such as terminal-restriction fragment length polymorphism (T-RFLP) and denaturing gradient gel electrophoresis (DGGE). DGGE and T-RFLP fingerprints of the bacterial communities in the mesenteric lymph nodes (MLN), jejunum, ileum, caecum and colon of six weaning piglets were screened for amplicons common in all samples. T-RFLP analysis of MLN revealed that the number of terminal restriction fragments (TRs) differed among the piglets, and only a few TRs were found in MLN and GI tract of all piglets. In comparison to T-RFLP, DGGE analysis detected approximately two times lower numbers of 16S rDNA amplicons of the MLN and GI tract samples. These results demonstrate high bacterial diversity in the MLN and reveal that specific parts of the microbiota in the gastrointestinal tract can be translocated from the intestine of weaning piglets.

P-6 **Taxonomy and role of *Butyrivibrio* and *Pseudobutyrivibrio* species in microbial ecosystem from the digestive tract of ruminants**. J. Kopečný[a], R. Marinšek-Logar[b] ([a] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Prague 10, Uhříněves, Czech Republic; [b] University of Ljubljana, Biotechnical Faculty, Domžale, Slovenia)

Butyrivibria are a major component of the ruminal microflora and have been isolated from the gastrointestinal tracts of various animals. They contribute to fiber digestion through degradation of plant hemicellulose, cellulose, starch and protein. Currently are recognized two butyrivibrio species: *Butyrivibrio fibrisolvens* and *Butyrivibrio crossotus*. Genetically closely related species are *Pseudobutyrivibrio ruminis* and *Clostridium proteoclasticum*. We have tested 62 "butyrivibrio" isolates from the rumen of the cow and sheep. There were estimated fermentation products, morphology, substrate utilization, enzyme production, composition of cellular fatty acids, RFLP of 16S DNA, DNA-DNA hybridization and 16S rDNA sequences. Obtained data enabled us to describe two new species: *Butyrivibrio hungatei* and *Pseudobutyrivibrio xylanovorans*, which were the most common species in our set of isolates. All bacteria tested were clustered into two genetically related groups: Butyrivibrio group included *Butyrivibrio fibrisolvens*, *Clostridium proteoclasticum* and *Butyrivibrio hungatei* isolates. Pseudobutyrivibrio group consisted of species *Pseudobutyrivibrio ruminis*, *Butyrivibrio crossotus* and *Pseudobutyrivibrio xylanovorans*. Pseudobutyrivibrio group included another cluster of bacteria isolated mainly from the rumen fluid of deer and sheep, which is not taxonomically defined. Both Pseudobutyrivibrio and Butyrivibrio group can be distinguished with specific PCR primers. *Pseudobutyrivibrio xylanovorans* isolates showed the highest ability to degrade plant polysaccharides. Beside that, they were producing, together with isolates of *Clostridium proteoclasticum*, the highest proteinase activity. All other species tested were utilizing metabolic intermediates of fiber degradation only. (The project – 524/99/0602 – was supported by The Czech Grant Agency).

P-7 **Molecular ecology of *Oscillospira guillermondii* – a large, morphologically conspicuous, but uncultured rumen bacterium.** R.I. Mackie[a], R.I. Aminov[a], W. Hu[a], M.A. Olsen[a,b], A.V. Klieve[c], Y. Kamagata[d] ([a] Department of Animal Sciences, University of Illinois, Urbana, IL 61801, USA; [b] Department of Arctic Biology and Institute of Medical Biology, University of Tromso, 9037, Tromso, Norway; [c] Queensland Beef Industry Institute, Queensland Department of Primary Industries, Moorooka, Australia; [d] National Institute of BioScience and Human Technology, Agency of Industrial Science and Technology, Tsukuba, Ibaraki 305-8566, Japan)

Detection and identification of microbial populations are the most basic prerequisites for microbial ecology studies. Although *Oscillospira* have been observed microscopically, based on their large size and conspicuous morphology, for over 90 years, they remain uncultured and their phylogeny unknown. Based on PCR-retrieved 16S rDNA sequences of *Oscillospira* spp., we have developed PCR, PCR-DGGE and FISH techniques for cultivation-independent monitoring of *Oscillospira* spp. to estimate the occurrence of this bacterium in different ruminant species, during diet shifts in cattle, and to evaluate the level of its genetic diversity. *Oscillospira*-specific sequences were detected in a broad range of geographically distant ruminant species: North American domestic cattle, sheep from Australia and Japan, and Norwegian reindeer. Phylogenetic analysis of the sequences obtained enabled

us to define three operational taxonomic units (OTUs) within the *Oscillospira* assemblage. Consistent with this genetic diversity, we observed other, atypical morphotypes of *Oscillospira*, detected with the use of the *Oscillospira*-specific FISH probe. Despite the visual disappearance of typical *Oscillospira* morphotypes during the switch from green pasture to indoor housing, its presence was still detected by *Oscillospira*-specific PCR. Together with their detection in geographically distant ruminant species fed a range of diets, these observations suggest the ubiqitous presence of *Oscillospira* species in various rumen ecosystems with the numbers, and types (possibly species) responding to diet and geographic location.

P-8 **Development of a competitive PCR for detection and enumeration of *Butyrivibrio* and *Pseudobutyrivibrio* strains in the rumen ecosystem.** J. Mrázek[a], J. Kopečný[a], G. Avguštin[b] ([a] Institute of Animal Physiology and Genetics, Prague 10, Czech Republic; [b] University of Ljubljana, Domžale, Slovenia)

Motile and butyrate-producing anaerobic bacteria in the rumen are represented mainly by *Butyrivibrio* and *Pseudobutyrivibrio* strains. They play an important role in the degradation of proteins as well as structural and storage plant polysaccharides. Recent studies based on 16S rDNA sequential analysis showed that those species are clustered into six distinct groups. The aim of this project was to develop a competitive PCR, which could easily distinguish *Butyrivibrio* and *Pseudobutyrivibrio* strains and quantify them in environmental samples. Twenty strains of butyrivibria were divided into two main groups on the basis of 16S rDNA sequences comparison. The primers specific for both groups were designed and tested on 50 strains of butyrivibria and pseudobutyrivibria. The highest specificity for butyrivibria was found in case of 71f primer (5- CGG AGA ATT TAC GCT GAT GAA G –3), and for pseudobutyrivibria was the most suitable primer F2 (5- AAT TTT CTA CGA TCC CTT CGG GG –3). The internal controls for cPCR systems for both groups were prepared using a "double primer method". Standard curves were constructed using a known number of cells from a pure culture of butyrivibria (counted by flow cytometer) and a serial dilution of internal control. The Agilent bioanalyzer 2100 was used for the analysis of cPCR products. The standard curves can be used for enumeration of the number of cells of butyrivibria in the range from 4.1×10^7 to 1×10^6 cells per mL and for pseudobutyrivibria in the range from 6.1×10^8 to 6.1×10^5 cells per mL of rumen fluid.

P-9 **An optimised RNA extraction protocol for rumen samples.** S. Muetzel, K. Becker (University of Hohenheim, Inst. for Animal Production in the Tropics and Subtropics, Dept. for Animal Nutrition and Aquaculture, 70593 Stuttgart, Germany)

For the analysis of complex microbial ecosystems quantitative recovery of nucleic acids is a prerequisite. Contamination of the extracted nucleic acids with carbohydrates, protein or polyphenols and the lysis of recalcitrant organisms are the two main problems during nucleic acid extraction. RNA recovery from rumen fluid samples was compared using two RNA extraction methods. The method that yielded higher group specific RNA concentrations was further modified to allow extraction of RNA in the presence of tannins. Furthermore the lysis protocol based on bead mill disruption was optimised in order to recover a maximum of RNA from recalcitrant and fragile organisms from rumen fluid samples. Quantification of the RNA was done by densitometry from polyacrylamide and agarose gels in the study of RNA-tannin interactions and by membrane hybridisation with group specific RNA probes targeting total rRNA, Bacteria, Archaea, Eukarya, *Ruminococcus flavefaciens* and the genus *Fibrobacter* for the lysis procedure study. The RNA extraction procedure presented is a rapid, inexpensive method with good reproducibility, yielding high amounts of RNA suitable for 16S rRNA targeted membrane hybridisation.

P-10 **Culture independent molecular analysis of the elderly faecal microflora reveals an extreme complexity.** K. Saunier[a], K. Tuohy[b], M. Sutren[a], A. Cresci[c], J. Doré[a] ([a] Institut National de la Recherche Agronomique, UEPSD, 78350 Jouy-en-Josas, France; [b] Unit of Food