hexalactone, (A + B) remains lower than 50% but (A + C) is above 90%.

For medium and highly polar compounds, losses are negligeable, which agrees with the results of Rapp and Knipser (1980). For less polar compounds such as terpene hydrocarbons, not studied by these authors, losses can be up to 20%.

The reproducibility of the method is good. Coefficients of variation are below 15%. In comparison with the results of Williams et al. (1978) for synthetic mixtures, it is better than with a purging technique but worse than with piston displacement.

By this method ethyl alcohol is not extracted and other alcohols are weakly extracted. Therefore, this method is of great interest to study the aroma of fermented beverages as there is no saturation of the extract by the alcohols formed during fermentation. It means that the time of extraction can be increased to recover a greater amount of esters and high-boiling compounds. No saturation has been noticed in the trapping step due to solvent rectifying. Owing to the good reproducibility of this method, low variations of volatiles among different samples can easily be revealed even if they are not quantitatively recovered.

ACKNOWLEDGMENT

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Registry No. Freon 11, 75-69-4; 4-methyl-1-pentanol, 626-89-1; 1-hexanol, 111-27-3; 2-heptanone, 110-43-0; α -pinene, 80-56-8; β -pinene, 127-91-3; ethyl hexanoate, 123-66-0; γ -hexalactone, 695-06-7; linalool, 78-70-6; 4–ethylphenol, 123-07-9; nerol, 106-25-2; 2–phenylethyl acetate, 103-45-7; γ –nonalactone, 104-61-0; damascenone, 23726-93-4; humulene, 6753-98-6; ethyl cinnamate, 103-36-6; β –ionone, 79-77-6.

LITERATURE CITED

Bertuccioli, M.; Viani, R. J. Sci. Food Agric. 1976, 27, 1035. Brown, R. M.; Purnell, C. J. J. Chromatogr. 1979, 178, 79.

Butler, L. D.; Burke, M. F. J. Chromatogr. Sci. 1976, 14, 117. Guichard, E. Sci. Aliments 1984, 2, 317.

- James, H. A.; Steel, C. P.; Wilson, I. J. Chromatogr. 1981, 208, 89.
- Jennings, W. G.; Wohleb, R. H.; Lewis, M. J. J. Food Sci. 1972, 37, 69.

Loyaux, D.; Roger, S.; Adda, J. J. Sci. Food Agric. 1981, 32, 1254. Murray, K. E. J. Chromatogr. 1977, 135, 49.

- Noble, A. C. In "Analysis of Foods and Beverages; Headspace Techniques"; Charalambous G., Ed.; Academic Press: New York, 1978; p 203.
- Noble, A. C.; Flath, R. A.; Forrey, R. R. J. Agric. Food Chem. 1980, 28, 346.
- Novak, J. J. Chromatogr. 1981, 209, 494.
- Novak, J. J. Chromatogr. 1982, 241, 434.
- Rapp, A.; Knipser, W. Chromatographia 1980, 13, 698.
- Simpson, R. F.; Miller, G. C. Vitis 1983, 22, 51.
- Tsugita, T.; Imai, T.; Doi, Y.; Kurata, T.; Kato, H. Agric. Biol. Chem. 1979, 43, 1351.
- Williams, A. A.; May, H. V.; Tucknott, O. G. J. Sci. Food Agric. 1978, 1041.
- Williams, P. J. J. Chromatogr. 1982, 241, 432.
- Williams, P. J.; Strauss, C. R. J. Inst. Brew. 1977, 83, 213.

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Hydrolysis of Wheat Straw Hemicelluloses and Heteroxylan (Larchwood) by Human Colon *Bacteroides ovatus* B4-11 Enzymes

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An intracellular enzyme fraction that catalyzes the degradation of wheat straw hemicelluloses and heteroxylan (larchwood) was isolated from *Bacteroides ovatus* B4-11. The intracellular enzyme(s) that degraded hemicelluloses and heteroxylan was (were) present at low levels in B4-11 grown on glucose or xylose. The hydrolytic activity was markedly increased when B4-11 was grown in the presence of hemicelluloses or heteroxylan. The percent hydrolysis calculated from the reducing sugar data was substantially higher than that calculated from HPLC data. The intracellular enzyme(s) of B4-11 grown on crude hemicellulose hydrolyzed and released about 13, 22, 4, 19, and 2% of the total sugars, respectively, from heteroxylan, crude hemicellulose, hemicellulose A, hemicellulose B, and wheat straw in 18 h. Hemicellulose A induced enzymes released about 5% of the total sugars from hemicellulose B. Heteroxylan-induced enzymes released about 5% of total sugars from hemicellulose B. Heteroxylan in 18 h. Xylose was the predominant sugar in addition to substantial amounts of oligomers in the hydrolysates.

Hemicellulose ranks second to cellulose in abundance in agricultural waste residues such as wheat straw and oat hulls. The hemicellulose content in plants changes with growth and maturity (Wilkie, 1979). Hemicelluloses are heteropolysaccharides and made up of at least two to four different types of sugar residues depending upon the source (Aspinall, 1970; Dekker, 1979; Wilkie, 1979). For example, hemicellulose A of wheat straw consists of three neutral sugar residues (xylose, arabinose, and glucose) and glucuronic acids, whereas hemicellulose B has four neutral sugar residues (xylose, arabinose, glucose, and galactose) and glucuronic acids (Bishop, 1953; Aspinall and Meek, 1956; Reddy et al., 1983).

Digestion of polysaccharides by rumen bacteria has been investigated in some detail (Dehority, 1973; Dekker, 1976; Chesson, 1981; Brice and Morrison, 1982; Williams and Withers, 1982a,b), but relatively little is known about hemicellulose degradation by human colon bacteria. *Bacteroides* sp. account for approximately 20% of the normal human fecal flora (Moore and Holdeman, 1974; Holdeman et al., 1976) and are known to ferment a wide

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variety of plant polysaccharides (Salyers et al., 1979). The enzymes responsible for the degradation of polysaccharides can be rapidly induced in *Bacteroides* sp. (Salyers et al., 1982; Balascio et al., 1981; Dekker and Palmer, 1981). Salyers et al. (1982) recently studied the hydrolysis of a commercial xylan (a larchwood heteroxylan) by three human colonic *Bacteroides* species that were grown in media containing xylan. They reported that the larchwood heteroxylan degrading enzymes of these strains were cell associated (intracellular) rather than extracellular. In contrast, Balascio et al. (1981) found that about 92% of the polysaccharidase activity was in the extracellular fluid, when a *Bacteroides ovatus* strain (ATCC 8433) was grown on guar gum.

In an earlier study (Reddy et al., 1983), 12 strains of Bacteroides sp. were screened for their ability to ferment wheat straw hemicelluloses and a larchwood heteroxylan, and B. ovatus B4-11 was selected for further study. The objective of the present study was to examine this strain for its ability to produce polysaccharide-degrading enzymes when grown in media containing wheat straw hemicelluloses, larchwood heteroxylan (heteroxylan), xylose, and glucose. Further, the hydrolysis of hemicelluloses and heteroxylan was also investigated, and the monosaccharides released from wheat straw and its hemicellulosic fractions and heteroxylan by enzymes from B. ovatus B4-11 were tentatively identified and quantitated by both HPLC and the reducing end assay method.

MATERIALS AND METHODS

Growth Media and Conditions. B. ovatus B4-11 was selected from an earlier study (Reddy et al., 1983) on the basis of its ability to grow on wheat straw hemicelluloses. Stock cultures were maintained in prereduced chopped meat broth (CMB) until used (Holdeman et al., 1977). The composition and preparation of the prereduced defined growth medium were described earlier (Reddy et al., 1983). Wheat straw hemicelluloses, heteroxylan, xylose, and glucose were substituted for carbohydrate in the medium and served as the sole source of carbohydrates.

Cultures for enzyme induction were prepared by inoculating 6 mL of CMB with 0.1 mL of a stock culture of *B. ovatus* B4-11 and incubated at 37 °C for 12 h. The 12-h culture was diluted 1:100 in prereduced peptone dilution blanks and 0.1 mL of this was inoculated into CMB and incubated for 18 h at 37 °C. The 18-h culture was inoculated (0.5% v/v) into the prereduced growth medium (10 mL) containing either wheat straw hemicelluloses, heteroxylan, xylose, or glucose (0.5% w/v). The inoculated tubes were incubated at 37 °C for 20 h for enzyme induction. All culture inoculations were done under oxygen-free CO₂ by using the VPI Anaerobe Culture System (Bellco Glass, Inc., Vineland, NJ).

Source and Preparation of Wheat Straw Hemicelluloses and Heteroxylan. A heteroxylan isolated from larchwood (Sigma Chemical Co., St. Louis, MO, lot no. 128C-03641) was used without further purification. Crude hemicellulose and hemicelluloses A and B from wheat straw (Arthur variety) were prepared by alkaline extraction as previously described (Reddy et al., 1983). The hemicellulose fractions of wheat straw were air-dried at room temperature to a moisture content of about 10% and ground in a Wiley mill to obtain 40-mesh powder. The milled powder was stored in screw-capped bottles at room temperature until used.

Preparation of Microbial Enzyme Fraction. Preparation of the intracellular enzyme fraction from *B. ovatus* B4-11 is outlined in Figure 1. The intracellular enzyme fraction was stored at 4 °C until ready for use.

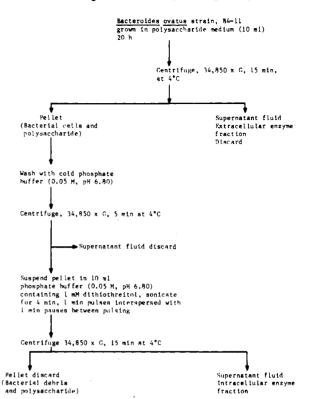


Figure 1. Preparation of the microbial intracellular enzyme fraction of B. ovatus B4-11. The intracellular enzyme fraction was adjusted to 10 mL.

Assay of Microbial Enzyme Fractions and Hydrolysis Products. Crude hemicellulose, hemicelluloses A and B, heteroxylan, and ball-milled wheat straw were used for assaying intracellular enzyme activity and hydrolysis. The substrate (40 mg) was weighed into individual test tubes and 4.0 mL of phosphate buffer (0.05 M, pH 6.80) was added. These tubes were autoclaved for 15 min at 121 °C. Less than 1% of sugars were detected in the autoclaved phosphate buffer medium. One volume (4.0 mL) of the intracellular enzyme fraction was added to 1 volume (4.0 mL) of the cooled substrate solution. The enzyme-substrate mixture was incubated at 37 °C for up to 18 h and then boiled for 15 min to terminate the reaction. Controls for each variable were prepared identically except that they were boiled immediately after addition of the enzyme. Following boiling and cooling, the samples were centrifuged at 34850g for 10 min at 4 °C. The supernatant fluid was placed in a vial and stored at -15 °C until analyzed. Total released sugars were measured by the method of Dygert et al. (1965) and HPLC. One unit of intracellular enzyme activity was defined as 0.1 μ mol of xylose equivalent produced per mL of enzyme preparation per 18 h at 37 °C.

For HPLC analysis, 1.0 mL of supernatant fluid was stirred with a mixed bed resin (AG-501-X8, 100-200 mesh, hydrogen and hydroxide form, 1:1, Bio-Rad Laboratories, Richmond, CA) and filtered through a 0.45- μ m pore size filter. The sugars in the filtrate were quantitated by HPLC (Palmer, 1979). The column employed was Aminex-HPX-87P Heavy Metal (Bio-Rad Laboratories, Richmond, CA). The sugars were separated by elution with water at 85 °C at a flow rate of 0.5 mL/min. Detection was via a postcolumn reactor in which sugars are reacted with sodium bicinchoninnate (Mopper, 1978) at 85 °C, and the resulting colored product was monitored at 546 nm. The minimum detectable concentration was about 0.1 μ g/mL (0.1 ppm), when injecting 100 μ L. Individual sugars were quantitated from HPLC peak area measurements relative

 Table I. Intracellular Enzyme Activity^a of B. ovatus B4-11

 Grown on Xylose, Glucose, and Various Polysaccharides

		induced on ^{b}							
substrate	xy- lose	glu- cose	het- er- oxy- lan	crude hemi- cellulose	hemi- cellulose A	hemi- cellulose B			
heteroxylan crude hemi- cellulose	0.7 0.2	0.7 0.0	5.2 3.3	9.5 7.9	4.7 6.6	4.8 3.5			
hemicellulose A	0.4	0.2	1.8	2.4	3.4	1.9			
hemicellulose B	1.2	0.2	4.4	10.4	8.4	6.6			

^a Each value is the average of three readings. Units per milliliter; 1 unit = 0.1 μ mol of xylose equivalents released per 18 h of hydrolysis per mL of enzyme fraction used in this assay. ^b Cells were grown for 20 h at 37 °C in the presence of the indicated carbohydrate.

to peak areas of authentic standards.

RESULTS AND DISCUSSION

Inducibility of Enzymes in B. ovatus B4-11 by Xylose, Glucose and Polysaccharides. B. ovatus B4-11 was grown in the defined growth medium containing either xylose, glucose, or various polysaccharides to determine if the intracellular enzymes were constitutive or induced. The intracellular enzyme fraction of B4-11 grown on xylose or glucose showed lower enzyme activity (released substantially smaller amounts of xylose equivalents) than cultures grown in the presence of polysaccharides (Table Previous researchers (Salvers et al., 1982) did not D. detect xylanase or β -xylosidase activity in B. ovatus grown on xylose or glucose. The levels of the intracellular enzyme fraction of B4-11 grown on polysaccharides (heteroxylan and wheat straw hemicelluloses) were much higher and released large amounts of xylose equivalents from heteroxylan and wheat straw hemicelluloses in 18 h at 37 °C, indicating that the enzyme activity levels were induced by the various polysaccharides (Table I). In most cases, Bacteroides enzymes released appreciable amounts of xylose (determined by TLC) from hemicelluloses, indicating the presence of β -xylosidase in addition to xylanase in the intracellular enzyme fraction.

Hydrolysis and Release of Sugars. The intracellular enzyme fraction of *B. ovatus* B4-11 was used for hydrolysis of wheat straw and its hemicellulose fractions and heteroxylan since the extracellular enzyme fraction has little hydrolytic activity (Reddy et al., 1983). The hydrolysis of the above substrates by B4-11 intracellular enzymes was followed both by measuring an increase in reducing sugars as xylose equivalents by the Dygert et al. (1965) method and by HPLC determination of predominant sugars such as xylose, arabinose, and glucose. Hydrolysis was calculated as percent of total sugars released from a particular substrate. Total sugars in wheat straw, crude hemicellulose, hemicellulose A, hemicellulose B, and heteroxylan are 61.5, 38.9, 64.9, 67.2, and 79.8%, respectively (Reddy et al., 1983).

The percent hydrolysis calculated from the reducing sugar data was substantially higher than that calculated from the HPLC data (see Tables II-VIII). The reason was apparent from the HPLC charts of polysaccharide hydrolysates, which showed peaks corresponding to both short chain (DP₂ or DP₃) and/or long chain (DP₄ and higher) oligomers. The presence of such oligomers was comfirmed by TLC. Thus, the enzymic hydrolysis yielded substantial quantities of oligomers, which were measured

Table II. Hydrolysis and Release of Sugars from Crude Hemicellulose^a by Crude Hemicellulose Induced Enzymes

incubation time, min	sug	gars released	hydro- lyzed, ^b	hydro- lyzed, ^c	
	xylose	arabinose	glucose	%	~ <u>%</u>
0	0.0	0.0	0.0	0.0	0.0
15	0.8ď			0.8	6.2
60	4.2			4.2	12.2
240	7.2	1.3		8.5	19.7
660	13.8	3.4		17.2	25.1
1080	17.7	4.4		22.1	30.5

^a Total sugar content of crude hemicellulose was 38.90%. ^b Obtained by the HPLC method by measuring individual released sugars, xylose, arabinose, and glucose. ^c Obtained by the Dygert et al. (1965) method measuring as xylose equivalents. ^d Each value is the average of three determinations.

 Table III. Hydrolysis and Release of Sugars from

 Hemicellulose A^a by Hemicellulose A Induced Enzymes

incubation time, min	sug	gars released	hydro- lyzed, ^b	hydro- lyzed,°	
	xylose	arabinose	glucose	%	%
0	0.0	0.0	0.0	0.0	0.0
15	0.4 ^d			0.4	0.7
60	0.4			0.4	1.6
240	2.4			2.4	4.9
660	3.3	0.9		4.2	6.5
1080	3.5	1.1		4.6	7.9

^a Total sugar content of hemicellulose A was 64.9%. ^bObtained by the HPLC method by measuring individual released sugars, xylose, arabinose, and glucose. ^cObtained by the Dygert et al. (1965) method measuring as xylose equivalents. ^d Each value is the average of three determinations.

Table IV. Hydrolysis and Release of Sugars from Hemicellulose B^a by Hemicellulose B Induced Enzymes

incubation	sug	gars released	hydro- lyzed, ^b	hydro- lyzed,°	
time, min	-		glucose	%	%
0	0.0	0.0	0.0	0.0	0.0
15	0.1 ^d			0.1	4.9
60	0.4			0.4	5.1
240	1.7	0.5	0.2	2.4	9.6
660	2.1	1.9	0.9	4.9	10.7
1080	3.3	2.6	1.6	7.5	14.7

^a Total sugar content of hemicellulose B was 67.2%. ^bObtained by the HPLC method by measuring individual released sugars, xylose, arabinose, and glucose. ^cObtained by the Dygert et al. (1965) method measuring as xylose equivalents. ^dEach value is the average of three determinations.

Table V. Hydrolysis and Release of Sugars from Various Polysaccharides^a and Wheat Straw^a by Heteroxylan Induced Enzymes during 18 h of Incubation

	sugar	s releas	sed, %		hydro- lyzed,' %
polysaccharide	xylose	ara- bi- nose	glucose	hydro- lyzed, ^b %	
heteroxylan	2.5 ^d	2.1		4.6	9.8
crude hemicellulose	1.7	1.3	0.5	3.5	12.7
hemicellulose A	0.6	0.3		0.9	4.2
hemicellulose B	1.8	1.7		3.5	9.8
wheat straw					0.5

^a Total sugar content of heteroxylan, crude hemicellulose, hemicellulose A, hemicellulose B, and wheat straw was 79.8, 38.9, 64.9, 67.2, and 61.5%, respectively. ^bObtained by the HPLC method by measuring individual released sugars, xylose, arabinose, and glucose. ^cObtained by the Dygert et al. (1965) method measuring as xylose equivalents. ^dEach value is the average of three determinations.

as increases in monosaccharides in the reducing sugar assay. Unfortunately, it has not been possible to identify and quantitate these oligomers by HPLC due to a lack of

Table VI. Hydrolysis and Release of Sugars from Various Polysaccharides^a and Wheat Straw^a by Crude Hemicellulose Induced Enzymes during 18 h of Incubation

	sugar	sugars released, %			hydro- lyzed,° %
polysaccharide	ara- bi- xylose nose glucose		hydro- lyzed, ^b %		
heteroxylan	7.3 ^d	4.1	1.8	13.2	17.8
crude hemicellulose	17.7	4.4		22.1	30.5
hemicellulose A	2.9	1.0		3.9	5.6
hemicellulose B	12.9	4.5	1.4	18.8	23.2
wheat straw	1.5		0.3	1.8	2.9

^aSee footnote *a* under Table V. ^bObtained by the HPLC method by measuring individual released sugars, xylose, arabinose, and glucose. ^cObtained by the Dygert et al. (1965) method measuring as xylose equivalents. ^dEach value is the average of three determinations.

Table VII. Hydrolysis and Release of Sugars from Various Polysaccharides^a and Wheat Straw^a by Hemicellulose A Induced Enzymes during 18 h of Incubation

	sugars released, %				hydro- lyzed,° %
polysaccharide	ara- bi- xylose nose glucose			hydro- lyzed, ^b %	
heteroxylan	2.7 ^d	2.1	0.9	5.7	8.8
crude hemicellulose	11.9	2.7	0.6	15.2	25.5
hemicellulose A	3.5	1.1		4.6	7.9
hemicellulose B	12.1	3.5		15.6	18.8
wheat straw	0.9		0.1	1.0	1.0

^aSee footnote *a* under Table V. ^bObtained by the HPLC method by measuring individual released sugars, xylose, arabinose, and glucose. ^cObtained by the Dygert et al. (1965) method measuring as xylose equivalents. ^dEach value is the average of three determinations.

Table VIII. Hydrolysis and Release of Sugars from Various Polysaccharides^a and Wheat Straw^a by Hemicellulose B Induced Enzymes during 18 h of Incubation

	sugar	rs releas			
polysaccharide	ara- bi- xylose nose glucose		hydro- lyzed, ^b %	hydro- lyzed,' %	
heteroxylan	1.7 ^d	2.7	1.2	5.6	9.0
crude hemicellulose	3.1	2.3	1.2	6.6	13.5
hemicellulose A	1.1	0.4		1.5	4.4
hemicellulose B	3.3	2.6	1.6	7.5	14.7
wheat straw	0.4		0.2	0.6	1.7

^aSee footnote a under Table V. ^bObtained by the HPLC method by measuring individual released sugars, xylose, arabinose, and glucose. ^cObtained by the Dygert et al. (1965) method measuring as xylose equivalents. ^dEach value is the average of three determinations.

appropriate standards. However, the presence of these oligomers emphasizes the necessity for determining the individual products of hydrolysis, especially in the situation where a particular monosaccharide (xylose in the present case) is the desired end product. The hydrolysis data obtained by reducing sugar assay and HPLC are presented in Tables II–VIII to show the differences and to point out the magnitude of error that can occur when using reducing sugar assays for quantitating the products in a polysaccharide hydrolysate. Characterization of the oligomer product could provide information on the mechanism(s) of hydrolysis and the rate-limiting steps in enzymic hydrolysis.

The intracellular enzyme fraction from B4-11 grown on crude hemicellulose was incubated with the same substrate for varying periods of time (0-1080 min) at 37 °C to de-

termine the hydrolysis and release of sugars. Similarly, hemicelluloses A and B were also used for induction of enzymes and hydrolysis. Hydrolysis and release of sugars from hemicelluloses by intracellular enzymes increased substantially as the incubation time progressed (Tables II-IV). Traces of glucuronic acid were detected in all enzyme hydrolysates by TLC. In most cases, the intracellular enzymes released mainly xylose, with smaller amounts of arabinose. During 1080 min of incubation, intracellular enzymes from B4-11 grown on crude hemicellulose hydrolyzed and released from crude hemicellulose about 22.1% of total sugars. In hemicellulose A, the intracellular enzymes of B4-11 grown on hemicellulose A liberated 4.6% (least amount) of total sugars in 1080 min with xylose representing 75.2% of the total (Table III). In the case of hemicellulose B, intracellular enzymes of B4-11 grown on hemicellulose B released nearly equal amounts of xylose and arabinose and smaller amounts of glucose. About 7.5% of total sugars were released from hemicellulose B during 1080 min of incubation (Table IV). Arabinose was released from hemicelluloses after 240 min of incubation, which suggests that the xylose-releasing enzymes, especially β -xylosidase and other xylanases, are more active during first 240 min of incubation followed by arabinose-releasing enzymes. Dekker and Palmer (1981) reported that the xylose and uronic acids are the predominant products released from peanut cell wall polysaccharides by intracellular enzymes of human fecal Bacteroides grown on the same substrate. They identified xylose and uronic acids by TLC and GC and estimated relative quantities from color intensity of TLC spots and/or size of GC peaks. Salyers et al. (1977) investigated the inducibility of laminarinase activity growing Bacteroides thetaiotaomicron, Bacteroides distasonis, and Bacteroides fragilis on laminarin, a linear polysaccharide. They reported that the products of laminarinase activity vary with species and glucose is the major product released, however. Differences in the hydrolysis of hemicelluloses by B4-11 intracellular enzymes could be due to (i) the structural composition and arrangement of sugar residues in hemicelluloses, (ii) the type of enzymes in intracellular fraction induced by B. ovatus B4-11, (iii) the presence of inhibitors such as metallic ions, phenolics, and nitrogenous compounds in the hemicelluloses and their association or complexation with hemicelluloses, which may impede the hydrolysis, (iv) the presence of large amounts of end products such as xylose, arabinose, and glucose, which inhibit their own enzyme that results in decreased hydrolysis (Salyers et al., 1977, 1982), (v) the lack of availability of linkages (α or β) for enzymic hydrolysis, and (vi) the solubility of the hemicelluloses in the medium (i.e., phosphate buffer was used for preparation of substrates). Wheat straw hemicelluloses and heteroxylan were least soluble, and less than 1% of free sugars were detected in the autoclaved phosphate buffer medium (0 h). Salyers et al. (1982) reported that solubility of the polysaccharide in the medium plays a role in the hydrolysis by intracellular enzymes of Bacteroides sp. Dekker and Palmer (1981) reported only 11.0% hydrolysis of peanut cell wall polysaccharides in 18 h, when they incubated peanut cell wall polysaccharides with intracellular enzymes induced by Bacteroides of human fecal origin.

B. ovatus B4-11 was grown on heteroxylan for induction of enzymes. Crude hemicellulose, hemicelluloses A and B, heteroxylan, and ball-milled wheat straw were incubated with heteroxylan-induced intracellular enzymes of B4-11 for estimating their extent of hydrolysis and release of sugars. Likewise, similar experiments were done by using crude hemicellulose and hemicelluloses A and B for inducing enzymes and hydrolysis. Heteroxylan-induced intracellular enzymes liberated aproximately equal amounts of xylose and arabinose from hemicellulose B and heteroxylan (Table V). In contrast, Salyers et al. (1982) found that the xylose is the major product released from heteroxylan by enzymes from *Bacteroides* grown on heteroxylan. However, they detected traces of arabinose and glucose. In crude hemicellulose, heteroxylan-induced enzymes released small amounts of glucose in addition to large amounts of xylose and arabinose. No sugars were released from ball-milled wheat straw (Table V).

Crude hemicellulose induced enzymes liberated significant amounts of xylose, arabinose, and glucose from heteroxylan and hemicellulose B (Table VI), while only xylose and arabinose were released from hemicellulose A. Appreciable amounts of xylose and glucose were released from ball-milled wheat straw by crude hemicellulose induced enzymes. Hemicellulose A induced enzymes released large amounts of xylose from crude hemicellulose and hemicellulose B (Table VII). A total of 15.2 and 15.6% sugars, respectively, were released from crude hemicellulose and hemicellulose B by hemicellulose A induced enzymes. Hemicellulose B induced enzymes released 1.5 and 6.6% of total sugars from hemicellulose A and crude hemicellulose respectively (Table VIII). Crude hemicellulose and hemicelluloses A and B induced enzymes all released appreciable amounts of xylose, arabinose, and glucose from heteroxylan compared to heteroxylan-induced enzymes, which released only xylose and arabinose.

Overall, less than 2.0% of total sugars were released from ball-milled wheat straw by hemicellulose-induced enzymes. This suggests that the enzymes induced by the hemicelluloses cannot hydrolyze intact wheat straw. Crude hemicellulose induced enzymes hydrolyzed and released large amounts of sugars from heteroxylan, hemicelluloses A and B, and crude hemicellulose. Currently, studies are under way to identify the individual enzymes present in the intracellular enzyme fraction of B. ovatus B4-11, with the goal of increasing the degree of hydrolysis of the hemicelluloses. Further studies are needed on (i) the effect of pretreatment such as steam explosion on wheat straw hydrolysis by human colon Bacteroides enzymes, (ii) the hydrolysis of hemicellulose fractions from other agricultural residues such as oat hulls and corn cobs by human colon Bacteroides enzymes, and (iii) similar hydrolysis studies with the rumen microorganisms enymes.

Registry No. Heteroxylan, 9014-63-5; hemicellulose, 9034-32-6; hemicellulose A, 63100-39-0; hemicellulose B, 63100-40-3; xylose, 58-86-6.

LITERATURE CITED

- Aspinall, G. O. "Polysaccharides"; Pergamon Press: Oxford, U.K., 1970; pp 1-43.
- Aspinall, G. O.; Meek, E. G. J. Chem. Soc. 1956, 3830.
- Balascio, J. R.; Palmer, J. K.; Salyers, A. A. J. Food Biochem. 1981, 5, 271.
- Bishop, C. T. Can. J. Chem. 1953, 31, 134.
- Brice, R. E.; Morrison, I. M. Carbohydr. Res. 1982, 101, 93.
- Chesson, A. J. Sci. Food Agric. 1981, 32, 745.
- Dehority, B. A. Fed. Proc., Fed. Am. Soc. Exp. Biol. 1973, 32, 1819.
- Dekker, J.; Palmer, J. K. J. Agric. Food Chem. 1981, 20, 480.
- Dekker, R. F. H. In "Carbohydrate Research in Plants and Animals"; Landbouwhogeschool: Wageningen, Netherlands, 1976; Vol. 12, p 43.
- Dekker, R. F. H. In "Polysaccharides in Foods"; Blanshard, J. M. V.; Mitchell, J. R., Eds.; Butterworth and Co: London, U.K., 1979; pp 93-108.
- Dygert, S.; Li, H.; Florida, D.; Thoma, J. A. Anal. Biochem. 1965, 13, 367.
- Holdeman, L. V.; Cato, E. P.; Moore, W. E. C. "Anaerobic Laboratory Manual", 4th ed.; VPI & SU: Blacksburg, VA, 1977.
- Holdeman, L. V.; Good, I. J.; Moore, W. E. C. Appl. Environ. Microbiol. 1976, 31, 359.
- Moore, W. E. C.; Holdeman, L. V. Appl. Microbiol. 1974, 27, 961.
- Mopper, K. Anal. Biochem. 1978, 85, 528.
- Palmer, J. K. In "GLC and HPLC Determination of Therapeutic Agents"; Tsuji, K., Ed.; Marcel Dekker: New York, 1979; Part III, pp 1317-1339.
- Reddy, N. R.; Palmer, J. K.; Pierson, M. D.; Bothast, R. J. J. Agric. Food Chem. 1983, 31, 1308.
- Salyers, A. A.; Balascio, J. R.; Palmer, J. K. J. Food Biochem. 1982, 6, 39.
- Salyers, A. A.; Palmer, J. K.; Balascio, J. R. In "Dietary Fibers: Chemistry and Nutrition"; Inglett, G. E.; Falkeheg, S. I., Eds.; Academic Press: New York, 1979; pp 193-201.
- Salyers, A. A.; Palmer, J. K.; Wilkins, T. D. Appl. Environ. Microbiol. 1977, 33, 1118.
- Wilkie, K. C. B. Adv. Carbohydr. Chem. Biochem. 1979, 36, 215.
- Williams, A. G.; Withers, S. E. J. Appl. Bacteriol. 1982a, 52, 377.
- Williams. A. G.; Withers, S. E. J. Appl. Bacteriol. 1982b, 52, 389.

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