detection and differentiation of the anaerobic gut fungi. We have also developed a chitin synthase gene marker for both phylogenetic and hybridisation studies.

A comparison of the effectiveness of both these gene sequences for the phylogenetic studies and the hybridisation-based differentiation of the various anaerobic fungal genera will be discussed. The potential for further development of these molecular approaches to the characterisation of the anaerobic gut fungi will be considered. In particular, the use of hybridisation-based assays for the direct detection, enumeration and differentiation of anaerobic gut fungal zoospores in rumen fluid and gut fungal thalli attached to plant biomass in rumen digesta will be discussed.

Grouping of anaerobic fungi isolated from the digestive tract of herbivores by PCR-RFLP analysis of ITS. V Julliand[1], M Hosny[1], H Dulien[1], G Fonty[2] *([1]INRA-ENESAD, Laboratoire de Microbiologie Anaérobie, BP 1607, 21036 Dijon, France; [2]INRA, Laboratoire de Microbiologie, CR Clermont Ferrand-Theix, 63122 Saint-Genès-Champanelle, France)*

The anaerobic fungi *Piromonas communis* and *P. citronii* are natural inhabitants of the rumen of ruminants and of the caecum of equids, respectively, These two species are usually differentiated by their morphological and metabolic characteristics [1]. In this study the PCR-RFLP analysis of the intergenic transcribed spacers (ITS) of the ribosomal RNA gene was used to discriminate between closely related strains isolated from the digestive tract of herbivores.

Seven strains of *P. citronii*, isolated from the ass caecum (A_2, A_3, A_4, A_5) and from the pony caecum (P_2, P_3, P_4) were compared to three ruminal strain of *P. communis*, isolated from a cow (V_1, V_2) and from a sheep (M_1). After extraction and purification of DNA from each strain, the ITS of the ribosomal unit together with the 5.8 S gene were specifically amplified by PCR using the universal primers ITS1 and ITS4. Amplification products were then digested by 13 restriction endonucleases (RFLP). The length of the amplified fragments was the same for all strains (720 Kpb) except for A_5 (780 Kpb) and M_1 (770 Kpb). Five enzymes (*Alu*I, *Eco*RI, *Nde*I, *Hind* III and *Hae*III) did not cut the ITS. *Taq*I yielded three fragments (340, 180 and 140 bp) after digestion of the ITS region of the equine strains and only two (370 and 320 bp) or one (370 bp) from the ITS of the ruminal *P. communis* strains. *Rsa*I generated one fragment (650 bp), and two (640 and 615 pb) from the ITS of *P. citronii*. *Hin*fI yielded one fragment of 360 bp from the ITS of the two ruminal bovine strains and one (380 bp) from the ITS of the ruminal ovine strain. *Mse*I, *Sau*3a, *Mbo*I, *Nde*II and *Taq*I also discriminated the bovine strains from the ovine strain by the number of the length of fragments yielded. *Hin*fI, *Mse*I, *Rsa*I, *Sau*3a, *Mbo*I, *Nde*II and *Dde*I discriminated A_5 from the other six equine strains. For example, *Mse*I generated one fragment of 280 bp from A_5 and two fragments (220 and 85 pb) from A_2, A_3, A_4, P_2 and P_4. Among the *P. citronii* strains, two groups were discriminated by *Hin*fI which yielded three fragments (360, 250 and 150 bp) from A_2, A_3, A_4, P_2 and only one (360 bp) from P_3 and P_4. *Mse*I

and *Taq*I differentiated the three ruminal strain from the seven equine strains. In consequence five groups of strains were differentiated: group I include A_2, A_3, A_4 and P_2, group II: A_5, group III: P_3 and P_4, group IV: V_1 and V_2, group V: M_1.

Thus, the ITS analysis allowed differentiation of the strains according to their origin.

1. Gaillard-Martinie B, Breton A, Dusser M, Julliand, V (1995) *FEMS Microbiol Lett* 130, 321-326

Attachment to cellulose and subsequent development of of the anaerobic rumen fungus *Neocallimastix frontalis* strain RE1. AJ Richardson[1], GW Gooday[2], CS Stewart[1] *([1]Rowett Research Institute, Bucksburn, Aberdeen, AB21 9SB, UK; [2]Department of Molecular and Cell Biology, Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen, AB25 2ZD, UK)*

Anaerobic fungi colonize a wide range of plant materials present in ruminant diets including maize, lucerne, straw and grasses. Degradation of cellulosic substrates by anaerobic fungi in the rumen involves the attachment and encystment of zoospores on plant surfaces and their subsequent maturation into zoosporangia with rhizoidal systems which penetrate and degrade the plant material. Relatively little is known about the mechanism of adhesion by rumen fungi. Radiolabelling has been used to quantify the effects of plant secondary metabolites on attachment of anaerobic fungal biomass to cellulose [1]. Using a similar technique we have quantified the attachment to cellulose of separated, ^{14}C-labelled zoospores and zoosporangia of the anaerobic rumen fungus *Neocallimastix frontalis* strain RE1 Both zoospores and zoosporangia were found to make a contribution to the colonization process *in vitro*.

Anaerobic fungi belong to the class Chytridiomycetes, order Spizellomycetales. In the Blastocladiales, an aerobic order of the Chytridiomycetes, almost all of the genera survive adverse conditions by producing resistant sporangia with cell walls which are frequently thickened and melanized [2]. The isolation of anaerobic fungi from samples of fresh and air-dried faeces and saliva from various herbivores has led to the suggestion that an aero-tolerant resistant stage similar to the resistant structures produced in aerobic chytrids may be responsible for the survival of these fungi outside the rumen. The formation of pigmented, melanized resistant structures in a *Neocallimastix* sp.has been reported [3] and these were considered to be the aero-tolerant resistant stage. When *N. frontalis* strain RE1 was grown on filter paper dark brown pigmented zoosporangia appeared after 4-5 d but these differed in some respects from those previously described in both aerobic and anaerobic chytrids. The cell walls of the zoosporangia were not thickened and the pigment was present in the cytoplasm which contracted into a compact mass within the cell. Histochemical tests showed that the pigment was not melanin. Furthermore pigmented zoosporangia developed when strain RE1 was incubated in the presence of tricyclazole, PP389 or glyphosate, compounds which block melanin biosynthesis in fungi. Nonetheless, these structures may represent a resting stage in the life-cycle of strain RE1.

1. Moniello G, Richardson AJ, Duncan SH, Stewart CS (1996) *Appl Environ Microbiol* 62, 4666-4668