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Metagenome-Predicted Growth Rate And Metatranscriptomic Analysis Reveal A Slow Growth Rate Of And High Butyrate Formation By *Faecalibacterium Prausnitzii* In The Rumen Of Low Methane-Emitting Sheep

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Short Report

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Abstract

Background: Methane emissions from ruminants contribute to global warming and lead to energy loss from the ingested feed. Reducing methane emissions while increasing feed efficiency is one of the most important challenges facing the livestock industry. Previous studies have reported differences in lactate-metabolizing bacteria in the rumen between low-methane yield (LMY) and high-methane yield (HMY) sheep. It was hypothesized that methane emissions might also be related to the growth of certain bacteria. The objective of this study was to investigate the correlation between the growth and metabolism of rumen bacteria and methane emissions in sheep.

Results: The growth rates of 21 species-level and 12 genera-level rumen bacterial populations were predicted based on the peak-to-trough ratio of their metagenomic sequences that were generated from two groups of sheep differing in methane yield (LMY vs. HMY). The growth rate of Faecalibacterium prausnitzii was found to be significantly different between the LMY sheep and the HMY sheep. The relative abundance of F. prausnitzii was significantly lower in the LMY sheep than the HMY sheep, whereas the relative abundance of Intestinibaculum porci and Megasphaera elsdenii was significantly higher in the LMY sheep than the HMY sheep. Metatranscriptomic analysis showed that in the LMY sheep the expression of energy-related genes of F. prausnitzii, including those involved in butyrate production, was significantly upregulated compared to the HMY sheep.

Conclusions: The current study revealed a negative association between the growth rate of F. prausnitzii in the rumen and methane yield in sheep and between the growth rate of F. prausnitzii and the expression of its genes involved in energy metabolism including butyrate production. Our result also showed enrichment of lactate-producing bacteria (i.e., I. porci) and lactate-utilizing bacteria (i.e., M. elsdenii) in the LMY sheep. Together with the reported metabolic response of F. prausnitzii to lactic acid bacteria, our study corroborates the association between lactate-metabolizing bacteria and methane emissions via promoting butyrate production. M. elsdenii, I. porci, and F. prausnitzii may serve as biomarkers of methane yield from sheep, and possibly other ruminants.

Background

Ruminants depend on their rumen microbiome to efficiently convert ingestible (by the animals) plant carbohydrates into volatile fatty acids (VFA) and microbial protein as the direct sources of energy and nutrients [1–3]. Methane is, however, inevitably produced by the rumen microbiome as a byproduct during rumen fermentation [4]. Methane emissions from ruminants not only contribute to global warming but also lead to an approximately 10% loss of gross dietary energy [5]. Because the rumen microbiome is directly responsible for most of the methane produced by ruminants, many researchers have attempted to determine and understand the relationship between methane emissions and rumen microbiome composition and functions. The rumen microbiome consists of many hundreds or thousands of species of microbes, primarily bacteria, and they differ in their ability to utilize different nutrients and play different roles in nutrient degradation, animal productivity, and contributions to waste outputs, including

methane emissions [6, 7]. Numerous studies have compared the rumen microbiome of animals differing in methane emissions and tried to identify the rumen microbes that were associated with methane emissions [8–10]. Other studies explored and evaluated different strategies to mitigate methane emissions from ruminants, which include methane inhibitors, oils and fats, oilseeds, electron sinks (e.g., nitrate), and tanniferous forages [11–13]. A group of microbes is considered associated or correlated with methane emissions when its relative abundance has a positive or negative correlation with methane emissions. The abundance of individual microbial taxa and functional genes, and transcripts in the rumen microbiome can be determined using qPCR [14, 15], metataxonomics, metagenomics, and metatranscriptomics [9, 16, 17]. The growth rate of individual microbes directly reflects their metabolic activity, but abundance data may not reflect the actual growth rates because of differences in passage rate (feed particle-associated microbes have a lower passage rate than planktonic microbes), autolysis, infection with phages and lysis, etc. among microbes [18]. Determination of growth rates of individual microbes in the rumen can provide new insights into their relationship with animal traits including methane emissions.

Individual bacteria grow their populations by binary division. Populational growth of bacteria is determined using cultivation-based methods (e.g., the optical density of broth cultures, plate counts, most probable number) or cultivation-independent methods (primarily qPCR). None of the above methods can be used to determine the growth rate of individual bacteria represented by meta-omic sequence data. Hence, no meta-omic study has explored the relationship between methane emissions and the growth of individual microbes in the rumen. This knowledge gap hinders the identification of the important factors that may affect methane emissions by ruminants and hurdles the understanding of the microbiological underpinning of the methane emissions from ruminants. Metagenomic sequence data can be used to infer the growth rate of the bacteria, especially those represented by metagenome-assembled genomes. Before one bacterial cell divides into two, its chromosome (mostly one and circular, except for a few) is replicated starting from a single origin (*ori*) and terminating at the terminus (*ter*) [19]. In rapidly growing bacteria, the next round of chromosome replication initiates before the previous one finishes (Fig. 1, adapted from [20]). Korem T, *et al.* [20] found that the pattern of metagenomic sequencing read coverage for different microbial genomes contained a single trough and a single peak, and the peak and trough coincided with the *ori* and the *ter* regions, respectively. They demonstrated that the peak to trough coverage ratio (PTR) was highly correlated ($r = 0.95$) with the growth rate of individual bacteria measured by optical density. They concluded that PTR could serve as a quantitative measure of the growth rates of individual bacteria represented by metagenomic sequencing data. Further, Gao Y, *et al.* [21] developed a multi-sample algorithm, dynamic estimator of microbial communities (DEMIC), based on contigs and sequencing coverage values. The DEMIC algorithm can be used to calculate the PTR of individual metagenome-assembled genomes (MAGs), which represent individual bacteria, using a sequence of statistic techniques such as principal component analysis (PCA) and linear regression. We hypothesized that the growth rates, in addition to the abundance, of some rumen bacteria might be correlated with methane production in the rumen. The objectives of the current study were to determine the growth rates of rumen bacteria based on the metagenomic data generated from two groups of sheep (low-methane

yield, LMY, and high-methane yield, HMY) that differed in methane yield and to assess the correlations of the growth rate of rumen bacteria, together with their gene expression, with methane yields. We showed that the growth rate, abundance, and transcriptomic activities of some rumen bacteria were associated with methane emissions from sheep.

Methods

Metagenomic and metatranscriptomic data and analysis

The metagenomic, metatranscriptomic, and methane emission data from eight sheep with low methane yield (referred to as LMY sheep) and eight sheep with high methane yield (HMY) were published by Kamke J, *et al.* [8]. The metagenomic data (891.33 Gb in total) is publicly available in the Sequence Read Archive database (accession number: SRA075938) of the National Centre for Biotechnology Information (NCBI) and were downloaded. The metagenomic data were assembled using MEGAHIT v1.2.9 [22]. The metagenomic sequencing reads were mapped to the assembled contigs using BowTie2 v2.3.5 and samtools v1.13 [23, 24] to calculate the mapping rate and assess the assembly quality. MAGs were further constructed using MetaBat v2.15 [25]. The completeness and contamination of the MAGs were checked using CheckM v1.0.13 [26], and only those with completeness greater than 70% and a contamination rate less than 10% were retained and further analyzed. The MAGs were taxonomically classified using Kraken2 v2.1.2 [27]. The abundance of a MAG in a sample was calculated based on scaffolds in the MAG using the following equation:

$$\frac{\sum \mathrm{lengthofascaffoldinaMAG} \times \mathrm{depthofthescaffold}}{\sum \mathrm{lengthofascaffoldinaMAG}}$$

Then, the relative abundance of each MAG was calculated as the percentage of its abundance over the total abundance of the MAGs in each sample. The growth rate of individual bacteria represented by the MAGs was calculated as PTR using DEMIC v1.0.2 [21]. The DEMIC software has dependencies on the lme4 package [28] and FactoMineR package [29] in R v3.3.1 [30]. For the MAGs that were classified to a known species, their PTR and relative abundance were calculated at the species level. Otherwise, PTR and relative abundance were clustered at the genus level.

Metatranscriptomic data (62.82 Gb) of the same set of samples reported in the same study by Kamke J, *et al.* [8] were also retrieved from NCBI. The RNA reads were trimmed using Cutadapt v3.2 [31] and then filtered with a Q score > 20. Paired-end RNA reads were then merged using FLASH v1.2.11[32]. The merged RNA sequences were mapped to the UniRef90 database [33] using HUMAnN v3 [34] for annotation. The reference genomes (4 in total, Suppl Table S2) and gene annotations of *Faecalibacterium prausnitzii* were downloaded from the Integrated Microbial Genomes (IMG) database [35]. The expressed genes were annotated using the IMG database for IMG ID, the KEGG database for KEGG Orthology (KO), Enzyme Commission (EC) numbers, and Clusters of Orthologous Groups (COG) of proteins [36]. The merged RNA sequences were then mapped to the reference genomes using BBmap v38.18 [37]. Individual RNA sequences were normalized to transcripts per million (TPM) reads mapped

[38]. Then RNA sequences were also grouped by their KEGG descriptions and EC number. A pyruvate-to-butyrate fermentation pathway with EC annotations was manually reconstructed and adapted from the KEGG butanoate metabolism (map00650) pathway [36].

Statistical analysis

Growth rates and the relative abundance of individual bacteria, the expression of the *F. prausnitzii* genes mapped to the IMG database, and expressions of the *F. prausnitzii* genes grouped to KEGG descriptions were compared between the HMY and the LMY sheep groups using the Wilcoxon Rank Sum (WRS) tests in R [30]. All results presented are based on FDR adjusted [39] *P*-values. To increase the robustness of the results while avoiding reporting potentially spurious associations, we also used the TopKLists package [40] to integrate the results obtained from the WRS test, t-test, and ANOVA test with the gene expression data (Supple file 4). Since the result from the TopKLists showed high consistency with that from the WRS test, we focused only on the results from the WRS test. Statistical significance was declared when $P \leq 0.05$, while tendency was declared with $0.05 \leq P \leq 0.10$. The log of fold change (Log FC) for the gene expression data was calculated as $\log_2 \frac{(\text{meanofgeneexpressionsinLMY} + 1)}{(\text{meanofgeneexpressionsinHMY} + 1)}$, where 1 was added to avoid invalidity when the divisor was 0.

Results

Relative abundance and bacterial growth estimated from peak-to-trough ratio of metagenomes

The relative abundance of 79 genera or species differed ($P \leq 0.05$), while that of another 158 genera or species did not differ ($P > 0.05$) between the HMY and the LMY sheep (Suppl Table S1). The relative abundance of *F. prausnitzii* significantly differed ($P = 0.043$) between the HMY and the LMY sheep, with the LMY sheep having a significantly lower relative abundance than the HMY sheep (Fig. 2B). The relative abundance of *Intestinibaculum porci* was significantly higher in the LMY sheep compared to the HMY sheep ($P = 0.011$, 21.5% vs. 1.5%). Also, the relative abundance of *Megasphaera elsdenii* was significantly higher in the LMY sheep compared to the HMY sheep ($P = 0.018$).

We obtained 1,291 MAGs, each of which was at least 70% complete and had a contamination rate of less than 10%. The PTR (representing growth rates) of 55 MAGs was calculated. These 55 MAGs were assigned into 33 taxa, with 21 being assigned to species of bacteria, while the remaining 12 being only assigned to known genera (Table 1). Based on the WRS test with FDR-adjusted *P*-values, the PTR of *F. prausnitzii* differed ($P = 0.034$) between the LMY and HMY sheep (Table 1), with the LMY sheep having a lower PTR than the HMY sheep (Fig. 2A).

Table 1
Peak-trough ratio (means and standard deviations) of the MAGs classified to species or genera of rumen bacteria.

Taxa	HMY sheep	LMY sheep	Adjusted *P*-value
Faecalibacterium prausnitzii	1.724 (0.140)	1.525 (0.102)	0.034
Bacillus pumilus	1.515 (0.096)	1.415 (0.071)	0.176
Aminipila	1.74 (0.073)	1.669 (0.119)	0.176
Corynebacterium	2.048 (0.168)	2.138 (0.178)	0.214
Ruminococcus champanellensis	1.828 (0.193)	1.679 (0.162)	0.287
Pseudobutyrivibrio xylanivorans	1.725 (0.082)	1.823 (0.146)	0.337
Bacteroides	1.733 (0.112)	1.802 (0.077)	0.337
Bacillus	3.826 (0.178)	3.987 (0.228)	0.379
Olsenella	1.951 (0.272)	1.811 (0.150)	0.379
Prevotella	1.755 (0.069)	1.688 (0.111)	0.379
Oscillibacter	3.893 (0.434)	3.691 (0.087)	0.427
Streptomyces	9.775 (0.470)	9.419 (0.291)	0.427
Butyrivibrio hungatei	1.642 (0.107)	1.689 (0.115)	0.558
Clostridium	1.423 (0.175)	1.317 (0.134)	0.558
Blautia hansenii	1.678 (0.133)	1.744 (0.121)	0.689
Blautia producta	5.681 (0.323)	5.81 (0.226)	0.689
Blautia argi	1.453 (0.072)	1.492 (0.131)	0.759
Intestinibaculum porci	1.549 (0.100)	1.527 (0.119)	0.759
Butyrivibrio proteoclasticus	1.733 (0.137)	1.746 (0.180)	0.807
Anaerobutyricum hallii	1.654 (0.081)	1.642 (0.090)	0.807
Butyrivibrio fibrisolvens	6.702 (0.441)	6.645 (0.237)	0.807
Lachnoclostridium phocaeense	3.201 (0.209)	3.182 (0.205)	0.902
Eggerthella	7.548 (0.372)	7.468 (0.345)	0.902
Lactobacillus terrae	1.724 (0.197)	1.744 (0.211)	0.953
Microbacterium testaceum	1.765 (0.117)	1.797 (0.146)	0.953
Streptococcus parauberis	1.686 (0.125)	1.716 (0.119)	0.953

Taxa	HMY sheep	LMY sheep	Adjusted *P*-value
Alistipes	5.633 (0.224)	5.61 (0.265)	0.953
Clostridium butyricum	1.657 (0.252)	1.67 (0.222)	0.992
Ruminococcus albus	1.748 (0.245)	1.749 (0.085)	0.992
Anaerocolumna	1.858 (0.102)	1.865 (0.084)	0.992
Blautia	9.128 (0.262)	9.134 (0.344)	0.992
Desulfovibrio vulgaris	2.015 (0.152)	2.015 (0.129)	1.000
Selenomonas ruminantium	1.662 (0.094)	1.652 (0.085)	1.000

Annotation of metatranscriptomic sequences to F. prausnitzii **and comparison between sheep with different methane emissions.**

A total of 1,566 unique transcripts were mapped to the genome of *F. prausnitzii* across all the samples (Suppl. Table S2). Those expressed genes were grouped into 369 KO descriptions (Suppl. Table S3). In total, six of the genes were differentially expressed between the LMY and the HMY sheep (Table 2). The expression of IMG Gene 2836652010 encoding K00656 (pyruvate formate lyase [EC: 2.3.1.54]), IMG Gene 2836650584 encoding K01715 (enoyl-CoA hydratase [EC:4.2.1.17]), IMG Gene 2836648021 encoding DNA repair photolyase, and IMG Gene 2836648020 encoding Bacteriophage protein gp37 was significantly higher ($P < 0.05$) in the rumen of the LMY sheep compared to the HMY sheep. Also, the expressions of IMG Gene 2815274858 encoding K01572 (oxaloacetate decarboxylase, beta subunit [EC:4.1.1.3]), IMG Gene 2836652211 encoding K02747 (Phosphotransferase system, N-acetylgalactosamine-specific IID component), IMG Gene 2815275791, and IMG Gene 2836649234 tended to be upregulated ($P = 0.098$) in the LMY sheep compared to the HMY sheep. On the other hand, the expression of IMG Gene 2815278666 encoding K17318 (putative aldouronate transport system substrate-binding protein) and IMG Gene 2836651018 was significantly lower ($P < 0.05$) in the LMY sheep compared to the HMY sheep.

Table 2
Genes of *Faecalibacterium prausnitzii* mapped to the IMG database differentially expressed between the HMY and the LMY sheep.

IMG Gene ID	KO ID	KO Description	COG Function[1]	Adjusted *P*-value[2]	Log FC[3]
2815278666	K17318	Putative aldouronate transport system substrate-binding protein	-	0.041	-2.997
2836651018	-	-	Membrane protease subunit, stomatin/prohibitin family, contains C-terminal Zn-ribbon domain	0.041	-2.163
2836652010	K00656	Formate C-acetyltransferase [EC:2.3.1.54]	Pyruvate-formate lyase	0.041	1.892
2836648021	-	-	DNA repair photolyase	0.041	1.951
2836650584	K01715	Enoyl-CoA hydratase [EC:4.2.1.17]	Enoyl-CoA hydratase/carnithine racemase	0.041	2.019
2836648020	-	-	Bacteriophage protein gp37	0.041	3.416
2815275791	-	-	Alcohol dehydrogenase, class IV	0.098	1.287
2815274858	K01572	Oxaloacetate decarboxylase, beta subunit [EC:4.1.1.3]	Na+-transporting methylmalonyl-CoA/oxaloacetate decarboxylase, beta subunit	0.098	1.443
2836649234	-	-	Activator of 2-hydroxyglutaryl-CoA dehydratase (HSP70-class ATPase domain)	0.098	3.602
2836652211	K02747	PTS system, N-acetylgalactosamine-specific IID component	Phosphotransferase system, mannose/fructose/N-acetylgalactosamine-specific component IID	0.098	6.796

[1] Clusters of Orthologous Genes

[2] based on Wilcoxon signed rank test and adjusted with false discovery rate.

[3] The log of fold change (Log FC) was calculated by $\log_2 \frac{(\text{mean of gene expressions in LMY} + 1)}{(\text{mean of gene expressions in HMY} + 1)}$

Based on KEGG annotations, five of the gene groups showed significant differential expressions. The expressions of the genes encoding pyruvate formate lyase [EC: 2.3.1.54], raffinose/stachyose/melibiose transport system permease and phosphotransferase system (PTS), and N-acetylgalactosamine-specific IID component were significantly higher ($P = 0.05$) in the LMY sheep than the HMY sheep. On the other hand, genes coding for putative aldouronate transport system proteins significantly expressed lower ($P = 0.05$) in the LMY sheep compared to the HMY sheep. Further, the expression of genes coding for enoyl-CoA hydratase [EC:4.2.1.17] was nearly significantly higher ($P < 0.06$) in the LMY compared to the HMY sheep (Table 3). The expression of the gene encoding simple sugar transport system ATP-binding protein [EC:3.6.3.17] tended to be expressed lower ($P = 0.057$) in the LMY sheep compared to the HMY sheep. The pyruvate formate lyase [EC: 2.3.1.54] enoyl-CoA hydratase [EC:4.2.1.17] are involved in pyruvate-to-butyrate fermentation (Fig. 3).

Table 3
Genes of *F. prausnitzii* grouped by KEGG descriptions that were differentially expressed between the HMY and the LMY sheep.

KO Description	HMY sheep	LMY sheep	Adjusted *P*-value[2]	Log FC[3]
Putative aldouronate transport system substrate-binding protein	518.512(312.405)	112.707(83.371)	0.05	-2.192
Putative aldouronate transport system permease protein	521.323(285.191)	114.986(64.783)	0.05	-2.171
Formate C-acetyltransferase [EC:2.3.1.54]	833.869(353.873)	2376.412(953.184)	0.05	1.51
Raffinose/stachyose/melibiose transport system permease protein	6.614(12.324)	105.774(44.726)	0.05	3.81
PTS system, N-acetylgalactosamine-specific IID component	3.686(10.424)	519.661(381.504)	0.05	6.796
Simple sugar transport system ATP-binding protein [EC:3.6.3.17]	3447.143(1484.889)	1220.057(568.151)	0.057	-1.498
Enoyl-CoA hydratase [EC:4.2.1.17]	369.966(173.265)	762.381(251.693)	0.057	1.041

[1] count per million (CPM), means and standard deviations

[2] based on Wilcoxon signed rank test and adjusted with false discovery rate.

[3] The log of fold change (Log FC) was calculated by $\log_2 \frac{(\text{meanofgeneexpressionsinLMY} + 1)}{(\text{meanofgeneexpressionsinHMY} + 1)}$

Discussion

The rumen microbiome is responsible for methane emissions from ruminants, including the huge number (> 1 billion) of sheep across the globe [41]. The growth, abundance, and metabolic activities are reflected by DNA replication at the DNA level and transcription at the RNA level. This study explored the potential associations of methane emissions from two groups of sheep (with low or high methane yield) with the growth rate of rumen bacteria, in addition to their abundance and transcriptional activities. The elevated relative abundance of *M. elsdenii* (a lactate utilizer [42]) in the LMY sheep reflects its enrichment in the rumen of LMY sheep, as reported by Kamke J, *et al.* [8]. In addition, our result showed that *I. porci*, which is a lactate producer recently isolated from the intestine of swine [43], was substantially more predominant in the LMY sheep than in the HMY sheep (21.5% vs. 1.5%). Kamke J, *et al.* [8] reported that the LMY rumen was highly enriched with *Sharpea azabuensis*, which was initially isolated from the feces of thoroughbred horses and produced lactate [44]. They used 16S rRNA gene sequences to calculate the relative abundance of individual bacteria, whereas in the present study we calculated the relative abundance of MAGs that were at least 70% complete, and none of the MAGs that met the MAG criteria was classified as *S. azabuensis*. The classification based on 16S rRNA gene sequences vs. MAGs might also be a reason for the discrepancy. *S. azabuensis* and *I. porci* are within the same order (*Erysipelotrichales*), and they share 91% similarity in their 16S rRNA gene sequences [43]. They also share the major fermentation product, lactic acid [43, 44]. Thus, even though the study presented here did not identify any MAGs (> 70% complete) that could be assigned to *S. azabuensis*, the enrichment of lactate-producing bacteria of the *Erysipelotrichales* in the rumen of the LMY sheep was consistent in both studies. In addition to *M. elsdenii* and *S. azabuensis*, *F. prausnitzii* and *I. porci* may serve as potential biomarkers of methane production by the rumen microbiome.

F. prausnitzii (formerly *Fusobacterium prausnitzii*) is a butyrate producer, and it does not produce hydrogen [45], which is the primary reducing power of hydrogenotrophic methanogenesis in the rumen when fermenting sugars. It can also utilize lactate and produce butyrate consuming hydrogen [46]. Intriguingly, the LMY sheep had a lower relative abundance and growth rate (as estimated as PTR from metagenomic sequence data) of *F. prausnitzii* compared to the HMY sheep. *F. prausnitzii* has not been detected as a major species of the rumen bacteria in metataxonomic studies. However, it was represented by contigs in a metagenomic study of the rumen microbiome of Holstein cows, and it was shown to decrease in response to methane-mitigating diets [47], which agrees with the low abundance of this species in the LMY sheep. Our metatranscriptomic analysis, interestingly, revealed the upregulation of genes involved in carbohydrate metabolism, including pyruvate-formate lyase, enol-CoA dehydrogenase, both of which are involved in butyrate production, alcohol dehydrogenase, and oxaloacetate decarboxylase in the LMY sheep compared to the HMY sheep. It is also intriguing that the LMY sheep had significantly upregulated expression of the gene encoding the bacteriophage protein gp37 (Table 2), which belongs to the superfamily Radical S-adenosylmethionine [48] that was reported to activate pyruvate formate-lyase [49]. The PTS was also upregulated in the LMY sheep. It is not known if all the genes of this butyrate fermentation pathway need to be upregulated to increase butyrate production or these two enzymes mediate the rate-limiting steps of the pathway, but the upregulation of these two enzymes might suggest increased butyrate production by *F. prausnitzii* in the rumen of the LMY sheep.

Nevertheless, the upregulated expression of the *F. prausnitzii* genes involved in carbohydrate metabolism is consistent with an *in-vitro* study [50], which showed that carbohydrate metabolism was upregulated while DNA replication was downregulated in *F. prausnitzii* when expressed to cell-free supernatant of lactic acid bacteria. Although the present study did not detect any significant downregulation of growth-related genes of *F. prausnitzii*, the lower growth rate of *F. prausnitzii* in the LMY sheep than in the HMY sheep suggests that the growth-related genes of *F. prausnitzii* were downregulated in the LMY sheep. Also, the expressions of two tRNA synthetase (i.e., isoleucyl − tRNA synthetase, EC: 6.1.1.1; and tyrosyl − tRNA synthetase, EC: 6.1.1.5) genes selected by the TopKLists analysis (Suppl Table 3) showed downregulation in the LMY group, which concur with the downregulation of growth-related genes of *F. prausnitzii*. The lactate concentration in the rumen of the LMY sheep was much higher than in the HMY sheep (~ 0.9 vs. 0.014 mM) [8]. *F. prausnitzii*, as shown in the present study and the study by Lebas M, *et al.* [50], might adjust its metabolism towards carbohydrate metabolism when exposed to lactate. Its ability to utilize lactate [46] might be one plausible explanation.

Based on the results of the present study and previous studies [8, 50], we proposed a working model to illustrate the growth-related mechanism to explain promoted butyrate formation by *F. prausnitzii* in the rumen enriched with lactate-producing bacteria and how they collectively influence methane production (Fig. 4). In this mechanism, lactate-producing bacteria, such as *S. azabuensis* and *I. porci*, were enriched in the rumen microbiome of the LMY sheep. The lactate produced (~ 0.9 mM) was then utilized by butyrate producers, such as *M. elsdenii* and *F. prausnitzii*, in producing butyrate, which reduced the metabolic hydrogen available for methanogenesis. Specifically, after metabolites (probably lactate) were produced by lactate-producing bacteria, the expression of carbohydrate metabolism-related genes of *F. prausnitzii* was a high priority, and butyrate production was increased, but the growth of *F. prausnitzii* might have been simultaneously suppressed, as demonstrated previously [50]. In contrast, in the rumen of the HMY sheep, butyrate formation was not promoted because the abundance of lactate-producing bacteria and lactate concentration (0.014 mM) were low. This could result in increased acetate production and methanogenesis. Indeed, the rumen of the HMY sheep had a higher concentration of acetate but a lower concentration of butyrate compared to the rumen of the LMY sheep, even though the differences did not reach statistical significance [8].

As sequencing cost continues to decrease, sequencing coverage depth in metagenomic studies has increased substantially, allowing for more and more MAGs to be obtained from complex microbiomes. Indeed, nearly 5,000 MAGs were recovered from the rumen [51] and more than 10,000 MAGs were recovered from different segments of the gastrointestinal tract, including the rumen [52]. The approach used in this present study can be used to infer the growth of individual bacteria represented by MAGs in metagenomes in future studies so that their population dynamics can be determined and taken into consideration in interpreting microbiome data. A recent study showed that the accuracy of the DEMIC method was high for fast-growing bacteria, but relatively low for slow-growing bacteria [53]. Continued improvement in bioinformatics and statistical tools can further improve the estimation of bacterial growth using metagenomic data.

Conclusion

The rumen microbiome of the LMY sheep was enriched with lactate-producing and lactate-metabolizing bacteria. Growth rates could be estimated from the metagenomic data of the rumen. Growth rate prediction combined with metatranscriptomic analysis revealed correlations among the growth and expression of the *F. prausnitzii* genes involved in carbohydrate metabolism including butyrate production, and methane emissions from sheep. Lactate-producing bacteria, such as *S. azabuensis* and *I. porci*, and butyrate-producing bacteria that can utilize lactate, such as *F. prausnitzii* and *M. elsdenii*, may play a bigger role in affecting methane production than previously thought. They may serve as biomarkers of methane emissions from ruminants. Future research is warranted to explore the associations of these species, especially with respect to their growth and transcriptional activities, with methane emissions from ruminants.

Abbreviations

Low methane yield (LMY), High methane yield (HMY), Metagenome-assembled genome (MAG), Wilcoxon Rank Sum (WRS), False discovery rate (FDR), Joint Genome Institute Integrated Microbial Genomes (JGI IMG), Kyoto Encyclopedia of Genes and Genomes (KEGG) KEGG Orthology (KO), Enzyme Commission (EC), Clusters of Orthologous Groups (COG), Transcripts per million (TPM)

Declarations

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Availability of data and material

The data used in this study have been published by Kamke et al. (reference 8, Microbiome 2016, 4:56. doi: 10.1186/s40168-016-0201-2) and are available at the NCBI SRA database (accession number: SRA075938). The R codes used in the statistical analyses are presented in Additional file 2 and at Github (https://github.com/boyangzhang1993/PTR_Methane_Bioinformatic).

Authors' contributions

BZ and ZY designed the study. BZ collected and analyzed the data and wrote the manuscript. BZ, SL, and ZY interpreted the results and revised the manuscript. All the authors read and approved the final

manuscript.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

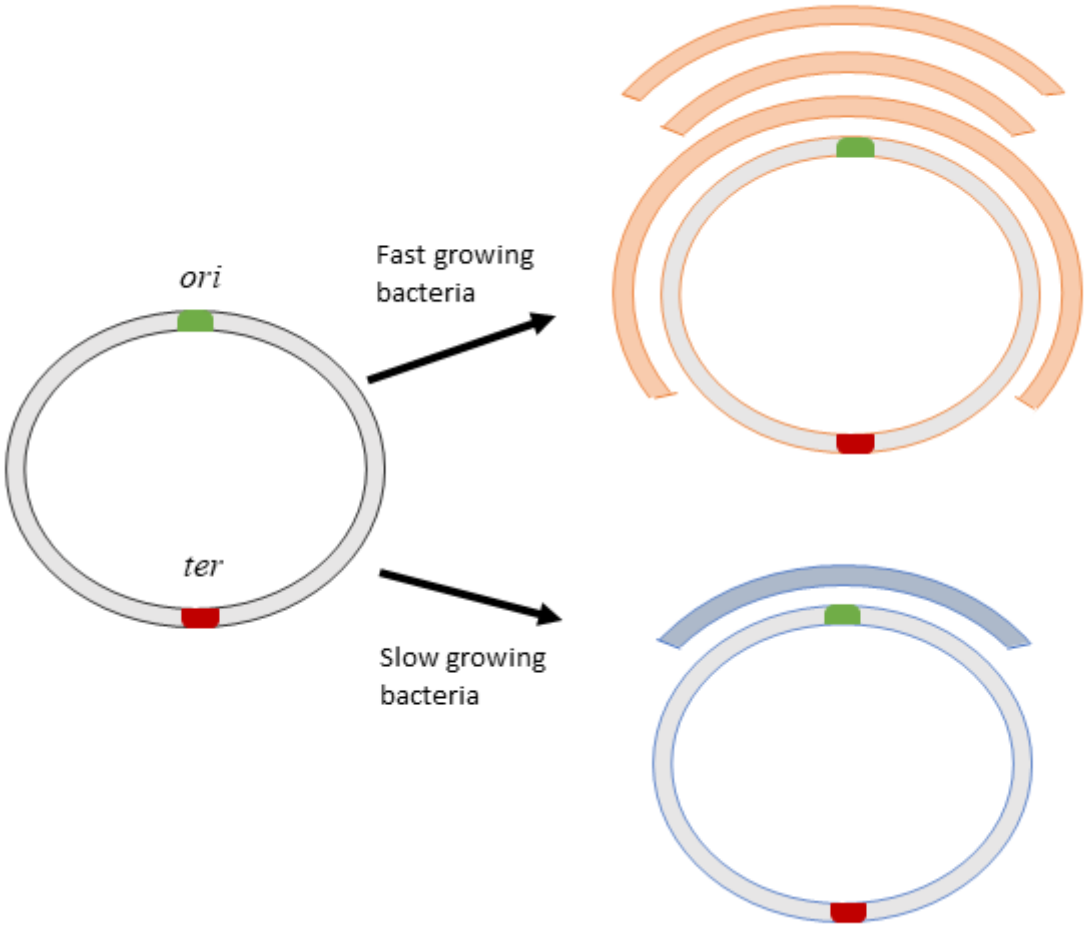

Figure 1

A schematic showing the replication of bacterial chromosome

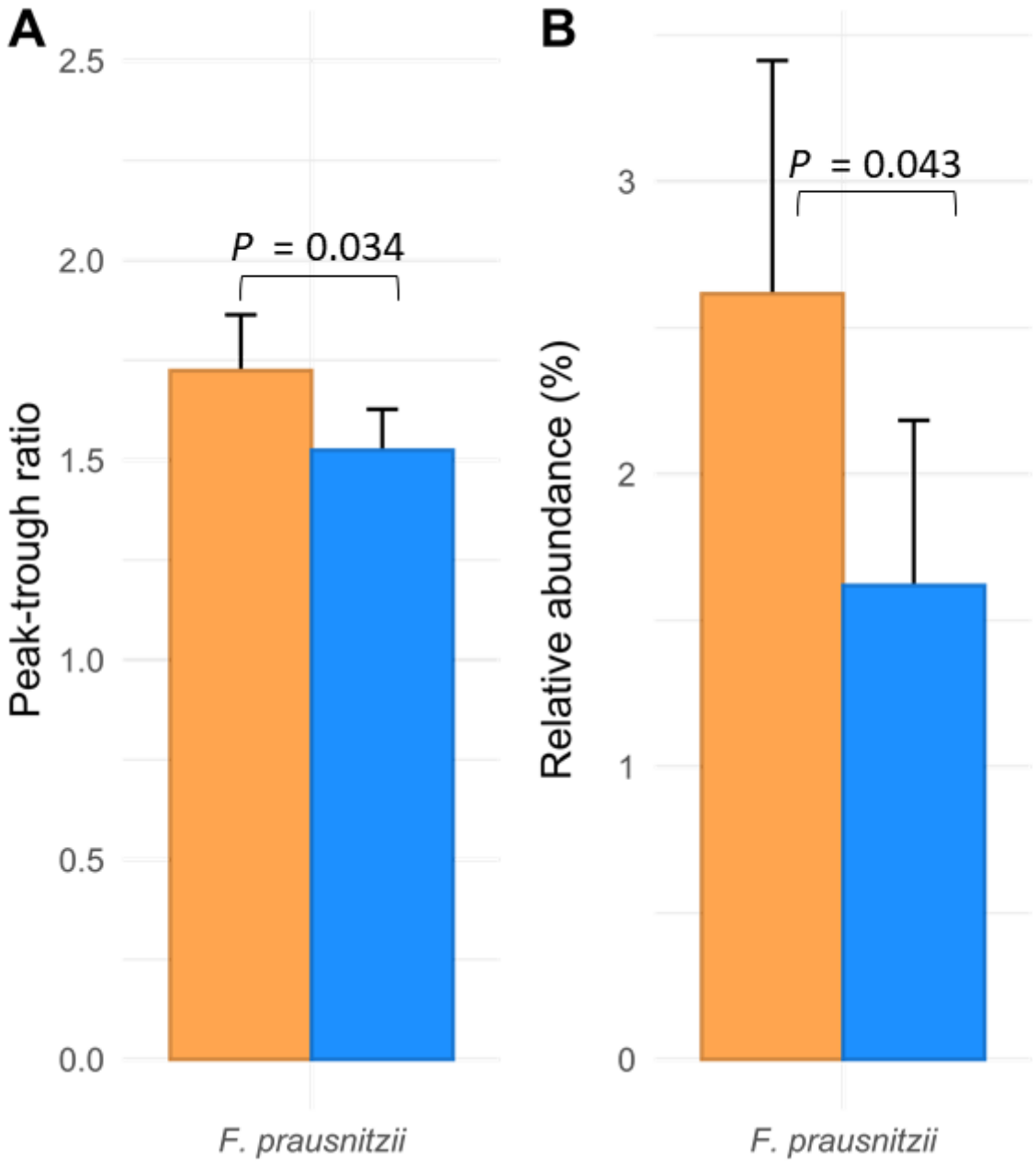

Figure 2

Growth rates as measured as peak-to-trough ratio of metagenome-assembled genomes (A) and relative abundances of *Faecalibacterium prausnitzii* in the rumen microbiome of the HMY (orange) and the LMY (blue) sheep. The error bars represent standard deviation.

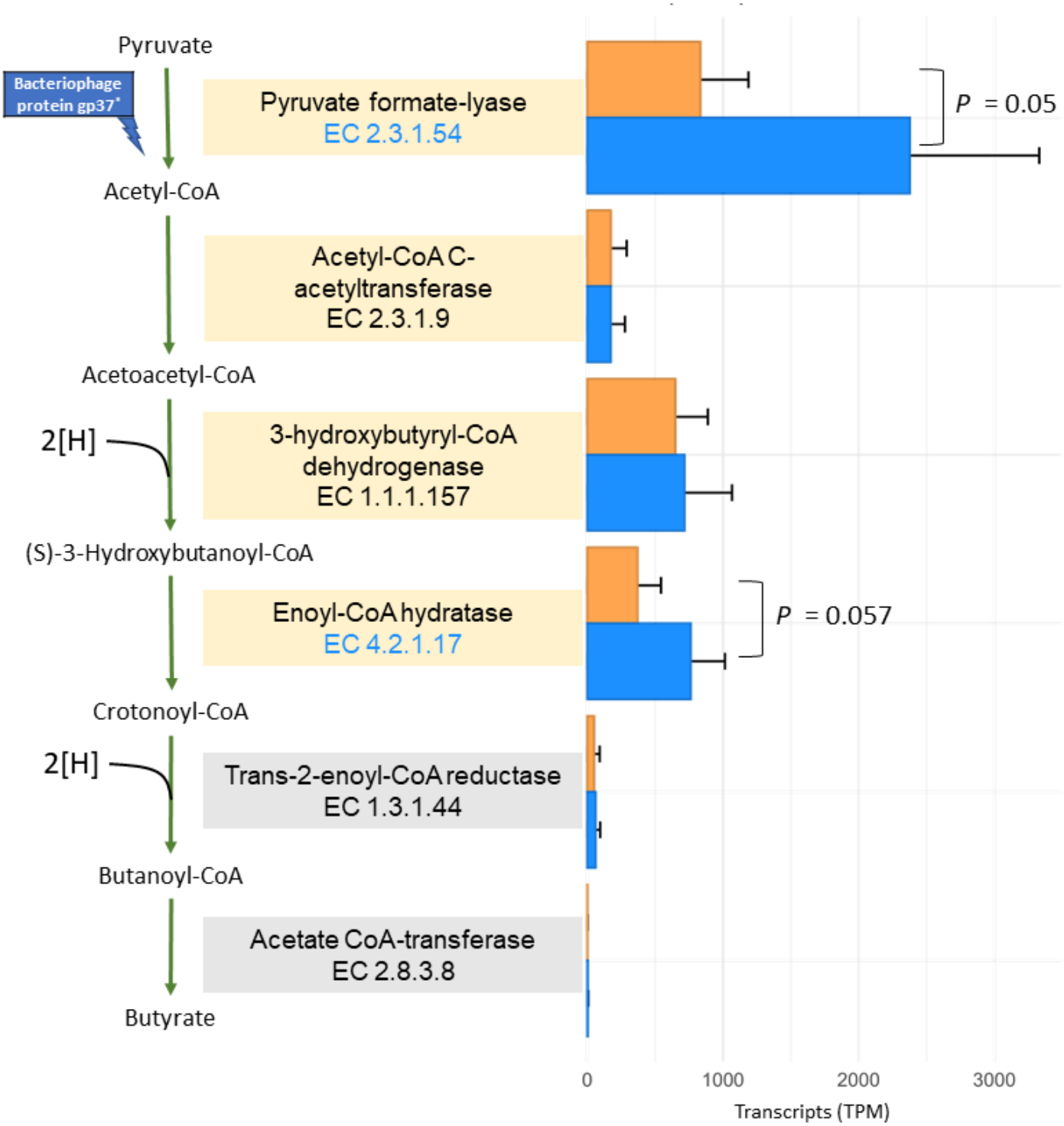

Figure 3

Expression of the genes of the butyrate fermentation pathway annotated to the genome of *Faecalibacterium prausnitzii* and/or the UniRef90 database between the HMY (orange) and LMY (blue) sheep. The yellow boxes indicate the RNAs mapped to the *F. prausnitzii* genome. The gray boxes indicate the RNA matched to the UniRef90 database. Blue EC numbers indicate the genes that were expressed nearly significantly different between the HMY and LMY sheep. The error bars in the bar plot represent

standard deviation. * Bacteriophage protein gp37 (IMG ID: 2836648020) can activate EC 2.3.1.54, and it was upregulated in LMY sheep.

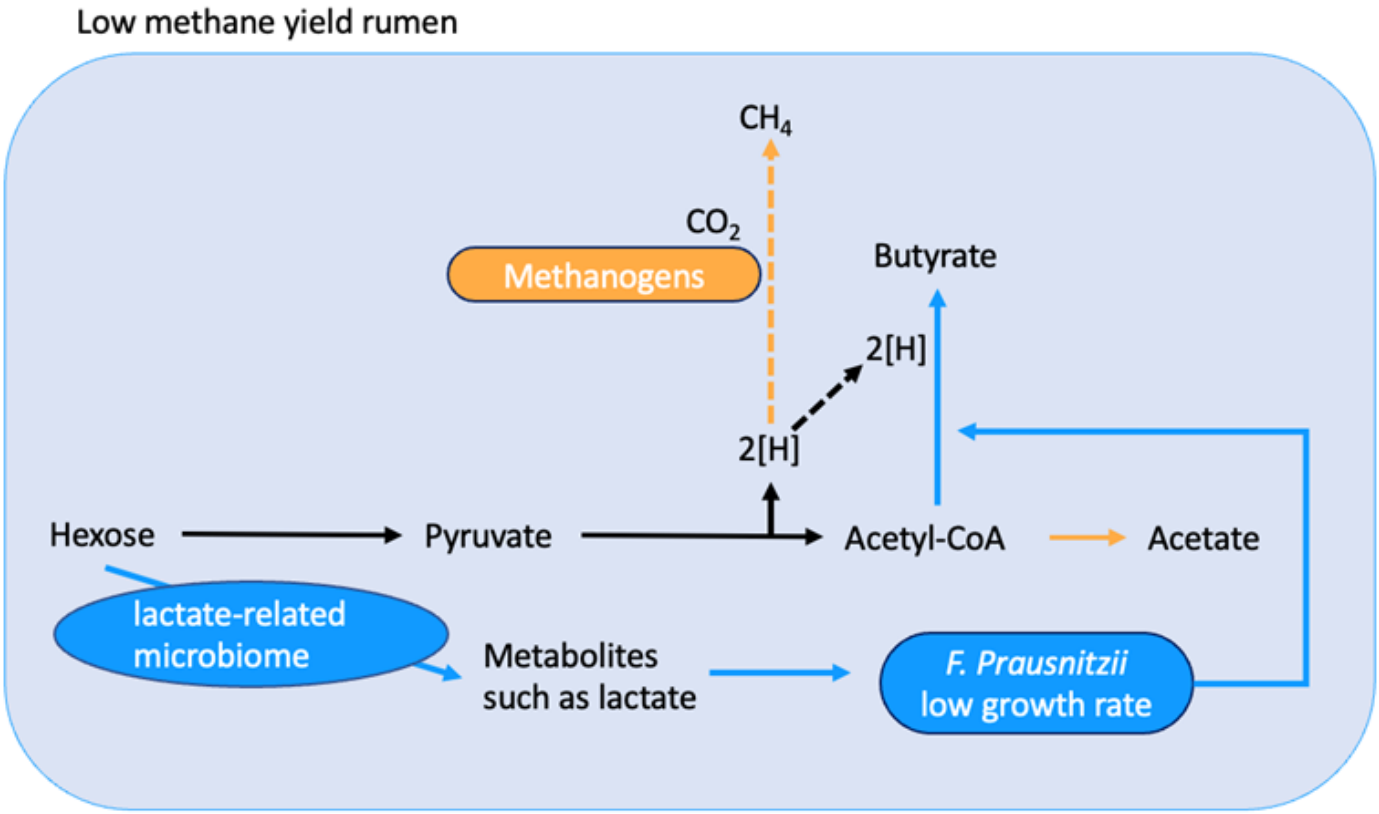

Figure 4

A working model showing how lactate-related metabolism and *Faecalibacterium prausnitzii* growth and butyrate formation affect methane production in the rumen of the LMY sheep. The yellow arrows indicate the formation of metabolic hydrogen formation and methane, and the blue arrows indicate the consumption of metabolic hydrogen and formation of butyrate. [H]: metabolic hydrogen.

Supplementary Files

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