Chitinase activity in recombinant *E. coli* strain XLOLR harbouring this chitinase plasmid did not increase in the presence of the lac promoter inducer IPTG. The activity in overnight cultures of *E. coli* carrying the chitinase plasmid is located mainly in the intracellular fraction (about 80%) with minor amounts in the periplasmic and cytoplasmic fractions. The chitinase, when assayed with 4-methylumbelliferyl-chitin substrates, seemed mainly to be the endo-type.

The pBluescript SK phagemid isolated from this clone contains a 1.7-kb fragment inserted at the *Eco*RI-*Xho*I cloning site. It contains a single open reading frame encoding a 60-kD polypeptide of 541 amino acids with high homology to chitinases from other organisms. The lack of an initiator methionine and alignment of the amino acid sequence with homologous proteins from other organisms suggests that the clone lacks a short stretch of sequence encoding the N terminal region of the chitinase protein. The ciliates *Tetrahymena, Paramecium* and *Euplotes* use some of the canonical stop codons to encode glutamine or cysteine. No departures from the universal code were invoked in the conceptual translation of the chitinase gene.

FIBRE DEGRADATION

Organisation of genes involved in xylan utilisation and xylan debranching in the rumen cellulolytic bacterium *Ruminococcus flavefaciens.*

V Aurilia[2], J Kirby[1], J Martin[1], S Ekinci[1] HJ Flint[1] ([1]*Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen, UK;* [2]*CNR-IABBAM, Via Argine, 1085 Napoli, Italy*)

Ruminococcus flavefaciens is a strictly anaerobic, Gram-positive bacterium that plays an important role in the degradation of plant cell wall polysaccharides in the rumen of domestic livestock. The degradation of plant structural polysaccharides requires the concerted action of many enzyme specificities including debranching enzymes in addition to enzymes that attack the main polysaccharide chains. So far genetic analysis in this species has revealed multiple cellulase and xylanase genes that are scattered around the genome [1]. Endoxylanases from *R. flavefaciens* were found to comprise a family of related enzymes of complex structure which often include more than one catalytic domain and a domain that is partially homologous with a putative thermostabilising domain found previously only in thermophilic bacteria [2]. In order to identify additional complementary activities we are investigating one of the few gene clusters so far found to be concerned with plant cell wall degradation in rumen microorganisms, while also screening directly for unlinked genes that encode xylan debranching activities. The *R. flavefaciens* chromosomal region recovered in the L9 phage clone [3] carries a gene encoding a bifunctional xylanase and β-(1,3-1,4)-glucanase enzyme. The upstream region contains a putative regulatory gene whose product has homology with AraC-type DNA binding proteins. Two downstream ORFs have been identified, one of which shows homology with family 3 β-glucosidases but is thought to be a β-xylosidase and α-arabinofuranosidase [4] and the other shows homology with xylose isomerases. This suggests strongly that the gene cluster is concerned with the utilisation of xylo-oligosaccharides as well as with xylan degradation. Expression of

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*.

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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-