

Xylanolytic activity of the rumen protozoan *Diploplastron affine**

K. Wereszka^{1,3}, T. Michałowski¹, C.J. Newbold² and N.R. McEwan²

¹The Kielanowski Institute of Animal Physiology and Nutrition,
Polish Academy of Sciences
05-110 Jabłonna, Poland

²The Institute of Rural Science, University of Wales
Aberystwyth Ceredigion SY23 3AL, United Kingdom

ABSTRACT

Cell free extract prepared from ciliates *Diploplastron affine* free from bacteria degraded xylan releasing reducing sugars at the rate of 117.8 ± 3.14 $\mu\text{M}/\text{mg}$ protein/h. Two xylan degrading enzymes have been identified in protozoal cell free extract by a zymogram technique it was also found that one of the identified enzymes degraded xylan to xylooligosaccharides i.e. was of endoxylanase character. Screening of the *Diploplastron affine* cDNA library resulted in the identification of a xylanase encoding gene consisting of 1670 bp.

KEY WORDS: *Diploplastron affine*, xylan, xylanase, xylanase gene

INTRODUCTION

Cellulose and xylan are carbohydrate components of plant cell wall utilized by ruminants due to fibrolytic activity of microbiota inhabiting the rumen and it is believed that ciliates *Diploplastron affine* are able to digest these polysaccharides (Williams and Coleman, 1992; Dehority, 1993). The aim of our studies was to examine the xylanolytic activity of ciliates *Diploplastron affine* and to confirm the ability of these protozoa to synthesize xylan degrading enzymes. Some of the results described in this paper were presented at the international microbiology symposium in Scotland (Wereszka et al., 2004).

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³ Corresponding author: e-mail: k.wereszka@ifzz.pan.pl

MATERIAL AND METHODS

The protozoa originated from the rumen of monofaunated sheep (Michałowski et al., 1999). Cell free extract was prepared from purified ciliates free of intracellular bacteria as described by Michałowski (1997). Degradation rate of xylan was determined according to Miller et al. (1960) by the quantification of reducing sugars released during the incubation of substrate with cell free extract for 1h at 40°C in 0.02 M sodium phosphate buffer (pH 6.0) while end products were identified by TLC after hydrolysis prolonged for 48 h. Zymographic study and fractionation of cell free extract by exchange chromatography on DEAE Sephadex A 50 were done as described by Kasperowicz and Michałowski (2001).

The cDNA library of *Diploplastron affine* (www.ercule.com) was used to identify and sequence gene(s) encoding xylanase enzymes. *E. coli* strains XL1-blue MRF' and XL0LR were used to screen and transform the phagmids with identified gene, respectively. The plasmids were then isolated using Wizard Plus SV Minipreps DNA Purification System Kit (Promega), and sequenced with Perkin Elmer DNA ABI Prism TM Big Dye Kit and DNA ABI Prism TM 377 XL Sequencer.

RESULTS AND DISCUSSION

The degradation rate of xylan by cell free extract reached 117.8 ± 3.14 μ M released xylose/mg protein/h and was almost two times greater as compared with *Epidinium ecaudatum* (Michałowski et al., 2001). Two xylan degrading protein bands were identified by zymogram technique and this was in agreement with the distribution of the xylanolytic activity over the fractionated cell free extract. Only oligosaccharides were found there as end products of xylan hydrolysis by the most active fraction and this suggests that the identified enzymes were “endo” mode of action (Figure 1).

Screening of the *Diploplastron affine* cDNA library resulted in the identification of a complete cDNA encoding for xylanase. Length of the identified gene determined by agarose gel electrophoresis was about 1.5 Kb (Figure 2), while sequencing of the gene following amplification by PCR revealed that the total number of nucleotides was 1670 (Figure 3) including 189 bp encoding the signaling peptide. Thus the gene encoding synthesis of endoxylanase in the cells of *Diploplastron affine* differs from xylanase genes identified in cDNA library of *Polyplastron multivesiculatom* (Devillard et al., 1999).



Figure 1. End products of xylan hydrolysis identified by TLC following digestion of substrate with either a cell free extract 1. or the fraction which exhibiting the highest xylanolytic activity 2. Lane 3 shows the position of xylose as a standard

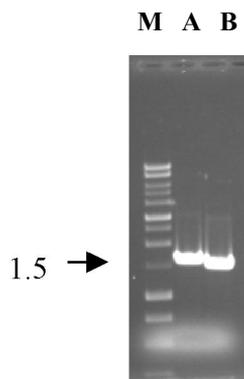


Figure 2. Clones encoding the xylanase gene from *Diploplastron affine*: A - complete cDNA encoding the xylanase, B - cDNA following removal of the putative signalling peptide sequence, and M - nucleotide size standards

5'CGCCTGCAGGTCGACACT**ATG**GATCCAAAAGAATTCGGCACGAGGCCTACTTTCAATTCTTCTTTC
 AAGCACCTTAAATAAAAAAATTCCTATTTTTGCGGAAGATGATGATATAATCTGTGAGACCAGTTTC
 GAAGATGGAGATTTTCAATGTTGTCACCCAGAGGTACTGATGAAGTCCTTGAAATAAGTACAGCT
 GGAGAAAAACCGGTGATAATTGTTAGCTGTTACAAAACGTGCCAATCTTGGAAATGGAGCACACA
 ATATAATCTTGAAAAAAGTTGTGAACCAAGGTGGTCAATATCTAGTCAAGGCATATGTAAAAGCCCAA
 TGGTATCCAATATTTGCCTAAGTATTCAATATACTGATGGTGATGGTGAAGATCATTATAACAACCTT
 AAAATGTGTATAAGTCAAGGAGATTGGGCTGAAAATCCAGAATACAAATTTTCTATGCCATCTGGA
 TGTACTGGTGATATATCTATTTTGAAAATACTGGAGGTACTAACGATTTTGGATTGATGATTTTCAG
 TTTAAAAAAGCTCCAGAAGGTCAAATCCAAGAAGATATTGTCTCATTAAAAAGATGTCTACAAAAA
 ATATTTCAAAAATTGAACTGCAACTACTGTTGCTGAAATTTTCCAAAAACTACCCAAAAATTAATT
 TTA AACATTTCAATAGCTTACACCTGGAAATGAGCTTAAACCTGATAGTTGTTAGATAAAGCTG
 GCACCATTTGAAGAATCAGAAAAACCGGAGATTACACAAAATCCAAAAGTTAAAAATGGTGCAGCT
 CAACCAATTTTAAATTTTGCCGCAGAAAATGATATCCCAGTTAGAGGCATATACTTAGTCTGGCATT
 CACAAACCCCTACCTGGTCTTCAAAGAAAAATGGGATGCTGATGGTGATGGGGTAGATAAAGAA
 ACTATGATAAAAAAGAATGGAAAATTATATTA AAAATCTATTCGATGCAGTTAAaGCTGCATATCTTAC
 TGTTAACTTTTATGCTTGGGATGTTGTCAATGAAGCCATTGTAGATGACGGTAAACCAAGAAATCC
 TGGTTCTCAAGAACAAAATCCAAATAATTCACCATGGGTTCAAATTTTCGgAGATAATTCATTTATT
 AAATATGCTTTCCAATTTGCTAAAAAATATGGAATTGAAGAATGCAAATATATTATAATGATTACA
 ATGAATATATGCCTCAAAAACTCCAGCAATTATCAAATGGTTAAAGAAGTTAATGAGGGTGAAC
 AATTAATGATGGTATTGGTTTACAATCTCATCTTGATGTTAATTTCCGGGATTAAGTGCTTATGAA
 AAGCCATTA AATTATTAAGTGA AACTGGACTTGATTTACAAATTACTGAATTAGACGCAACTACT
 CAAGACACTTCTGAAGCTGGATTTGAAAAGACAAGCAGAATATTATAGCGATATCATGGACGTAATT
 GTTAAATATTCTAAATCAATTTCTGCTGTTGTTTCTGGGGTACAACAGATGATCAAAGTTGGAGAG
 CTGATAGATGCCACTTCTTTTCAATGAAGATTATACTGCCAAACCTTGCTTCTACTCTATTGTTGA
 TGGACTTT**TAA**AAAAATTTAAAAATTATCAAACAACCTATGTTATAATTATTAATTAATAACA AAAATA
 GATTATATAAAATTAAAACCTTAAAGAGTAAATTAATATGAGTTAAATTA AAAAAAAAAAAAAA3'

Figure 3. Nucleotide sequence of cDNA encoding synthesis of xylanase in the cells of *Diploplastron affine*. Underlined ATG and TAA sequences in bold are start and stop codons, respectively

CONCLUSIONS

The presented study showed that the rumen ciliate *Diploplastron affine* is able to digest xylan. Zymographic and ion exchange chromatography suggest the presence of at least 2 different xylanolytic enzymes and one of them exhibited the properties of endoxylanase. The xylanolytic activity was present after elimination of intracellular bacteria. On the other hand genetic studies revealed the presence of xylanase gene in cDNA library of ciliates. These findings confirmed hypothesis that ciliates *Diploplastron affine* digest xylan using their own enzymes.

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