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Effects of dietary supplementation of active dried yeast on fecal methanogenic archaea diversity in dairy cows

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ABSTRACT

This study aimed to investigate the effects of dietary supplementation of different dosages of active dried yeast (ADY) on the fecal methanogenic archaea community of dairy cattle. Twelve multiparous, healthy, mid-lactating Holstein dairy cows (body weight: 584 ± 23.2 kg, milk produced: 26.3 ± 1.22 kg/d) were randomly assigned to one of three treatments (control, ADY2, and ADY4) according to body weight with four replicates per treatment. Cows in the control group were fed conventional rations without ADY supplementation, while cows in the ADY2 and ADY4 group were fed rations supplemented with ADY at 2 or 4 g/d/head. Real-time PCR analysis showed the populations of total methanogens in the feces were significantly decreased ($P < 0.05$) in the ADY4 group compared with control. High-throughput sequencing technology was applied to examine the differences in methanogenic archaea diversity in the feces of the three treatment groups. A total of 155,609 sequences were recovered (a mean of 12,967 sequences per sample) from the twelve fecal samples, which consisted of a number of operational taxonomic units (OTUs) ranging from 1451 to 1,733, were assigned to two phyla, four classes, five orders, five families and six genera. Bioinformatic analyses illustrated that the natural fecal archaeal community of the control group was predominated by *Methanobrevibacter* (86.9% of the total sequence reads) and *Methanocorpusculum* (10.4%), while the relative abundance of the remaining four genera were below 1% with *Methanosphaera* comprising 0.8%, *Thermoplasma* composing 0.4%, and the relative abundance of *Candidatus Nitrososphaera* and *Halalkalicoccus* being close to zero. At the genus level, the relative abundances of *Methanocorpusculum* and *Thermoplasma* were increased ($P < 0.05$) with increasing dosage of ADY. Conversely, the predominant methanogen genus *Methanobrevibacter* was decreased with ADY dosage ($P < 0.05$). Dietary supplementation of ADY had no significant effect ($P > 0.05$) on the abundances of genera unclassified, *Candidatus Nitrososphaera,* and *Halalkalicoccus.* In conclusion, supplementation of ADY to the rations of dairy cattle could alter the population sizes and composition of fecal methanogenic archaea in the feces of dairy cattle. The decrease in *Methanobrevibacter* happened with a commensurate increase in the genera *Methanocorpusculum* and *Thermoplasma.*

1. Introduction

Methane (CH_4) contribution to the global greenhouse effect has been estimated to be 18–20% and agriculture could be responsible for about 50% of CH_4 emissions, with 2/3 attributed to rice culture and livestock [1]. Daily excretion, mostly by eructation, of CH_4 is estimated to be 400–500 L in an adult dairy cow, representing an 8–12% loss of carbon and energy of the net energy in the ration [2]. Therefore, limiting CH_4 emission in livestock would not only be beneficial for environmental protection, but also would enhance productivity.

Recent studies have illustrated the feasibility to manipulate CH_4 production through nutritional regulation [3,4]. Dairy cattle fed rations with high a concentrate to forage ratio decreased CH_4 emissions [5,6] and cattle fed high quality forage resulted in lower

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CH_4 production than cattle fed low quality forage [7,8]. Furthermore, CH_4 mitigation through the inclusion of compounds that exhibit methanogen toxicity, such as saponin [9], allicin [10], and tannin [11], in the ration of ruminants has also been well documented.

Active dry yeast (ADY), usually *Saccharomyces cerevisiae*, is now considered worldwide as having beneficial effects on the production of livestock, whether dairy cattle [12] or beef cattle [13], when high starch diets are provided. Supplementation of ADY has been shown to improve feed efficiency, rumen maturity, and stabilization of ruminal pH through interactions with lactate-metabolizing bacteria [12,14]. *In vitro* experiments showed yeast addition (4 mg/g dry matter (DM)) decreased CH_4 production, and it was suggested that yeast can be used to mitigate CH_4 production from dairy calves fed a high concentrate diet [15]. However, it was reported that live yeast (*Saccharomyces cerevisiae*) had no influence on CH_4 emission in beef cattle [16]. Moreover, *in vitro* trials revealed that horse fecal inocula combined with *Biocell F53* increased CH_4 production at the dose of 2 mg/g DM, but decreased CH_4 production by 78% at 4 mg/g DM after 24 h incubation [17]. Therefore, it remains controversial whether ADY supplementation is effective in reducing methane production.

Methanogenic archaea are a specific group of anaerobic microbes that are able to synthesize CH_4 as an anaerobic fermentation end-product in the rumen by utilizing carbon dioxide/hydrogen, formate, methanol, acetate, and methylamines as substrates [18,19]. It is well known that high feed efficiency cattle produce less CH_4 than low efficiency cattle, but no difference was detected in the total populations of methanogens between cattle with different feed efficiencies. However, the abundance of *Methanosphaera stadtmanae* and *Methanobrevibacter* sp. strain AbM4 were higher in low feed efficiency animals [20], indicating that methanogenesis activity in ruminants is associated with the community structure of methanogens. Targeting ruminal methanogenic archaea may provide a long-term solution for CH_4 mitigation [21–23]. However, the effect of ADY on the community structures of methanogenic archaea in ruminants has not yet been documented. Therefore, the present study was carried out to determine the effects of ADY on the population and diversity of fecal methanogenic archaea using Illumina MiSeq sequencing technology.

2. Materials and methods

The experimental protocol used in the present study was approved by the Animal Policy and Welfare Committee of the Agricultural Research Organization of Sichuan Province, China, and was in accordance with the guidelines of the Animal Care and Ethical Committee of the Sichuan Agricultural University.

2.1. Experimental animals, design, and sample collections

Twelve multiparous, mid-lactating Holstein dairy cows (body weight: 584 ± 23.2 kg, milk produced: 26.3 ± 1.22 kg/d) were housed in individual pens at the animal housing facilities of Sichuan Agricultural University, China. The 12 cows were randomly assigned to one of three treatment groups (four cows per treatment, with one cow in each replicate) for the 40 d experiment. The control cows were fed conventional ration (constituents and nutritional values are shown in Table S1) without adding ADY and the cows in the two treatment groups were fed ADY at 2 or 4 g/d/head on the basis of conventional rations [16]. The ADY was donated from Angel Yeast Co. Ltd. (Yichang City, Hubei Province, China, 2×10^{10} CFU/g). The ADY was mixed thoroughly with 100 g total mixed ration to make sure ADY was ingested by the cows and was fed once daily at 0800 h. Cows were fed twice daily at 0800 and 1700 h in equal portions. All cows had free access to water at all times. The 40 d experiment period consisted of two weeks of an adaptation period during which cows were fed with conventional rations, three weeks of a formal experiment period, and a 5 d sample collection period. The fresh feces of individuals were collected, mixed together, and stored at −20 °C before DNA extraction.

2.2. DNA extraction

Stored fecal samples were freeze-dried and total DNA was extracted from 200 mg of fecal sample using a QIAamp DNA Stool Mini Kit (Qiagen, USA) according to the manufacturer's protocol. DNA extracts were dissolved in 200 μL EB buffer and the quality and quantity of the extracted DNA were determined by UV spectrophotometric analysis using a NanoDrop ND-1000 Spectrophotometer (Nyxor Biotech, Paris, France). Because most of the concentrations exceed 1000 ng/μL, the DNA extracts were diluted 10 times and 50 times in the EB buffer prior to performing PCR. Fecal samples were numbered: control (1, 2, 3 and 4), ADY2 (5, 6, 7 and 8), ADY4 (9, 10, 11 and 12). All extracted DNA samples were stored at −20 °C until being used as templates for real-time qPCR and Illumina sequence analyses.

2.3. Real-time PCR analysis

Real-time qPCR was used to determine the change in total methanogenic abundance for the twelve fecal samples. The abundance of methanogens was determined by calculating the copy numbers of 16S rRNA genes. The methyl coenzyme-M reductase (*mcrA*) gene was targeted for the detection of methanogenic archaea (F:5′-TTCGGTGGATCDCARAGRGC to target the conserved amino acid sequence FGGSQR, R: 5′-GBARGTCGWAWCCGTAGAATCC to target the conserved amino acid sequence GFYGYDL [24]). The *mcrA* gene was cloned into white *Escherichia coli* first and followed by amplification and purification. The white *Escherichia coli* M13 amplification product was diluted and a spectrophotometer (Qubit 2.0, Invitrogen, Life Technologies, CA, USA) was used to determine the plasmid concentration of the *mcrA* clone. The PCR assay was carried out using an ABI Gene Amp 9700 thermocycler in the following conditions: initial denaturation at 95 °C for 2 min followed by 30 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s. The reaction was terminated after an extension step at 72 °C for 5 min and a cooling step at 10 °C. The copy number was calculated based on the size of the fragments amplified using the equation $NA \times A \times 10^{-9}/(660 \times n)$, where NA is Avogadro's constant, 6.02×10^{23} molecules per mol, A is the molecular weight of the molecules in the standard, and n is the size of the fragment amplified [25]. The size of the PCR fragment analyzed in this study was ~800 bp. PCR efficiency (E) was calculated from the linear regression of the standard curve using equation $E = 10(^{-1/slope})-1$ and the data derived from reactions with efficiency between 90% and 110% were used for further analysis. The result of the quantitative real-time PCR is shown in Table 1.

2.4. Amplification and Illumina library generation

The variable region V3–V4 of the archaeal 16S rRNA gene was amplified using primers Arc915af (5′-AGGAATTGGCGGGGGAGCAC-3′) and Arc1386R (5′-GCGG TGTGTGCAAGGAGC-3′) [20]. A two-step PCR amplification procedure with different primer pairs was used in library construction. Fragments were amplified in the first step using interest-specific primers with overhang adapters attached (915F TTCCCTACACGACGCTCTTCCGATCTAGGAATTGGCGGGGGAGCAC, 1386R