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.

This work was partially supported by "Program for Promotion of Basic Research Activities for Innovation Biosciences" (PROBRAIN), Japan.

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.

Both rate and extent of bacterial growth from 8h onwards ranked S>P>M>CW ($P<0.001$). However, the proportion of adherent bacteria from 8 to 24h was higher in P than in the other three media ($P<0.01$), reaching values over 50% at 8 and 24h. The proportion of adherent bacteria for CW, M and S, changed little with time and only increased ($P<0.01$) in S after 24h. Specific enzymatic activity in the fermentation residue (per unit of attached bacteria) against CMC and X was higher in CW than in the other three media at all times ($P<0.01$). However, the greater attachment induced by the supplements showed higher total enzymatic activities (per unit of residual CW) than in CW from 20h onwards ($P<0.05$). There were no differences ($P>0.10$) among the supplemented media in total or specific activity. Addition of carbohydrates reduced specific fibrolytic enzymatic activity, but total activity was increased by a higher bacterial growth. Addition of starch promoted a higher bacterial growth than pectin, but the latter enhanced attachment to cell walls.

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Simultaneous metabolism of glucose and cellobiose in *F. succinogenes* S85 studied by in vivo ^{13}C-NMR. Evidence of glucose 6-phosphate accumulation. A-M Delort[1], C Matheron[1], T Liptaj[2], G Gaudet[3], E Forano[3] (*[1]Laboratoire Synthèse, Electrosynthèse et Etude de systèmes à Interêt Biologique UMR 6504 du CNRS, Université Blaise-Pascal, 63177 Aubière Cedex, France.; [2]Slovak Technical University, Faculty of Chemical Technology, 812 37 Bratislavia, Slovakia; [3] INRA, Station de Recherches sur la Nutrition des Herbivores, Centre de Clermont-Theix, 63122 Saint Genès-Champanelle, France)*

Fibrobacter succinogenes is a strictly anaerobic ruminal bacterium which degrades cellulose to glucose and cellobiose. The two sugars are transported across the cytoplasmic membrane through independent constitutive transporters. Previously, in vivo ^{13}C- and ^{1}H-NMR has been used to investigate *F. succinogenes* S85 metabolism. Futile cycling of glycogen [1] and a new metabolic pathway involving the presence of phosphoketolase and pyruvate formate lyase were found [2].

In this study the kinetics of (1-^{13}C) glucose utilization by resting cells in the presence or the absence of unlabelled cellobiose was monitored by ^{13}C-NMR [3]. The analysis of the percentage of labelling of the metabolites showed that glucose was preferentially used for glycogen storage and energy production, while part of cellobiose was used for cellodextrin synthesis. Both cellobiase and cellobiose-phosphorylase activities were assayed in cell-free extracts and it is suggested that the role of cellobiase is to synthesise cellodextrins while that of cellobiose-phosphorylase is to cleave cellobiose. Glucose 6-phosphate concentration was increased by over three-fold when cells metabolised cellobiose. A possible role for glucose 6-phosphate in the regulation of cellodextrins synthesis is suggested.

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