for hsp60 and hsp70 of the anaerobic fungi *Neocallimastix* sp. L2 and *Piromyces* sp. E2. Hsp60 and hsp70 are ubiquitous enzymes. In eukaryotes, hsp60 is localised in mitochondria and chloroplasts. Hsp70 is localized in the cytosol, in the endoplasmatic reticulum, in mitochondria and in chloroplasts. Phylogenetic analysis of the hsp60 and hsp70 genes will reveal whether mitochondrial hsp60 and hsp70 genes are recent.

Heterologous production of *Neocallimastix frontalis* xylanases from *Escherichia coli*, filamentous fungi and yeast. R Durand, C Rascle, M Fèvre (*Laboratoire de Biologie Cellulaire Fongique, Centre de Génétique Moléculaire et Cellulaire, CNRS UMR 5534, Bât 405, Université Claude Bernard Lyon I, 69622 Villeurbanne Cedex, France)*

Anaerobic fungi are potent producers of enzymes that degrade the various components of plant cell walls in the rumens of herbivorous animals. The secreted xylanolytic system of *Neocallimastix frontalis* is characterized by a multiplicity of activities, as revealed by zymogram analysis. In order to establish whether these polypeptides are the products of different xylanase genes, or the result of post-translational modifications of the products of a limited number of genes, we started upon the molecular characterization of the xylanolytic system.

Different cDNAs designated *xyn*3 and *xyn*4 were isolated from an expression library of *N. frontalis*, providing evidence for a multigene family in this organism. *Xyn*3 was further characterized and shown to contain a single open reading frame of 1821 bp coding for a protein XYN3 of M_r 66000. The predicted primary structure of XYN3 consisted of two large N-terminal reiterated regions of 223 amino acids showing high homology to each other (88.3%). Each domain of XYN3, XYN3A and XYN3B showed significant homology with fungal and bacterial xylanases belonging to endoxylanase family 11. Analysis of truncated forms of XYN3 confirmed that the full length protein contained two catalytic domains which displayed similar substrate specificity. XYN3A antiserum recognized similar polypeptides in the culture medium of two other rumen fungi *Piromyces rhizinflata* and *Caecomyces communis* indicating that the rumen fungi may secrete similar xylanolytic enzymes. A second cDNA, *xyn*4, that is not homologous to *xyn*3 was shown to encode an endoxylanase consisting of two catalytic domains (A and B) connected by a linker region. The different catalytic domains of *N. frontalis* xylanases were cloned in a bacterial expression plasmid harbouring a 6His-C terminal tag and the recombinant proteins XYN3, XYN3A and XYN4B were purified from *Escherichia coli.* The recombinant proteins hydrolysed xylan to xylo-oligosaccharides.

The availability of recombinant fungal xylanases offers a potentially attractive source of enzymes for industrial applications in the feed, food and paper industries. Chimeric enzymes containing the XYN3A catalytic domain and interdomain linker of the xylanase, fused to one or two XYN4B domains, were produced in *E. coli* cells and purified by affinity chromatography. Experiments are in progress to compare the substrate specificities of the parental domains and of the engineered enzymes.

The coding sequence of *xyn*3A was introduced into different vectors designed

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;*