Wheat Straw Hemicelluloses: Composition and Fermentation by Human Colon *Bacteroides*

N. Rukma Reddy,* James K. Palmer, Merle D. Pierson, and Rodney J. Bothast

The hemicellulosic components of wheat straw have been partially characterized, and the ability of selected anaerobic bacteria from the human colon to ferment wheat straw, its hemicellulose fractions, and commercial xylan was evaluated. Three strains of *Bacteroides ovatus*, which grew particularly well on media containing hemicellulose, were examined for the presence and cellular localization of the hemicellulose-degrading enzymes. Wheat straw contained about 75% carbohydrate on a dry weight basis. Xylose was the major sugar in the hemicelluloses, representing about 31% of crude hemicellulose and 50% of hemicelluloses A and B. Of the 12 strains of *Bacteroides* sp. tested, none fermented wheat straw. Of seven strains of *B. ovatus* evaluated, six fermented crude hemicellulose and hemicelluloses A and B. Of five strains representing *Bacteroides vulgatus*, *Bacteroides uniformis*, and *Bacteroides thetaiotaomicron*, none fermented hemicelluloses. The hemicellulose-degrading enzyme activity was predominantly intracellular.

Agricultural waste residues such as wheat straw, corn stover, and oat hulls contain a significant proportion of cellulose and hemicelluloses. These materials are potential sources for production of alcohol fuels and other chemical feedstocks. Detroy and Hesseltine (1978) reviewed some of the most promising and successful technological processes available for the production of chemicals from agricultural waste materials. Currently much attention is focused on the conversion of agricultural residues to alcohol fuels and other chemicals by using various microorganisms, including yeasts and fungi (Detroy et al., 1980, 1981, 1982; Slininger et al., 1982).

One approach that has not been explored is the degradaton of hemicelluloses from wheat straw or other agricultural residues to their component sugars by enzymes from the anaerobic bacterica of the human colon. This approach appears to be quite promising for production of chemical feedstocks. Digestion of polysaccharides (including pectin, xylan, and cellulose) from forage by rumen anaerobic bacteria has been investigated in some detail (Dehority, 1965, 1973; Williams and Withers, 1982a,b), but there is relatively little information on the degradation of such residues by human colon anaerobes.

Bacteroides sp. account for about 20% of the human fecal flora, but there are other major genera such as *Bifidobacterium*, *Peptostreptococcus*, *Lactobacillus*, *Ruminococcus*, *Coprococcus*, *Eubacterium*, and *Fusobacterium* that are also present in relatively high concentrations in the human colon (Holdeman et al., 1976; Moore and Holdeman, 1974). Most of these species are saccharolytic. Recent studies (Salyers et al., 1977a–c, 1978, 1979) reveal that certain strains of *Bacteroides* ferment a wide range of commercially available complex polysaccharides. Growth for as little as 4 h on a particular polysaccharide induces production of enzymes that are readily isolated and can be used to catalyze substantial degradation of the original inducing polysaccharide (Salyers et al., 1977a, 1982; Balascio et al., 1981; Dekker and Palmer, 1981). For example, selected colon *Bacteroides*, grown on laminarin, produce enzymes that degrade laminarin to a wide range of products, including glucose and higher oligomers of glucose. Enzymes from strains grown on xylan catalyze production of xylose when incubated with the original xylan (Salyers et al., 1978).

The studies described in the present report had two primary objectives. The first objective was to characterize wheat straw hemicelluloses in terms of method of isolation and composition. Earlier studies (Adams, 1952; Bishop, 1953) directed at characterizing wheat straw xylans provide considerable detail on particular xylan fractions but only approximate data on the overall hemicellulose composition. These studies also strongly emphasized that the composition of isolated hemicellulose(s) depends on the procedure employed. The second objective was to test 12 *Bacteroides* strains from the human colon, previously shown to be capable of fermenting complex polysaccharides, for their ability to ferment wheat straw and/or the isolated and characterized wheat straw hemicelluloses. When it was found that *Bacteroides ovatus* strains fermented the wheat straw hemicelluloses, the study was extended to obtain information on the enzymes responsible for hemicellulose degradation.

MATERIALS AND METHODS

Isolation of Hemicelluloses from Wheat Straw. The straw (soft winter wheat, Arthur variety) was obtained from Northern Regional Research Center, U.S. Department of Agriculture, Peoria, IL. It was ground in a Wiley mill to pass through a 1-mm screen. Crude hemicellulose and hemicelluloses A and B were isolated by alkaline extraction (Whistler and Feather, 1965).

"Crude hemicellulose", essentially a mixture of hemicelluloses A and B, was isolated from wheat straw by a simplified procedure. Wheat straw (100 g) was extracted with deoxygenated 10% sodium hydroxide solution containing 1 mg/mL sodium borohydride for 24 h under oxygen-free nitrogen at room temperature. The alkaline slurry was vacuum filtered through six layers of cheesecloth. The filtrate was then cooled and adjusted to pH 5.0 with acetic acid and mixed with 4 volumes of 95% ethanol. The resulting precipitated crude hemicellulose was removed by centrifugation at 16000*g*, washed with water, and air-dried at room temperature.

For preparation of hemicelluloses A and B, wheat straw was first treated with 80% ethanol for 10 h at room temperature to remove any soluble sugars. The residue was then washed 3 times with water and extracted for 10 h at room temperature with 2% sodium chloride to remove any

Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061 (N.R.R., J.K.P., and M.D.P.), and Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604 (R.J.B.).

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crude protein. The sodium chloride extracted residue was washed 5 times with water and air-dried. The air-dried residue was extracted for 24 h under oxygen-free nitrogen at room temperature with deoxygenated 10% sodium hydroxide solution containing 1 mg/mL sodium borohydride. The alkaline slurry was then vacuum filtered through six layers of cheesecloth. The hemicellulose A was precipitated from the filtrate by acidification to pH 5.0 with acetic acid and collected by centrifugation at 16000*g*. Hemicellulose B was isolated from the supernatant fluid by precipitation with 4 volumes of 95% ethanol and centrifugation. The precipitated hemicelluloses A and B were washed with water and air-dried at room temperature.

All the dried hemicellulose fractions were ground in Wiley mill (40 mesh) and stored in screw-capped bottles at room temperature until used.

Chemical Analysis. AOAC (1975) methods were employed for determination of moisture, ash, and nitrogen content of wheat straw, residue (remaining after extraction of crude hemicellulose), crude hemicellulose, and hemicelluloses A and B. Sodium, potassium, iron, calcium, and magnesium were determined by a Perkin-Elmer Model 403 atomic absorption spectrophotometer. The instrument settings and other experimental conditions were in accordance with the manufacturer's specifications. Methanol-extracted phenolic content was determined by the method of Burns (1971). Catechin was used for preparation of standard curves and as an internal standard.

For determination of total sugars and individual sugars, the samples were hydrolyzed according to the method of Bittner et al. (1980). Total sugars in the hydrolysate were estimated by the method of Dubois et al. (1956). A mixed sugar solution (40% arabinose, 40% xylose, 10% glucose, and 10% galactose) was used as a standard, since wheat straw hemicellulose contained large amounts of xylose and arabinose and small amounts of glucose and galactose. The Ahmed and Labavitch (1977) method was employed for determination of uronic acid content, utilizing galacturonic acid as a standard.

Gas chromatography (GC) and high-performance liquid chromatography (HPLC) were employed to identify and quantitate individual sugars in the hydrolysates of various wheat straw hemicellulose fractions, wheat straw, and residue. A 200-μL aliquot of acid hydrolysate was derivatized to alditol acetates and analyzed by the GC method of Bittner et al. (1980). For the HPLC method, 0.5 mL of acid hydrolysate was neutralized with a few drops of concentrated ammonia and diluted to 10 mL. A 1.0-mL aliquot of the diluted sample was deionized by stirring with about 0.1 mL of 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 μm pore-size filter. The sugars in the filtrate were quantitated by HPLC (Palmer, 1975, 1979, 1982). The column employed was Aminex-HPX-85 (Bio-Rad Laboratories, Richmond, CA). The sugars were separated by elution with water at 85 °C at a flow rate of 0.4 mL/min. Detection was via a postcolumn reactor (Palmer, 1982) in which the sugars are reacted at 85 °C with sodium bicinchoninate reagent (Mopper, 1978) and the resulting colored product is monitored at 546 nm. The minimum detectable concentration was about 0.1 μg/mL (0.1 ppm), when injecting 100 μL.

Bacterial Cultures. Previously isolated and identified human colon strains of *B. ovatus* (B4-11, C1-45, C2-26, C8-14, R3-39, T4-7, 0038-1), *Bacteroides vulgatus* (C16-16), *Bacteroides uniformis* (C20-25, 0061-1), and *Bacteroides thetaiotaomicron* (C11-15, 7330-1) were obtained from Dr. T. D. Wilkins of Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, VA. These strains were previously evaluated for their ability to degrade various commercial polysaccharides by Salyers et al. (1977b). The stock cultures were maintained in prereduced chopped meat broth (CMB) (Holdeman et al., 1977).

Prereduced Defined Medium for Fermentation. The defined medium of Varel and Bryant (1974) as adapted by Salyers et al. (1977a) was used for selecting hemicellulose-degrading organisms and for inducing enzymes. Agar and phenol red were deleted from the medium. Briefly, the basal growth medium contained the following: $(NH_4)_2SO_4$, 1.0 g/L; vitamin B_{12}, 5.0 μg/L; hemin, 5.0 mg/L; K_2HPO_4, 2.26 g/L; KH_2PO_4, 0.9 g/L; $FeSO_4 \cdot 7H_2O$, 4.0 mg/L; NaCl, 0.9 g/L; $CaCl_2 \cdot 2H_2O$, 0.027 g/L; $MgCl_2 \cdot 6H_2O$, 0.02 g/L; $MnCl_2 \cdot 4H_2O$, 0.01 g/L; $CoCl_2 \cdot 6H_2O$, 0.01 g/L; $Na_2CO_3 \cdot H_2O$, 0.5 g/L; cysteine hydrochloride, 0.5 g/L; carbohydrate (glucose), 5.0 g/L; resazurin, 1.0 mg/L. Wheat straw, hemicelluloses, or other fractions were substituted for glucose in the medium and served as the sole source of carbohydrates.

The defined medium was prepared anaerobically according to the procedures outlined by Holdeman et al. (1977). The medium was adjusted to pH 7.1 with 5 N sodium hydroxide, dispensed into anaerobic culture tubes (18 × 150 mm, Bellco, Glass, Inc., Vineland, NJ) containing the wheat straw or other fractions to yield a final concentration of 0.5% of the test material, and stoppered under oxygen-free nitrogen. The tubes were placed in a tube press (Bellco Glass, Inc., Vineland, NJ) and autoclaved for 15 min at 121 °C. The tubes were allowed to cool to room temperature prior to inoculation.

Fermentation Studies. The procedure involved inoculating 6 mL of CMB with 0.1 mL of stock culture and incubating at 37 °C for 24 h. These 24-h cultures were diluted 1:100 in prereduced 0.1% peptone dilution blanks, and 0.5 mL was inoculated into 10 mL of defined growth medium containing 0.5% wheat straw or fractions thereof. Inoculation was done under oxygen-free nitrogen using a VPI Anaerobe Culture System (Bellco Glass, Inc., Vineland, NJ). The inoculated tubes were incubated for 5 days at 37 °C to monitor fermentation of wheat straw or other fractions. Control tubes were prepared by adding 0.5 mL of sterile 0.1% peptone to 10 mL of defined medium containing wheat straw or other fractions and incubating as above. Tubes containing 0.5% commercial xylan (Sigma Chemical Co., St. Louis, MO) were inoculated and incubated similarly to serve as positive controls. The turbidity and the pH of the medium were monitored for evidence of fermentation. A drop in pH and/or visible bacterial growth during 5 days of incubation was interpreted as fermentation.

In some of the initial fermentation experiments, CO_2 gas was used as headspace, since a high CO_2 atmosphere would better simulate the growth of *Bacteroides* in the colon. Essentially the same patterns of fermentation results were obtained with CO_2 and N_2. However, N_2 gas was selected for routine use because the pH data were significantly more variable and inconsistent with CO_2.

Preparation of Microbial Enzyme Fractions. On the basis of the fermentation studies, three strains (B4-11, C1-45, and 0038-1) of *B. ovatus* were selected for further study and tested for the presence, inducibility, and cellular localization of hemicellulose-degrading enzymes. These three strains grew very well on the hemicellulose-based medium. The general procedures developed by Dekker (1979) and Dekker and Palmer (1981) were used. Briefly,

Table I. Proximate Composition[a] of Wheat Straw and Its Hemicellulose Fractions (in Weight Percent)

component	wheat straw	crude hemicellulose	hemicellulose A	hemicellulose B	residue[b]
Kjeldahl nitrogen	0.61	1.58	0.95	0.92	0.45
carbohydrate content	73.92	63.30	89.56	87.18	79.65
methanol-extracted phenolics[c]	0.31	0.42	0.28	0.33	0.00
ash	8.87	28.79	6.46	15.17	2.84
sodium	0.03	10.05	2.63	3.73	0.05
potassium	1.50	0.16	0.01	0.01	0.002
iron	0.19	0.02	0.01	0.004	0.005
calcium	0.07	0.004	0.002	0.001	0.15
magnesium	0.04	0.006	0.002	0.001	0.04

[a] Expressed on a moisture-free basis. [b] Residue refers to the material remaining after extraction of crude hemicellulose. [c] Methanol-extracted phenolic content is presented as catechin equivalents.

the strains of *B. ovatus* were grown on prereduced media containing 0.5% hemicellulose under a CO_2 atmosphere. After various periods of growth at 37 °C, extracellular and intracellular enzymes fractions were prepared as outlined in Figure 1.

Assay of Microbial Enzyme Fractions. Crude hemicellulose and hemicelluloses A and B were used as substrates for assaying extracellular and intracellular enzyme activity. The substrate (40 mg) was suspended in 4 mL of phosphate buffer (0.05 M, pH 6.80). The tubes containing the substrate–buffer mixture were autoclaved at 121 °C for 15 min and cooled prior to addition of 4 mL of the extracellular or intracellular enzyme preparation. The enzyme–substrate mixture was incubated at 37 °C for 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 34850*g* for 10 min at 4 °C. The supernatant fluid was placed in a vial and stored at −15 °C until analyzed for total realeased sugars by the method of Dygert et al. (1965). Activity was defined as xylose equivalents (μg) released from 10 mg of substrate per mL of enzyme in 18 h.

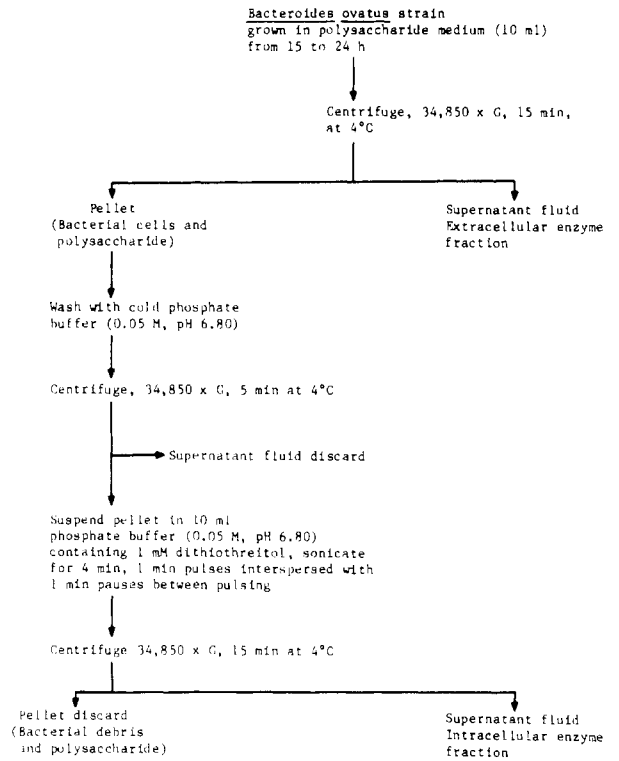

Figure 1. Preparation of microbial extracellular and intracellular enzyme fractions. The extracellular and intracellular enzyme preparations were adjusted to 10 mL in order to eliminate the differences in total volume.

RESULTS AND DISCUSSION

Proximate Composition. Carbohydrate was the major component in wheat straw and its hemicellulose fractions and residue, ranging from 63.3 to 89.6% (Table I). Crude hemicellulose, prepared by a simplified procedure involving alkaline extraction of wheat straw without pretreatment, had the lowest carbohydrate content and a substantially higher nitrogen content than hemicelluloses A and B. Crude hemicellulose also had high ash content, in part because of a high sodium content (Table I). Presumably this sodium was carried over from the alkaline extractant. Hemicellulose B had a high ash content, only partially accounted for by increased sodium. It seems likely that silica (not determined in the present study) accounts for much of the ash. Many agricultural residues contain considerable amounts of silica. For example, rice straw contains 18.6% ash, of which about 85% is silica (Han, 1975). Wheat straw itself had nearly 9% ash, and only 20% of this ash is accounted for by the elements determined (Table I). Wheat straw, crude hemicellulose, and hemicelluloses A and B contained appreciable amounts of methanol-extracted phenolics.

Yield of Hemicelluloses. Crude hemicellulose represented 51% of wheat straw on a dry weight basis; the residue represented 41%. Hemicelluloses A and B were isolated in yields of 13.5% and 15.8%, respectively, on a dry weight basis.

Sugar Content. Optimum conditions for acid hydrolysis at 95 °C were determined by HPLC measurement of the yield of individual sugars after 1, 2, 3, and 4 h of hydrolysis. Constant values (within the limit of experimental error) for the yield of xylose and arabinose for all times tested indicated that 1 h was sufficient to solubilize these sugars. However, glucose yields tended to increase continually and were all significantly higher at 4 h. For example, glucose in wheat straw went from 27.6% at 1 h to 34.3% at 2 h, 36% at 3 h, and 37.2% at 4 h. This undoubtedly reflects a somewhat incomplete hydrolysis of cellulose, and the 4-h values were taken as being closest to the actual value. Sloneker (1976) reported 40% cellulose in wheat straw. Overall, a hydrolysis time of 2–4 h was judged adequate for measurement of neutral sugar content.

The data on sugar and uronic acid composition are summarized in Table II. Xylose was the major sugar in the hemicelluloses and in commercial xylan. Hemicelluloses A and B contained appreciable arabinose and small amounts of glucose, while the xylan contained appreciable glucose and some arabinose. Glucose and xylose were the major sugars in wheat straw, reflecting the presence of cellulose and xylose-rich hemicellulose. The crude hemicellulose composition was distorted by the

Table II. Sugar Composition of Wheat Straw and Its Hemicellulose Fractions[a] (in Weight Percent)

	total carbohydrate	recovered carbohydrate[b]	uronic acid	arabinose	xylose	glucose	galactose	mannose	ratio of xylose/arabinose
wheat straw	73.9	64.5 (87.0)[e]	3.0	5.6	18.7	37.2	trace	trace	3.3
crude hemicellulose	63.3	42.2 (67.0)	3.3	4.9	31.4	2.6	trace		6.4
hemicellulose A	89.6	68.5 (76.0)	3.6	11.0	50.4	3.5			4.6
hemicellulose B	87.2	72.6 (83.0)	5.4	14.3	50.2	2.7	trace		3.5
commercial xylan[c]	86.8	79.8 (92.0)		6.0	59.7	14.1			4.2
residue[d]	79.7	69.1 (87.0)	2.0	trace	5.9	61.3			

[a] All values are expressed on a moisture-free basis. Total carbohydrate was determined by the method of Dubois et al. (1956); uronic acids were detected by the method of Ahmed and Labavitch (1977); traces of galactose and mannose were detected via GC. All other data were obtained via HPLC. [b] Sum of arabinose, xylose, glucose, and uronic acids. [c] Obtained from Sigma Chemical Co., St. Louis, MO. [d] Residue refers to the material remaining after extraction of crude hemicellulose. [e] Values in parentheses represent the percentage of total carbohydrate recovered.

presence of nearly 29% ash, but xylose was still the major sugar present. Uronic acid values averaged about 3% for all samples except hemicellulose B, which had nearly twice this amount. Note that sugar and uronic acid values in Table II are expressed as percent of original dry matter and that the recoveries of total carbohydrate are consistently below 100% of the total carbohydrate, determined by an independent method (Dubois et al., 1956) that is reported to measure both free sugars and polysaccharide sugars. Even the commercial xylan yielded only 92% of its weight as sugar.

Since there was only a small residue in the vials at the completion of hydrolysis, there must be some proportion of the carbohydrate that dissolves in the hydrolysis medium but that is not hydrolyzed to free sugars. This residual oligomeric material would not be detected (or determined) by the HPLC or GC methods employed, but it would be measured in the method of Dubois et al. (1956). Recently, Himmelsbach and Barton (1980) reported a similar phenomenon in hydrolysates of hemicelluloses from forages. They included some evidence that the dissolved but undetermined material is a complex of carbohydrate with phenolic constituents.

When the wheat straw and other fractions were analyzed via GC, the sugars recoveries were poor and variable (60.0–20.5%). These poor recoveries probably result from the presence of noncarbohydrate substances (minerals, phenolics, and nitrogenous components) in the hydrolysates, which interfere with the derivatization to alditol acetates. Therefore, the GC data were used only to confirm identity of the sugars and to provide additional information on the relative concentrations of sugars in the hydrolysates. In a few cases, the more sensitive GC method provided evidence for the presence of trace quantities of individual sugars that were not detected by HPLC.

For purposes of comparison with earlier studies, the data for sugars and uronic acids in the present report can be normalized to 100% carbohydrate, e.g., by dividing the determined values by 0.422 in the case of crude hemicellulose. If this is done, the crude hemicelluloses and the fractions A and B contain about 73 ± 4% xylose, 16 ± 4% arabinose, 5 ± 1% glucose, and 6.7 ± 1.5% uronic acid. Earlier data (Aspinall and Meek, 1956; Bishop, 1953) indicate a composition of about 78% xylose, 7% arabinose, 0% glucose, and 5% uronic acid for wheat straw crude hemicellulose. The significantly higher values for arabinose and glucose in the present study probably result from the protecting effects of the nitrogen and sodium borohydride used during isolation of the hemicellulose. The ratio of xylose/arabinose varied from 3.3 in wheat straw to 6.4 in crude hemicellulose. The ratio of xylose/arabinose was greater in crude hemicellulose than in hemicelluloses A and B and commercial xylan (Table II).

Fermentation of Wheat Straw and Its Fractions. Fermentation of various plant polysaccharides and agricultural residues by any microbe including human colon bacteria depends on several factors: (i) degree of branching and type and sequence of glycosidic linkages present; (ii) composition (sugars and uronic acids); (iii) accessibility of polysaccharides to bacterial enzymes; (iv) association of polysaccharides with inhibitory components such as polyphenolics, metallic ions, etc. Earlier studies (Salyers et al., 1977b) have shown that the *Bacteroides* strains selected for the present study will ferment commercial xylan.

None of the *B. ovatus* strains fermented wheat straw or the residue from crude hemicellulose extraction (Table III). Attempts were made to make the wheat straw more accessible to microbial attack and/or to remove substances such as phenolics that might be inhibitory to microbial growth. Pretreatment of the straw with cellulase and pectinase, extraction with 80% ethanol and 2% sodium chloride, cold water, hot water, and acidified (1% acetic acid) methanol had little or no effect on fermentability. Wheat straw clearly has some unique compositional and/or structural features that make it highly resistant to degradation by *Bacteroides* strains, which are capable of degrading a variety of model "cell wall" and storage polysaccharides, including xylans.

In contrast to the results with wheat straw, the selected *B. ovatus* strains (except T4-7) fermented crude hemicellulose and hemicelluloses A and B from wheat straw, as well as commercial xylan. This was evidenced by a drop in pH (0.8 unit or more within 5 days of incubation at 37 °C) and heavy bacterial growth in the prereduced, defined medium. Hemicellulose B and xylan were better substrates for fermentation by *B. ovatus* strains (except T4-7) than crude hemicelluloses and hemicellulose A. Strain T4-7 slightly degraded or utilized the commercial xylan but not the hemicelluloses of wheat straw. This was also observed by a moderate growth in the inoculated tubes containing xylan. Thus, the results of the present study indicating fermentation of hemicelluloses of wheat straw by strains of *B. ovatus* parallel those of Salyers et al. (1977a,b) for the fermentation of xylan with the exception of strain T4-7. The strains of other species, *B. vulgatus*, *B. uniformis*, and *B. thetaiotaomicron*, did not ferment wheat straw nor its hemicellulose fractions; however, they slightly degraded the commercial xylan. On the basis of the fermentation results, *B. ovatus* strains (B4-11, C1-45, and 0038-1) were randomly selected and further tested for the presence, cellular localization, and inducibility of hemicellulose-degrading enzymes.

Induction and Localization of Hemicellulose-Degrading Enzymes in *Bacteroides ovatus*. *Bacteroides*

Table III. Fermentation[a] of Wheat Straw and Its Hemicellulose Fractions and Commercial Xylan by Different Strains of *Bacteroides* from Human Colon

strains	wheat straw or residue[b]	crude hemicellulose	hemicellulose A	hemicellulose B	xylan
B. ovatus					
B4-11	6.82 ± 0.05[c]	5.84 ± 0.04	6.17 ± 0.02	5.32 ± 0.03	5.17 ± 0.03
C1-45	6.82 ± 0.04	5.83 ± 0.06	6.15 ± 0.05	5.36 ± 0.03	5.08 ± 0.08
C2-26	6.88 ± 0.02	5.86 ± 0.05	6.25 ± 0.05	5.37 ± 0.03	5.23 ± 0.05
C8-14	6.79 ± 0.03	5.86 ± 0.03	6.15 ± 0.05	5.29 ± 0.02	5.17 ± 0.02
R3-39	6.79 ± 0.02	5.77 ± 0.04	6.13 ± 0.02	5.37 ± 0.06	5.18 ± 0.04
T4-7	6.97 ± 0.01	6.95 ± 0.03	7.00 ± 0.01	6.95 ± 0.02	6.40 ± 0.06
0038-1	6.81 ± 0.03	5.83 ± 0.05	6.05 ± 0.05	5.28 ± 0.03	5.05 ± 0.03
B. vulgatus					
C16-16	6.97 ± 0.01	6.95 ± 0.01	7.00 ± 0.01	6.95 ± 0.02	6.56 ± 0.03
B. uniformis					
C20-25	6.90 ± 0.05	7.00 ± 0.05	7.00 ± 0.02	7.00 ± 0.05	ND[d]
0061-1	6.94 ± 0.01	6.96 ± 0.05	6.91 ± 0.001	6.89 ± 0.04	6.46 ± 0.04
B. thetaiotaomicron					
C11-15	6.98 ± 0.01	6.97 ± 0.02	7.00 ± 0.01	6.97 ± 0.01	6.49 ± 0.01
7330-1	6.93 ± 0.04	7.00 ± 0.002	7.00 ± 0.02	6.97 ± 0.03	ND

[a] Fermentation is represented by a pH drop from the initial pH of 7.00. Inoculated tubes were incubated for 5 days at 37 °C. [b] Residue refers to the material remaining after extraction of crude hemicellulose. [c] Mean pH ± standard deviation; each reading is the average of three different experiments. [d] Not determined.

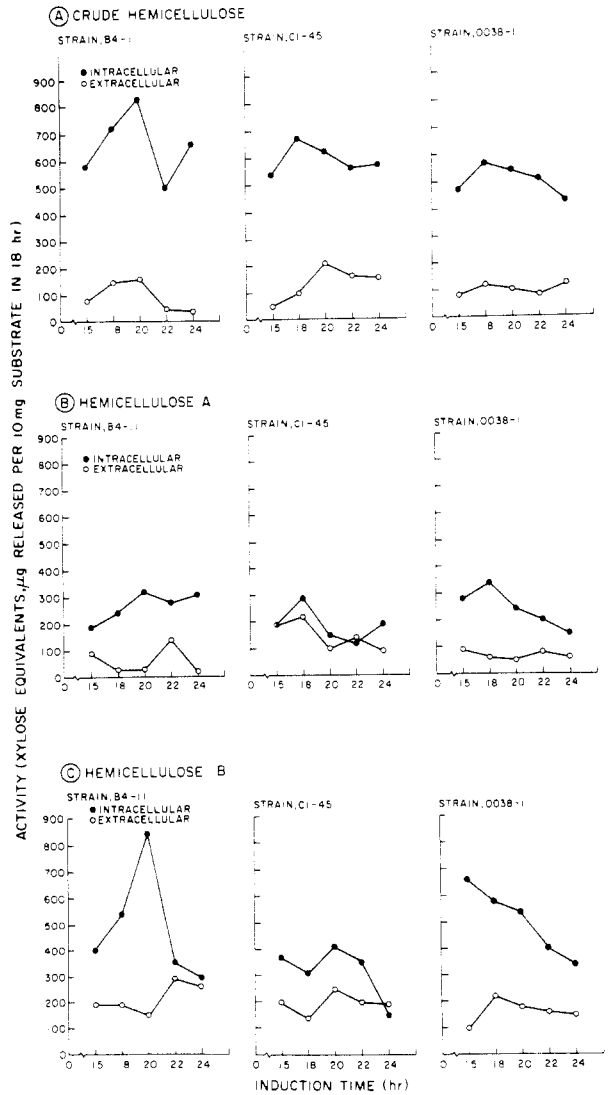

Figure 2. Induction and localization of hemicellulose-degrading enzymes in *B. ovatus* strains B4-11, C1-45, and 0038-1 when grown on crude hemicellulose, hemicellulose A, and hemicellulose B for various lengths of incubation at 37 °C.

ovatus strains B4-11, C1-45, and 0038-1 were grown on crude hemicellulose and hemicelluloses A and B to test for the presence of such enzymes. The extracellular and intracellular enzyme activities were measured as their ability to liberate reducing ends (calculated as xylose equivalents (μg) released from 10 mg of substrate in 18 h at 37 °C) from crude hemicellulose and hemicelluloses A and B. The extracellular and intracellular enzyme preparations were adjusted to equal volumes (10 mL) in order to eliminate volume differences. With all three strains, the intracellular enzyme fractions showed the highest level of activity (Figure 2). Earlier reports (Salyers et al., 1977a, 1982) indicate that xylan- and laminarin-degrading enzymes of *B. ovatus* strains are cell associated (intracellular) rather than extracellular; i.e., polysaccharidase activity is detected in sonically disrupted bacterial cells but not in the extracellular fluid. In contrast, Balascio et al. (1981) found that about 92% of the polysaccharidase activity is in the extracellular fluid, when a *B. ovatus* strain (ATCC 8433) was grown on guar gum based medium.

As shown in Figure 2, all three *B. ovatus* strains contained the maximum amount of intracellular enzymes when grown on crude hemicellulose and hemicelluloses A and B for 18–20 h at 37 °C. This increase in intracellular enzyme activity is evidence of enzyme induction. Growing beyond 20 h in the presence of each substrate did not result in an increase in enzyme levels (Figure 2). Of the three *B. ovatus* strains evaluated, B4-11 generally had the highest intracellular enzyme activity when grown on crude hemicellulose or hemicelluloses A and B for 20 h at 37 °C. It was selected for further study of the products and enzymatic mechanism of wheat straw hemicelluloses and xylan degradation. Those studies are under way.

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Is pH Drop a Valid Measure of Extent of Protein Hydrolysis?

Samuel M. Mozersky* and Reynold A. Panettieri

Utilizing nine foods as substrates, protein digestibility as determined by the two-step, pH-drop procedure of Satterlee et al. was compared to the extent of hydrolysis at the end of the incubation period. The extent to which the substrate protein was hydrolyzed was found to be much less than the calculated digestibility, as expected. However, the digestibility and extent of hydrolysis did not correlate with one another in any reasonable way. It is concluded that, although pH drop can be used to follow the course of hydrolysis of a single substrate, and in spite of a demonstrated correlation between pH drop and in vivo protein digestibility, pH drop alone cannot serve as a measure of extent of protein hydrolysis for the comparison of different substrates.

Since evaluation of the protein digestibility of food products by in vivo methods is very slow and costly, a quick, reliable in vitro procedure to replace them has been sought. Satterlee and co-workers (Satterlee and Kendrick, 1979; Satterlee et al., 1979, 1982) have proposed a procedure in which protein digestibility is calculated from the drop in pH obtained after in vitro digestion of the substrate, initially at pH 8.00, with pancreatic, intestinal, and bacterial enzymes. The correlation between their in vitro results and those obtained in vivo is impressive.

In our laboratory we observed that the digestibility of various substrates as determined by the procedure of Satterlee et al. seemed to bear little or no relation to the extent of protein hydrolysis after digestion of the same substrates with pepsin, Pronase, and kidney peptidase (Mozersky and Panettieri, 1983). There are two possible explanations of this: (1) The digestion procedure used by Satterlee et al. is very different from our own. (2) The pH drop obtained on hydrolysis of a protein is dependent on factors other than extent of hydrolysis and cannot be used as a measure of the extent of protein hydrolysis. The latter possibility is examined in the work presented here.

Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118.

MATERIALS AND METHODS

Materials. The trypsin, chymotrypsin, and peptidase used were the products specified by Hsu et al. (1977). The bacterial protease was Sigma Chemical Co. Type XIV (Pronase E) from *Streptomyces griseus*, the product specified by Satterlee et al. (1982).

Substrates are listed in Table I with their proximate compositions. All of the substrates are solids with a particle size sufficiently small to pass an 80-mesh screen, with the exception of the brain preparation, which was ground to pass a 2-mm screen.

Assays for Total Nitrogen and for Amino and Amide Group Content. Nitrogen content was determined by Kjeldahl digestion followed by assay with ninhydrin (Jacobs, 1962). Twenty-milligram samples of the substrates were digested according to the procedure of Willits