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ORIGINAL ARTICLE

Evaluation of group-specific, 16S rRNA-targeted scissor probes for quantitative detection of predominant bacterial populations in dairy cattle rumen

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Abstract

Aims: To develop a suite of group-specific, rRNA-targeted oligonucleotide scissor probes for the quantitative detection of the predominant bacterial groups within the ruminal microbial community with the rRNA cleavage reaction-mediated microbial quantification method.

Methods and Results: Oligonucleotides that complement the conserved sites of the 16S rRNA of phylogenetically defined groups of bacteria that significantly contribute to the anaerobic fermentation of carbohydrates in ruminal ecosystems were selected from among published probes or were newly designed. For each probe, target-specific rRNA cleavage was achieved by optimizing the formamide concentration in the reaction mixture. The set of scissor probes was then used to analyse the bacterial community in the rumen fluids of four healthy dairy cows. In the rumen fluid samples, the genera *Bacteroides/Prevotella* and *Fibrobacter* and the *Clostridium coccoides-Eubacterium rectale* group were detected in abundance, accounting for 44–48%, 2·9–10%, and 9·1–10% of the total 16S rRNA, respectively. The coverage with the probe set was 71–78% of the total bacterial 16S rRNA.

Conclusions: The probe set coupled with the sequence-specific small-subunit rRNA cleavage method can be used to analyse the structure of a ruminal bacterial community.

Significance and Impact of the Study: The probe set developed in this study provides a tool for comprehensive rRNA-based monitoring of the community members that dominate ruminal ecosystems. As the ruminal microbial community can be perturbed, it is important to track its dynamics by analysing microbiological profiles under specific conditions. The method described here will provide a convenient approach for such tracking.

Introduction

Ruminant animals harbour a complex microbial community comprising a diverse array of bacteria, archaea, protozoa and fungi in the rumen. For decades, the importance of these micro-organisms for rumen function has been studied intensively with regard to, e.g. the emission of methane from livestock and its suppression (Dumitru *et al.* 2003; Eun *et al.* 2004; Mohammed *et al.* 2004; Sar *et al.* 2004). However, there are still methodological obstacles to precisely and rapidly monitor the entire ruminal microbial community at the species and genus

