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

genomic DNA was isolated from these centrifuged cell pellets and used for amplification of either the V3 or V9 region of the 16S rDNA gene. When the different PCR profiles obtained from amplification of the V3 region of the 16S rDNA were compared for samples from animals fed the grass-legume hay diet, the patterns were remarkable similar. The DGGE profiles demonstrated at least 16 distinguishable bands, with five predominant. PCR profiles obtained from amplification of the V3 region of samples from animals fed the different corn based diets, showed profiles which were distinctly different from those of the animals fed the grass-legume hay diet. The profiles from each of these four corn-based diet fed animals also differed from each other. In general, these profiles were less complex, with four to five predominant bands and, depending on the animal, at least eight distinguishable bands of lesser intensity. Samples from the animals fed modified corn fiber or distillers dried grains gave profiles that were most similar. In comparison to these two animals, the sample from the animal fed the corn gluten diet was only slightly different in its profile, lacking two of the predominant species. The profile obtained from the sample from the animal fed the cornstarch diet was distinctly different from profiles from samples from the other three animals, with three different predominant species being present. Analysis of PCR profiles obtained from amplification of the V9 region of the 16S rDNA were in general less complex than PCR profiles obtained from the V3 region. Nonetheless, distinct profiles were obtained. These results demonstrate that this technique will contribute to our understanding of the genetic diversity and community structure of the rumen ecosystem.

A 16S rDNA-based molecular profiling approach for studying relative changes in ruminal bacterial populations. J Wood[1], KP Scott,[1], G Avguštin[2], CJ Newbold[1], F McIntosh[1] HJ Flint[1] ([1]*Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK;* [2]*Biotechnical Faculty, University of Ljubljana, Grobje 3, 61230 Domzale, Slovenia)*

16S rRNA-based oligonucleotides targetted at particular groups of rumen microbes have been used successfully to follow specific populations in rumen samples [1]. While this approach gives valuable information, a range of probes is needed to gain simultaneous information on different groups, and probe sequences are defined mainly from cultured organisms. Random sequencing of PCR-amplified material can provide information on uncultivated organisms but is not a practical approach for analysing large numbers of samples. As an alternative approach, we have used a primer specific for *Bacteroides-Prevotella* spp together with a universal eubacterial primer to selectively amplify 16S rRNA sequences of this group from rumen-extracted DNA. Restriction enzyme cleavage of the PCR product yields profiles that can be correlated with patterns obtained for isolated strains or from database information [2]. By radioactively labelling one primer with ^{32}P we were able to accurately quantify the relative abundance of particular bands in these profiles, and hence estimate their relative contributions to total *Bacteroides-Prevotella* rDNA. Representation of *Bacteroides-Prevotella* rDNA among total eubacterial rDNA was also estimated by a probing approach. Bands characteristic of *P. bryantii*, *P. brevis* and *P. ruminicola* [3] were detected in