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Fermentation of Xylans by *Butyrivibrio fibrisolvens* and Other Ruminal Bacteria

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The ability of *Butyrivibrio fibrisolvens* and other ruminal bacteria (6 species, 18 strains) to ferment a crude xylan from wheat straw or to ferment xylans from larchwood or oat spelts was studied. Liquid cultures were monitored for carbohydrate utilization, cell growth (protein), and fermentation acid production. *B. fibrisolvens* 49, H17c, AcTF2, and D1 grew almost as well on one or more of the xylans as they did on cellobiose-maltose. *B. fibrisolvens* 12, R28, A38, X10C34, ARD22a, and X6C61 exhibited moderate growth on xylans. Partial fermentation of xylans was observed with *Bacteroides ruminicola* B14, *Bacteroides succinogenes* S85, *Ruminococcus albus* 7, *Ruminococcus flavefaciens* C94 and FD1, and *Succinivibrio dextrinosolvens* 22B. All xylans tested appeared to have a small fraction of carbohydrate that supported low levels of growth of nonxylanolytic strains such as *Selenomonas ruminantium* HD4. Compared to growth on hexoses, the same array of fermentation acids was produced upon growth on xylans for most strains; however, reduced lactate levels were observed for *B. fibrisolvens* 49 and *Selenomonas ruminantium* HD4. Measurements of enzyme activities of *B. fibrisolvens* AcTF2, 49, H17c, and D1 indicated that the xylobiase activities were cell associated and that the xylanase activities were predominantly associated with the culture fluid. The pattern of expression of these enzymes varied both between strains and between the carbon sources on which the strains were grown.

The major polysaccharides found in plant materials include cellulose, hemicellulose, pectin, and starch. After cellulose, hemicellulose is the most abundant polysaccharide in forages, accounting for 20 to 40% of the total carbohydrate fraction. Unlike cellulose, hemicellulose does not have a homogeneous chemical composition; the predominant polymer (50% or more) is a pentosan or xylan composed of xylose with arabinose side chains. Depending on the given forage, smaller amounts of glucose, galactose, rhamnose, or glucuronic acid may be present (9). Overall, xylans represent a major energy source for microbial fermentation within ruminants and other forage-degrading animals.

It was shown over 30 years ago that suspensions of mixed ruminal microorganisms are capable of degrading xylans to xylose, arabinose, xylobiose, xylotriose, xylotetraose, xylopentose, and a series of higher oligosaccharides (14). By using media containing xylan as the only added carbohydrate source, active xylan-fermenting bacterial strains were isolated (13) which conformed to the description of *Butyrivibrio fibrisolvens* (4). Extensive studies by Dehority and coworkers (5–10) and by Williams and Withers (22) established that a number of other ruminal bacteria, including *Bacteroides ruminicola*, *Bacteroides succinogenes*, *Ruminococcus flavefaciens*, and *Ruminococcus albus*, were capable of extensively hydrolyzing and/or fermenting a wide variety of xylans. However, these workers did not establish the nature of the fermentation products made during growth of these bacterial species on xylans.

The extent to which forage materials are digested in the rumen varies considerably with the type of carbohydrate fraction. Starches and pectins undergo extensive digestion, whereas the fiber fractions (cellulose and xylan) are digested only to the extent of 40 to 60% in most ruminants. Numerous treatments of forages prior to ingestion have been developed to increase the extent of forage digestion in the animal, but many of these treatments have been only partially successful or too expensive to use or both. Recently, a technique for treating straws with hydrogen peroxide at pH 11.5 has been developed which effectively separates the straw into a particulate fraction, containing mostly cellulose with some xylan and lignin components, and a liquid fraction, containing mostly solubilized xylan and some lignin components (11). The particulate, cellulosic fraction was found to be highly digested by ruminants (16). On the other hand, it is not known whether the xylan-rich liquid fraction can be fermented by xylan-degrading ruminal bacteria.

The first objective of the current study was to compare the abilities of various ruminal bacteria to degrade xylans from larchwood, oat spelts, and peroxide-treated wheat straw under similar growth conditions. The second objective was to determine the cell growth yields and fermentation products made relative to growth on soluble hexoses.

MATERIALS AND METHODS

Organisms and growth conditions. All bacterial strains used were characterized in previous publications. These strains were from the author's (R.B.H.) stock culture collection or were obtained from M. P. Bryant, B. A. Dehority, N. O. VanGylswyk, or R. A. Mackie. For comparative purposes, all organisms were grown under anaerobic conditions at 37°C on RGM medium (Table 1) containing cellobiose-maltose or the appropriate xylan source. Larchwood and oat spelt xylans (LX and OX, respectively) were purchased commercially (Sigma Chemical Co., St. Louis, Mo.) and were determined, by neutral-sugar analysis, to be composed mostly (85% [wt/wt]) of xylose-arabinose. Treatment of wheat straw with alkaline hydrogen peroxide (11) yielded a liquid xylan fraction (APSH) containing 5.0% (wt/wt) carbohydrate (50% xylose, 35% arabinose, 15% glucose), 1.7% (wt/wt) UV-light-absorbing (260 nm) materials (lignins), and 4.9% (wt/wt) ash. After it was autoclaved, this

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TABLE 1. Composition of RGM medium

Component(s)	Amt/100 ml
Carbohydrate solution (5% [wt/vol])[a,b]	5.0 ml
Trypticase[c]	0.3 g
Yeast extract	0.2 g
Mineral solution no. 1[d]	5.0 ml
Mineral solution no. 2[d]	5.0 ml
Trace mineral solution (R-1 salts)[e]	0.1 ml
Short-chain volatile fatty acids[f]	3.0 ml
Resazurin (0.1% [wt/vol])	0.1 ml
L-Cysteine hydrochloride–sodium sulfide · $9H_20$ (2.5% [wt/vol] each)[b]	1.0 ml
Sodium carbonate (8% [wt/vol])	2.0 ml
Hemin-naphthoquinone (0.01% [wt/vol] each)	1.0 ml
Distilled water	78.0 ml

[a] LX, OX, APSH, or 2.5% each of cellobiose and maltose.
[b] Autoclaved separately and added to cooled medium. Final pH of medium was 6.8 under a 20:80 (vol/vol) carbon dioxide-nitrogen atmosphere.
[c] BBL Microbiology Systems, Cockeysville, Md.
[d] From data of Bryant and Burkey (3).
[e] From data of Hespell and Canale-Parola (12).
[f] Prepared by mixing 6.85 ml of acetic acid, 3.0 ml of propionic acid, 1.85 ml of butyric acid, 0.55 ml of 2-methylbutyric acid, 0.5 ml of isobutyric acid, 0.55 ml of valeric acid, and 700 ml of 0.2 M NaOH. The solution was adjusted to pH 7.5 with NaOH and to a final volume of 1 liter.

APSH fraction was added directly to the media as a carbohydrate source.

Growth in cultures was monitored by measurement of the optical density at 660 nm (Spectronic 70; Bausch and Lomb, Inc., Rochester, N.Y.). However, these measurements were not reliable with xylan-grown cultures until after the xylans were solubilized. Thus, final cell concentrations were determined by measurement of cell protein by the method of Lowry et al. (17) after hydrolysis of the cells (0.1 M NaOH, 70°C, 30 min). Cytochrome *c* (horse heart; Sigma) was used as the protein standard.

Carbohydrate assays. Hexose concentrations were determined by the phenol-sulfuric acid method (2), with glucose as the standard. Total xylan concentrations were determined as xylose equivalents by using the orcinol assay (21). Soluble and insoluble xylans were differentiated by acid ethanol precipitation methods as described by Dehority (7). Neutral-sugar compositions of LX and OX were determined as alditol acetate sugar derivatives (1).

Fermentation products. Fermentation acids were quantitated by gas-liquid chromatography of the free acids (18) or of the butyl esters (20). Hydrogen and other fermentation gases were measured by gas chromatography (18).

Xylanase and xylobiase assays. Xylanase and xylobiase activities present in cells, culture fluids, or total cultures were measured routinely with cultures grown to mid- or late exponential phase. Cell suspensions were prepared anaerobically from cultures (18) and suspended in 10 mM phosphate–10 mM dithiothreitol buffer. This buffer and all components of the xylanase assay were made with oxygen-free distilled water and stored under nitrogen gas. The assay mixture contained 0.3 ml of water, 0.1 ml of 10 mM dithiothreitol, 0.1 ml of 0.1 M potassium phosphate (pH 6.8) buffer, and 0.4 ml of 5% (wt/vol) LX in 25 mM potassium phosphate buffer (pH 6.8). The assay was initiated by adding 0.1 ml of enzyme source and incubating the mixture at 37°C under a nitrogen atmosphere. After 60 min, the reaction was terminated by adding 3 ml of ice-cold acid alcohol (ethanol-glacial acetic acid; 95:5, vol/vol) and cooling the mixture on ice for 30 min. The mixture was centrifuged (15,000 × *g*, 20 min, 4°C), and the supernatant fluid was analyzed for xylose equivalents by using the orcinol assay (21). This assay most likely underestimates true xylanase activity as measured by the increase in the number of sugar polymers having a reducing sugar at their termini. Because of the reducing agents in the medium, this procedure could not be used.

Xylobiase activities were measured with *para*-nitrophenyl-β-D-xylopyranoside (NPX) as the substrate under assay conditions similar to those used for measurement of xylanase activities. The assay mixture contained 0.05 ml of 5 mM NPX, 0.1 ml of 10 mM dithiothreitol, and 0.25 ml of 50 mM potassium phosphate (pH 6.8). The reaction was initiated by adding 0.1 ml of enzyme source. After 60 min at 37°C, 0.25 ml of 2% (wt/vol) sodium carbonate was added, and the A_{405} was determined spectrophotometrically. *para*-Nitrophenol was used as the standard, with a molar extinction coefficient of 18,800 for basic solutions.

For both of the assays described above, controls were run to ensure that the reaction rates were linear with time and the amount of enzyme source used. Where necessary, corrections were made for nonspecific absorbances by background materials in the culture fluid.

The data presented in all of the tables are the averages of duplicate samples from two replicate cultures.

RESULTS

Xylan degradation and cell yields. All ruminal bacteria tested were able to grow rapidly to high cell densities on RGM medium containing a mixture of maltose and cellobiose as the energy sources (Table 2). When one of the xylans was used as the energy source, the amount of xylan utilized varied with both the xylan type and the bacterial strain. Only small amounts of carbohydrate disappeared upon growth of *Bacteroides ruminicola* and *Bacteroides succinogenes*, and in addition the cell yields were quite low (Table 3). Only

TABLE 2. Carbohydrate utilization by ruminal bacteria upon fermentation of xylans or cellobiose-maltose[a]

Species and strain(s)	Amt of carbohydrate utilized[b]			
	CM	APSH	LX	OX
Bacteroides ruminicola B14	7.4	2.9	2.7	3.3
Bacteroides succinogenes S85	6.1	3.0	3.9	2.0
Butyrivibrio fibrisolvens				
AcTF2	9.3	13.9	6.8	5.9
D1	8.9	8.8	8.0	3.8
49	8.1	13.4	8.3	6.3
H17c	8.6	4.4	5.2	7.2
12	3.1	2.9	0.5	2.3
R28	4.6	3.2	1.1	1.9
A38	9.2	0.7	8.8	3.9
X10C34	9.6	ND	7.0	6.3
ARD22a	9.0	2.1	4.1	0.2
X6C61	8.8	ND	5.1	2.7
Butyrivibrio-like sp. strain B385-1	8.4	1.0	6.1	2.2
Ruminococcus albus 7	6.1	4.8	0.8	1.4
Ruminococcus flavefaciens				
C94	5.8	3.2	3.8	5.4
FD1	3.1	0.4	0.7	0.2
Succinivibrio dextrinosolvens 22B	6.2	5.1	3.4	1.6
Selenomonas ruminantium HD4	9.3	1.9	0.9	2.2

[a] The inocula (5% [vol/vol]) were from cultures grown with the same carbohydrate source.
[b] Values are expressed as micromoles of hexose or pentose used per milliliter after 18 h of growth in RGM medium. Initial values for cellobiose-maltose (CM), APSH, LX, and OX were 10.1, 17.5, 11.2, and 9.9, respectively, with average amounts used being 7.3, 4.5, 4.3, and 3.3, respectively. ND, No utilization detected.

TABLE 3. Cell protein yields of ruminal bacteria upon fermentation of xylans or cellobiose-maltose

Species and strain(s)	Cell protein yield from[a]:			
	CM	APSH	LX	OX
Bacteroides ruminicola B14	26.2	ND	ND	14.5
Bacteroides succinogenes S85	6.1	3.1	1.4	3.6
Butyrivibrio fibrisolvens				
AcTF2	12.2	7.7	12.9	13.3
D1	15.6	7.8	10.3	11.7
49	11.6	8.8	8.2	14.6
H17c	19.6	13.1	6.9	6.0
12	11.9	13.1	4.7	18.9
R28	13.3	20.7	19.8	24.0
A38	17.5	29.2	15.5	22.7
X10C34	16.1	tr	11.8	9.5
ARD22a	13.5	3.1	2.8	tr
X6C61	7.5	ND	5.7	15.9
Butyrivibrio-like sp. strain B385-1	9.1	8.1	10.8	13.0
Ruminococcus albus 7	20.1	4.1	14.9	13.5
Ruminococcus flavefaciens				
C94	7.0	ND	ND	1.1
FD1	7.5	ND	13.3	5.4
Succinivibrio dextrinosolvens 22B	9.1	1.8	3.9	8.6
Selenomonas ruminantium HD4	7.5	7.2	4.7	6.4

[a] Values are expressed as micrograms of cell protein formed per micromole of hexose or pentose fermented after 18 h of growth in RGM medium with the appropriate substrate added. Abbreviations: CM, cellobiose-maltose; ND, no cell protein detected (less than 0.1 μg); tr, trace amounts of cell protein detected (0.1 to 0.3 μg).

marginal amounts of carbohydrate disappeared upon growth of *Selenomonas ruminantium*, a sugar-fermenting, non-xylanolytic bacterial species, but higher cell yields were found with this organism. Almost all strains of *B. fibrisolvens* were able to grow fairly well on at least one of the xylans tested. In particular, strains AcTF2, D1, 49, and H17c showed very good degradation of all three xylans. The cell yields were also high but were not as great as those seen on maltose-cellobiose (Table 3). *Ruminococcus albus* 7 could degrade any one of the xylans to a small extent, and the cell yields were low. *Ruminococcus flavefaciens* C94 and FD1 appeared to ferment the xylans to some extent, but little or no growth occurred. Similar results were seen with *Succinivibrio dextrinosolvens*. Little or no growth on any of the xylans was observed with *B. fibrisolvens* S2, *Bacteroides ruminicola* 23, *Selenomonas ruminantium* D, and *Streptococcus bovis* JB1.

Fermentation products. Although the degradation of xylans by numerous ruminal bacteria has been observed, information on the type of fermentation products made has not been reported. In terms of hydrogen gas formation, those strains that produced substantial amounts during growth on hexoses also produced substantial amounts during growth on the pentoses from xylan degradation (Table 4). However, there was considerable variation between xylans for any given strain, although growth on APSH generally resulted in the least hydrogen gas production. With *Ruminococcus albus*, virtually no hydrogen gas was produced upon growth on any xylan.

The types of fermentation acids produced during growth on cellobiose-maltose and on the xylans were determined for several species (Table 5). As might be expected, butyrate was a major acid produced by *B. fibrisolvens* strains, and, generally, lesser amounts were produced by xylan-fermenting cultures as compared with cellobiose-maltose-fermenting cultures. With strain 49, lactate production was also reduced, and no lactate formation was detected with the low levels of growth of *Selenomonas ruminantium* on the xylans. *Ruminococcus flavefaciens* produced little succinate when grown on xylans, but the carbon recoveries were quite low.

Enzyme activities. The degradation and fermentation of xylans requires the concerted action of a number of enzyme activities, including xylanases and xylobiases. The activities of these two enzymes in several *B. fibrisolvens* strains were determined by measurement of the release of reducing sugars from xylan and of the hydrolysis of NPX, respectively. With strains AcTF2, 49, H17c, and D1, the xylanase activities were predominantly associated with the culture fluid, whereas the xylobiase activities were cell associated (Table 6). When grown on glucose or cellobiose-maltose, strain AcTF2 produced low levels of xylanase; xylanase levels increased about 60-fold when this strain was grown on LX. In contrast, this strain produced xylobiase activity under all three growth conditions. Strains 49 and H17c behaved similarly in that xylanase was produced at high levels by glucose- or cellobiose-maltose-grown cells and increased only twofold upon growth on LX. However, in contrast to strain AcTF2, these strains produced high levels of xylobiase only when grown on LX. A different pattern was observed with strain D1 in that both xylanase and xylobiase were always produced, but these activities did increase three- to fivefold with growth of strain D1 on LX as compared with growth on the sugars.

DISCUSSION

This study, in conjunction with previous studies (5, 6, 9, 10), has shown that a variety of the major ruminal bacteria have some ability to degrade xylans. The APSH fraction was fermented reasonably well by a number of species, suggesting that it would be fermented in the rumen if included in the feed. Regardless of whether the strains were initially isolated on xylan-containing media, almost all *B. fibrisolvens* strains were capable of extensive degradation of one or more of the

TABLE 4. Hydrogen gas production by ruminal bacteria upon fermentation of xylans or cellobiose-maltose

Species and strain(s)	H_2 production from[a]:			
	CM	APSH	LX	OX
Bacteroides ruminicola B14	1,098	378	1,487	388
Bacteroides succinogenes S85	79	ND	ND	tr
Butyrivibrio fibrisolvens				
AcTF2	146	191	245	334
D1	76	74	181	151
49	779	435	1,027	776
H17c	216	166	188	401
12	1,442	1,178	ND	1,409
R28	366	281	268	444
A38	571	107	276	56
X10C34	35	ND	tr	363
ARD22a	147	131	463	250
X6C61	1,196	ND	844	1,368
Butyrivibrio-like sp. strain B385-1	967	950	658	575
Ruminococcus albus 7	1,856	ND	tr	tr
Ruminococcus flavefaciens				
C94	85	tr	tr	tr
FD1	105	900	ND	ND
Succinivibrio dextrinosolvens 22B	ND	ND	tr	tr
Selenomonas ruminantium HD4	ND	ND	88	120

[a] Values are expressed as nanomoles of hydrogen gas per micromole of hexose or pentose fermented after 18 h of growth in RGM medium. Abbreviations: CM, cellobiose-maltose; ND, none detected (less than 1 nmole); tr, trace amounts detected (1 to 10 nmol).

TABLE 5. Fermentation acids produced by ruminal bacteria upon growth on xylans or cellobiose-maltose

Species and strain(s)	Growth substrate	Amt of fermentation acid produced[a]						
		F	A	P	L	B	S	V
Butyrivibrio fibrisolvens								
AcTF2	CM[b]	2.24	0.51	0.00	0.00	0.96	0.00	0.05
	APSH	0.66	0.55	0.00	0.00	0.53	0.00	0.01
	LX	1.10	0.53	0.00	0.00	0.48	0.00	0.04
	OX	1.12	0.80	0.00	0.00	0.54	0.00	0.02
49	CM	0.54	0.00	0.04	1.09	1.07	0.00	0.01
	APSH	0.23	0.00	0.01	1.61	0.67	0.00	0.01
	LX	0.40	0.00	0.02	0.09	1.00	0.00	0.00
	OX	0.50	0.00	0.00	0.24	1.07	0.00	0.00
D1	CM	2.36	0.51	0.02	0.00	1.05	0.00	0.00
	APSH	0.85	1.06	0.03	0.00	0.47	0.00	0.00
	LX	1.14	0.59	0.00	0.00	0.53	0.00	0.00
	OX	0.96	0.58	0.00	0.00	0.48	0.00	0.00
Ruminococcus flavefaciens C94	CM	0.10	0.39	0.00	0.00	0.00	0.89	0.00
	APSH	0.31	0.60	0.14	0.00	0.00	0.03	0.05
	LX	0.00	0.00	0.00	0.00	0.00	0.08	0.02
	OX	1.36	0.76	0.00	0.00	0.00	0.03	0.00
Succinivibrio dextrinosolvens 22B	CM	0.32	0.72	0.00	0.00	0.00	0.91	0.00
	APSH	0.00	0.20	0.00	0.00	0.00	0.19	0.00
	LX	0.38	0.66	0.00	0.00	0.00	0.71	0.00
	OX	0.19	0.70	0.01	0.00	0.00	0.74	0.00
Selenomonas ruminantium HD4	CM	0.00	0.31	0.32	1.10	0.00	0.05	0.00
	APSH	0.00	0.72	1.16	0.00	0.00	0.30	0.00
	LX	0.00	2.71	2.96	0.00	0.00	0.00	0.08
	OX	0.00	0.44	0.85	0.00	0.00	0.05	0.12

[a] Values are expressed as micromoles of acid formed per micromole of hexose or pentose fermented and are corrected for 0 h values. F, A, P, L, B, S, and V represent formate, acetate, propionate, lactate, butyrate, succinate, and valerate, respectively.
[b] CM, Cellobiose-maltose.

xylans APSH, LX, and OX (Table 2). In most instances, these strains displayed efficiencies of growth on xylans that approached those observed upon growth on cellobiose-maltose, and with strains R28 and A38 the efficiencies were higher (Table 3). These results are in general agreement with those of previous studies (4, 9, 10) which conclude that *B. fibrisolvens* is the most important ruminal bacterium involved in the digestion of xylans (hemicelluloses). In contrast, sugar-fermenting species such as *Selenomonas ruminantium* grew poorly on the xylans examined. However, the fact that some growth occurred suggests that there may be breakdown products, such as xylooligosaccharides, present in the xylan preparations initially or after autoclaving or at both times. These breakdown products might be used for growth by nonxylanolytic species. This possibility is similar to the findings of Russell (19), who has shown that a number of noncellulolytic species, including *Selenomonas ruminantium*, *Streptococcus bovis*, and *Bacteroides ruminicola*, are capable of growing on and degrading cellulooligosaccharides.

TABLE 6. Xylanase and xylobiase activities of *B. fibrisolvens* strains

Growth substrate	Strain	Xylanase activity in[a]:			Xylobiase activity in[b]:		
		Total culture	Cells	Culture fluid	Total culture	Cells	Culture fluid
Glucose	AcTF2	0.09	0.01	0.00	73.70	13.80	0.20
	49	96.60	0.21	14.86	1.90	0.30	0.01
	H17c	70.19	0.13	9.77	1.40	0.20	0.01
	D1	7.76	0.03	1.64	27.80	5.90	0.06
Cellobiose-maltose	AcTF2	0.08	0.01	0.00	49.30	10.30	0.30
	49	50.55	0.07	7.36	0.60	0.20	0.01
	H17c	33.99	0.11	5.16	0.90	0.10	0.01
	D1	12.37	0.04	2.22	28.30	5.00	0.17
LX	AcTF2	4.43	0.21	0.36	37.10	4.60	0.20
	49	199.12	1.82	12.71	89.10	6.70	0.05
	H17c	244.89	3.47	19.55	66.50	7.60	0.05
	D1	42.16	1.63	3.56	233.20	28.30	0.39

[a] Expressed as nanomoles of pentose released per microgram of cell protein per hour (total culture) or micromoles of pentose released per milliliter of culture per hour (cells or culture fluid).
[b] Expressed as nanomoles of nitrophenyl formed per microgram of cell protein per hour (total culture) or micromoles of nitrophenyl formed per milliliter of culture per hour (cells or culture fluid).

Bacteroides succinogenes, *Ruminococcus albus*, and *Ruminococcus flavefaciens* are cellulolytic species but were found to grow to only a small extent on the xylans and with rather low growth efficiencies (Tables 2 and 3). Dehority (6) has shown that cellulolytic ruminal bacteria often can degrade hemicelluloses extensively but that only a few strains are capable of growing on these substrates. Compared with *Butyrivibrio* or *Bacteroides* species, rates of hemicellulose degradation by *Ruminococcus* species are often slower, and soluble oligosaccharides accumulate in the growth media because the rates at which these compounds are used are slow (7, 8). Thus, the maximum extent to which these species could degrade any one xylan was probably underestimated in our studies (Table 2) owing to the 18-h incubation

times used. However, all of the species described above which exist in the natural environment probably act synergistically to degrade the polysaccharides in plant cells on the basis of work with cultures of mixed pure species (9).

The fermentation of hemicelluloses or xylans involves mostly cellular metabolism of pentoses, mainly xylose and arabinose. A comparison of the fermentation products made from xylans with those made from the hexoses, cellobiose and maltose, shows no major changes with the highly xylanolytic strains of *B. fibrisolvens* (Tables 4 and 5). Although the xylans used were predominantly composed of pentoses, hexoses in the form of uronic acids and glucose were also present. Because of this substrate heterogeneity, carbon recoveries and oxidation/reduction balances could not be accurately determined. However, the data suggest that the hexose monophosphate pathway for pentose metabolism predominated, as one would expect considerably more acetate formation and less hydrogen gas production if the phosphoketolase route were the major pathway. With the weakly xylanolytic species, there were shifts towards lower rates of succinate formation by *Ruminococcus flavefaciens* and *Succinivibrio dextrinosolvens* and lower rates of lactate formation by *Selenomonas ruminantium* (Table 5). Whether these changes are related to inhibitory substances in the xylans or to changes in growth rates or to both is not known.

An examination of xylanase and xylobiase activities in selected *B. fibrisolvens* strains showed the former activity to be mostly extracellular and the latter activity to be cell associated. Early studies by Howard et al. (15) with a *Butyrivibrio* species indicated that both of these enzyme activities were expressed when cells were grown on xylan or wheat flour pentosan but not glucose, arabinose, or xylose. This pattern is similar to that observed with *B. fibrisolvens* D1, but it appears that the regulation is not strictly one of inducibility because the enzyme activities only increased about fivefold upon growth on xylan (Table 6). On the other hand, with strain AcTF2 the xylanase activity clearly is induced by growth on xylan, but the xylobiase activity appears to be constitutively produced. An enzyme pattern inverse to that of strain AcTF2 was observed with strains 49 and H17c. Preliminary experiments with crude xylanase preparations from strain H17c indicate that xylobiose through at least xylohexaose can be formed via LX degradation. These xylooligosaccharides may be involved in the induction of the xylobiase activity as was found with the *Butyrivibrio* strain studied by Howard et al. (15).

The results of the current study indicate that strains of *B. fibrisolvens* are almost uniformly highly xylanolytic. Studies in progress in our laboratories are currently focusing on delineation of the types and numbers of enzymes involved in xylan degradation by this organism as well as on the physiological and genetic regulation of these enzymes. These studies should provide information towards understanding the basis for the rather low digestibility of xylans and hemicelluloses in the rumen and possible genetic modification of *B. fibrisolvens* or other ruminal species for increased xylan degradation in the rumen or for fermentation of agricultural biomass wastes by these species.

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