APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1988, p. 878–883
0099-2240/88/040878-06$02.00/0

Vol. 54, No. 4

Neutral Sugar Composition of Extracellular Polysaccharides Produced by Strains of *Butyrivibrio fibrisolvens*†

ROBERT J. STACK

Northern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois 61604

Received 5 October 1987/Accepted 13 January 1988

The extracellular polysaccharides (EPSs) produced by 37 isolates presently classified as *Butyrivibrio* species (or more specifically as *Butyrivibrio fibrisolvens*) were purified from glucose-grown cultures. The neutral sugar compositions of these EPSs were determined by both thin-layer and gas-liquid chromatographic techniques. Results showed that while the neutral sugar composition of the EPS was constant for a given strain, it varied considerably between strains. In addition, several acidic components in the EPS, of both known and unknown structure, were detected artifactually as acetylated lactones, the acetylated alditols derived from these lactone(s), or both. Two novel components, L-altrose and the acidic sugar 4-*O*-[1-carboxyethyl]-D-galactose, were common constituents of the EPS from some strains of *B. fibrisolvens*. These and other EPS compositional features were used to sort isolates of *B. fibrisolvens* into groups which may have taxonomic significance. A scheme for sorting isolates into these groups, and the relative relationships between groups, is proposed.

Butyrivibrio fibrisolvens is a commonly isolated bacterial species found in both ruminal and cecal portions of the gastrointestinal tracts of mammals. The species has been described (4) as an anaerobic, curved, rod-shaped, butyric acid-forming bacterium, which stains gram-negatively but which apparently has a thin gram-positive cell wall (6). A large number of isolates fitting this general description have been obtained from the gastrointestinal tracts of cattle, sheep, goats, pigs, Alaskan reindeer, and other ruminant animals.

The various isolates placed in the genus *Butyrivibrio*, or more specifically identified as *B. fibrisolvens*, display tremendous diversity in morphology, nutritional requirements, substrates fermented, and fermentation products produced. Strains have been described that are cellulolytic (27), xylanolytic (9), amylolytic (4), pectinolytic (10), lipolytic (17), and proteolytic (14). Most strains evidently possess a single polar flagellum; however, nonflagellated strains have also been reported (27). This heterogeneity has prompted several studies which have attempted to classify isolates into relatedness groups based on one or more phenotypic characteristics.

Thus, Shane et al. (27) organized cellulolytic strains into two groups based on differences observed in the patterns of short-chain volatile fatty acids produced or consumed during growth. Other studies have tried to exploit differences in nutritional requirements (26) or immunochemical properties (18, 23, 24, 29) as a means of further differentiating these isolates. It seems clear that the taxonomic criteria (25) that describe the genus *Butyrivibrio* and differentiate the two species in this genus (*B. fibrisolvens* and *B. crossatus*) are not very precise and will probably be revised. Several investigators have suggested that comprehensive biochemical analyses, genetic analyses, or both will be required to sort out these isolates and to define further species or genera as necessary (4, 11, 27). A simple and reliable means of categorizing representative strains from laboratories around the world, along with comparing new isolates with these previously described strains, is sorely needed.

Preliminary studies undertaken in this laboratory showed that most strains of *B. fibrisolvens* produced extracellular polysaccharides (EPSs) when grown on a defined medium. Compositional analysis showed that several unusual sugars were constituents of these EPSs and suggested that these may serve as useful traits for sorting out the diverse group of organisms presently classified in the genus *Butyrivibrio*. The present study was initiated with the goal presented above kept in mind. EPSs from a large number of representative isolates were purified, and their neutral sugar components were identified and quantitated. In this report the results of this approach are described.

MATERIALS AND METHODS

Organisms. Representative isolates classified in the genus *Butyrivibrio* or specifically as *B. fibrisolvens* were obtained from various laboratories as follows: M. P. Bryant, Department of Dairy Science, University of Illinois, Urbana, Ill.: strains D1, A38, and 49 (5); *Butyrivibrio*-like strains B385-1 and B385-2-33 (3); and strain C-14; B. A. Dehority, Agricultural Research and Development Center, The Ohio State University, Wooster, Ohio: strains H4a, H10b, H13b, and H17c (9); strains D16f and D30g (10), strains ARD-22a and ARD-23c (11); strains E9a, E21c, and E46a (12); and strains CF1b, CF2d, CF3, CF3a, CF3c, and CF4c (21); G. P. Hazlewood, Department of Biochemistry, Agricultural Research Council, Institute of Animal Physiology, Babraham, Cambridge, England: strains 1L6-31 and NOR-37 (29), strain B835 (19), strain LM8/1B (16), strain S-2 (17), and strains PI-7 and PI-26 (18); R. B. Hespell, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Ill.: strain R-28 and duplicates of strains D1, A38, and 49; R. I. Mackie, Animal and Dairy Science Research Institute, Irene, Republic of South Africa: strains CE51 and CE52 (27); N. O. Van Gylswyk, Council for Scientific and Industrial Research, National Chemical Research Laboratory, Pretoria, Republic of South Africa: strain AcTF2 (20), strain 12 (32), and strains X6C61 and X10C34; and M. J. Allison, National Animal Disease Laboratory, U.S. Department of

† This paper is dedicated to Marvin P. Bryant for his outstanding contributions to the study of *B. fibrisolvens* and to the field of ruminant microbiology.

Agriculture, Ames, Iowa: duplicates of strains D1, A38, and 49. Two isolates were also obtained from the American Type Culture Collection, Rockville, Md.: *Butyrivibrio* sp. strain C-3 (7) (ATCC 29550) and an additional duplicate of strain D1 (ATCC 19171).

Media and growth conditions. The chemically defined medium of Cotta and Hespell (8) was used for all studies, with 1% glucose present as the carbon and energy source. This medium was supplemented with 0.3% Trypticase (BBL Microbiology Systems, Cockeysville, Md.) for a few strains which would not grow on the defined medium alone. A few selected strains were also grown with 1% xylose in place of glucose to test whether the carbon source affected polysaccharide production. Cultures generally were grown in 500 ml of anaerobic medium in 1,000-ml round-bottom flasks and were grown to the stationary phase at 37°C.

Polysaccharide purification. Cells were removed from the stationary-phase cultures by centrifugation (6,000 × *g*, 30 min, 4°C) and discarded. Culture supernatants were lyophilized to dryness and reconstituted in 50 to 100 ml of water. These reconstituted supernatants were placed in dialysis bags (molecular weight cutoff, 6,000 to 8,000; Spectrum Medical Industries, Los Angeles, Calif.) which had been boiled twice to remove contaminating polysaccharides, metals, and sulfides, and then were dialyzed against three to four changes of 40 liters of water over 3 days at 4°C. Dialysates were ultracentrifuged (185,000 × *g*, 2.5 h, 4°C) to remove protein precipitates and other insoluble materials. The resulting supernatants were lyophilized to dryness and designated as crude EPS. More purified preparations of EPS (pure EPS) were obtained by phenol extraction of reconstituted crude EPS by the procedure of Westphal and Jann (33).

Neutral sugar determinations. The neutral sugar composition of either crude or pure EPS was obtained by gas-liquid chromatography (GLC) of alditol acetates, which were prepared as previously described (30) by using a modification of the procedure described by Albersheim et al. (1). Monosaccharides were also analyzed by thin-layer chromatography on silica gel plates as described previously (30).

Miscellaneous analyses and chemicals. The yield of either crude or pure EPS was determined by the anthrone procedure (13), with glucose used as the standard. Protein determinations were made as described by Lowry et al. (22), with bovine serum albumin used as a standard. Trifluoroacetic acid, inositol, sodium borohydride, and D-altrose were from Sigma Chemical Co. (St. Louis, Mo.). All other chemicals and media components were reagent grade or better.

RESULTS

The defined medium of Cotta and Hespell (8) supported good growth and EPS production for nearly all strains of *B. fibrisolvens* used in this study. Strains 20-32, B385-1, CF2d, and X10C34 were exceptions and required supplementation with Trypticase (final concentration, 0.3% [wt/vol]) before satisfactory growth was obtained. Strains H13b and E46a required supplementation with both Trypticase and yeast extract (final concentration, 0.2% [wt/vol]) for satisfactory growth. However, the mannans in yeast extract copurified with the EPS from these two strains and greatly complicated data analysis. Thus, these two strains were not studied further.

Strains of *B. fibrisolvens* varied widely in their ability to produce EPSs. Yields ranged from a low of 2 to 4 mg/liter (strains B385-2-33, B385-1, and 20-32) to over 500 mg/liter (strain CF3), with yields of most strains falling in the range of 100 to 400 mg/liter. Unfortunately, yields were often variable for a given strain (data not shown). The cultures of the highest EPS-producing strains were extremely viscous, and considerable difficulty was encountered in sedimenting the cells during centrifugation. Generally, a 1:1 dilution of culture supernatant with water followed by recentrifugation sufficed for complete cell removal from cultures.

Dialysis of the reconstituted culture supernatants against water was generally accompanied by the formation of a flocculent precipitate. Measurement of carbohydrate and protein content in dialyzed supernatant fluid and in both the pellet and supernatant fluid from ultracentrifuged dialysates showed the flocculent precipitate to be proteinaceous material. For most strains, this simple dialysis and ultracentrifugation step removed 50 to 80% of the extracellular protein, leaving 90 to 100% of the carbohydrate in the supernatant (data not shown).

The low EPS-producing strains were not adequately deproteinized by this treatment, and crude EPS preparations were phenol extracted to obtain more purified EPS preparations. Other strains (CF3, D1, 49, X6C61, LM8/1B, ARD-22a, and others) were also phenol extracted to obtain purified EPS for comparison of carbohydrate composition with crude EPS. In all cases, no discernible difference in the relative monosaccharide composition was noted between crude or pure EPS for a given strain (data not shown).

The composition and molar ratios of neutral sugar constituents in the EPS of each strain inferred from both alditol acetate and TLC data are presented in Table 1. Although the yield of EPS for a given strain sometimes varied, as noted above, the relative ratio of neutral sugar components was constant and strain specific. However, tremendous strain-to-strain variations were encountered in both the nature and relative amount of neutral sugar components in the EPS from *B. fibrisolvens*. Most strains produced a galactose-containing EPS; thus, sugar compositions in Table 1 are generally given relative to those for galactose. This was not appropriate for group III or group V strains; hence, altrose and mannose, respectively, were used as references (Table 1).

Several unknown acidic components of the EPS from strains of *B. fibrisolvens* were also detected as their acetylated lactones; the corresponding acetylated neutral sugars, which were derived from artifactual reduction of these lactones; or both (unpublished data). These, when present, are indicated with a "Yes" in Table 1.

Twelve isolates of *B. fibrisolvens* produced EPS that commonly contained rhamnose, galactose, and glucose. The EPSs from strains C-3 and 1L6-31 contained no additional neutral sugar components, whereas those from strains B835, D16f, E9a, E21c, LM8/1B, NOR-37, and S-2 also contained mannose. The EPSs from strains D1 (the type strain of the species) (5), AcTF2, and A38 contained fucose as well as mannose.

The EPSs from eight isolates commonly contained galactose, glucose, and an unknown component (subsequently identified as 4-*O*-[1-carboxyethyl]-D-galactose [R. J. Stack, T. M. Stein, and R. D. Plattner, submitted for publication]) that was detected by GLC as its acetylated lactone and designated as lactyl-galactose in Table 1. The EPS from strain X6C61 also contained rhamnose, which was found in much lower amounts in the EPS from strains 12, CE51, CE52, D30g, H10b, 49, and H17c. An additional unknown component, designated as Unk-1 in Table 1, was detected by GLC analysis of alditol acetates prepared from the EPSs of strains 49 and H17c (Fig. 1). The identity of the component giving rise to this peak is presently under investigation.

TABLE 1. Relative neutral sugar composition and acidic components detected in the EPS from strains of *B. fibrisolvens*[a]

Group	Type and strain	No. of independent replicates	Relative composition of the following neutral sugar:						Presence of the following acidic component:				
			Rha	Fuc	Alt	Man	Gal	Glu	Lactyl-Gal	Unk-1	Unk-2	Unk-3	IdUA
I	A												
	D1	4	2.9	1.0	—	2.7	1.0	3.5	—	—	—	—	—
	AcTF2	2	2.6	1.2	—	2.0	1.0	5.8	—	—	—	—	—
	A38	2	2.0	0.9	—	0.3	1.0	4.6	—	—	—	—	—
	B												
	B835	2	1.7	—	—	0.6	1.0	2.9	—	—	—	—	—
	D16f	1	0.8	—	—	0.4	1.0	4.8	—	—	—	—	—
	E9a	1	1.2	—	—	0.2	1.0	1.1	—	—	—	—	—
	E21c	1	1.5	—	—	0.2	1.0	1.6	—	—	—	—	—
	LM8/1B	1	2.5	—	—	0.6	1.0	1.8	—	—	—	—	—
	NOR-37	4	3.0	—	—	0.6	1.0	2.1	—	—	—	—	—
	S-2	1	2.1	—	—	0.7	1.0	2.1	—	—	—	—	—
	C												
	C-3	2	0.4	—	—	tr	1.0	0.5	—	—	—	—	—
	1L6-31	1	2.4	—	—	tr	1.0	0.8	—	—	—	—	—
II	A												
	C-14	2	1.4	1.3	1.5	0.2	1.0	6.0	Yes	—	—	—	—
	PI-26	2	2.1	0.6	0.2	0.7	1.0	1.9	tr	—	—	—	—
	ARD-22A	2	1.7	0.3	0.1	tr	1.0	1.3	tr	—	—	—	—
	B												
	H4a	1	0.2	0.1	0.6	—	1.0	1.3	Yes	—	—	—	—
	R-28	2	0.2	—	0.5	—	1.0	1.1	Yes	—	—	—	—
	ARD-23c	2	0.2	0.1	0.9	—	1.0	2.6	Yes	—	—	—	—
	PI-7	2	0.1	tr	0.3	tr	1.0	0.6	tr	—	—	—	—
III													
	CF1b	1	tr	—	1.0	—	tr	2.4	Yes	—	—	Yes	—
	CF2d	1	tr	—	1.0	—	tr	3.7	Yes	—	—	Yes	—
	CF3	4	tr	—	1.0	—	tr	2.9	Yes	—	—	Yes	—
	CF3a	1	tr	—	1.0	—	tr	2.9	Yes	—	—	Yes	—
	CF3c	1	tr	—	1.0	—	tr	2.4	Yes	—	—	Yes	—
	CF4c	1	tr	—	1.0	—	tr	2.5	Yes	—	—	Yes	—
IV	A												
	X6C61	11	0.7	—	—	—	1.0	1.0	Yes	—	—	—	Yes
	B												
	49	5	tr	—	—	—	1.0	2.1	Yes	Yes	—	—	—
	H17c	2	tr	—	—	—	1.0	2.1	Yes	Yes	—	—	—
	C												
	12	2	tr	—	—	tr	1.0	1.2	Yes	—	—	—	—
	CE51	1	tr	—	—	—	1.0	1.2	Yes	—	—	—	—
	CE52	1	tr	—	—	—	1.0	1.2	Yes	—	—	—	—
	D30g	1	0.1	—	—	—	1.0	2.2	Yes	—	—	—	—
	H10b	2	tr	—	—	—	1.0	1.0	Yes	—	Yes	—	—
V													
	B385-1	4	—	—	—	1.0	0.1	0.4	—	—	—	—	—
	B385-2-33	3	—	—	—	1.0	0.1	0.7	—	—	—	—	—
	X10C34	2	0.4	—	—	1.0	0.2	2.7	—	—	—	—	—
	20-32	2	2.0	—	—	1.0	0.5	0.2	—	—	—	—	—

[a] Abbreviations: Rha, rhamnose; Fuc, fucose; Alt, altrose; Man, mannose; Gal, galactose; Glu, glucose; Lactyl-Gal, lactyl-galactose; Unk-1, Unk-2, and Unk-3, unknowns 1, 2, and 3, respectively; IdUA, iduronic acid; —, not found; tr, trace, defined as less than 0.1.

Similarly, the EPS from strain H10b contained one additional, as yet unidentified, component designated as Unk-2 in Table 1.

The EPS from six isolates (CF1b, CF2d, CF3, CF3a, CF3c, and CF4c) contained altrose, glucose, lactyl-galactose, and one additional unknown component designated as Unk-3 in Table 1. The combined area of the altritol hexaacetate and 2,3,4-tri-*O*-acetyl-1,6-anhydroaltrose peaks were used to calculate relative compositions, as previously described (30).

The EPSs from seven isolates commonly contained glucose, altrose, galactose, and rhamnose. However, the EPSs from strains R-28, ARD-23c, PI-7, and H4a generally contained much lower relative amounts of rhamnose than did strains C-14, PI-26, and ARD-22a and were also generally devoid of mannose. Fucose and lactyl-galactose were sometimes also detected.

The four remaining isolates (strains B385-1, B385-2-33, 20-32, and X10C34) all produced very low amounts of EPS and were not well-suited to these kinds of analyses. Nonetheless, the compositional data of these EPSs are also shown in Table 1. An overall scheme which was developed and used to sort the strains into the groups shown in Table 1 is presented in Fig. 2.

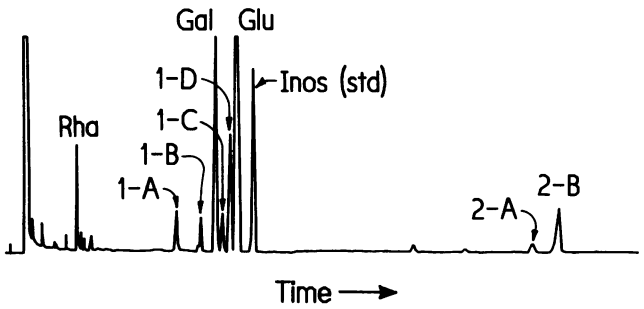

FIG. 1. GLC analysis of alditol acetates prepared from the EPS of *B. fibrisolvens* 49. Peaks corresponding to rhamnose (Rha), galactose (Gal), glucose (Glu), and inositol (Inos; internal standard [std]) are indicated. Peaks 1-A, 1-B, and 1-C represent different acetylated lactones derived from the acidic sugar designated Unk-1. Peak 1-D represents the corresponding acetylated alditol derived from borohydride reduction of the lactone(s) formed from Unk-1 (unpublished data). Similarly, peak 2-B represents the acetylated lactone derived from 4-*O*-[1-carboxyethyl]-D-galactose, and peak 2-A represents the corresponding artifactually generated acetylated alditol derived from this compound (Stack et al. submitted).

The substitution of xylose for glucose in the growth media did not affect the types or relative amounts of neutral sugar components in the EPSs obtained from strains D1, 49, or X6C61 (data not shown). In addition, strain D1 from four independent sources (R. B. Hespell, M. P. Bryant, M. J. Allison, and the American Type Culture Collection) yielded EPSs with nearly equivalent compositions. Similarly, the EPSs from strain 49 obtained from R. B. Hespell, M. P. Bryant, and M. J. Allison were indistinguishable on the basis of neutral sugar composition (data not shown).

DISCUSSION

Gutierrez et al. (15) first described EPS production from the glycosidic moiety of saponin by strains of *B. fibrisolvens*. In the present study it has been shown that EPS production in the genus *Butyrivibrio* is a general phenomenon, with 33 of 37 strains examined producing significant amounts of EPS. The neutral sugar compositions of these EPSs varied greatly (Table 1) and again illustrates the considerable heterogeneity of the isolates presently classified in the genus *Butyrivibrio*. Nevertheless, strains could be sorted into clusters, or groups, according to EPS compositional data by means of the scheme shown in Fig. 2.

The alditol acetate procedure described by Albersheim et al. (1) is reportedly specific for determining neutral sugar components of polysaccharides. However, this was not a completely satisfactory method for analyzing and determining neutral sugar components of the EPS produced by *B. fibrisolvens*, as several acidic components were detected artifactually as acetylated lactones, the corresponding acetylated alditols, or both. For example, iduronic acid, a constituent of the EPS from *B. fibrisolvens* X6C61, formed sodium borohydride-reducible lactones which, on acetylation, yielded iditol hexaacetate (unpublished data). Similarly, 4-*O*-[1-carboxyethyl]-D-galactose readily formed lactones; however, these were not appreciably reduced by the reduction step and were detected by GLC as acetylated lactones (Stack et al., submitted; see also Fig. 1). The other unknown components detected by GLC in the EPS of *B. fibrisolvens* (Unk-1 in strains 49 and H17c, Unk-2 in strain H10b, and Unk-3 in group III strains) were all acidic sugars of the lactyl ether type, which yielded acetylated lactones, the corresponding acetylated neutral sugar (unpublished data), or both. Determination of the exact structures of these compounds is presently being pursued. Since lactonization is not necessarily quantitative or reproducible, the exact amount of these acidic constituents relative to that of other EPS components cannot readily be ascertained (see Table 1). Therefore, quantitation of the acidic components shown in Table 1 was not possible when the described methodologies were used, and the data in Table 1 should not be taken as a comprehensive listing of acidic components in the EPS of *B. fibrisolvens*.

Isolates of *B. fibrisolvens* classified as group I strains produced EPS that contained galactose, glucose, and rhamnose. The isolates could be divided into three types based on the additional presence of fucose, mannose, or both. The EPS from type A strains (D1, AcTF2, and A38) contained both fucose and mannose, while that from type B strains

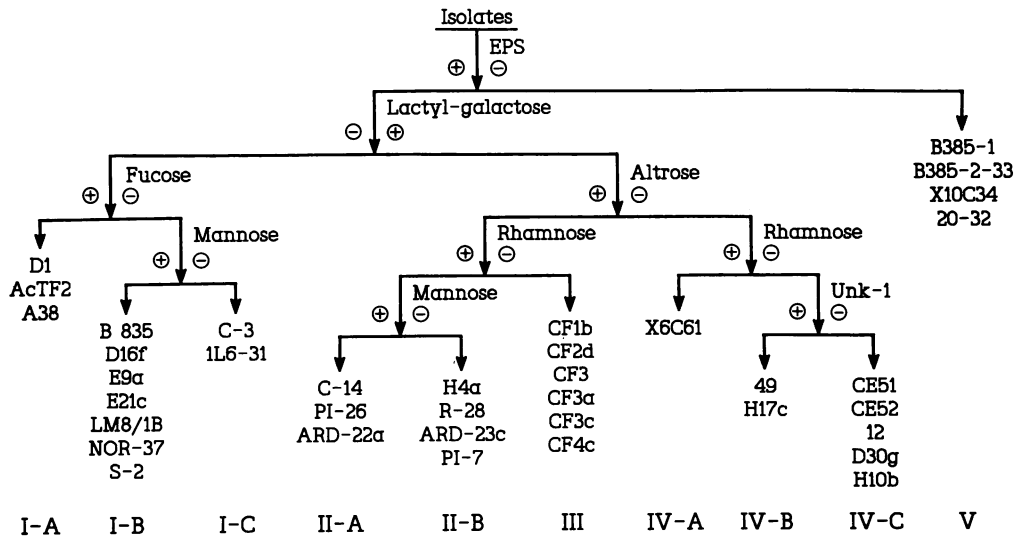

FIG. 2. Proposed scheme for sorting isolates of *B. fibrisolvens* into relatedness clusters, or groups, based on EPS compositional data.

(B835, D16f, E9a, E21c, LM8/1B, NOR-37, and S-2) contained only mannose. Type C strains (C-3 and 1L6-31) yielded EPS with no additional components.

Group II isolates all produced an altrose-containing EPS, with galactose, glucose, and rhamnose as additional components. The altrose in the EPS of strains ARD-23c, C-14, H4a, PI-26, and R-28 was previously shown to have the L-configuration (R. J. Stack and L. D. Ericsson, submitted for publication). This unusual hexose is the C-5 epimer of D-galactose, and thus far it has only been found in the EPS of *B. fibrisolvens* (30). Similarly, the EPS of group II strains contained 4-*O*-[1-carboxyethyl]-D-galactose, an unusual acidic sugar that thus far has also been found only in the EPS from *Butyrivibrio* sp. (Stack, et al., submitted). This compound, as mentioned above, was detectable as its acetylated lactone, as shown for strain 49 in Fig. 1. The EPS from strains PI-7, PI-26, and ARD-22a contained only small amounts of this compound. However, strain PI-7 was classified along with strains H4a, R-28, and ARD-23c as group II-type B strains because of the lower relative contents of rhamnose, fucose, and mannose compared with those in group II-type A strains (C-14, PI-26, and ARD-22a).

Group III isolates were all ovine cecal isolates (21) and, in general, produced the highest amounts of EPS of the strains examined. The EPS from these strains (CF1b, CF2d, CF3, CF3a, CF3c, and CF4c) contained altrose, glucose, 4-*O*-[1-carboxyethyl]-D-galactose, and an additional unidentified acidic component of the lactyl ether type (Unk-3). The altrose in the EPS of strain CF3 was previously shown to have the L-configuration (30); presumably, this is also true for the other strains in group III. A relationship between group II and group III strains was inferred from the common occurrence of two very unusual sugars, namely, L-altrose and 4-*O*-[1-carboxyethyl]-D-galactose.

Group IV isolates produced EPS that also contained 4-*O*-[1-carboxyethyl]-D-galactose, as well as galactose and glucose. Strain X6C61 was uniquely classified as a group IV-type A isolate because, in addition to rhamnose, the EPS of this strain contains iduronic acid (unpublished data). This relatively rare and unusual acidic component was detected artifactually as iditol hexaacetate, as mentioned above. The EPSs of strains 49 and H17c contained an additional acidic sugar of the lactyl ether type (Unk-1), and were thus classified as group IV-type B strains. The rest of the isolates in this group (strains 12, CE51, CE52, D30g, and H10b) were classified as group IV-type C strains. The EPS from strain H10b uniquely contained Unk-3, still another as yet unidentified acidic component of the lactyl ether type (unpublished data). Groups II, III, and IV strains all produced 4-*O*-[1-carboxyethyl]-D-galactose-containing EPS, from which it can be inferred that these groups may be more closely related to each other than they are to group I strains.

Group V was created for those strains that produced very low amounts of EPS (2 to 4 mg/liter) and is best viewed as a miscellaneous category of isolates not particularly well-suited to these kinds of analyses. As such, strains placed in this group are not necessarily related or unrelated to each other, and the relationship of this group to the other groups is uncertain. Strains B385-1, B385-2-33, X10C34, and 20-32 were placed in this group.

While compositional analyses of extracellular polysaccharides have not been used extensively in bacterial taxonomy, strains and species of *Rhizobium* can be differentiated in this manner (31). Similarly, a chemotaxonomic system for fungi based on the analysis of fungal cell wall polysaccharides and first proposed by Bartnicki-Garcia (2) has been supported by much subsequent genetic and biochemical data. Marine red algae of the genus *Gracilaria*, which produce the cell wall polysaccharide commonly known as agar, can be similarly classified into species at least partly on the basis of the compositional and structural features of the agar that is produced (34).

Similarly, serological and immunochemical techniques are commonly used and widely accepted taxonomic methods. These techniques usually employ production of a specific antibody against an antigenic determinant, which is commonly a carbohydrate component of a bacterial cell. On this basis, EPS compositional analysis is somewhat comparable in approach and, in fact, may yield more detailed information on the species in question. There are limitations to this approach, as there are to all single-method taxonomic procedures. Neutral sugar compositional analysis alone does not define polysaccharide structure, just as amino acid composition and guanosine and cytosine content do not define protein and DNA structure, respectively. Moreover, it has been well-documented that polysaccharide structure (unlike protein or DNA structure) can vary with media composition, stage of growth, temperature, carbon source, etc. (31). These variables were defined and controlled as much as possible in the present study; and even when deliberately altered (i.e., substitution of xylose for glucose), they appeared to have little effect on the EPS composition from strains of *B. fibrisolvens*. EPS compositions of *B. fibrisolvens* similarly did not seem to vary significantly with stage of growth, at least for strains D1, 49, and X6C61 (Stack and Cotta, unpublished data). Thus, these factors may not affect the composition of the EPS from strains of *Butyrivibrio* as much as they can for other organisms.

Results from previous studies provide some additional support for the groups presented in Table 1. For example, Roche et al. (26) found that the nutritional requirements of strains 12 and CE51 (both group IV-type C) were similar (27). Strains CE51 and CE52 are both cellulolytic and were previously placed in a group 1 defined by Shane et al. (27). A possible relationship between strains 12 and 49 was inferred by Sharpe et al. (28), who immunochemically detected glycerol teichoic acids in both of these strains (NOR-37 and 1L6-31 were also found to be similar in this regard).

In a detailed study by Hazlewood et al. (18), 63 presumptive butyrivibrios were screened for their ability to cross-react with monoclonal antibodies raised against *B. fibrisolvens* PI-7 (group II-type B). Strains 1L6-31, LM8/1B, and B835, all of which were classified as group I-type B in the present study, showed a weak cross-reaction to a specific monoclonal antibody against strain PI-7. However, the same antibody neither reacted with strain NOR-37 (another group I-type B strain) nor strain 49 (group IV-type B). Sharpe and Reiter (29) have demonstrated that cells of NOR-37 contain the O-4 antigen of the genus *Salmonella*, implying that an abequosyl-mannosyl-rhamnosyl-galactose repeat unit exists as part of the ultrastructure of this strain. While the relationship of the antigenic determinant(s) examined in the previous study (29) to the EPS analyzed in the current study is unclear, it is interesting that the EPS of strain NOR-37 contains rhamnose, mannose, and galactose. Sharpe and Reiter (29) also noted that cells of strain 1L6-31 (group I-type C) cross-reacted with a polyvalent O-antiserum, which may imply a relationship between this strain and NOR-37. Since polysaccharides often represent important antigenic determinants, it is interesting to speculate that the considerable diversity encountered in the genus *Butyrivibrio* by other workers who used immunochemical techniques (18, 23, 24)

may be a reflection of the diversity in EPS composition reported here.

Results of the present study confirm the general diversity of the isolates characterized as *Butyrivibrio* species, but definitively organizes them into groups that have well-defined EPS phenotypic characteristics. EPS compositions of additional strains can be easily obtained by the described methodologies, allowing a quick and presumptive identification and comparison with other better-studied strains. Similarly, the groups in Table 1 provide a useful basis for testing the relatedness of these isolates by other comprehensive genetic or biochemical means.

ACKNOWLEDGMENTS

I gratefully acknowledge the excellent technical assistance of Linda Ericsson and Robert Wolf. Helpful suggestions and consultations on the preparation of this manuscript with G. L. Cote, R. B. Hespell, and M. A. Cotta are also gratefully acknowledged.

LITERATURE CITED

1. **Albersheim, P., D. J. Nevins, P. D. English, and A. Karr.** 1967. A method for the analysis of sugars in plant cell wall polysaccharides by gas-liquid chromatography. Carbohydr. Res. **5:** 340–345.
2. **Bartnicki-Garcia, S.** 1968. Cell wall chemistry, morphogenesis, and taxonomy of fungi. Annu. Rev. Microbiol. **22:**87–108.
3. **Bryant, M. P.** 1956. The characteristics of strains of *Selenomonas* isolated from bovine rumen contents. J. Bacteriol. **72:** 162–167.
4. **Bryant, M. P.** 1984. *Butyrivibrio*, p. 641–643. *In* N. R. Krieg (ed.), Bergey's manual of systemic bacteriology, 9th ed., vol. 1. The Williams & Wilkins Co., Baltimore.
5. **Bryant, M. P., and N. Small.** 1956. The anaerobic monotrichous butyric acid-producing curved rod-shaped bacteria of the rumen. J. Bacteriol. **72:**16–21.
6. **Cheng, K. J., and J. W. Costerton.** 1977. Ultrastructure of *Butyrivibrio fibrisolvens*: a gram-positive bacterium? J. Bacteriol. **129:**1506–1512.
7. **Cheng, K. J., G. A. Jones, F. J. Simpson, and M. P. Bryant.** 1969. Isolation and identification of rumen bacteria capable of anaerobic rutin degradation. Can. J. Microbiol. **15:**1365–1371.
8. **Cotta, M. A., and R. B. Hespell.** 1986. Proteolytic activity of the ruminal bacterium *Butyrivibrio fibrisolvens*. Appl. Environ. Microbiol. **52:51–58.**
9. **Dehority, B. A.** 1966. Characteristics of several bovine rumen bacteria isolated with xylan medium. J. Bacteriol. **91:**1724–1729.
10. **Dehority, B. A.** 1969. Pectin-fermenting bacteria isolated from the bovine rumen. J. Bacteriol. **99:**189–196.
11. **Dehority, B. A.** 1975. Characterization studies on rumen bacteria isolated from Alaskan reindeer (*Rangifer tarandus* L.), p. 228–240. *In* Proceedings of the First International Reindeer and Caribou Symposium. University of Alaska, Anchorage.
12. **Dehority, B. A., and J. A. Grubb.** 1977. Characterization of the predominant bacteria occurring in the rumen of goats (*Capra hircus*). Appl. Environ. Microbiol. **33:**1030–1036.
13. **Dische, E.** 1962. Color reactions of carbohydrates, p. 477–512. *In* R. L. Whistler and M. L. Wolfrom (ed.), Methods in carbohydrate chemistry, vol. 1. Academic Press, Inc., New York.
14. **Fulghum, R. S., and W. E. C. Moore.** 1963. Isolation, enumeration, and characteristics of proteolytic ruminal bacteria. J. Bacteriol. **85:**808–815.
15. **Gutierrez, J., R. E. Davis, and I. L. Lindahl.** 1959. Characteristics of saponin-utilizing bacteria from the rumen of cattle. Appl. Microbiol. **7:**304–308.
16. **Hazlewood, G. P., and R. M. C. Dawson.** 1975. Isolation and properties of a phospholipid-hydrolyzing bacterium from ovine rumen fluid. J. Gen. Microbiol. **89:**163–174.
17. **Hazlewood, G. P., and R. M. C. Dawson.** 1979. Characteristics of a lipolytic and fatty acid-requiring *Butyrivibrio* sp. isolated from the ovine rumen. J. Gen. Microbiol. **112:**15–27.
18. **Hazlewood, G. P., M. K. Theodorou, A. Hutchings, D. J. Jordan, and G. Galfre.** 1986. The preparation and characterization of monoclonal antibodies to a *Butyrivibrio* sp. and their potential use in the identification of rumen butyrivibrios, using an enzyme-linked immunosorbent assay. J. Gen. Microbiol. **132:**43–52.
19. **Henderson, C.** 1973. The effects of fatty acids on pure cultures of rumen bacteria. J. Agric. Sci. Camb. **81:**107–112.
20. **Henning, P. A.** 1979. Examination of methods for enumerating hemicellulose-utilizing bacteria in the rumen. Appl. Environ. Microbiol. **38:**13–17.
21. **Lewis, S. M., and B. A. Dehority.** 1985. Microbiology and ration digestibility in the hindgut of the ovine. Appl. Environ. Microbiol. **50:**356–363.
22. **Lowry, O. H., N. J. Roseborough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
23. **Margherita, S. S., and R. E. Hungate.** 1963. Serological analysis of *Butyrivibrio* from the bovine rumen. J. Bacteriol. **86:**855–860.
24. **Margherita, S. S., R. E. Hungate, and H. Storz.** 1964. Variation in rumen *Butyrivibrio* strains. J. Bacteriol. **87:**1304–1308.
25. **Moore, W. E. C., J. L. Johnson, and L. V. Holdeman.** 1976. Emendation of *Bacteroidacea* and *Butyrivibrio* and descriptions of *Desulfomonas* gen. nov. and ten new species in the genera *Desulfomonas*, *Butyrivibrio*, *Eubacterium*, *Clostridium*, and *Ruminococcus*. Int. J. Syst. Bacteriol. **26:**238–252.
26. **Roche, C., H. Albertyn, N. O. van Gylswyk, and A. Kistner.** 1973. The growth response of cellulolytic acetate-utilizing and acetate-producing butyrivibrios to volatile fatty acids and other nutrients. J. Gen. Microbiol. **78:**253–260.
27. **Shane, B. S., L. Gouws, and A. Kistner.** 1969. Cellulolytic bacteria occurring in the rumen of sheep conditioned to low-protein teff hay. J. Gen. Microbiol. **55:**445–457.
28. **Sharpe, M. E., J. H. Brock, and B. A. Phillips.** 1975. Glycerol teichoic acid as an antigenic determinant in a gram-negative bacterium *Butyrivibrio fibrisolvens*. J. Gen. Microbiol. **88:**355–363.
29. **Sharpe, M. E., and B. Reiter.** 1972. Common antigenic determinant in a rumen organism and in *Salmonellae* containing the antigen O4. Appl. Microbiol. **24:**615–617.
30. **Stack, R. J.** 1987. Identification of L-altrose in the extracellular polysaccharide from *Butyrivibrio fibrisolvens* strain CF3. FEMS Microbiol. Lett. **48:**83–87.
31. **Sutherland, I. W.** 1985. Biosynthesis and composition of gram-negative bacterial extracellular and wall polysaccharides. Annu. Rev. Microbiol. **39:**243–270.
32. **van Gylswyk, N. O., and C. E. G. Roche.** 1970. Characteristics of *Ruminococcus* and cellulolytic *Butyrivibrio* species from the rumens of sheep fed differently supplemented teff (*Eragrostic tef*) hay diets. J. Gen. Microbiol. **64:**11–17.
33. **Westphal, O., and K. Jann.** 1965. Bacterial lipopolysaccharide extraction with phenol-water and further applications of the procedure, p. 83–91. *In* R. L. Whistler (ed.), Methods in carbohydrate chemistry, vol. 5. Academic Press, Inc., New York.
34. **Yaphe, W., and M. Duckworth.** 1972. The relationship between structures and biological properties of agar, p. 15–22. *In* Proceedings of the International Seaweed Symposium, vol. 7. University of Tokyo Press, Tokyo.