (d), propionate (e), and butyrate (f) in the Standard ($n = 37$), Rice ($n = 21$), corn silage (CS; $n = 8$), and corn silage–rice (CSR; $n = 8$) groups during the fattening periods are shown. Bar colors indicate the fattening periods: early (blank), mid (gray), and late (black). Error bars represent standard deviation of the mean; [abc] represents means differing within the dietary group ($p < 0.05$, calculated by Tukey's honest significant differences [HSD] test)

TABLE 2 Alpha diversity indices of rumen microbiota in Japanese Black cattle during early, mid- and late fattening periods

Item	Dietary group	Fattening period			p values[§]
		Early[‡]	Mid[‡]	Late[‡]	
Chao1[†]	Standard	387.9 ± 112.3[a]	246.0 ± 104.1[c]	324.4 ± 80.6[b]	<0.001
	Rice	227.3 ± 106.0[b]	257.1 ± 87.2[b]	382.0 ± 86.0[a]	<0.001
	CS	621.0 ± 83.3[a]	493.7 ± 95.0[b]	489.4 ± 80.1[b]	0.008
	CSR	548.8 ± 152.7	465.0 ± 152.7	491.4 ± 63.5	0.266
Shannon[†]	Standard	5.01 ± 0.44[a]	4.59 ± 0.51[b]	4.95 ± 0.31[a]	<0.001
	Rice	4.45 ± 0.54[b]	4.42 ± 0.45[b]	4.99 ± 0.30[a]	<0.001
	CS	5.66 ± 0.14[a]	5.49 ± 0.13[ab]	5.38 ± 0.23[b]	0.010
	CSR	5.57 ± 0.40	5.42 ± 0.40	5.36 ± 0.11	0.283

Note: [abc] indicates means differing within the dietary group ($p < 0.05$, calculated by Tukey's honest significant differences [HSD] test).
Abbreviations: CS, corn silage; CSR, corn silage–rice.
[†]Calculated using Phyloseq Bioconductor package.
[‡]Values are shown as mean ± SD.
[§]p values were calculated by ANOVA within the respective dietary groups.

the factors affecting the distribution of the core rumen bacterial members, PCoA plots and permutational ANOVA (based on Bray–Curtis dissimilarity matrices) were employed (Figure S1). Although the composition of core rumen bacterial members was affected both by the fattening period ($R^2 = 0.16$; $p < 0.01$) and the dietary group ($R^2 = 0.08$; $p < 0.01$), a higher correlation coefficient was observed for the fattening period than the dietary group.

To study the population dynamics patterns of the core rumen bacterial taxa during the fattening periods, correlation analysis was performed. Figure 3a represents the correlation matrix based on

the abundances of the core rumen bacterial taxa in the Standard group during the fattening period. The abundance of *Prevotella* spp. was negatively correlated ($p < 0.05$) with that of the other core members, except for the unclassified Bacteroidales. Similar to *Prevotella* spp., unclassified Bacteroidales showed a negative correlation ($p < 0.05$) with many of the core members (Figure 3a). In contrast, the distributions of unclassified Clostridiales, unclassified Ruminococcaceae, *Ruminococcus* spp., and unclassified Christensenellaceae were positively correlated ($p < 0.05$) with each other (Figure 3a).
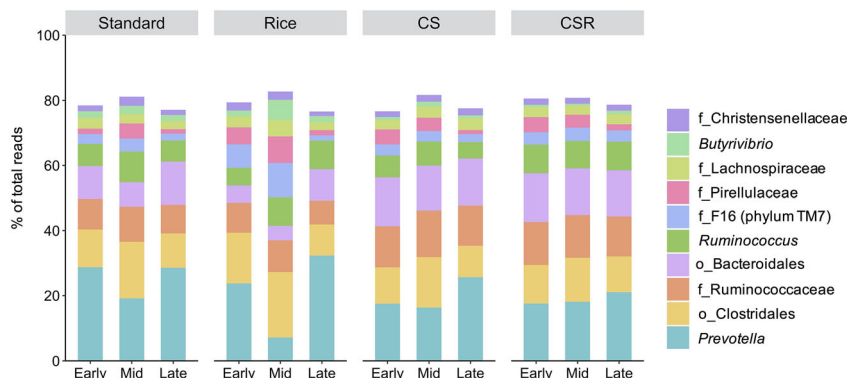
FIGURE 2 Distribution of the core rumen bacterial taxa in Japanese Black cattle determined by amplicon sequencing of 16S rRNA gene. The relative abundances of bacterial taxa at the genus-level showing >1% average relative abundance and >95% prevalence across all samples are depicted as stacked bar graphs. Unclassified bacterial orders and families are prefixed with "o_" and "f_," respectively
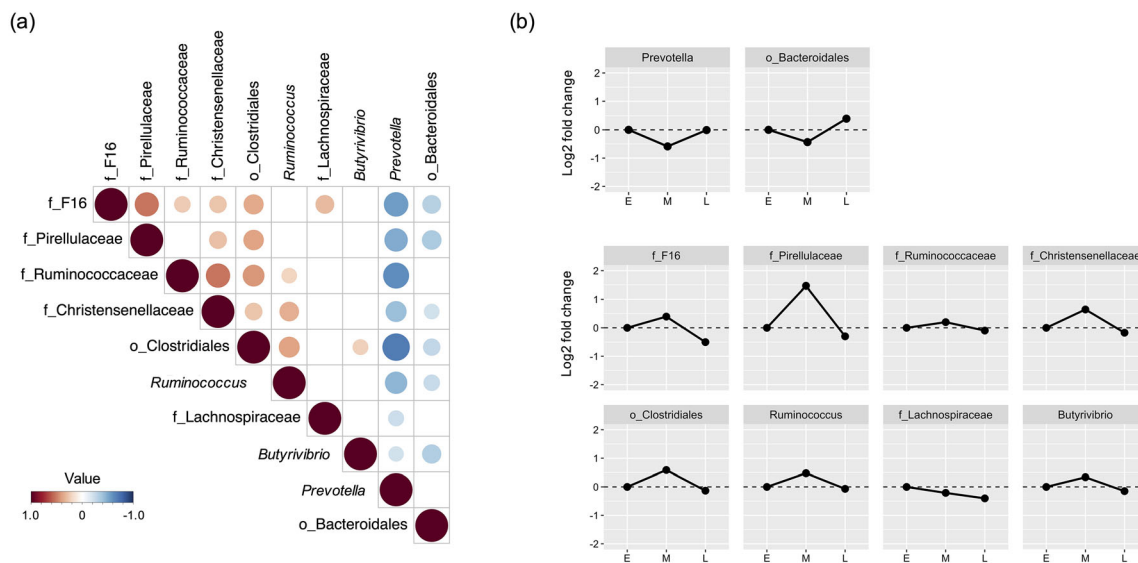
FIGURE 3 Fluctuations in the abundances of the core rumen bacterial taxa of Japanese Black cattle in the Standard group. (a) Pearson correlation among the core rumen bacterial members in the Standard group during fattening period. The values of relative abundances (percentage of the total reads) of respective bacterial taxa during early, mid-, and late fattening periods were used for calculations. Only the plots showing statistical significance ($p < 0.05$) are indicated in the panel. The scale colors indicate the correlation: positive (closer to 1: red circle) or negative (closer to −1: blue circle). (b) The relative changes in the abundance of each core rumen bacterial taxa in early, mid-, and late fattening periods. The values are expressed as $\log_2$ fold changes against the abundances in early fattening period. E, M, and L in the x-axis indicate early, mid-, and late fattening periods, respectively. Unclassified bacterial orders and families are prefixed with "o_" and "f_," respectively

The population dynamics of each core member during the fattening period in the Standard group are presented in Figure 3b. *Prevotella* spp. and unclassified Bacteroidales decreased during the mid-fattening period, while the other core members, except for the unclassified Lachnospiraceae, increased during the same period.
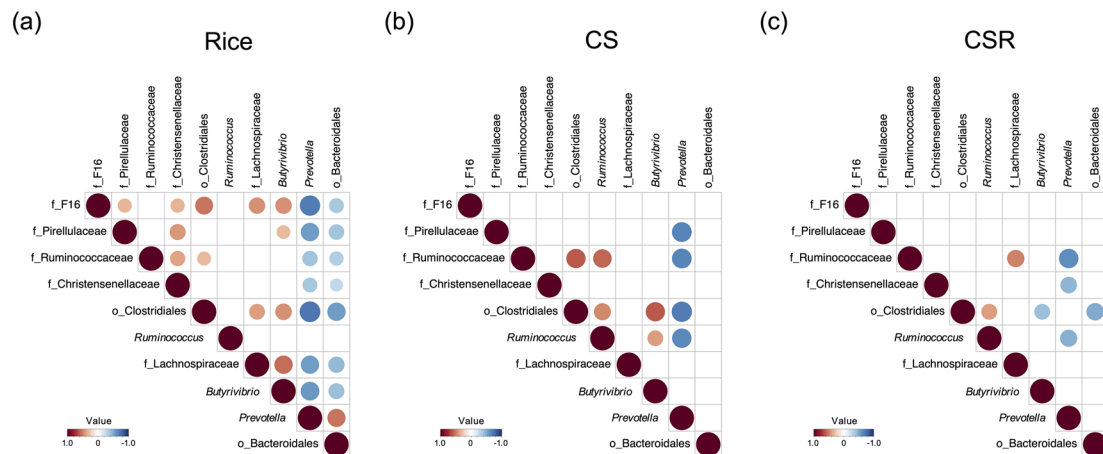
3.4 | Correlation among the abundances of the core rumen bacterial taxa during the fattening period

Correlation analyses between the core rumen bacterial taxa in the Rice, CS, and CSR groups were performed in the same manner as the Standard group, and the results are shown in Figure 4. The abundance of *Prevotella* spp. consistently showed a negative correlation ($p < 0.05$) with that of the other core members except for unclassified

Bacteroidales. Fewer significant correlations among the abundances of core rumen bacterial members were observed in the CS and CSR groups compared with the Standard and Rice groups (Figure 4b,c).
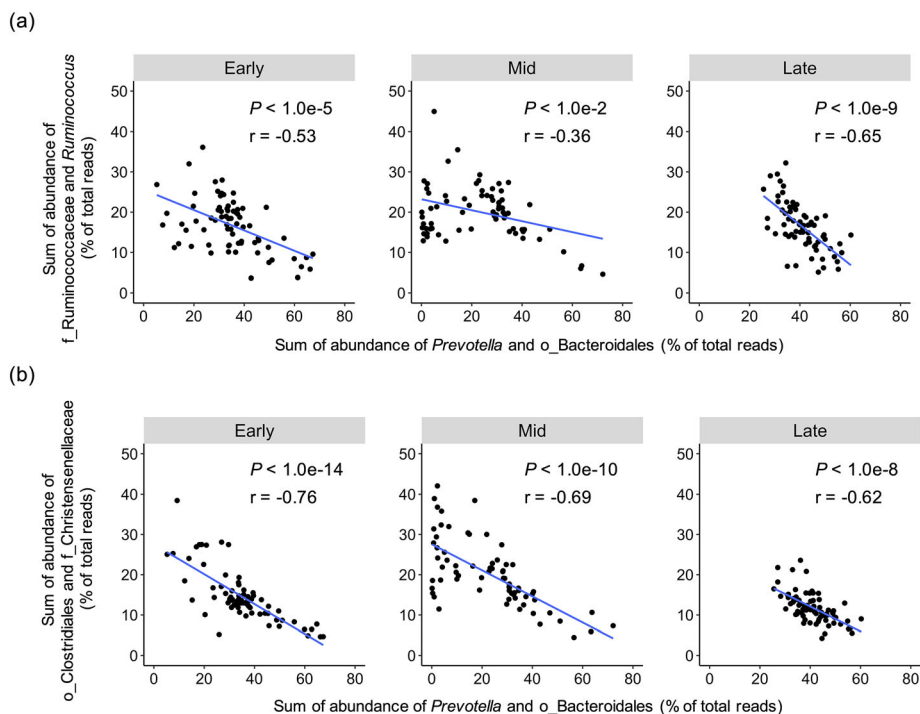
The abundances of *Prevotella* spp. and unclassified Bacteroidales had a negative correlation with those of unclassified Ruminococcaceae, *Ruminococcus* spp., unclassified Clostridiales, and unclassified Christensenellaceae under almost all dietary conditions (Figures 3 and 4). Therefore, we performed correlation analysis between them (Figure 5). The sum of abundances of unclassified Ruminococcaceae and *Ruminococcus* spp., both of which belong to the family Ruminococcaceae, showed a negative correlation ($p < 0.05$) with the sum of abundances of *Prevotella* spp. and unclassified Bacteroidales, irrespective of the fattening period (Figure 5a). Similarly, a negative correlation ($p < 0.05$) between the sum of abundances of unclassified Clostridiales and unclassified Christensenellaceae, both of which

FIGURE 4 Pearson correlation analysis between the relative abundances of the core rumen bacterial taxa in the Rice (a), corn silage (CS) (b), and corn silage–rice (CSR) (c) groups. The values for relative abundances (percentage of the total reads) of respective bacterial taxa during early, mid-, and late fattening periods in the rumen of cattle in each dietary group were used for calculations. Only the plots showing statistical significance ($p < 0.05$) are indicated in the panel. The scale colors indicate the correlation: positive (closer to 1: red circle) or negative (closer to −1: blue circle). Unclassified bacterial orders and families are prefixed with "o_" and "f_," respectively

FIGURE 5 Correlation analyses between the abundances of the core rumen bacterial taxa showing a negative relationship in their distribution during the fattening period. The x-axis indicates the sum of relative abundances of _Prevotella_ spp. and unclassified Bacteroidales, and the y-axis corresponds to the sum of relative abundances of unclassified Ruminococcaceae and _Ruminococcus_ spp. (a) or unclassified Clostridiales and unclassified Christensenellaceae (b). Individual plots represent the rumen samples from each cattle during the respective fattening period. Blue lines in each panel represent linear regression during the respective fattening period

belong to the family Christensenellaceae, and the sum of abundances of _Prevotella_ spp. and unclassified Bacteroidales was observed in the respective fattening periods (Figure 5b).

4 | DISCUSSION

Stable rumen fermentation is one of the most important factors for the production of high-quality meat in Japanese Black cattle. Therefore, identification of the core rumen bacterial taxa contributing to stable rumen fermentation is important to achieve high-quality meat production in this breed through appropriate feeding management. In the present study, rumen fermentation parameters of the Standard and Rice groups showed normal changes (the typical responses against an increase in concentrate diet), while those of the CS and CSR groups were stable during the fattening period. Less fluctuations in the rumen fermentation parameters of the CS and CSR groups may be attributed to TMR feeding. Overall, the fermentation parameters' values were within the normal range irrespective of the dietary groups and the fattening periods. Therefore, the animals used in the present study maintained stable rumen fermentation throughout the fattening period in all dietary groups. This finding was partly supported by alpha diversity indices of rumen microbiota; although lower Chao1 and Shannon indices were observed in the mid-fattening period, the

extent of decrease was not remarkable. Therefore, the stability of rumen microbiota was maintained irrespective of dietary conditions and fattening periods, and slight decreases of rumen microbial diversity in the mid-fattening period were probably the normal response against the increase in concentrate diet.

By exploring the rumen microbiota of 74 fattening Japanese Black cattle, we observed a steady distribution of 10 bacterial taxa, namely *Prevotella* spp., unclassified Clostridiales, unclassified Ruminococcaceae, unclassified Bacteroidales, *Ruminococcus* spp., unclassified F16, unclassified Pirellulaceae, unclassified Lachnospiraceae, *Butyrivibrio* spp., and unclassified Christensenellaceae, irrespective of the location (farm), dietary conditions, and fattening periods. Therefore, it can be proposed that these bacterial groups form the core rumen bacterial community in Japanese Black cattle. Ogata et al. (2019, 2020) reported a high abundance of unclassified Ruminococcaceae, unclassified Lachnospiraceae, and *Prevotella* spp. in the rumen of Japanese Black cattle. Our observations validate their findings in a larger number of animals. Besides, we also identified unclassified Clostridiales, unclassified Bacteroidales, *Ruminococcus* spp., unclassified F16, unclassified Pirellulaceae, *Butyrivibrio* spp., and unclassified Christensenellaceae as the core members of the rumen microbiota in Japanese Black cattle.

The proportion of each core bacterial taxon and the population dynamics during the fattening period were somewhat different among the four dietary conditions in the present study. Although unclassified Bacteroidales appeared to be slightly correlated with ruminal acetate proportion in CS and CSR groups (data not shown), the effect of dietary conditions on the distribution of core bacterial taxa was not clearly detected. Because the main focus of the present study was to identify the core rumen bacterial taxa distributing regardless of farms and dietary conditions, not to evaluate the dietary effect on rumen microbiota, there was a limitation in the experimental design to determine the factors affecting their distribution in the rumen. As Henderson et al. (2015) reported, dietary conditions are the most significant factor affecting the composition of rumen microbiota. The dietary factors that impact the distribution of core rumen bacterial taxa in Japanese Black cattle need to be further elucidated to understand the key for stable rumen fermentation.

Of the core rumen bacterial taxa identified in the present study, *Prevotella* spp., unclassified Clostridiales, unclassified Ruminococcaceae, unclassified Bacteroidales, *Ruminococcus* spp., unclassified Lachnospiraceae, and *Butyrivibrio* spp. are reported as the dominant rumen bacterial groups in ruminant species (Henderson et al., 2015). Although the functions of these dominant rumen bacteria are not fully understood, their high abundance indicates their role in rumen fermentation. Three bacterial taxa, namely, unclassified Pirellulaceae, unclassified F16, and unclassified Christensenellaceae, are relatively new phylogenetic groups compared to the other core rumen bacterial taxa. However, all of these have been previously reported in the rumen of different animals (Baek et al., 2020; Henderson et al., 2015; Lima et al., 2015). The possible roles of Pirellulaceae and unclassified F16, belonging to phylum TM7, in carbohydrate degradation have been reported in the animal gut (Kindaichi et al., 2016; Parata et al., 2020). Christensenellaceae has been recently recognized as an important hydrogen-producing

bacterial group, which is utilized for methane production in the human gut and rumen (Greening et al., 2019; Waters & Ley, 2019). Moreover, some species belonging to this family have been reported to utilize structural carbohydrates (Morotomi et al., 2012). Based on these previous reports, unclassified Pirellulaceae, unclassified F16, and unclassified Christensenellaceae could be involved in carbohydrate digestion in the rumen of Japanese Black cattle.

The core rumen bacterial taxa constituted the majority of the rumen microbiota (approximately 80% of the total bacteria) irrespective of the dietary groups or the fattening periods, and its composition seemed to vary during the fattening periods. PCoA analyses combined with permutational ANOVA indicate that the distribution of the core rumen bacterial taxa was affected more by the fattening period than by the dietary conditions. This finding indicates that the increase in the concentrate diet during the fattening period is the main factor affecting the population dynamics of the core rumen bacterial taxa in Japanese Black cattle. Based on these results, we focused on the population dynamics of core rumen bacterial taxa during the fattening period. Correlation analysis revealed that the abundance of *Prevotella* spp. and unclassified Bacteroidales was negatively correlated with the other core rumen bacterial taxa. In particular, the distributions of the families Ruminococcaceae (unclassified Ruminococcaceae and *Ruminococcus* spp.) and Christensenellaceae (unclassified Clostridiales and unclassified Christensenellaceae) were negatively correlated with that of *Prevotella* spp. and unclassified Bacteroidales. This was attributed to a decrease in the abundances of *Prevotella* spp. and unclassified Bacteroidales in the mid-fattening period and an increase in the abundances of the families Ruminococcaceae and Christensenellaceae during the same period. It is well known that the abundance of gram-positive bacteria, including families Ruminococcaceae and Christensenellaceae, increases, whereas that of gram-negative bacteria, including *Prevotella* spp. and unclassified Bacteroidales, decreases during rumen acidosis (Monteiro & Faciola, 2020). Gram-negative bacteria are generally more sensitive to low rumen pH compared with gram-positive bacteria. In fact, the abundances of core members belonging to Bacteroidales and Clostridiales correlated positively and negatively, respectively, with ruminal pH ($p < 0.05$) (Figure S2). Although the cattle used in the present study did not suffer from rumen acidosis, rumen pH was lower in the Standard and Rice groups during the mid-fattening period of the study. Therefore, a drop in pH due to an increase in the concentrate diet in the mid-fattening period could be a possible cause of the change in population dynamics of the core rumen bacterial taxa.

A negative correlation between the abundances of *Prevotella* spp. and unclassified Bacteroidales and the families Ruminococcaceae and Christensenellaceae suggests a competitive or complementary relationship between them. Based on the reverse population dynamics between the early and mid-fattening periods (decrease in *Prevotella* spp. and unclassified Bacteroidales and increase in the families Ruminococcaceae and Christensenellaceae) and the mid- and late fattening periods (increase in *Prevotella* spp. and unclassified Bacteroidales and decrease in the families Ruminococcaceae and Christensenellaceae), a complementary relationship likely developed among these bacterial groups. *Prevotella* spp. are the most abundant

genera in the rumen (Henderson et al., 2015) and exhibit versatile functions, including the degradation and fermentation of both roughage and concentrate (Cotta, 1988; Flint, 1997). Ruminococcaceae includes *Ruminococcus bromii*, which is a major amylolytic bacterium in the rumen of cattle fed with a high-concentrate diet (Khongpradit et al., 2020; Klieve et al., 2007). Christensenellaceae, containing the Clostridiales R-7 group, was recently identified as one of the dominant bacterial groups and is possibly involved in xylan and xyloglucan degradation in the rumen (Seshadri et al., 2018). Therefore, these bacterial groups could play vital roles in rumen fermentation. The complementary population dynamics of core members results in maintaining the niche for all core rumen bacterial taxa, which ensures stable rumen fermentation during the fattening period in Japanese Black cattle.

A long-term fattening with high concentrate feeding in the production of Japanese Black cattle increases the risk of dysbiosis, leading to abnormal rumen fermentation, such as rumen acidosis. Under high concentrate feeding, some lactic acid bacteria including *Streptococcus bovis* and *Lactobacillus* spp. exhibit rapid growth, and it is a trigger for rumen acidosis. Therefore, preventing the overgrowth of these lactic acid bacteria is important for normal rumen fermentation. It can be assumed that the rumen microbiota dominated by the core bacterial taxa in a complementary manner could increase the stability of the rumen ecosystem contributing to the prevention of the overgrowth of lactic acid bacteria in the rumen of Japanese Black cattle. The complementary population dynamics in the core bacterial taxa during the fattening period were relatively smaller in TMR feeding groups (i.e., CS and CSR in the present study) than those in other dietary groups. This observation indicates the stability of rumen microbiota in TMR feeding groups and partly explains less fluctuation of rumen fermentation parameters in these groups. Although the complemental relationship among the core rumen bacterial taxa needs to be further validated, the negative correlation between Bacteroidetes and Firmicutes (the former contains *Prevotella* spp. and unclassified Bacteroidales, and the latter includes the families Ruminococcaceae and Christensenellaceae) is well recognized in the animal gut (Filippo et al., 2010). Besides, it is reported that *Prevotella* spp. showed a negative correlation with *Butyrivibrio* spp. and *Shuttleworthia* spp. belonging to Clostridiales in the rumen of Holstein dairy cows (Jami et al., 2014).

In conclusion, the present study identified the core rumen bacterial taxa that account for approximately 80% of the total bacteria in the rumen of Japanese Black cattle. The distribution of these core members was mainly affected by the increase in the concentrate diet during the fattening period. Additionally, the population dynamics of the core rumen bacterial members is likely to be complementary, resulting in their stable distribution during the fattening period. Therefore, the key findings of the present study provide an insight into the key players in stable rumen fermentation in Japanese Black cattle.

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CONFLICT OF INTEREST

Authors declare no conflict of interests for this article.

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