lated from the rumen ciliate, *Entodinium caudatum,* maintained *in vivo* in monofaunated sheep. The enzyme was purified using a combination of cation exchange chromatography on carboxymethylcellulose and size exclusion gel filtration on Sephadex G-75. The isolated enzyme resembled lysozyme in that it was a basic protein which rapidly degraded *Micrococcus lysodeikticus* cell walls. The enzyme had an isoelectric point of pH 9. It displayed optimal activity at pH 6.5, an ionic strength of 0.05M and at a temperature of 40°C. It had an apparent affinity constant (K_{app}) of 400mg *M. lysodeikticus* l^{-1}. The protein had a molecular weight of 14 kDa as determined by SDS-PAGE. Based on the N-terminal amino acid sequence the protein showed some similarity to a distinct class of lysozymes found in *Streptomyces* species, the fungi *Chararopsis* and the protozoal parasite *Entamaoeba histolytica* [2].

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2. Jacobs T, Leippe M (1995) *Eur J Biochem* 231, 831-838

Molecular cloning of a chitinase gene from the rumen protozoon *Entodinium caudatum*. K Komatani[1], DP Morgavi[1], R Onodera[1], BA White[2], I Cann[2] KM Karrer[3] *([1]Miyazaki University, Laboratory of Animal Nutrition and Biochem, Miyazaki 889-21, Japan; [2]University of Illinois, Department of Animal Science, 1207 W Gregory Dr, Urbana, IL 61801, USA; [3]Marquette University, Deptartment of Biology, Milwaukee, WI 53201, USA)*

The predatory activity of ruminal protozoa profoundly affects the microbial population in the rumen and consequently influences the degradation of food and availability of nutrients to the host animal. It is thought that chitinases, lysozymes and other hydrolytic enzymes are of fundamental importance in the protozoal digestion of engulfed fungi and bacteria, and this process, if well understood, may lead to the design of strategies to improve ruminant performance. The aim of the present study was to clone a gene from the rumen protozoon *Entodinium caudatum* encoding a chitinase in order to better understand the role that this hydrolytic enzyme plays in the interrelationship between protozoa and fungi in the rumen.

E. caudatum, harvested from a monofaunated goat fed a concentrate and hay cube ration, were washed by centrifugation and total RNA was isolated using a single-step guanidinium method. Poly(A)$^+$ RNA was purified on oligo(dT)-cellulose resin and used as template for the construction of a cDNA library in λphage by using a commercial kit (ZAP-cDNA® synthesis kit, Stratagene). The pBluescript phagemids containing the cloned inserts from the entire library were in vivo excised from the λphage vector (Uni-ZAP XR) following the manufacturer's instructions; these phagemids were used to transform *E. coli* strain SORL. Transformants grown on LB ampicillin agar plates in the presence of IPTG were covered with 0.7% agar containing 0.02% glycol chitin and incubated at 37°C for 6 to 8h. After the incubation period, plates were stained with a 1% solution of Congo Red.

Several colonies showing a clear halo, indicating chitin degradation, were isolated and re-screened. After a third screening, the clone producing the most perceptible clearing of chitin was chosen.

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.*

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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