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Archaeal community structure diversity in the rumen of faunated and defaunated sheep

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Abstract. Elimination of protozoa from the rumen is usually accompanied by a decrease in methane production. However, the possible changes brought about by defaunation upon the structure of the methanogenic population are not known. Archaeal communities of rumen samples from 2 faunated (F), 2 recently defaunated (3 months, D−), and 2 long-term defaunated (+1 year, D+) sheep were profiled by PCR-denaturing gradient gel electrophoresis (DGGE) using 16S rDNA primers. Dendrogram analysis of the whole densitometric curves separated F and D+ in different clusters. Samples from D− animals did not show high similarity and were not separated into an independent cluster although they tended to be placed at an intermediate distance between F and D+. Principal components analysis (PCA) also confirmed these results. Bands explaining most of the differences in PCA could be correlated ($P<0.05$) to the presence/absence of protozoa. As measured by the Shannon index (H) based on DGGE operational taxonomic units, D+ populations had a lower diversity than F or D− populations ($P<0.05$). Shifts in the archaeal community structure of the rumen in the presence or absence of protozoa were identified. Population changes that follow defaunation seem to develop slowly as evidenced by the differences between recent and long-term defaunated animals.

Keywords: Protozoa; Archaea; Diversity; Rumen ecosystem; Defaunation

1. Introduction

Anaerobic microbial fermentation of feed components generates CH_4 in the rumen. The production of this gas represents a lost in energy for the animal of 6% to 8% of the dietary intake. It is also detrimental for the environment as ruminants are the main agricultural source of this potent greenhouse gas [1]. Methanogens produce CH_4 primarily from H_2,

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CO_2, and acetate but in the rumen the hydrogenotrophic pathway predominates. Protozoa liberate H_2 as one of the major end products of their metabolism and consequently play a key role in the interspecies hydrogen transfer and methane production in the rumen. In addition, there is a close physical association between protozoa and methanogens, which are found both on the surface and inside the protozoal cell [2,3]. Elimination of protozoa or defaunation has been shown to reduce CH_4 production by up to 50% depending on the diet (reviewed in [4]), and has been proposed as a way to mitigate emissions [4,5]. Using animals in which the defaunation period differed in length, we have also found that the absence of protozoa decreases methane emissions both in vitro and in vivo when defaunation was 3 months or less. However, animals defaunated for more than 1 year emitted similar amount of CH_4 than faunated animals ([6] and unpublished data). The objective of this work was to determine the diversity and distribution of archaea in the rumen of these faunated and defaunated sheep using the PCR-DGGE community fingerprinting technique.

2. Materials and methods

Six wethers fitted with rumen cannulae were used as donors of rumen fluid. Two animals had a regular mixed protozoal population (F), while the other four animals had previously been defaunated by rumen emptying and washing following the method of Jouany and Senaud [7]. At the time of experimentation, two of these animals had been kept defaunated for 6 to 12 weeks (short-term defaunation, D−) while the other two had been kept in that state for more than 1 year (long-term defaunation, D+). Animals were fed a maintenance diet twice daily. Rumen samples were taken 3 h after the morning feeding and whole rumen contents were strained through a polyester monofilament fabric to obtain liquid and solid fractions.

Total DNA was extracted using the UltraClean Fecal DNA Kit from MoBio Laboratories, Inc. (Solana Beach, CA) and a region of the 16S rDNA gene was amplified by PCR using archaeal primers 0348aF (5′-TCCAGGCCCTACGGG-3′) and 0690aR (5′-TTACAGGATTTCACT-3′) [8], the forward primer had a GC clamp at its 5′ end. DGGE was performed on an 8% (w/v) polyacrylamide gel in 0.5 mM TAE with a denaturant gradient of 25–65% run at 200 V for 5 h at 60 °C. Gels were silver stained and images were acquired and analysed using LabScan v5 and ImageQuant TL v2003 softwares (GE Healthcare). The similarity matrix for the profiles was evaluated by the Pearson product–moment correlation and clustered using the unweighted pair group method with arithmetic averages (UPGMA) in SAS v8 (SAS Inst., Inc.) with results presented as dendrograms. Principal components analysis (PCA) was made in Minitab 13 (Minitab SARL, France). Single bands were used for correlation analysis between band intensity and other biological descriptors such as volatile fatty acids, pH, Eh, and presence/absence of protozoa. Indices of biological diversity, the Shannon index of diversity, and the indices of evenness and dominance, were calculated for each sample and tested for differences using one-way ANOVA (SAS Inst., Inc.).

3. Results and discussion

Cluster analysis of the whole densitometric curves revealed that F and D+ samples were separated at the fist node (Fig. 1a). Animals from D−, which had a low within group similarity, were not separated into an independent cluster, although they tended to be