

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].

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**The fate of *Escherichia coli* 0157 isolates under simulated rumen conditions and the use of a *gfp*-labelled isolate for ecological studies.** SH Duncan<sup>1</sup>, KP Scott<sup>1</sup>, CS Stewart<sup>1</sup>, HJ Flint<sup>1</sup>, F Thompson-Carter<sup>2</sup>, TH Pennington<sup>2</sup>  
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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-

liferation of *E. coli* in the ruminant gut requires further knowledge of the behaviour of this bacterium in this ecosystem.

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### **Production and characteristics of bacteriocins of rumen-associated enterococci.**

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Enterococci represent the group of bacteria which form the obligate microflora of traditional cheeses in most Mediterranean countries. These species are also a part of the normal flora of the gastrointestinal tract and faeces of humans, various animals and poultry. In ruminants, enterococci are among the first bacterial groups colonizing the rumen wall as well as rumen fluid. On the other hand, the presence of enterococci in milk and milk products can be considered as an indication of in-

adequate sanitation during the production and processing of milk. They also often cause nosocomial gastrointestinal or urinary infections. Enterococci thus are an important group of microorganisms. Several strains are producers of bacteriocins [1]. In general, bacteriocins can be characterized as a heterogeneous group of proteins of varying molecular mass and biochemical properties with bactericidal effect against strains and species usually closely related to the producer culture. Bacteriocin producers have been isolated from food, silage or human sources. Newer bacteriocins from ruminal strains have been detected and characterized. Therefore, this study is relevant both to basic research and appropriate applications in veterinary medicine or the food industry.

Three small (3-10kDa) thermostable, hydrophobic bacteriocin-like substances, susceptible to proteases, were isolated. Two were produced by *Enterococcus faecium* strains CCM 4231 and EF3 of ruminal origin (calf and sheep). The third was produced by *E. casseliflavus* EC24, isolated from the rumen contents of deer. All proteineous substances showed a broad antimicrobial spectrum of activity (200 - 1600AU ml<sup>-1</sup>). They inhibited the growth of Gram positive indicator bacteria. The best characterized was enterocin CCM 4231, which was also active against the G<sup>-</sup> indicator organism *Proteus mirabilis*. The optimum pH, for enterocin CCM 4231 production was pH 4.0 - 7.5. Using controlled buffered systems (pH7) for cultivation of the producer strain, production of bacteriocin was constitutive. On the basis of their properties, these bacteriocins could be allotted to bacteriocin class II [2].