sequence-specific enzymes: (1) a restriction endonuclease that specifically cleaves (phage) DNA within the recognition sequence and (2) a modification methyltransferase that specifically methylates DNA. It has been shown that a large number of R-M systems can be found in natural bacterial populations. The widespread presence of R-M systems in bacteria would be a consequence of the selective advantage of having a defense tool against phage infection. The analysis of R-M activities in seven phenotypically different rumen strains of *Megasphaera elsdenii* revealed the presence of GATC-specific restriction-modification systems (MboI isoschizomers) in all of the strains tested. A complete lack of other restriction and/or modification enzymes previously characterized in closely related *Selenomonas ruminantium* was confirmed by a methylation protection assay. Based on the results of our experiments, it could be assumed that *M. elsdenii,* as compared to *S. ruminantium,* uses a strategy different from R-M systems for bacteriophage protection.

Diversity of rumen methanogens from sheep in Western Australia and Queensland identified by 16S clone libraries. C. Pimm, A.F. Toovey, A.J. Williams, B. Winder, S. Rodgers, K. Smith, A.-D.G. Wright (CSIRO Livestock Industries, Private Bag 5 Wembley WA 6913, Australia).

Individual 16S clone libraries were prepared from the rumen contents of 17 Merino sheep in Western Australian fed 3 different diets (grazing pasture vs. oaten hay vs. lucerne hay). Amongst the 733 clones examined, 65 phylotypes were found to be similar to cultivated methanogens of the order *Methanobacteriales*. The diversity of rumen methanogens was the greatest in the sheep grazing pasture. *Methanobrevibacter* strains SM9, M6 and NT7 accounted for over 90% of the 733 clones examined, with M6 being more prevalent in grazing sheep and SM9 more prevalent in sheep fed the lucerne-based diet. Five new species were identified, two of which have very little sequence similarity to any cultivated methanogens. In contrast, an investigation into the molecular diversity of rumen methanogens from pooled rumen fluid contents from five Merino sheep in Queensland, Australia revealed that only eight of the 34 phylotypes from 89 clones were similar to methanogens belonging to the order *Methanobacteriales*. The remaining 26 phylotypes represented a new taxonomic order of methanogens, atypical for the rumen environment, some 15–20% dissimilar to *Methanobacteriales*.

Implications for disease of colonic bacterial diversity: A culture independent analysis of the microbial community from the colon of individuals with IBD and colon cancer. P. Scanlan, F. Shanahan, J. Marchesi (Dept. of Microbiology, University College Cork, Cork, Eire).

The large intestine is the most heavily colonised part of the gastrointestinal (GI) tract with numbers reaching 10^{12} bacteria per gram of luminal contents. Micro-organisms are essential to maintaining the normal functioning of the gut, and as such have a profound effect on the health status of their host from an immunological, dietary and physiological perspective. Thus, the potential role bacteria hold in various disease states of the GI tract is widely appreciated. In this study the total bacterial diversity and the diversity of specific bacterial genera of colon cancer ($n = 40$), polyp ($n = 40$) and inflammatory bowel disease (IBD) patients, on specific probiotic trials, were analysed. Molecular techniques (ribosomal intergenic spacer analysis and denaturing gradient gel electrophoresis) widely used in the microbial ecology field were employed to investigate the bacterial populations at given time points. The results obtained were compared to those of normal subjects and are discussed here. The methods employed illustrated not only considerable variation between individuals but also intra-individual variation in certain sub-groupings of the trials.

Fe-hydrogenases from bovine rumen: a metagenomic approach. E. Severing, A. Ederveen, G.W.M. van der Staay, S.Y. Moon-van der Staay, R.M. de Graaf, Th.A. van Alen, N. McEwan*, C.J. Newbold*, J.-P. Jouany#, T. Michałowski*, P. Pristas*, J. Fried*, G. Ricard*, M.A. Huynen*, J.H.P. Hackstein (Dept. Evolutionary Microbiology, Fac. Sci., University of Nijmegen, Toernooiveld 1, NL6525ED Nijmegen, The Netherlands and the EU projects # ERCULE/*CIMES).

The evolution of eukaryotic Fe-hydrogenases is still poorly understood. It has remained unclear until now as to whether these hydrogenases represent an old eukaryotic heritage or whether they were acquired by bacterial-to-eukaryote gene transfer. Here we describe the recovery of a set of DNA sequences encoding the H-cluster of Fe-hydrogenases from rumen ciliates. The rumen ciliates were isolated from the rumen fluid of a cow by electromigration. The DNA of the total rumen ciliate population was purified and used to amplify the H-clusters of Fe-hydrogenases by PCR with degenerated primers. For the identification of the corresponding ciliates, PCR was performed on DNA from type-strain ciliates. Phylogenetic studies revealed the presence of a monophyletic group of eukaryotic Fe-hydrogenases in the bovine rumen. Supported by the EU Contract QLRI-CT-2000-01455 "ERCULE" and Contract QLK3-2002-02151 "CIMES".

Study of two simple techniques for cryopreservation of rumen ciliate protozoa. E. Nsabimana[a], D. Macheboeuf[a], C.J. Newbold[b], J.-P. Jouany[a] ([a] INRA, CR de Clermont/Theix, 63122 St-Genès-Champanelle, France; [b] University of Wales, Aberystwyth, UK).

Rumen ciliates are difficult to cryopreserve. The two-step freezing technique, which has been applied successfully (Nsabimana et al. 2003, Appl. Environ. Microbiol., 69, 3826–3832), is complex and needs a special equipment to be performed. Two simple techniques using Bicell® and M.Frosty® freezing cells and allowing the respective cooling rates of 0.5 °C/min and 1 °C/min, were tested for their ability to cryopreserve the following ciliate species: *Dasytricha ruminantium, Entodinium caudatum, Epidinium ecaudatum caudatum, Eudiplodinium maggii, Isotricha intestinalis, Isotricha prostoma, Metadinium medium*. The ciliates were isolated from sheep monofaunated with each species. Centrifuged fresh rumen fluid was used as the freezing and thawing media. Equilibration with DMSO (0.56 M) was set at 25 °C or 30 °C for 5 or 10 min before freezing in the two tested freezing cells. Tubes containing the ciliates were plunged into liquid nitrogen when the freezing cells reached the temperature –80 °C. The survival rate (SR) of ciliates was determined from their motility. After two weeks of storage in liquid nitrogen, the highest SR were obtained with *D. ruminantium* (100%), *I. prostoma* (100%), *I. intestinalis* (100%), *E. ecaudatum caudatum* (87%) for the two freezing cells. Bicell® allowed a higher SR for *M. medium* (87 vs. 80%) and for *E. maggii* (94 vs. 76%), while M.Frosty® gave better results for *E. caudatum* (64 vs. 40%). In conclusion, the two simple freezing techniques Bicell and M.Frosty can be applied to cryopreserve the rumen ciliates with an acceptable rate of survival. This project was supported by EU infrastructure grant ERCULE (QLRI-CT-2000-01455) www.ercule.com.

The effect of temperature on the in vitro viability of the mixed rumen protozoal population. G. de la Fuente[a], M. Pérez-Quintana[b], J.A. Cebrián[c], M. Fondevila[a] ([a] Departamento de Producción Animal y Ciencia de los Alimentos y, Universidad de Zaragoza, Spain; [b] Departamento de Química y Biología, Universidad de Matanzas, Matanzas, Cuba; [c] Departamento de Bioquímica y Biología Molecular y Celular; Universidad de Zaragoza, Miguel Servet 177, 50013 Zaragoza, Spain).

Survival of rumen protozoa is reduced at low temperatures, making their storage in refrigeration difficult and hampering their study. A mixed rumen protozoal population (type A) from sheep was anaerobically diluted 1:100 in culture medium and kept at 38, 15 and 5 °C for 2, 4 or 6 h to determine the effect of temperature on cell viability, according to an estimation of membrane damage by a double-stain fluorescence method (5 tubes per treatment). Temperature was reduced from 38 °C to 15 °C at 3 °C·min^{-1} and from 15 to 5 °C at 0.6 °C·min^{-1}. Apparently, all genera responded to both stains, and *Ophryoscolex* showed an effect of temperature (80.3, 55.4 and 54.5% viability at 38, 15 and 5 °C; $P < 0.01$). However, only *Entodinium* cells were in a number high enough to be strictly compared. The viability of the *Entodinium* species in the rumen inoculum was 93.8 ± 2.69, and remained between 90.5 and 79.8% when cultivated at 38 °C for 2 to 6 h. The proportion of non damaged cells after 2 h at 15 or 5 °C did not diminish (87.3 and 86.2%; $P > 0.05$). However, viability was reduced ($P < 0.05$) to 61.3 and 59.9 % after 6 h at 15 °C and 5 °C (s.e.m. = 3.78). The viability of *Entodinium* was reduced at refrigeration temperatures; around 60% of the