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

Species	*Disapperance*			*Recovered*		*Degraded*	
	DM	*PCA*	*FA*	*PCA*	*FA*	*PCA*	*FA*
Fibrobacter succinogenes S85	7.0	9.4	12.1	2.2	6.2	7.2	5.9
Ruminococcus albus 20	9.3	9.0	19.6	1.0	10	8.0	9.6
Neocallimastix frontalis 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.

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Gene expression of cellulase and xylanase in tobacco and cell wall digestion by domain-shuffled enzymes. K Ohmiya [1], J-L Sun[1], S Karita[2], T Kawazu[3], T Kimura[1] K Sakka[1] ([1]*Mie University School of Bioresources, Kamihama-cho, Tsu 514, Japan;* [2]*Center for Molecular Biology and Genetics, Mie University, Kamihama-cho, Tsu 514, Japan;* [3]*Oji 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 CO_2 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

and energy sources. This phenomenon suggests that the rate of grass solubilization by bacteria and their cellulolytic enzymes limits the rate of ruminant growth. From this point of view, we are interested in looking for pretreatment methods that will enhance the biodegradation of grass. In addition, effective solubilization of cellulosic materials is also a world wide problem that must be solved in order to overcome food and energy shortages in the near future. In this paper, we report the expression of the cellulolytic enzyme genes from anaerobic bacteria in tobacco plants and the construction of chimeric enzymes by domain shuffling designed to enhance cell wall digestion.

Cellulase and xylanase genes from *Ruminococcus albus* and *Clostridium stercorarium* were expressed in tobacco cells (BY2) under the control of cauliflower mosaic virus (CaMV) 35S promoter. The xylanase gene product reached more than 4% of the total cell protein in tobacco cells, although cellulase expression was very low. The growth of the transformed tobacco cells was inhibited to a negligible extent compared with wild type cells. Extracts from tobacco cells transformed with the xylanase gene digested powdered barley straw. A cellulose-binding domain from a *C. stercorarium* xylanase was fused to a cellulase gene. The chimeric cellulase acquired an enhanced capability in digesting insoluble substrates such as ball-milled cellulose but not soluble carboxymethyl cellulose. These results suggest that such domain-shuffled enzymes can contribute to effective digestion of grass and other cellulosic materials.

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Microbial growth and polysaccharidase activity against straw cell walls in response to the nature of added carbohydrates. A Barrios-Urdaneta,[2], M Fondevila[1], SM Martín-Orúe, JC Machado-Nogueira[3] ([1]*Dpto. Producción Animal y Ciencia de los Alimentos, Universidad de Zaragoza, M. Servet 177, 50013 Zaragoza, España;* [2]*Facultad de Agronomía, Universidad del Zulia, Aptdo. 526, Maracaibo, Venezuela;* [3]*Facultade de Zootecnia e Engenharia de Alimentos, Universidade de Sao Paulo, Caixa Postal 23, Pirassununga, S.P., CEP 13630-000, Brasil)*

The nature of carbohydrates added to the media affects both the rate of adhesion and the extent of digestion of cell wall polysaccharides by mixed rumen bacteria [1]. In addition to the rate of bacterial attachment to cell walls, the enzymatic activity produced against structural polysaccharides must also be considered in determining the effect of added carbohydrates on the degradation of plant cell walls. Culture media [2] were prepared with straw cell walls (0.5% w/v) as substrate, either unsupplemented (CW) or with 0.27% w/v starch (S), pectin (P) or soluble sugars (M) and dispensed (8ml) anaerobically in tubes. These were inoculated (1ml) with rumen fluid diluted 1:10, and incubated at 39°C. In a first experiment, bacterial growth was estimated after 4, 8, 12, 20 and 24h by the concentration of purine bases in both the solid residue of fermentation and the liquid media. In a second experiment, the residue after 4, 8, 12, 20, 24 and 30h was hydrolysed with lysozyme, and the released enzymes tested against carboxymethylcellulose (CMC) and xylan (X). CW residues and attached bacteria, were also determined.