for expression in fungi. A protease-deficient strain of *Penicillium roqueforti* was transformed with an expression cassette containing the coding sequence of *xyn*3A 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 *xyn*3 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 Gorgerddan, 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.

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Screening of anaerobic gut fungi for effective plant biomass degradation using gas production. W-Y Zhu[1], MK Theodorou[1], BB Nielsen[1,2], APJ Trinci[2] ([1]*Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK;*

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The fermentation of cellulosic substrates by gut anaerobic fungi is usually determined in batch culture by gravimetric measurements of dry matter loss or by quantification of fermentation end-products [1]. These techniques involve destructive sampling of the cultures, thereby requiring many replicate cultures in time-course studies. Recently, a simple gas production technique employing a pressure transducer has been developed [2]. This new technique can be used to follow the growth of gut anaerobic fungi on particulate substrates without destructive sampling and thus it is possible to obtain an entire growth curve from a single culture bottle.

In the present study, 30 gut fungal isolates, belonging to the genera *Neocallimastix* and *Piromyces*, were used to ferment wheat straw (10g dry matter (DM) l^{-1}). DM losses were determined after harvest at the end of a 160h fermentation period, with the values ranging from 38-60%. Gas production was measured at 4-24h intervals, with total cumulative values ranging from 100-150ml gas. The ranking order for total cumulative gas production was similar to that for DM loss, suggesting that the gas production profiles were reliable indicators of fungal growth. Cumulative gas production profiles were fitted to the model described by France *et al.* [3], and predicted values for the growth parameters, asymptotic gas pool size, and specific growth rate at the time of half final gas pool were obtained. Lag-times ranged from 16-26h and all isolates, except one, showed similar specific growth rates, with values ranging from 0.031-0.042h^{-1}. The difference between the measured and asymptotic final gas pool size was found to be less than 3% for all isolates. From the experiment, 16 isolates were identified as good digesters of large amounts of plant material and these isolates also produced high levels of gas. The growth parameter values and DM loss data were also subjected to a Canonical Variate Analysis [4] and a hierarchical cluster tree was constructed. The results showed that of 30 isolates 20 can be formed into two main groups at the 90% similarity level and the other 10 isolates grouped at lower levels of similarity. Generally, these groupings were similar to those observed from DM loss and gas production data.

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RUMEN PROTOZOA

Effect of sampling site on concentration and fibrolytic activity of protozoa in bovine rumen contents.

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An apparent heterogeneity or stratification within the ingested food materials and