Distribution of glycoside hydrolase activities in different cell fractions of xylan-grown *P. bryantii* B_14

Cell fraction	*Xylanase*	*CMCase*	*pNP-arabino-furanosidase*	*pNP-xylosidase*
	Specific activity (nmol.min^{-1}/mg protein)			
Washed cells	105.24	25.53	38.92	0.09
	% Total cell-associated activity			
Osmotic release (includes periplasm)	62.0	99.5	0.0	0.0
35,000 g supernatant (soluble)	4.5	0.5	24.3	12.9
35,000 g pellet (includes membranes)	33.4	0.0	75.7	87.1

xylosidase activities were found in the crude membrane fraction. Less than 30% of the total xylanase and CMCase activity was found to be extracellular. HPLC separation of B_14 proteins released by osmotic shock was performed using DEAE based compact porous disks [6] and four peaks with endoxylanolytic activity were recovered. More work is needed to unravel the xylanolytic systems of *P. bryantii* which apparently includes not only cell surface [7] but also periplasmic and membrane components.

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Xylooligosaccharide utilization by the ruminal bacterium *Selenomonas ruminantium.* MA Cotta, TR Whitehead (*USDA, ARS, National Center for Agricultural Utilization Research, 1815 N. University St, Peoria, IL, 61604, USA)*

Xylan is an abundant polysaccharide in plant cell walls and, as such, comprises a significant portion of the diets of ruminant livestock. The digestion of xylan is incomplete and this contributes to the inefficient conversion of feed into animal products. The enzymatic hydrolysis of xylan is accompanied by the formation of xylose, arabinose, and methyl-glucuronic acid containing oligosaccharides. These oligosaccharides can be utilized by several species of xylanolytic ruminal bacteria and by *Selenomonas ruminantium*, a non-xylanolytic species. Co-culture experiments with *S. ruminantium* and xylanolytic ruminal microorganisms demonstrated that *S. ruminantium* reduced the accumulation of xylooligosaccharides, in some cases leading to enhancement of xylan degradation. The objective of the current work was to study the fermentation of xylooligosaccharides by strains of *S. ruminantium* and examine the enzymes and genes that may be important in the

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 xylotetraose 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 oatspelt 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 Recherchès 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