transcripts from the L9 region is strongly regulated in response to xylan. *R. flavefaciens* 17 was also found to produce activities likely to be involved in xylan debranching including esterases and arabinofuranosidases, whose expression was regulated by xylan. A gene clone, that expresses activity against β-naphthyl acetate, has been isolated from a λEMBL3 phage library that is possible candidate for deacetylation of xylan. Cloned genes from *R. flavefaciens* have so far been analysed mainly in *E. coli* where proteolysis and internal starts were found often to result in anomalous products [3]. The *xynD* gene has now been expressed from its own promoter in Gram-positive hosts including *Enterococcus faecalis, Lactococcus lactis* and the rumen species *Streptococcus bovis*.

1. Flint HJ, McPherson CA, Bisset J (1989) *Appl Environ Microbiol* 55, 1230-1233
2. Zhang J-X, Martin J, Flint HJ (1994) *Mol Gen Genet* 245, 260-264
3. Flint HJ, Martin J, McPherson CA, Daniel A, Zhang J-X (1993) *J Bacteriol* 175, 2943-2951
4. Margolles-Clark E, Tenkanen M, Nakari-Setala T, Penttila M (1996) *Appl Environ Microbiol* 62, 3840-3846

Some genetic and biochemical aspects of *Prevotella bryantii* B_14 xylanases. R Marinšek-Logar[1], A Gasparic[1], FV Nekrep[1], HJ Flint[2] ([1]*University of Ljubljana, Biotechnical faculty, Groblje 3, 61230 Domzale, Slovenia;* [2]*The Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, Scotland, UK)*

Prevotella spp. are recognised as one of the most numerous groups of culturable bacteria inhabiting the rumen. Potentially significant activities include the degradation of plant cell wall polysaccharides, starch, proteins and peptides. Because of high degree of genetic diversity within the former *P. ruminicola* four new species were proposed recently, including *P. bryantii* which comprises B_14 - related strains [1].

P. bryantii B_14 is not cellulolytic but is actively xylanolytic and carries multiple xylanase genes. Four regions encoding xylanase activity have been identified, one of which corresponds to a broad-specifity endoglucanase [2]. Of the remaining regions, one encodes activities against *p*-nitrophenyl-β-xyloside (pNPX) and *p*-nitrophenyl-α-L-arabinofuranoside(*p*NPA) in addition to xylanase activity. This region carries at least two linked genes (*xynA* and *xynB*), one of which encodes an endo-xylanase while the other encodes a novel oxygen-sensitive exo-xylanase that has associated *p*NPX and *p*NPA activities [3]. The gene *xynC* encodes an endoxylanase [4]. These enzymes probably act sequentially in the breakdown of xylan.

Xylanolytic activity in *P. bryantii* was found to be strongly inducible, the specific activity of cells grown on xylan being increased at least 20-fold by comparison with cells grown on glucose [2]. SDS PAGE xylanograms of cell proteins revealed two clear endoxylanolytic bands at 26 - 29kDa and 63 - 66kDa and the latter is thought to correspond to XynC. Fractionation procedures based on osmotic shock [5] were applied to late exponential phase cells grown with xylan as energy source. The majority of the cell-associated endoxylanase and carboxymethyl cellulase (CMCase) activity was found in the frac tion released by osmotic shock while most of the α-L-arabinofuranosidase and β-

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.

1. Avguštin G, Wallace RJ, Flint HJ (1997) *Int J Syst Bacteriol* 47, 284-288
2. Gasparic A, Marinšek Logar R, Martin J, Wallace RJ, Nekrep, FV, Flint, HJ (1995) *FEMS Microbiol Lett* 125, 135-142
3. Gasparic A, Martin J, Daniel A, Flint HJ (1995) *Appl Environ Microbiol* 61, 2958-2964
4. Flint HJ, Whitehead TR, Martin JC, Gasparic A (1997) *Biochim Biophys Acta* 1337, 161-165
5. Huang L, Forsberg CW (1987) *Appl Environ Microbiol* 53, 1034-1041
6. Štrancar A, Koselj P, Schwinn H, Josic D (1996) *Anal Chem* 681, 3483-3488
7. Gardner RG, Wells JL, Russell JB, Wilson DB (1995) *Appl Environ Microbiol* 61, 3288-3292

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