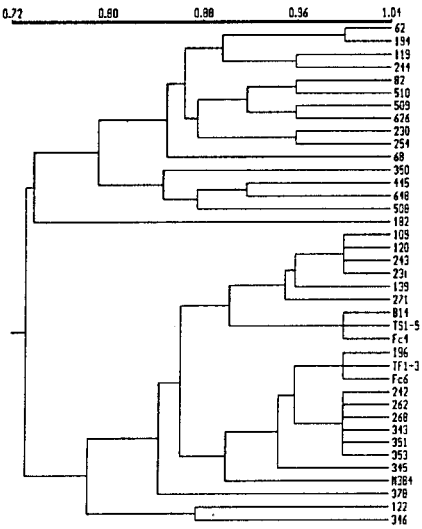

Dendrogram based on restriction enzyme cleavage patterns obtained with *Taq*I, *Hha*I, *Alu*I and *Dde*I for PCR-amplified 16SrDNA from clones recognised by the BacPre probe. An unweighted pair group method analysis (UPGMA) was performed. Profiles from cultured *Prevotella* strains $B_1$4, M384, 23, TF1-3, TS1-5, FC2, FC4) are included for comparison.

of *Prevotella* strains present in a single animal.

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An investigation of microbial diversity in the rumen of dairy cattle using comparative sequence analysis of cloned 16S rRNA genes. RJ Forster, MF Whitford, CE Beard, J Gong (*Centre for Food and Animal Research, Agriculture and Agri-Food Canada, Ottawa, ON, Canada, K1A OC6)*

The concentration of bacterial cells in the rumen may reach 10^{11}-10^{12} cells ml^{-1}, but it is usually possible to culture only a small fraction of these numbers. Bacteria in the rumen have generally been studied after isolation on selective media. Bacteria which are not able to grow on selective or non-selective media may include cells of known types that are inviable or unable to reproduce. Bacteriocins, which have been shown to be prevalent amongst the genus *Butyrivibrio* [1], would also inhibit the growth of many strains in vitro. However it may also be that most in vitro culture conditions are simply not able to support the growth of many rumen bacteria. The extent to which unfamiliar bacterial strains contribute to the rumen ecosystem is therefore unknown. This is of concern to rumen microbiologists, especially when effects of novel or genetically engineered rumen bacteria on the rumen ecosystem need to be evaluated.

In preliminary attempts to address this problem we have directly amplified 16S rRNA gene sequences from ruminal fluid samples of dairy cattle. Total DNA was extracted from the rumen fluid of ten cattle fed haylage/corn silage/concentrate rations at two different times, using a bead-beating, phenol/chloroform extraction method. Primers which are homologous to most 16S rRNA genes were used in PCR reactions to amplify almost complete 16S rRNA gene sequences (approx. 1450 bp). One set of DNA extractions was amplified using either 12 or 30 cycles of PCR in order to examine biases introduced during

the reactions. The PCR products were cloned into plasmids using blunt-end ligations and some of the resulting transformants were sequenced.

Approximately 30 sequences have been obtained from the first set of clones from the first five cows sampled. From the second set of cattle approximately 75 sequences have been obtained from DNA amplified using 12 cycles and 45 sequences from PCR using 30 cycles. Sequences from the 5′ end of the 16S rRNA gene were compared to existing sequences in Ribosomal Database Project and to sequences determined from common rumen bacterial species produced in our laboratory. The first set of clones produced a data set in which approximately 50% of the sequences were similar to low G+C gram positive bacteria related to the genus *Clostridia*, the majority of which were closely related to bacteria in Cluster XIV of Collins [2]. Approximately 30% of the cloned sequences were related to bacteria in the *Prevotella-Bacteroides* group. The second set of clones produced a data set in which the majority of sequences were related to the *Prevotella-Bacteroides* group, regardless of the number of cycles of PCR.

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Microbial community structure of the rumen as assessed by denaturing gradient gel electrophoresis of polymerase chain-reaction amplified 16S rDNA genes. SA Kocherginskaya, JM Simpson, and BA White *(Department of Animal Sciences, University of Illinois, 1207 West Gregory Drive, Urbana, IL 61801, USA)*

A new approach for directly determining the genetic diversity of complex microbial populations is based on separation of polymerase chain-reaction (PCR)-amplified 16S rDNA products in linear denaturing gradient polyacrylamide gels. In denaturing gradient gel electrophoresis (DGGE), DNA fragments of the same length but with differing base-pair sequences, can be separated. This procedure has been applied to the analysis of PCR fragments derived from variable regions of the 16S rDNA. Fragments obtained after PCR amplification of 16S rRNA genes from genomic DNA of uncharacterized mixtures of microorganisms can be rapidly and easily analyzed. Subsequent hybridization with group-specific oligonucleotide probes can be used to identify particular constituents of the population. This procedure allows the direct identification of the presence and relative abundance of different species and provides the means to profile microbial populations in both a qualitative and semiquantitative way.

We have applied this procedure to rumen samples from steers fed different diets to determine the utility of this technique for the analysis of a very complex microbial community. Four steers were fed a diet of medium-quality grass-legume hay at maintenance and four other steers were fed a diet of 20% hay, 52% corn, 5% corn steep liquor, 3% miscellaneous, and 20% of either corn starch, corn gluten feed, modified corn fibre, or distillers dried grains. Rumen samples were harvested approximately 1 h prior to feeding. Total