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The ability of the rumen ciliate *Anoplodinium denticulatum* to utilize hemicellulosic material for *in vitro* growth

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ABSTRACT

Xylan was found to stimulate the growth of a population of *Anoplodinium denticulatum* when supplied at a rate of 0.03 and 0.06 mg/ml culture/d. The protozoa were able to digest xylan since the crude enzyme preparation from the cells of ciliates incubated with this polysaccharide released over 811 μM xylose/mg protein/h. An increase in the VFA concentration followed the incubation of ciliates with xylan in the presence of antibiotics with the main fermentation products being acetic and butyric acids. The production rate of total VFA was 6.4 pM/protozoan/h.

KEY WORDS: rumen ciliates, xylan, xylanases, digestion, fermentation

INTRODUCTION

Digestion of structural carbohydrates by ruminants results entirely from the fibrolytic activity of the microorganisms inhabiting their digestive tract, mainly the rumen. Apart from significant progress in studies on the protozoal enzymes catalysing degradation of cellulose and hemicellulose (Devilliard et al., 1999; Michałowski et al., 2001; Wereszka et al., 2004) the role of ciliates in ruminal fibrolysis is much less known as compared with bacteria and fungi. The objective of this study was to examine the ability of the ciliate *Anoplodinium denticulatum,* to digest and ferment xylan, which is a component of plant cell wall hemicellulose.

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MATERIAL AND METHODS

Ciliates *Anoplodinium denticulatum* were isolated from the rumen fluid of sheep and maintained *in vitro* as bath or continuous cultures (Michałowski 1979; Michałowski et al., 1989) or were grown in the rumen of selectively faunated sheep. The cultivation medium for *in vitro* culture was composed of, g/l: $KH_2PO_4$1, $NaHCO_3$5, NaCl 6.0, $CaCl{\cdot}H_2O$0.2, $Mg_2SO_4{\cdot}7H_2O$0.2 (Hungate, 1942). The growth experiment lasted 28 d during which the ciliates were counted every 4 d. The food consisted of only hay and wheat gluten (control cultures) or was supplemented with three doses of xylan (experimental cultures) The particular components were supplied in the proportion of 0.3 (hay), 0.08 (wheat gluten) and 0.03, 0.06 and 0.12 (xylan) mg/ml culture/d, respectively. Bacteria-free ciliates were used in fermentation and enzymatic experiments. Bacteria were eliminated by incubation with ampicillin, streptomycin and chloramphenicol (50 μg/ml). In fermentation experiments, the ciliates were incubated for 12 h with xylan and antibiotics (experimental culture) or with antibiotics only (control cultures). Volatile fatty acid (VFA) concentrations and protozoa counts were determined every 3 h using gas chromatography and microscopy counting methods, respectively. The 3,5-dinitrosalicylic acid reagent (Miller et al., 1960) was used to assay the reducing sugars released from xylan incubated for one hour with the protozoal crude enzyme preparation.

RESULTS AND DISCUSSION

The population densities of *Anoplodinium denticulatum* grown in the media supplemented with xylan are presented in Figure 1. A stimulating effect was found when xylan was supplied in an amount of either 0.03 or 0.06 mg/ml culture/d ($P<0.05$). The both doses may have been effectively utilized by the protozoa.

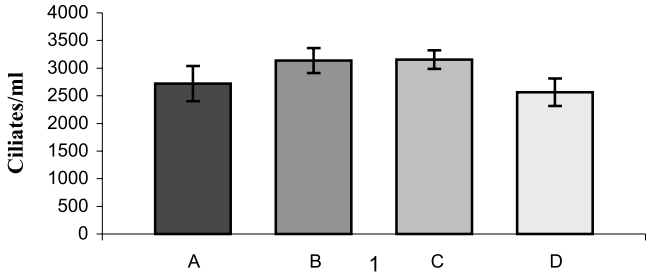

Figure 1. Mean numbers of ciliates *Anoplodinium denticulatum* in control cultures (A) fed hay and wheat gluten at a rate of 0.3 and 0.08 mg/ml/d, respectively, and in experimental cultures given the same food supplemented with xylan at a rate of 0.03 (B), 0.06 (C) and 0.12 (D) mg/ml/d

This suggestion is supported by the high xylanolytic activity of the crude enzyme preparation. The digestion rate was 811.5 μM released xylose/mg protein/h. It markedly exceeded the xylanolytic activity of the enzyme preparations obtained from *Epidinium ecaudatum* and *Diploplastron affine* (Michałowski et al., 2001; Wereszka et al., 2004). The effect of the highest xylan dose tended to be negative, perhaps due to unfavourable changes in the culture medium resulting from the accumulation of acidic products of carbohydrate metabolism (Michałowski et al., 1989).

Table 1. Fermentation pattern of xylan by the rumen ciliates *Anoplodinium denticulatum* during incubation *in vitro*

Item	Control	Experimental
Production rate of total VFA, pM, VFA/protozoan/h	2.8 ± 1.42	6.4 ± 3.48**
Acetate, % of total VFA	74.3 ± 3.64[a]	75.6 ± 4.53[a]
Propionate, % of total VFA	11.6 ± 2.70[b]	10.6 ± 2.79[b]
Butyrate, % of total VFA	14.1 ± 1.67[c]	13.8 ± 2.47[c]

values with different letters differ significantly ($P<0.05$)
* $P<0.01$

Ciliates incubated with xylan produced more VFA ($P<0.01$) compared with starved ones (Figure 2). The presented data also showed that the main fermentation products were acetate, followed by butyrate (Table 1). Such a fermentation pattern was also characteristic for the mixed population of ciliates in the rumen (Michałowski, 1987). The main sugar fermented by protozoa was presumably the digestion product of xylan, i.e. xylose. The presented findings thus confirm the ability of *Anoplodinium denticulatum* to utilize pentoses as a source of energy. Such a property has not been satisfactorily documented to date in relation to ciliates from the family *Ophryoscolecidae* (Williams and Coleman, 1992).

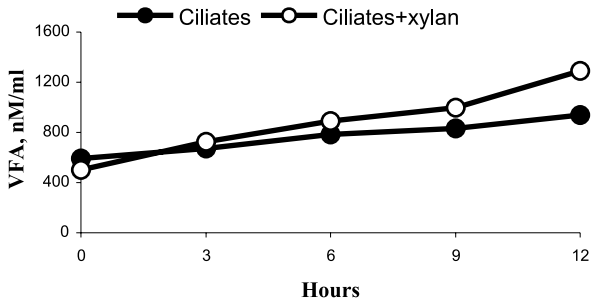

Figure 2. Changes in the concentration of VFA during *in vitro* incubation of ciliates *Anoplodinium denticulatum* with xylan

CONCLUSIONS

The rumen ciliate, *Anoplodinium denticulatum,* digested and fermented xylan to volatile fatty acids, which suggests that it was able to utilize xylose to cover energy requirements. The digestive and fermentative properties were exhibited even after treating the ciliates with antibiotics. Both properties therefore seem to be of protozoan origin.

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