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

samples from seven animals, accounting for 30-80% of total *Bacteroides-Prevotella* rDNA or 5-25% of total eubacterial 16rDNA. A fourth species *P. albensis* was detected in two animals by this method. At the same time, we found evidence that the most abundant strains were often distantly related to the type strains, as is also shown by random cloning and sequencing studies [4].

1. Stahl DA, Flesher B, Mansfield HR, Montgomery L (1988) *Appl Environ Microbiol* 54, 1079-1084
2. Wood J, Avguštin G, Scott KP, Flint HJ (1995) In: *Beijerinck Centennial, Abstracts Book, The Hague* 100-101
3. Avguštin G, Wallace RJ, Flint HJ (1996) *Int J Syst Bacteriol* 47, 284-288
4. Avguštin G, Ramšak A, Peterka M, Nekrep FV, Flint HJ (1997) (This meeting, page 27)

The fate of *Escherichia coli* 0157 isolates under simulated rumen conditions and the use of a *gfp*-labelled isolate for ecological studies. SH Duncan[1], KP Scott[1], CS Stewart[1], HJ Flint[1], F Thompson-Carter[2], TH Pennington[2]
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Ingesting a small number of cells of *Escherichia coli* strain 0157 can produce severe human enteric disease. The ruminant gut acts as a reservoir of these bacteria, but little is known about how verocytotoxic *E. coli* colonize this habitat and survive the protective effects of the commensal rumen microbial population.

Commensal strains of *E. coli* are sensitive to the presence of volatile fatty acids and it has been suggested that 0157 strains show superior tolerance to these acids. A batch culture system simulating the rumen was used to examine the inhibitory effects on *E. coli* 0157 of VFA at rumen-like concentrations and the potentially stimulatory effects of nutrients present in yeast extract. The results showed that *E. coli* 0157 strains were no more tolerant of acids than were commensal ruminal strains of *E. coli*, in agreement with some previous findings [1]. The presence of acids was clearly not the only factor controlling the growth of 0157 strains in this system. Competition for nutrients with commensal anaerobes also seemed likely to influence the numbers of *E. coli* present.

Colicins play an important role in the evolution and ecology of habitat invasion and colonisation by strains of *E. coli* [2] and some isolates of *E. coli* 0157 are colicinogenic. Several commensal strains of *E. coli* and other bacteria from the rumen of sheep were shown to produce bacteriocins and some of these strains were shown to inhibit the growth of 0157 strains. Studies are in progress to demonstrate whether such inhibitors play a role in the natural defence of the rumen population against colonization by this bacterium.

Over 75% of rumen bacteria are associated with biofilms which colonize the digesta and the rumen wall. Biofilm-associated bacteria are notoriously difficult to kill by use of drugs and other toxic compounds [3]. To facilitate investigations of whether *E. coli* strains associate with biofilms in the rumen, a plasmid carrying the gene encoding green fluorescent protein (*gfp*) from the jellyfish *Aequorea victoria* has been introduced into *E. coli* 0157 (NCTC 12900) by transformation. Selecting strategies to control the pro-