Journal of Integrative Agriculture 2017, 16(5): 1130–1137

Available online at www.sciencedirect.com

ScienceDirect

RESEARCH ARTICLE

Comparison of rumen archaeal diversity in adult and elderly yaks (*Bos grunniens*) using 16S rRNA gene high-throughput sequencing

WANG Li-zhi, WANG Zhi-sheng, XUE Bai, WU De, PENG Quan-hui

Animal Nutrition Institute, Sichuan Agricultural University, Ya'an 625014, P.R.China

Abstract

This study was conducted to investigate the phylogenetic diversity of archaea in the rumen of adult and elderly yaks. Six domesticated female yaks, 3 adult yaks ((5.3±0.6) years old), and 3 elderly yaks ((10.7±0.6) years old), were used for the rumen contents collection. Illumina MiSeq high-throughput sequencing technology was applied to examine the archaeal composition of rumen contents. A total of 92 901 high-quality archaeal sequences were analyzed, and these were assigned to 2 033 operational taxonomic units (OTUs). Among these, 974 OTUs were unique to adult yaks while 846 OTUs were unique to elderly yaks; 213 OTUs were shared by both groups. At the phylum level, more than 99% of the obtained OTUs belonged to the Euryarchaeota phylum. At the genus level, the archaea could be divided into 7 archaeal genera. The 7 genera (i.e., *Methanobrevibacter*, *Methanobacterium*, *Methanosphaera*, *Thermogymnomonas*, *Methanomicrobiu*, *Methanimicrococcus* and the unclassified genus) were shared by all yaks, and their total abundance accounted for 99% of the rumen archaea. The most abundant archaea in elderly and adult yaks were *Methanobrevibacter* and *Thermogymnomonas*, respectively. The abundance of Methanobacteria (class), Methanobacteriales (order), Methanobacteriaceae (family), and *Methanobrevibacter* (genus) in elderly yaks was significantly higher than in adult yaks. In contrast, the abundance of *Thermogymnomonas* in elderly yaks was 34% lower than in adult yaks, though the difference was not statistically significant. The difference in abundance of other archaea was not significant between the two groups. These results suggested that the structure of archaea in the rumen of yaks changed with age. This is the first study to compare the phylogenetic differences of rumen archaeal structure and composition using the yak model.

Keywords: yak, archaea, rumen, diversity, high-throughput sequencing

1. Introduction

Yak (*Bos grunniens*) is a unique species, which exclusively lives in alpine and subalpine regions at altitudes ranging from 3 000 to 5 000 m (Wang *et al.* 2006). There are 15 million yaks in the Qinghai-Tibetan Plateau of China, accounting for more than 90% of the total yak population worldwide (Zhang *et al.* 2014). The Qinghai-Tibetan Plateau is located at high altitude with severe weather and poor natural conditions. Yaks are normally managed under free grazing conditions

Received 16 May, 2016 Accepted 14 July, 2016
WANG Li-zhi, Mobile: +86-13882438306, E-mail: 184750130@qq.com; Correspondence PENG Quan-hui, Tel: +86-835-2885125, Fax: +86-835-2885065, E-mail: pengquanhui@126.com

doi: 10.1016/S2095-3119(16)61454-5

all year round with no concentrate supplementation, even in the winter when grass is covered by snow (Ding *et al.* 2007). To adapt to this harsh natural environment, yaks have evolved physiologically to survive in such poor conditions (Ishizaki *et al.* 2005; Wang *et al.* 2009; Shao *et al.* 2010). It was documented that yaks produce less enteric methane to reduce energy loss compared with other ruminants (Ding *et al.* 2010). The methane (CH_4) energy loss accounts for over 7% of the total energy intake in ruminant animals given forage-based diets (Yan *et al.* 2000). Although the CH_4 emission rate may be relatively low, yaks remain a significant source of CH_4 production in China due to their large population (Xue *et al.* 2014). The reduction of enteric CH_4 emission has been recognized as an effective approach to lower the global methane emission, which can also enhance the livestock production efficiency (Johnson *et al.* 1993). Methane that is emitted by ruminants is produced by archaea in the rumen. To reduce ruminant methane emissions, it is essential to understand the archaeal community composition in the rumen and to characterize its phylogeny.

Previous research indicated that archaeal methanogen diversity in the gastrointestinal tract could vary between animal species (Pei *et al.* 2010; Luo *et al.* 2013), and animals of different ages could have different microbial communities in the rumen (Jami *et al.* 2013). However, currently, there is little information on the diversity of archaea in the rumen of yaks and on the effects of ageing process on their ruminal archaea composition. We hypothesized that the structure and composition of archaea in the rumen of yaks might be different compared with other ruminants, and changes would happen with ageing. Therefore, the present work was conducted to (i) examine the diversity of archaea in the rumen of yaks and to (ii) compare the differences in archaeal composition in the rumen of adult and elderly yaks using the high-throughput gene sequencing technology. This comprehensive knowledge can promote the understanding of the factors that affect the archaeal diversity in the rumen and lay the foundation to study the widely applicable and long-term regulation of the rumen methane production.

2. Materials and methods

2.1. Animals and sampling

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

Six female domesticated yaks were used in the present study — 3 adult yaks ((5.3±0.6) years old and (214.5±18.3) kg of live weight) and 3 elderly yaks ((10.7±0.6) years old and (294.3±17.3) kg of live weight). One month before sample collection, all yaks grazed on a single natural grassland sward with no concentrate supplementation at the commercial farm at the Ganzi-Tibetan Autonomous Prefecture, Sichuan Province, China. The average altitude of this farm was 3 500 m with a plateau monsoon climate. Rumen content samples (approximately 100 mL) were collected from different positions of the rumen (Petri *et al.* 2013) immediately after the yaks were slaughtered at the commercial abattoir. The rumen content was filtered through 4 layers of cheese cloth, and the rumen fluid was immediately placed in liquid nitrogen (Guo *et al.* 2015). The samples were taken to the laboratory and stored at –80°C until the DNA extraction.

2.2. DNA extraction

Total DNA was extracted separately from the rumen contents using a commercially available kit (Tiangen, China) according to the manufacturer instructions. Each sample was analyzed in triplicates and finally pooled. DNA samples were purified using a PCR Clean-Up system (Promega, Madison, USA) and then stored at −20°C for further processing.

2.3. PCR and amplicon sequencing

The archaea-specific primers, U519F (5′-CAGYMGC CRCGGKAAHACC-3′) and U806R (5′-GGACTACHVGG GTWTCAAT-3′), were used to amplify the V4 hypervariable region of the archaeal 16S rRNA gene (Shehab *et al.* 2013). Three replicates of the DNA extract from each sample were amplified using PCR. PCR was carried out using a PCR thermal cycler Model C1000 (Bio-Rad, Richmond, CA) with the following thermal cycling conditions: initial denaturation at 94°C for 3 min, followed by 30 cycles at 94°C for 30 s, annealing at 56°C for 30 s and at 72°C for 30 s. The reaction was terminated after the extension step at 72°C for 5 min. The total volume of the reaction mixture was 50 μL, which consisted of 0.5 μL of each primer (50 pmol each), 5 μL of 2.5 mmol L^{-1} dNTP mixture, 5 μL of 10× *Ex Taq* buffer (20 mmol L^{-1} Mg^{2+}; TaKaRa, Dalian, China), 0.25 μL of *Ex Taq* DNA polymerase (TaKaRa), 1 μL of an environmental DNA template, and 37.75 μL milli-Q water. The PCR products were visualized using a 2% agarose gel electrophoresis and purified using a PCR Purification Kit (QIAGEN, Australia). The purified PCR product was quantified using a Quant-iTPicoGreen dsDNA Reagent Kit (Life Technologies, USA) according to the manufacturer instructions and combined in equimolar ratios into a single tube. Then, the samples were sent to Macrogen Inc. (South Korea) for sequencing