

utilization of xylooligosaccharides by this organism.

Strains of *S. ruminantium* varied considerably in their capacity to ferment xylooligosaccharides prepared by partial hydrolysis of oat spelt xylan (50mM phosphoric acid, 121°C, 15min). This ability ranged from strain GA192, which completely utilized xylose through xyloketetraose and was able to ferment considerable quantities of larger oligosaccharides, to strain HD4 which used only the simple sugars present in the hydrolysate (xylose and arabinose). Lactic acid was the major fermentation product formed by all strains. The ability of *S. ruminantium* strains to utilize xylooligosaccharides was correlated with the presence of xylosidase and arabinosidase activities (measured by hydrolysis of appropriate *p*-nitrophenyl glycosides). The production of these activities appears to be regulated. Both arabinosidase and xylosidase were induced by growth on xylose or xylooligosaccharides, but no activity was detected in glucose or arabinose grown cultures. A genetic locus from *S. ruminantium* GA192 was cloned into *Escherichia coli* JM83 using pUC18, that produced both xylosidase and arabinosidase activities. Analyses of crude extracts from the *E. coli* clone and *S. ruminantium* GA192 using native polyacrylamide gel electrophoresis and methylumbelliferyl substrates indicated that a single protein was responsible for both activities. The enzyme expressed in *E. coli* was capable of degrading xylooligosaccharides derived from oat spelt xylan. DNA sequencing of the locus demonstrated the presence of an open reading frame which encodes for a protein of 61,174 molecular weight. Attempts are underway to introduce the xylosidase-arabinosidase gene into *S. ruminantium*

strains lacking these enzyme activities, and also into xylooligosaccharide fermenting strains for enhanced degradation of the oligosaccharides.

Hydrolysis and degradation of esterified phenolic acids from the maize cell wall by rumen microbial species. I Giraud, JM Besle, G Fonty (INRA, Station de Recherches sur la Nutrition des Herbivores, Centre de Clermont-Theix, 63122 Saint Genès-Champanelle, France)

Several rumen bacteria and fungi possess *p*-coumaroyl and feruloyl esterases [1]. In addition, several species are able to degrade monoaromatics [2]. The aim of this work was to characterise the ability of two bacteria, *Fibrobacter succinogenes* S85 (Fs) and *Ruminococcus albus* 20 (Ra), and a fungus, *Neocallimastix frontalis* MCH3 (Nf), to release and degrade the esterified phenolic acids from maize cell walls.

The cell wall residue (CWR) from maize stems (cv LG11, silage stage) was autoclaved and fermented in triplicate in a suitable medium [3] for two and five days either uninoculated (control) or inoculated with Fs, Ra or Nf. The phenolic acid content of the residues and the fermented supernatants was determined [4] after alkaline hydrolysis (1M NaOH 20h, 20°C). The CWR contained 25.8 and 6.4g kg⁻¹ of *p*-coumaric (PCA) and ferulic (FA) acids, respectively. After two days incubation, the dry matter disappearances (DMD) were low, but higher with the bacteria than with the fungus. PCA and FA losses were 6.6 and 7.5% of initial content in the control.

The net disappearance (subtracting

Dry matter disappearance and distribution of phenolic acids after 48h incubation expressed as % initial content.

Species	Disappearance			Recovered		Degraded	
	DM	PCA	FA	PCA	FA	PCA	FA
<i>Fibrobacter succinogenes</i> S85	7.0	9.4	12.1	2.2	6.2	7.2	5.9
<i>Ruminococcus albus</i> 20	9.3	9.0	19.6	1.0	10	8.0	9.6
<i>Neocallimastix frontalis</i> MCH3	4.7	8.1	18.9	1.2	7.7	6.9	11.2

control losses) of PCA was comparable for all species (8-9%) but relatively greater for Nf when compared to the DM disappearance. Those of FA were greater than those of PCA, especially for Ra and Nf. The released phenolic acids were mainly found in free form (90% of the recovered soluble fraction), except for Ra which released about 40% of both phenolic acids still esterified to soluble cell wall fragments. In the released fraction (RF), the degraded (unrecovered) fraction in the supernatants was nil in the controls and lower for FA (49 to 59% RF) than for PCA (77 to 89% RF). No peak from the degradation pathway of the phenolic acids was detected. The degrading capacity of Nf was greatest for FA and as high as that of Ra for PCA. A similar degradation pattern was observed after five days incubation.

The phenylesterase activity of the bacteria in this study seemed relatively greater than that previously reported [1]. The strong phenylesterase and degrading activity of the fungus is an advantage in utilizing grass cell walls.

1. Borneman WS, Akin DE (1990) In: *Microbial and Plant Opportunities to Improve Lignocellulose Utilization by Ruminants* (Akin, DE, Ljungdahl, LG, Wilson, JR, Harris, PJ, eds) Elsevier, New York, 325-339 2.
2. Besle JM, Cornu A, Jouany JP (1995)

FEMS Microbiol Rev 16, 33-52

3. Joblin KN (1981) *Appl Environ Microbiol* 42, 1119-1122
4. Bernard-Vailhé MA, Besle JM, Doré, J (1995) *Appl Environ Microbiol* 61, 379-381

Gene expression of cellulase and xylanase in tobacco and cell wall digestion by domain-shuffled enzymes. K Ohmiya¹, J-L Sun¹, S Karita², T Kawazu³, T Kimura¹, K Sakka¹ (¹Mie University School of Bioresources, Kamihama-cho, Tsu 514, Japan; ²Center for Molecular Biology and Genetics, Mie University, Kamihama-cho, Tsu 514, Japan; ³Oji Paper Co Ltd, Forestry Research Institute, Nobono-cho, Kameyama, 519-02, Japan)

The effective utilization of the cellulosic material of plant cell walls, photosynthesized from CO₂ and water by solar energy, still presents a problem because of the resistance of this material to biodegradation. A well-established natural system for biodegradation is found in the rumen of cattle, where cellulases and xylanases from strictly anaerobic bacteria solubilize grass and forage effectively. The soluble sugars produced by enzyme action, however, are detected only at low concentrations in the rumen fluid indicating that the solubilized products are either immediately utilized by bacteria or absorbed by cattle as carbon