

for expression in fungi. A protease-deficient strain of *Penicillium roqueforti* was transformed with an expression cassette containing the coding sequence of *xyn3A* under the control of the *Penicillium* aspartyl protease promoter. Xyn3A was produced in the culture medium of recombinant strains in an active but hyperglycosylated form. The *xyn3* gene was cloned into a multicopy episomal plasmid downstream the strong PGK promoter and expressed in the yeast *Kluyveromyces lactis*. The recombinant yeast strains produced and secreted the *N. frontalis* xylanase into the culture medium as an active enzyme.

Esterases and the gut fungi. S Rogers (School of Biological Sciences, Stopford Building, University of Manchester, Oxford Road, Manchester. M13 9PT. UK and IGER, Plas Gogerddan, Aberystwyth, Dyfed. SY23 3EB. UK)

Anaerobic rumen fungi are thought to be the primary colonisers of lignocellulose in the rumen. This initial colonisation by the gut fungi is quickly followed by secondary colonisation by other rumen microorganisms. A number of plant cell wall degrading enzymes from the gut fungi which are important in the occupation of this ecological niche have been studied. Cellulases, mannanases and xylanases with extremely high specific activity have been extensively studied and along with xylanases are present as a multienzyme complex which is believed to be secreted [1]. To date, little information about another group of enzymes, the phenolic acid esterases, has been obtained. Such enzymes would be expected to play an important role in the liberation of utilizable sugars within the rumen ecosystem.

Phenolic acid esters are particularly abundant in grasses where they are components of both primary and secondary cell walls. The plant uses phenolic acid esters to prevent degradation of the arabinose side chain of hemicellulose. These ester linkages also provide a means of attachment between the lignified secondary and primary cell walls. The enzymes produced by the anaerobic gut fungi enable the separation of the lignin and the non-lignified tissues encouraging efficient degradation within the rumen. The esterase activity of the anaerobic rumen fungi has been characterised using standard protein chemistry approaches [2]. However, a comparative ecological approach to the production of this enzyme activity has not previously been attempted.

We have looked at the growth and enzymatic activity of three different species of fungal isolate using gas production and HPLC techniques. Preliminary data suggests there are significant differences in both the profile and the resulting phenolics produced between all isolates studied. The relevance of the enzymatic difference will be discussed with reference to the role of fungal genera within the rumen ecosystem.

1. Fannutti C, Ponyi T, Black GW, Hazelwood GP, Gilbert HJ (1995) *J Biol Chem* 270, 29314-29322
2. Borneman WS, Ljungdahl LG, Hartley RD, Akin DE (1992) *Appl Environ Microbiol* 58, 3762-3766

Screening of anaerobic gut fungi for effective plant biomass degradation using gas production. W-Y Zhu¹, MK Theodorou¹, BB Nielsen^{1,2}, APJ Trinci² (¹Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK;