APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1989, p. 1016–1022
0099-2240/89/041016-07$02.00/0

Vol. 55, No. 4

Production of Xylanase by the Ruminal Anaerobic Fungus *Neocallimastix frontalis*

DOUGLAS O. MOUNTFORT* AND RODNEY A. ASHER
Cawthron Institute, Private Bag, Nelson, New Zealand

Received 5 July 1988/Accepted 5 January 1989

Xylanase (1,4-β-D-xylan xylanohydrolase, EC 3.2.1.8) production was investigated in the ruminal anaerobic fungus *Neocallimastix frontalis*. The enzyme was released principally into the culture fluid and had pH and temperature optima of 5.5 and 55°C, respectively. In the presence of low concentrations of substrate, the enzyme was stabilized at 50°C. Xylobiose was the principal product of xylanase action, with lesser amounts of longer-chained xylooligosaccharides. No xylose was detected, indicating that xylobiase activity was absent. Activities of xylanase up to 27 U ml^{-1} (1 U represents 1 μmol of xylose equivalents released min^{-1}) were obtained for cultures grown on xylan (from oat spelt) at 2.5 mg ml^{-1} in shaken cultures. No growth occurred in unshaken cultures. Xylanase production declined with elevated concentrations of xylan (>2.5 mg ml^{-1}), and this was accompanied by an accumulation of xylose and, to a lesser extent, arabinose. Addition of either pentose to cultures grown on low levels of xylan in which neither sugar accumulated suppressed xylanase production, and in growth studies with the paired substrates xylan-xylose, active production of the enzyme occurred during growth on xylan only after xylose had been preferentially utilized. When cellobiose, glucose, and xylose were tested as growth substrates for the production of xylanase (each initially at 2.5 mg ml^{-1}), they were found to be less effective than xylan, and use of xylan from different origins (birch wood or larch wood) as the growth substrate or in the assay system resulted in only marginal differences in enzyme activity. However, elevated production of xylanase occurred during growth on crude hemicellulose (barley straw leaf). The results are discussed in relation to the role of the anaerobic fungi in the ruminal ecosystem, and the possible application of the enzyme in bioconversion processes is also considered.

Lignocellulose constitutes the largest biomass source on earth and is mainly composed of lignin, cellulose, and hemicellulose of which xylan is the major component. Xylan also composes a substantial proportion of herbage, and it has been known for some time that the rumen has xylanolytic activity. Xylanases have been described for a number of ruminal bacteria (9, 10, 21, 22), and ruminal protozoa have also been shown to possess xylanolytic activity (23). For a long time, these organisms were considered to be the major degraders of xylan in the rumen. However, the discovery more than 10 years ago that anaerobic fungi are also inhabitants of the rumen and occur in high numbers when ruminants are fed a high-fiber diet (4, 5) led to the possibility that these organisms, too, possess xylanase activity. The enzyme has subsequently been shown to be produced by several ruminal fungi (14, 19, 20, 27), but its significance in ruminal fiber degradation is not certain.

Apart from their role in natural ecosystems, xylanases are now being increasingly recognized as having application in the bioconversion of hemicellulose to fermentable sugars and in other industries such as paper making. Consequently, there has been considerable interest in selecting for organisms which produce novel xylanases and which give high yields of the enzyme (12, 25, 28).

In this communication, we describe the production of xylanase by *Neocallimastix frontalis* and determine the conditions necessary for optimal production of the enzyme together with the regulatory constraints involved. The results are discussed in relation to the role the anaerobic fungi play in the ruminal ecosystem, and because the enzyme possesses a number of characteristics relevant to bioconversion processes, its possible industrial application is also considered.

* Corresponding author.

MATERIALS AND METHODS

Organism. *N. frontalis* PN-1 was obtained from the culture collection at the Department of Scientific and Industrial Research, Palmerston North, New Zealand.

Culture media. Anaerobic techniques for the preparation of culture media were based on those of Hungate (13) as modified by Balch and Wolfe (3).

Salt solutions were used in the preparation of all media. Solution A contained the following (wt/vol):KH_2PO_4, 0.3%; NaCl, 0.6%; $(NH_4)_2SO_4$, 0.3%; $CaCl_2$, 0.03%; and $MgSO_4$, 0.03%. Solution B contained 0.3% (wt/vol) K_2HPO_4.

Complex medium for growth of *N. frontalis* was based on that of Bauchop and Mountfort (6) and had the following composition: solution A, 165 ml; solution B, 165 ml; cell-free ovine ruminal fluid, 170 ml; distilled water, 500 ml; $NaHCO_3$, 5 g; yeast extract (BBL Microbiology Systems, Cockeysville, Md.), 1 g; Bacto-Peptone (Difco Laboratories, Detroit, Mich.), 1 g; cysteine hydrochloride, 0.2 g; $Na_2S \cdot 9H_2O$, 0.1 g; and resazurin, 0.001 g.

Medium for maintenance or experimental cultures was boiled and then dispensed in 10-ml amounts into culture tubes (18 by 150 mm) or in 60-ml amounts into 120-ml bottles modified for use with black rubber septum stoppers and aluminum serum-cap closures. The gas phase was 70% N_2–30% CO_2. Tubes or flasks were sealed, and media were sterilized by autoclaving. Sterile sodium sulfide reducing agent was added to media several hours before use.

For preparation of maintenance medium (10 ml), sisal (fiber from leaves of *Agave sisalona* L. commercially available as twine) was added to culture tubes in 100-mg amounts prior to addition of culture medium. Unless otherwise

stated, experimental growth media (60 ml) contained type 1 xylan in the range from 0.6 to 10 mg ml^{-1} or other carbohydrates at 2.5 or 3 mg ml^{-1}.

Culture techniques. The techniques described previously by Hungate (13) as modified by Balch and Wolfe (3) were adapted for the maintenance and subculturing of *N. frontalis*.

Experimental cultures were inoculated by transferring 3 ml of 72-h-old cultures grown on xylan. The inoculum was equivalent to between 1.5×10^3 and 2.0×10^3 CFU of fungus ml^{-1}. The numbers of CFU of the fungus were determined after 4 days of incubation in roll tubes inoculated with 5×10^{-2} to 5×10^{-4} ml of culture, and glucose (0.1% [wt/vol]) was used as the growth substrate.

All incubations were at 37°C. Tubes for the maintenance of cultures were kept in a vertical position without shaking, and experimental cultures were incubated by shaking on a Grant (model SS-30) water bath at 80 oscillations per minute.

Culture purity. Cultures of *N. frontalis* were routinely checked for purity by examining wet mounts and by roll tubing in complex media as described above or in AC medium (Difco).

Time course of xylan degradation and xylanase production. The time course of xylan degradation and xylanase release by *N. frontalis* was determined by periodic sampling of the growth medium. Growth was determined by measuring H_2 production as previously described (16, 17). Normally, a 4-ml sample of culture medium was removed by syringe and then centrifuged at 6,000 × *g* at 0 to 2°C for 15 min. The supernatant was carefully removed, and a 2-ml portion was kept at <2°C for assay of xylanase while the remainder was stored at −18°C for the determination of sugars.

Determination of xylan hydrolytic products in time course experiments. Qualitative analysis of xylan hydrolytic products in growth culture supernatants was achieved by descending paper chromatography on Whatman no. 1 paper. Sugars were separated with ethyl acetate-pyridine-water (8:2:1 [vol/vol/vol]) solvent and identified after chromatograms were dipped in alkaline silver nitrate solution (26).

Quantitative analysis of sugars was achieved by high-performance liquid chromatography. Supernatant was mixed with an equivalent volume of acetonitrile-H_2O (85:15 [vol/vol]) which was filtered. A sample (20 μl) was injected into a carbohydrate analysis column (30 cm by 3.9 mm) (Waters Associates, Inc., Milford, Mass.), and separation was achieved with a mobile phase of acetonitrile-H_2O (85:15 [vol/vol]) at a flow rate of 1 ml min^{-1}. Eluted sugars were detected by refractive index and were identified and quantitated by comparison of retention times and peak areas with those of authentic samples.

Xylan in the culture fluid was determined by a modification of the anthrone method (1) adapted for measurement of pentoses, and values were corrected for the presence of the hydrolytic products determined above.

Assay of xylanase. Xylan solution used in the xylanase assay was prepared by suspending 1% (wt/vol) xylan (type 1, unless otherwise stated) in 0.05 M sodium acetate buffer (pH 5.5). About 0.7% (wt/vol) of the xylan was dissolved after the mixture was heated to 80°C for approximately 15 min, followed by cooling and centrifuging (6,000 × *g* for 10 min at 2°C) (24). For the determination of xylanase, 0.2 ml of culture supernatant was added to 4.8 ml of acetate buffer containing dissolved xylan, and the reaction mixture was incubated at 55°C for 5 min. The reaction was stopped in an ice bath, and reducing sugar released was determined by the dinitrosalicylic acid method (15) modified as previously described (17).

TABLE 1. Distribution of xylanase in various fractions of a xylan-grown culture of *N. frontalis*

Fraction	Activity (U ml^{-1})[a]
Supernatant	26.6
Fungal rhizoid	2.7
Sonicated rhizoid	4.6
TET-treated rhizoid	4.0
Sonicated TET-treated rhizoid	4.7

[a] Values represent the activity of various fractions per milliliter of culture of maximal growth on xylan (2.5 mg ml^{-1}) and are means of duplicate determinations. Xylanase associated with the fungal rhizoid was determined after the pellet collected by centrifugation of a 60-ml culture was dispensed in 20 ml of 0.05 M sodium acetate buffer (pH 5.5) with a Teflon piston homogenizer. Activity of the sonicated fraction was determined after a portion of fungal suspension had been sonicated for 2 min at 2°C with a cell disrupter (Heat Systems Ultrasonics Inc.) at 50% load and with an output energy of 4. TET-treated rhizoid was obtained by addition of a toluene–ethanol–Triton X-100 (1:4:0.2 [vol/vol/vol]) mixture to a portion of fungal suspension to give a final concentration of 5% (vol/vol).

One unit of xylanase activity was defined as the amount of enzyme that produced reducing sugar equivalent to 1 μmol of xylose min^{-1} under the conditions described above.

Qualitative analysis of hydrolysis products of xylan in assay mixture. For analysis of xylan hydrolysis products, contents from an assay system which had been incubated for 5 min were cooled to 2°C and then passed through a column of AG-50W-X2(H^+) ion-exchange resin (Bio-Rad Laboratories, Richmond, Calif.) to remove cations. The eluate was concentrated by lyophilization, and the contents were dissolved to 1/10th of the original assay volume. Samples (50 μl) were spotted onto precoated Silica Gel 60 (E. Merck AG, Darmstadt, Federal Republic of Germany) plates together with standards of glucose, xylose, L-arabinose, and xylobiose. Separation was achieved with a 7:2:1 (vol/vol/vol) isopropanol-acetic acid-water solvent system, and chromatograms were developed with aniline-diphenylamine reagent (11).

Chemicals. Xylan type 1 (stated origin, oat spelt) was obtained from Fluka Chemicals (Buchs, Switzerland) and contained <2% L-arabinose. Xylan type II (stated origin, birch wood) with an average molecular weight of 25,000 was purchased from Roth (Karlsruhe, Federal Republic of Germany). It contained 0.5% arabinose and glucose. Type III xylan (stated origin, larch wood) was obtained from United States Biochemical Corp. (Cleveland, Ohio). Xylobiose was obtained from Pfanstiehl Laboratories (Waukegan, Ill.). All other chemicals were obtained from commercial sources.

RESULTS

Location and general properties of xylanase. When *N. frontalis* was grown on xylan, xylanase was present mainly in the culture fluid. Table 1 shows that only about 10% of the activity was found to be associated with the fungal rhizoid. Treatment of a suspension of fungal rhizoid with toluene–ethanol–Triton X-100 (1:4:0.2 [vol/vol/vol]) mixture (TET) resulted in release of xylanase amounting to 20% of the total activity, and sonication in the absence and presence of TET likewise resulted in the release of the same amount of enzyme. Because xylanase was found to be mainly released into the culture fluid, activities were subsequently determined in the culture supernatants.

Xylanase activity was found to be unaffected by oxygen tension within the range from 0 to 20% of the gas phase at

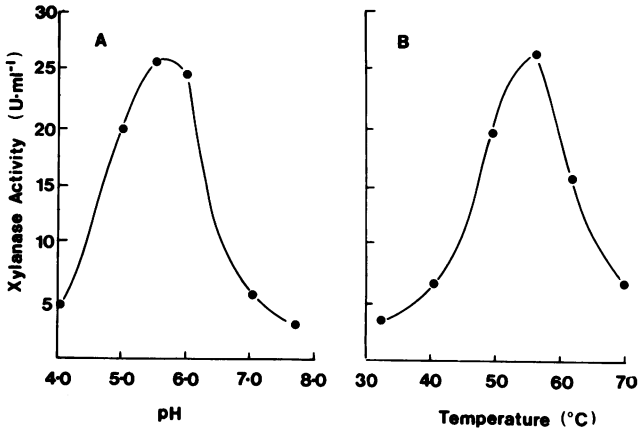

FIG. 1. pH (A) and temperature (B) profiles of xylanase from *N. frontalis*. Activity was determined in the supernatant from cultures grown on 2.5 mg of xylan ml^{-1}. For the pH profile, activities in the range from pH 4 to 6 were determined with 0.1 M sodium acetate assay buffer, and at pHs of >6.0, 0.1 M citrate-sodium phosphate (McIlvaine) buffer was used.

ambient pressure. The pH optimum of the enzyme was 5.5, and the temperature optimum was 55°C (Fig. 1A and B). The enzyme was found to be virtually stable at 50°C for 1 h in the presence of 1% xylan, but 30% of the activity was lost after incubation for the same time period in the absence of substrate (Fig. 2). The presence of substrate made little difference to stability at 55°C, and almost 80% of the initial activity was lost after incubation at this temperature for 1 h. Xylanase was stable at 2°C for up to 100 h, with little loss of activity (<5%) occurring upon freezing and thawing.

Confirmation that the enzyme was a xylanase was obtained after identification of the products by thin-layer chromatography. The major product was xylobiose, with lesser quantities of longer-chained xylooligosaccharides. No xylose was produced, indicating that xylobiase was absent.

Optimization of xylanase production on xylan and influence of pentoses. When cultures of *N. frontalis* were grown on xylan at different concentrations, maximal (or highest) activities obtained in the time course corresponded to maximal growth (Fig. 3A and B). Maximal activities were highest when the initial xylan was at 2.5 mg ml^{-1}, and increasing xylan beyond this level led to a decline in xylanase production.

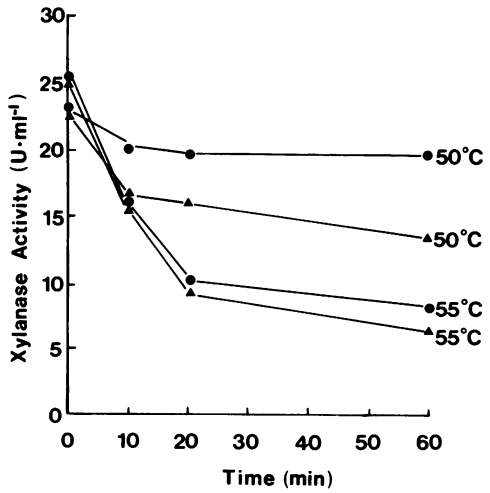

FIG. 2. Effect of xylan on thermal stability of xylanase from *N. frontalis*. Supernatant from a culture grown on 2.5 mg of xylan ml^{-1} was incubated in the presence and absence of 1% xylan for 1 h at the temperatures indicated. The residual activities were determined under standard conditions. Symbols: ●, plus xylan; ▲, control incubation with no xylan added.

A number of possibilities could have explained the decline in enzyme production at elevated xylan concentrations. The first could have been adsorption of enzyme onto xylan that had not been utilized in the culture fluid. To test whether this was likely, we incubated supernatant from cultures fully grown on xylan at various initial concentrations (2.5 to 10 mg ml^{-1}) with different amounts of xylan (range, 2.5 to 10 mg ml^{-1}) for 2 h at 37°C. Adsorbed xylanase was determined by comparison with controls incubated in the absence of xylan. In most cases, <10% of the enzyme was absorbed and an increase in xylan did not lead to a substantial increase in absorption. Thus, absorption onto substrate could not adequately account for the decreased xylanase production at high xylan concentrations in the culture media.

Another possible explanation for decreased xylanase production was that it was due to accumulation of soluble sugars. To test this, we analyzed culture supernatants by paper chromatography at various stages in the time course of enzyme production. Xylose and arabinose were found to accumulate only in cultures grown on xylan at levels ≥5 mg ml^{-1}, and no other sugars were detected. The accumulation of xylose but not arabinose was transitory, as is shown in Fig. 4 for growth on 10 mg of xylan ml^{-1}. The results on pentose accumulation together with those showing a decline in xylanase activity during growth on elevated xylan concentrations suggested that the sugars are in some way involved in the regulation of the enzyme.

Evidence for a regulatory role of pentose sugars in xylanase production was obtained when either xylose or L-arabinose was added to cultures growing on xylan which did not accumulate the sugars and which had actively begun to produce xylanase. Production of the enzyme decreased upon the addition of these sugars (Fig. 5A and 6A), and with xylose added at 4.5 mg ml^{-1}, complete cessation of enzyme production occurred. Growth was markedly stimulated by xylose addition even though enzyme production declined (Fig. 5B), and the increase in growth presumably reflected utilization of xylose. Addition of L-arabinose, however, resulted in a decrease in growth (Fig. 6B), which is consistent with the inability of *N. frontalis* to utilize this substrate (16).

The results on xylose addition were substantiated by paired-substrate growth experiments in which the pentose was found to be preferentially utilized over xylan during diauxic growth on the two substrates (Fig. 7C). Utilization of xylan and concomitant xylanase production in the absence of xylose was complete in about 70 h (Fig. 7A), whereas in paired-substrate growth, there was a delay in xylan utilization and xylanase production until growth on and utilization of xylose was nearly complete. In the absence of xylan, rapid growth occurred on xylose but xylanase production was minimal (Fig. 7B).

Examination of xylanase associated with the fungal rhizoid immediately prior to active xylanase production in the paired-substrate experiments or at the end of incubation in the xylose- or arabinose-treated systems revealed an activity of <4.0 U ml of $culture^{-1}$, indicating that the decreased levels of enzyme in the culture fluid were not due to its increased association with the fungal rhizoid. Furthermore, decreased xylanase activity in the presence of elevated

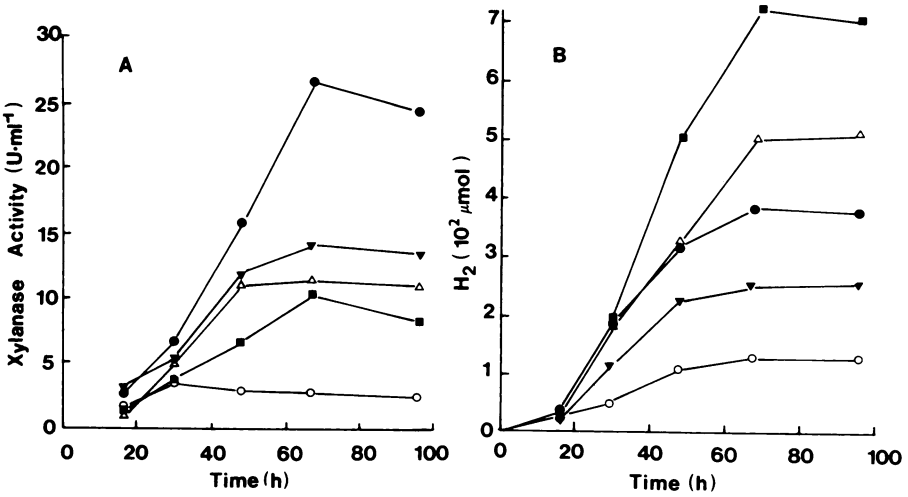

FIG. 3. Time course for xylanase (A) and hydrogen (B) production by *N. frontalis* grown on various concentrations of xylan. Symbols: ○, 0.6; ▼, 1.25; ●, 2.5; △, 5.0; ■, 10 mg of xylan ml^{-1}. Xylanase activity was measured in the culture supernatant, and values are means of duplicate determinations.

xylose or arabinose concentrations could not have been due to inhibition by these sugars because their addition to actively xylanolytic supernatants from cultures grown on xylan only, at the same concentrations as added in the growth experiments (Fig. 5 and 6), failed to alter enzyme activity. Likewise, dialysis of xylanase preparations (obtained from cultures treated with pentose) against 50 mM sodium acetate (pH 6.0) for 24 h failed to produce any difference in activity from the undialyzed preparations.

Comparison of effects of various carbohydrates on xylanase production. The concentration of xylan in the culture medium which gave the highest xylanase production was 2.5 mg ml^{-1}. This concentration was therefore selected to determine the effects of other growth substrates on xylanase production. From Table 2 it is clear that xylose, glucose, and cellobiose were less effective than xylan as substrates for the production of xylanase. The activity from xylose was almost twice that obtaned from cellobiose or glucose. Xylanase production was not determined with cellulose as a growth substrate.

Little difference in xylanase activity was obtained after growth of *N. frontalis* on various xylan preparations, the exception being barley straw leaf, with which a significant increase in enzyme production occurred (Table 3). Similarly, the use of different xylan types in the assay system resulted in only small differences in activity.

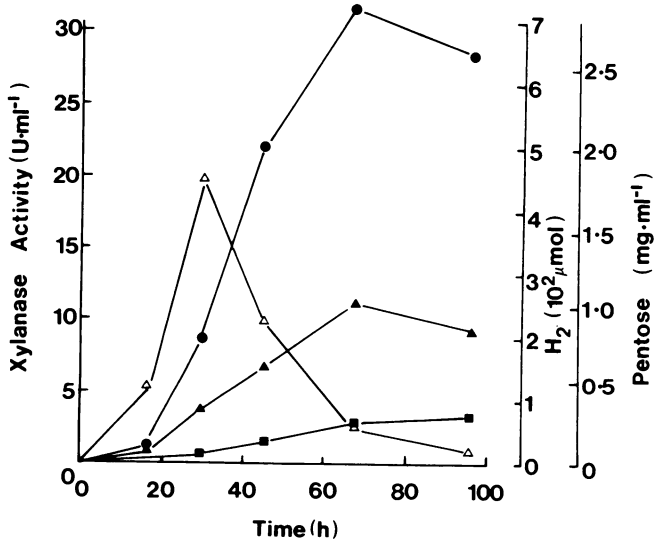

FIG. 4. Time course for xylanase production during growth of *N. frontalis* on xylan at 10 mg ml^{-1}. Symbols: ▲, xylanase; ●, hydrogen; △, xylose; ■, arabinose.

DISCUSSION

N. frontalis released xylanase mainly into the culture fluid, and only a small proportion of the enzyme was found to be associated with the fungal rhizoid (10%). This value increased somewhat after solubilization of rhizoid by treatment with TET or by sonication. Our results on the distribution of xylanase differed from those described by Lowe et al. (14) which showed that a substantial proportion of xylanase was associated with the rhizoid of an anaerobic fungus similar to *N. frontalis*. They also differed from those reported for ruminal bacteria which indicated that xylanase was mainly cell associated (9, 21, 22). The mode of xylanase release by *N. frontalis* appeared to be similar to that of endo-acting polysaccharidases previously reported for this organism (17, 18).

Xylanase action was endo hydrolytic, as confirmed by the presence of mainly xylobiose and lesser quantities of xylo-oligosaccharides as products. No xylose was detected, indicating the absence of xylobiase activity. The effect of the presence of low concentrations of xylan in stabilizing xylanase at 50°C indicated protection against moderately elevated temperatures through interaction with substrate, although nonspecific effects could not be ruled out. To our knowledge, there have been no previous reports of the stabilizing effect of xylan on xylanases, and it may be important to further study this effect on other xylanases, particularly if there is an interest in the commercial application of these enzymes.

Xylanase activity was highest when *N. frontalis* grew on xylan at 2.5 mg ml^{-1} in the culture medium. The activity of 27 U ml of culture^{-1} approached that obtained by Tan et al.

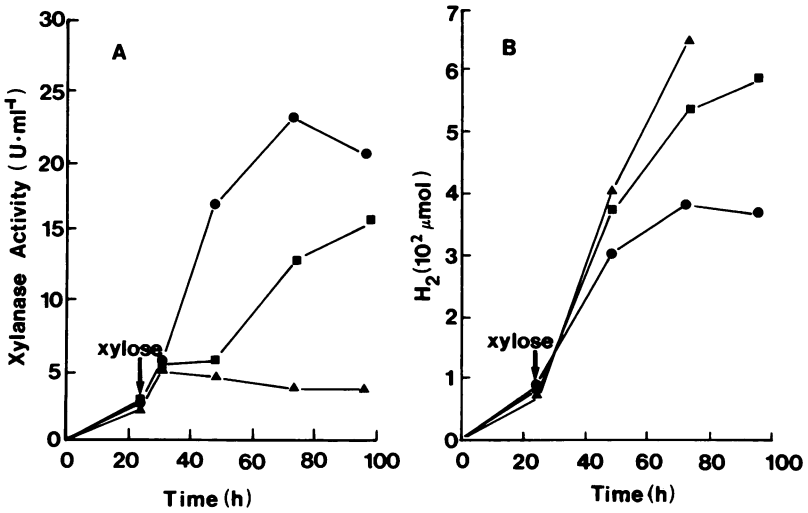

FIG. 5. Effect of xylose addition (arrow) on xylanase production (A) and hydrogen production (B) by *N. frontalis* growing on 2.5 mg of xylan ml^{-1}. Symbols: ●, 0; ■, 3.0; ▲, 4.5 mg of xylose ml^{-1}. Xylanase activity was measured in the culture supernatant, and values are means of duplicate determinations. The concentration of L-arabinose and xylose in the culture fluid immediately prior to addition was <0.1 mg ml^{-1}.

(25) for the highly active xylanase released in cultures of *Trichoderma harzianum* but was somewhat lower than that reported by Yu et al. (28) for xylanase in filtrates from cultures of the thermophilic fungus *Thermoascus aurantiacus*.

At elevated xylan concentrations, xylanase production decreased and this was accompanied by an accumulation of xylose and, to a lesser extent, arabinose in the culture medium. Since xylobiase activity was absent in the culture fluid, these sugars must have been produced by a cell-associated enzyme. No accumulation of xylose or arabinose occurred in cultures which were actively xylanolytic; thus, a regulatory role for these sugars on xylanase production was considered. The likely regulatory role of these sugars on xylanase production was evident from pentose addition experiments and from the paired-substrate growth studies. Low levels of xylanase associated with the fungal rhizoid in the pentose-treated cultures ruled out the possibility that the decreased activities found in the culture fluid could be due to a redistribution of enzyme. Inhibition of xylanase by pentose was also ruled out because addition of the sugars to active xylanase preparations failed to alter enzyme activity. It therefore appears that xylose and arabinose are involved in the regulation of xylanase by a mechanism other than inhibition, and further studies will be required to elucidate this. We have already reported the regulation of α-amylase (18) and carboxymethylcellulase (17) production by glucose in *N. frontalis*, and it appears that these mechanisms together with the pentose regulatory system function to facilitate the preferential utilization of simple sugars over polysaccharides, providing the fungi with a competitive advantage in situations in which there is an abundance of soluble sugars. We point out, however, that in the rumen this situation is rare and the levels of soluble sugars are usually

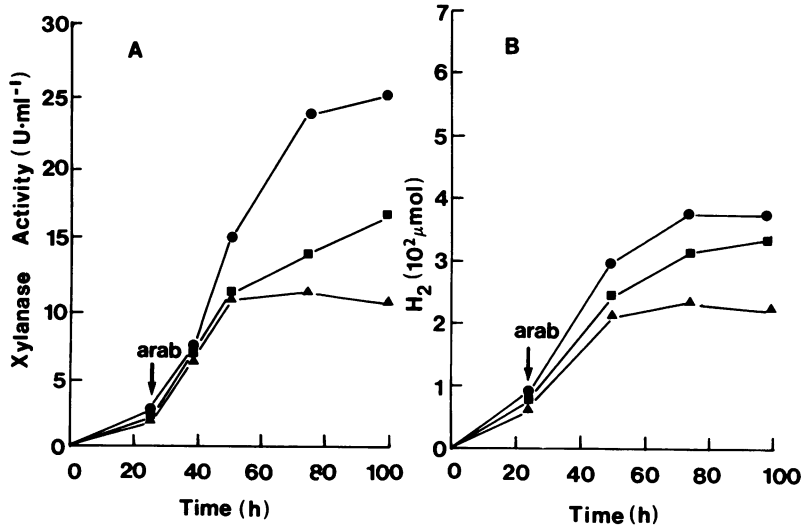

FIG. 6. Effect of L-arabinose addition (arrow) on xylanase production (A) and hydrogen production (B) by *N. frontalis* growing on 2.5 mg of xylan ml^{-1}. Symbols: ●, 0; ■, 3.0; ▲, 4.5 mg of L-arabinose ml^{-1}. Xylanase activity was measured in the culture supernatant, and values are means of duplicate determinations. The concentration of L-arabinose and xylose in the culture fluid immediately prior to addition was <0.1 mg ml^{-1}.

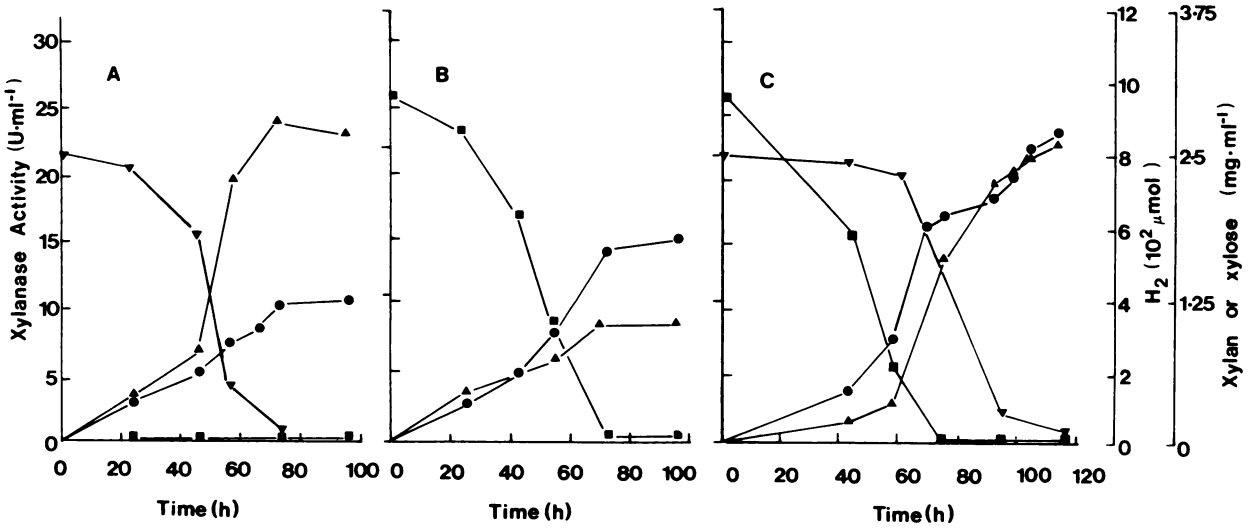

FIG. 7. Time courses for xylanase production by *N. frontalis* during growth on xylan at 2.5 mg ml^{-1} (A), xylose at 3 mg ml^{-1} (B), and a mixture of xylan and xylose at 2.5 and 3 mg ml^{-1}, respectively (C). Cultures were inoculated with xylan-grown organisms and were incubated as described in Materials and Methods. Symbols: ▲, xylanase activity; ●, hydrogen; ■, xylose; ▼, xylan.

very low, even shortly after feeding (8), reflecting their rapid utilization owing to intense competition for these substrates by ruminal microflora.

Xylan was found to be the most effective of all the substrates tested for the production of xylanase. Less effective as growth substrates were xylose, glucose, and cellobiose, and the relative activities were similar to those obtained by Lowe et al. (14) for an unnamed anaerobic fungus growing on the same substrates. The finding here that at least some activity was present after growth on cellobiose suggests that xylanase is partly constitutive. That the enzyme is also inducible is evident from the considerably elevated activities obtained after growth on xylan.

Different xylan growth substrates did not produce much variation in xylanase activity, and it is probable that the xylanase produced after growth on these substrates was the same enzyme. It was of interest that relatively high levels of xylanase were produced after growth on barley straw leaf, and further studies will be required to determine whether the characteristics of this enzyme are the same as for xylanase obtained after growth on xylan.

Xylanase is the most active of all the endo-acting polysaccharidases so far studied in *N. frontalis*, and its production at levels comparable to those obtained with organisms which actively produce the enzyme (25, 28) could be of significance in ruminal digestion. Ruminant feed often contains a substantial proportion of hemicellulose and with some herbage it represents the major component of the dry weight (2). Because of its ability to release substantial quantities of xylanase, it would appear that *N. frontalis* is very well suited to degrading such a substrate. Such a feature may assist in the penetrative mode of fungal growth in plant tissues and would be distinct from the surface-associated xylanolytic activity of most ruminal bacteria in which immediate juxtaposition of the organism to the substrate would be required to facilitate digestion (7). Both modes of attack have presumably developed to allow either type of organism effective participation in ruminal plant fiber degradation.

The xylanase of *N. frontalis* represents one of the few xylanolytic enzymes described for a ruminal fungus. Because of its stability at moderately elevated temperatures, its elevated production from crude hemicellulose, and its easy accessibility (it is not cell associated), there seems some justification for considering its possible application in industry, such as in the improvement of fermentability of residual hemicellulosic material. We are currently evaluating the suitability of the fungal enzyme in hemicellulose bioconversion.

TABLE 2. Comparison of various carbohydrates in the production of xylanase

Growth substrate[a]	Growth[b] (µmol of H_2, 10^2)	Xylanase (U ml^{-1})[c]
Xylan	4.1 (3)	27.0
Xylose	3.9 (3)	9.8
Glucose	7.2 (3)	6.7
Cellobiose	5.2 (5)	5.8

[a] The initial concentration of substrate in growth media was 2.5 mg ml^{-1}.
[b] Values in parentheses refer to the number of days required to reach maximum growth.
[c] Values are for supernatants from 60-ml cultures at maximum growth and are means of duplicate determinations.

TABLE 3. Comparison of various xylan substrates for the production of xylanase

Growth substrate[a]	Activity (U ml^{-1})[b] with the following assay substrate[c]:			Growth (µmol of H_2, 10^2)[d]
	Xylan (type I)	Xylan (type II)	Xylan (type III)	
Xylan (type I)	25.0	25.0	21.4	3.7 (3)
Xylan (type II)	25.8	23.6	24.3	3.8 (3)
Xylan (type III)	30.6	27.0	26.2	3.6 (3)
Barley straw leaf	38.2			4.2 (3)

[a] The initial concentration of substrate in growth media was 2.5 mg ml^{-1}. Designations for xylan type are: type I, oat-spelt xylan; type II, birch-wood xylan; type III, larch-wood xylan.
[b] Values are for supernatants from 60-ml cultures at maximum growth and are means of duplicate determinations.
[c] Xylanase was assayed under standard conditions with different assay substrates. The designation for xylan type is as described in footnote *a*.
[d] Values in parentheses refer to the number of days required to reach maximum growth.

ACKNOWLEDGMENT

We thank David White for technical assistance.

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