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# Some Chemical and Physical Properties of Extracellular Polysaccharides Produced by *Butyrivibrio fibrisolvens* Strains

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**Most strains of *Butyrivibrio fibrisolvens* are known to produce extracellular polysaccharides (EPs). However, the rheological and functional properties of these EPs have not been determined. Initially, 26 strains of *Butyrivibrio* were screened for EP yield and apparent viscosities of cell-free supernatants. Yields ranged from <1.0 to 16.3 mg per 100 mg of glucose added to the culture. Viscosities ranged from 0.71 to 5.44 mPa · s. Five strains (CF2d, CF3, CF3a, CE51, and H10b) were chosen for further screening. The apparent viscosity of the EP from each of these strains decreased by only 50 to 60% when the shear rate was increased from 20 to 1,000 $s^{-1}$. Strain CE51 produced the EP having the highest solution viscosity. A detailed comparison of shear dependency of the EP from strain CF3 with xanthan gum showed that this EP was less shear sensitive than xanthan gum and, at a shear rate of 1,000 $s^{-1}$, more viscous. EPs from strains CF3 and H10b were soluble over a wide range of pH (1 to 13) in 80% (vol/vol) ethanol-water or in 1% (wt/vol) salt solutions. The pH of 1% EP solutions was between 4.5 and 5.5. Addition of acid increased solution viscosities, whereas addition of base decreased viscosity. EPs from strains CF3, CE51, and H10b displayed qualitatively similar infrared spectra. Calcium and sodium were the most abundant minerals in the three EPs. The amounts of magnesium, calcium, and iron varied considerably among the EPs, but the potassium contents remained relatively constant.**

The genus *Butyrivibrio* is composed of obligately anaerobic, curved, rod-shaped, butyric acid-forming bacteria found in both ruminal and cecal portions of the gastrointestinal tracts of mammals (9). Most strains of *Butyrivibrio fibrisolvens* are known to produce extracellular polysaccharides (EPs) when grown on a defined medium. EPs from several strains have been characterized on the basis of sugar composition (20). The compositional analysis of EPs produced by 37 strains of *B. fibrisolvens* showed that unusual sugars such as L-altrose (19, 21), 4-*O*-(1-carboxyethyl)-D-galactose (23), and 4-*O*-(1-carboxyethyl)-L-rhamnose (24) are common constituents of the EPs. However, the rheological and functional properties of these EPs have not been studied.

The commercial utility of polysaccharides known as hydrocolloids is based on their wide range of functional properties. The property of thickening or imparting viscosity to a solution is fundamental to hydrocolloids (6, 8, 16, 17). Generally, the functional properties of a given polysaccharide can be influenced by both the sugar composition and the spatial structure of its basic units. The unusual sugar constituents of EPs produced by *Butyrivibrio* strains suggest that these EPs might have unique rheological and functional properties. Therefore, this study was undertaken to screen strains of the strictly anaerobic ruminal bacterium *B. fibrisolvens* for EP production on a chemically defined medium containing glucose. Second, EPs were isolated from selected strains and their rheological properties were determined.

## MATERIALS AND METHODS

**Organisms and growth conditions.** All bacterial strains used were obtained from R. B. Hespell's stock culture collection (National Center for Agricultural Utilization Research, U.S. Department of Agriculture, Peoria, Ill.) and are representative strains isolated from diverse sources (13). A chemically defined medium (2) with 1% glucose as the carbon source was used for all studies. Cultures were prepared and grown anaerobically at 37°C. For routine screening of EP production and cultural viscosities, 10-ml test tube culture broths were used. To detect the presence of extracellular polysaccharides during growth, samples of the culture were removed, mixed with India ink, and observed with a phase light microscope.

**Crude EP preparation.** For large-scale EP preparations, 0.5- to 2.0-liter cultures were used. Cells were removed from stationary-phase cultures by centrifugation (10,000 × *g* for 1 h at 4°C) and discarded. Culture supernatants were dialyzed against three to four changes of 15 volumes of distilled water over 3 days at 4°C. Dialysates were recentrifuged to remove protein precipitates and other insoluble materials. The resulting supernatant fluids were lyophilized, and the resulting fluffy white material was designated as crude EP.

**Viscometry.** Apparent viscosities of total cultures, cell-free culture fluids, or crude EP solutions were determined with a Haake viscometer equipped with an M500 measuring drive unit and an NV sensor system. For crude EPs, the dried material was dissolved in distilled water by stirring and hydration for 24 h at ambient temperature prior to testing. All viscosity measurements were repeated at least twice, using these freshly prepared solutions. A Wells-Brookfield cone/plate viscometer (model RVTVP) equipped with a CP-40 cone spindle (0.8°) and a Wells-Brookfield viscometer (model LVTDV-II) equipped with a small-sample adaptor (SC4-18) were also used to determine viscosity of EP solutions at different shear rates.

**Infrared spectroscopy.** KBr pellets of the EPs were prepared by freezing 1.3 mg of EP under liquid nitrogen in a stainless-steel vial containing two stainless-steel balls. The vial was shaken rapidly on a Wig-L-Bug amalgamator for 20

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s, refrozen in liquid nitrogen, and shaken again for 20 s to convert EP into a fine powder. The powdered EP was then mixed for 30 s with 400 mg of powdered KBr in the sample vial, and a 300-mg portion of the mixture was pressed to form a KBr disk. The KBr-EP disks were analyzed by Fourier transform infrared (FTIR) spectroscopy, using a Laser Precision Analytical RFX-75 spectrometer. Spectra with 4-$cm^{-1}$ resolution were obtained by averaging 32 interferometer scans, using Happ-Genzel apodization.

**Atomic absorption spectroscopy.** Samples were prepared by wet-ashing of freeze-dried EPs (5, 15). Calcium, magnesium, iron, sodium, and potassium were determined by atomic absorption, using a Perkin-Elmer 303 spectrophotometer.

## RESULTS

**Initial screening of strains.** *Butyrivibrio* strains were screened on the basis of apparent viscosity of the cell-free culture fluids. Actual EP production and optical density of cultures were also considered as selection parameters. Strains which did not grow well in the defined medium with 1% glucose or which did not produce enough EP for viscosity measurement were eliminated from further screening. Table 1 shows the results of these initial screening procedures.

The data show considerable variation between strains in the amount of EP produced and in the apparent viscosities of the cell-free supernatants. Yields of EP ranged from <1.0 (i.e., nonproducing strains) to 16.3 mg per 100 mg of glucose added to the culture. Viscosities ranged from 0.71 to 5.44 mPa · s. In general, strains belonging to groups I and II produced small amounts of EPs and exhibited low supernatant viscosities, whereas the opposite was observed with group III strains. Cultures of CF strains, originally isolated from sheep ceca (12), were viscous, and longer centrifugation times were needed to sediment the cells. Some strains of group IV also made reasonable amounts of EP, but the cells could be easily centrifuged out of these cultures. India ink staining of culture samples of various strains and CF strains, in particular, showed the presence of an amorphorous EP matrix surrounding the cells. This EP matrix often was 10- to 100-fold greater in volume than the cell volume.

**Secondary screening.** Apparent viscosity measurement of four concentrations (0.10, 0.20, 0.50, and 1.00%) of EPs produced by seven selected strains showed that EP solutions were mildly pseudoplastic. Five of these strains (CF2d, CF3, CF3a, H10b, and CE51) were chosen for further screening. Viscosities of EPs at different shear rates are shown in Table 2 for these five strains. Strain CE51 produced an EP with the highest solution viscosity, while the EP from strain H10b showed the lowest solution viscosity. Increasing the shear rate from 20 to 1,000 $s^{-1}$ decreased the apparent viscosity by only 50 to 60% with all five EPs. A detailed comparison of shear dependency of xanthan gum and the EP of strain CF3 was made (Fig. 1). The data showed that the EP from strain CF3 was less shear sensitive than xanthan gum and, at shear rates of 1,100 $s^{-1}$, more viscous.

**Solution properties.** All EPs from *Butyrivibrio* strains were soluble in distilled water and produced a clear solution. Strains in groups II, III, and IV produce EPs that contain acid constituents (20), and these behaved like acidic polysaccharides in ethanolic solution. Two representatives of these polysaccharides, EPs from strains CF3 and H10b, were studied in more detail. Both polysaccharides were soluble over a wide range (1 to 13) of pH in 80% (vol/vol) ethanol-water or in 1% (wt/vol) salt solutions such as KCl, NaCl, $MgCl_2$, or $CaCl_2$. However, addition of ethanol (55%, vol/vol, in final concentration) to either EP dissolved in 1% KCl resulted in precipitation of the polysaccharide. The precipitated polysaccharides could be redissolved in distilled water, dialyzed, and recovered after lyophilization. The recov-

TABLE 1. Production and viscosity of EPs made by *Butyrivibrio* strains[a]

| EP group[b] | Strain | Growth[c] | EP wt (mg)[d] | Viscosity (mPa · s)[e] |
|---|---|---|---|---|
| I-A | AcTF2 | 1.20 | 1.90 | 0.96 |
| I-B | 835 | 1.30 | 1.00 | 0.87 |
| | D16f | 1.30 | 5.30 | 1.06 |
| | E21c | 1.30 | 2.60 | 0.92 |
| I-C | C3 | 0.58 | 1.00 | 1.13 |
| II-A | PI-26 | 0.52 | 1.00 | 1.18 |
| | ARD-22a | 0.61 | 1.00 | 0.71 |
| II-B | PI-7 | 0.56 | 1.00 | 0.79 |
| III | CF3 | 1.20 | 11.80 | 5.21 |
| | CF1b | 1.20 | 3.90 | 1.55 |
| | CF3a | 1.10 | 16.30 | 4.48 |
| | CF3c | 1.15 | 7.70 | 5.12 |
| | CF2d | 1.20 | 15.70 | 1.20 |
| | CF4c | 1.05 | 11.90 | 4.84 |
| IV-B | 49 | 1.15 | 6.10 | 2.00 |
| | H17c | 1.20 | 2.60 | 1.27 |
| IV-C | 12 | 1.30 | 1.44 | |
| | CE51 | 1.20 | 7.60 | 3.67 |
| | CE52 | 1.15 | 6.80 | 4.46 |
| | H10b | 0.78 | 10.00 | 5.44 |
| Other | 110 | 1.15 | 1.00 | 0.95 |
| | 111 | 1.30 | 1.00 | 1.17 |
| | 112 | 1.10 | 1.00 | 1.21 |
| | 113 | 1.45 | 1.00 | 0.94 |
| | 117 | 1.15 | 1.60 | 1.03 |
| | D23g | 1.10 | 4.10 | 1.21 |

[a] Strains examined that grew, but produced little EP, included 114, 12, ARD-31a, C14, D30g, H13b, IL-631, R28, and X6C61.
[b] Grouping based on neutral sugar compositions of EPs (20).
[c] Maximal optical density at 660 nm.
[d] Dry weight of crude EP from 10-ml culture.
[e] Apparent viscosity at shear rate ($D$) of 1,000 $s^{-1}$.

TABLE 2. Viscosity versus shear rate of 0.20% solutions of EPs produced by *B. fibrisolvens* CF2d, CF3, CF3a, CE51, and H10b

| Shear rate ($s^{-1}$)[a] | Viscosity (mPa · s) of given strain | | | | |
|---|---|---|---|---|---|
| | CF2d | CF3 | CF3a | CE51 | H10b |
| 18.75 | 11.4 | 9.8 | 11.4 | 16.4 | 7.4 |
| 37.5 | 7.2 | 9.8 | 9.2 | 11.1 | 7.2 |
| 75 | 5.9 | 8.7 | 8.8 | 9.8 | 6.7 |
| 150 | 5.6 | 8.3 | 8.7 | 9.2 | 6.5 |
| 375 | 5.5 | 6.9 | 7.9 | 8.3 | 6.3 |
| 750 | 5.3 | 6.6 | 7.0 | 7.2 | 5.8 |
| 1,000[b] | 4.4 | 5.3 | 5.0 | 5.4 | 4.0 |

[a] Determined by using Brookfield cone/plate-type viscometer.
[b] Determined by using Haake viscometer.

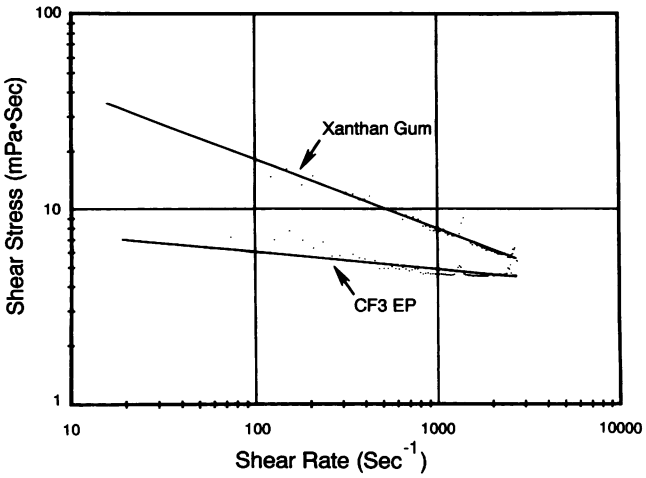


FIG. 1. Apparent viscosity versus shear rate curves of 0.20% solutions of EP from strain CF3 and xanthan gum, respectively.

ered polysaccharides displayed the original thickening properties.

The pH of 1.00% EP solutions ranged from 4.5 to 5.5. By using 1.0% EP solutions, addition of acid increased viscosities, whereas addition of NaOH generally decreased viscosity by 20 to 30% and increased the pH from 5 to 10. Addition of ethanol (67%, vol/vol, in final concentration) to an alkaline solution (>pH 12) of EP from strain H10b resulted in precipitation of the polysaccharide.

**Mineral-element content.** The mineral contents of dialyzed crude EPs after wet-ashing were determined by using atomic absorption spectroscopy (representative data are shown in Table 3). Calcium and sodium were most abundant in the EPs studied. The content of sodium and potassium did not vary greatly among the EPs. However, each EP showed differences in amounts of calcium, magnesium, and iron. The EP from strain CE51 showed the highest levels for all three divalent metals as well as the highest total mineral-element content. In view of its FTIR spectrum, which showed the most free carboxylic acid still present, the mineral level indicates that the original carboxylic acid content was also highest and reflects the very acidic nature of this EP.

**Infrared spectra.** The EPs from strains CF3, CE51, and H10b displayed similar FTIR spectra (Fig. 2). The spectra exhibited a broad O—H stretching absorption band centered around 3,420 cm$^{-1}$, a minor C—H stretching band at 2,930 cm$^{-1}$, and several C—O absorption bands in the 1,400- to 900-cm$^{-1}$ region, including a set of strong C—O stretching bands at 1,038 and 1,078 cm$^{-1}$ (7, 18). The spectra also displayed absorption bands of carboxylate groups at 1,598 to 1,616 and near 1,400 cm$^{-1}$. The first carboxylate band resulted from asymmetrical stretching. The second arose from symmetrical stretching (18). The intensity of C=O stretching of un-ionized carboxylic acid was different among EPs. The EP from strain CE51 displayed the strongest C=O band at 1,734 cm$^{-1}$, whereas that from strain H10b exhibited a minor band. Measured in absorbance units, the FTIR spectra provide estimates of components in the EPs. The absorbance spectra of EPs from strains CE51, H10b, and CF3 show respective increases of metal carboxylate (1,420 to 1,460 cm$^{-1}$) content as the free carboxylic acid (1,734 and 1,250 cm$^{-1}$) content decreases. The 1,250-cm$^{-1}$ band is due to C—O stretching in the free carboxylic acid. Both the C=O band and the C—O band absorb separately as parts of the same COOH structure. However, in the carboxylate form neither of these individual absorptions occurs due to the resonance structure of the $COO^-$ ion. Hence, the 1,734- and 1,250-cm$^{-1}$ bands increase or decrease together, as shown in Fig. 2.

TABLE 3. Mineral-element contents in EPs produced by *Butyrivibrio* strains[a]

| Strain | Content (ppm) | | | | |
|---|---|---|---|---|---|
| | Na | K | Mg | Ca | Fe |
| CE51 | 13,581 | 2,235 | 9,775 | 16,563 | 338 |
| H10b | 10,615 | 2,156 | 4,736 | 9,328 | 212 |
| CF3 | 12,521 | 2,056 | 6,260 | 9,364 | 72 |

[a] Determined by atomic absorption spectroscopy.

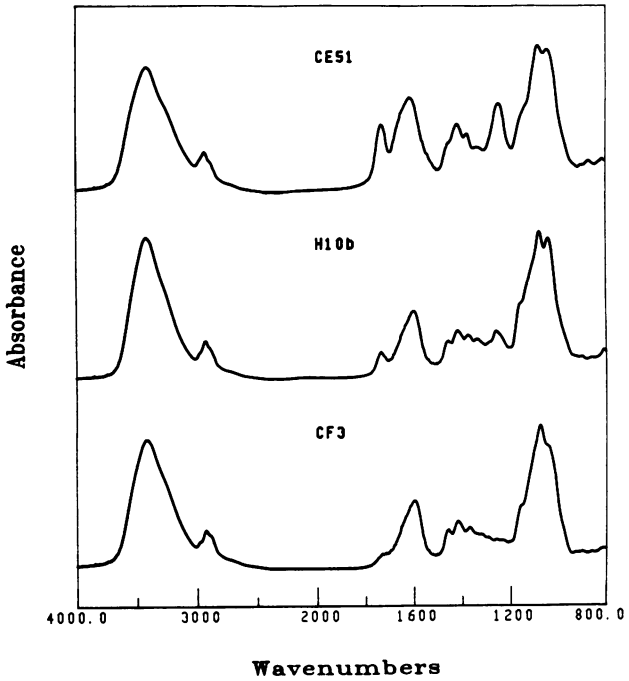


FIG. 2. FTIR spectra of EPs produced by *Butyrivibrio* strains CE51, H10b, and CF3.

## DISCUSSION

The results of this study show that the amounts of EP made by *B. fibrisolvens* vary greatly among strains. The highest EP-producing strains are the CF strains. Four of six strains examined converted 11 to 16% of the initial glucose to EP (Table 1). Solutions of the EPs made by these strains display considerable viscosity, which decrease by only 50 to 60% at high shear rates (Table 2). A detailed comparison of the EP made by strain CF3 with xanthan gum showed that the *B. fibrisolvens* EP was, in fact, less sensitive to shear, particularly at high shear rates (Fig. 1). Other comparisons of the EPs made by strains H10b and CE51 with xanthan gum also yielded similar, but less dramatic results (data not shown). The pseudoplasticity of these EPs is an advantageous flow property which may be useful, particularly when these EPs are mixed with other materials in commercial applications.

The differences in rheological properties among the EPs of H10b, CE51, and CF3 do not appear to be directly correlated to their neutral sugar compositions. The EPs of the CF strains contain L-altrose and glucose, whereas the EPs of the other two strains contain galactose and glucose (20). However, all of these EPs contain one or more acidic sugar components. The most common acidic sugar is a lactylgalactose, which is 4-*O*-(1-carboxyethyl)-D-galactose in the EP of *B. fibrisolvens* 49 (23). The infrared spectra of these polysaccharides (Fig. 2) clearly show the presence of carboxylate groups. Most likely, these carboxylate groups can serve as binding sites for divalent metal ions. The absorbance spectra also suggest that the carboxylic acid groups in the EPs are disproportionately neutralized by the basic metal ions. In the EP from strain CE51, the free carboxylic acid and metal-carboxylate conjugate exist in roughly equal amounts, whereas in the EP from CF3 nearly all of the ionizable groups exist in the neutralized carboxylate form. Because this disproportionation is itself pH dependent, the FTIR spectra agree with the observed pH dependence of the viscosities of these EPs. The spectra also suggest that some of the viscosity differences among the EPs may therefore be attributable to differences in the degree of neutralization. Hence, the rheological properties of these EPs may be adjustable by controlling pH.

Isolation of the EPs involving distilled water dialysis steps yielded EPs that varied in both amounts of magnesium, calcium, and iron and total amount of divalent metal ions bound (Table 3). Subsequent dialysis of these EPs, using EDTA-containing buffers, indicated that almost all of the divalent metal ions could be removed and much of the sodium or potassium could be replaced by one of these monovalent ions by dialysis against the appropriate buffer (data not shown). Further work is needed to determine whether the differences in divalent metal compositions of the EPs reflect differences in binding affinities or capacities or both. If the EPs are found to have high and/or selective affinities for these or other metal ions, the EPs may be commercially useful as chelating agents for removal of metals from aqueous or other solutions.

The solution properties were studied in more detail with the EPs from strains H10b and CF3. The data indicated that the EPs behaved like acidic polysaccharides. The EPs dissolved readily in water, 80% (vol/vol) ethanol, or 1% (wt/vol) salt solutions. Preliminary examination by gel filtration chromatography of the solubilized EPs suggest that they did not have a homogeneous molecular weight and displayed a polydispersity over the range of 300 to 800 kDa. Although the EPs were soluble from pH 1 to 13, the addition of ethanol (67%, vol/vol, final concentration) to alkaline solutions resulted in a precipitated EP which could be redissolved in water and displayed normal viscosity. The solution properties of these two EPs could be used in developing commercial procedures for large-scale preparation of the polysaccharides. While not examined in this study, the manipulation of cultural conditions may lead to enhancement of EP production. A previous study with *B. fibrisolvens* NYX has shown that EP production can be influenced by the nitrogen sources used for growth (26). The ability to grow most *B. fibrisolvens* strains on defined media suggests that further nutritional manipulations are possible.

As shown by this study and previous studies (1, 20), most *B. fibrisolvens* strains produce an EP. The natural functions of this EP are not clearly known. These organisms readily attach to cellulose fibers (14) and to feed and other particles in the rumen (3, 4). Presumably, the EP aids in this association and possibly hinders diffusion of the diverse extracellular enzymes such as xylanases (9), proteases (2), or esterases (10) made by *B. fibrisolvens*. The EP may also serve in a protective role since the presence of unusual sugars might make the EP more resistant to the action of glycanases (19, 24). Regardless of the natural roles, the EPs of some *B. fibrisolvens* strains may have potential commercial applications on the basis of their rheological properties, as shown in this study. These applications could be similar to the ones currently being used for xanthan gum. Alternatively, the carboxylate groups of the EPs of *B. fibrisolvens* strains might be used as functional groups to link these EPs to starch or man-made polymers to form new polysaccharides having unique properties. In addition, some EPs could serve as a source of rare sugars such as L-altrose (CF strains [19]) or L-iduronic acid (strain X6C61 [22]).

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## REFERENCES

1. **Cheng, K.-J., and J. W. Costerton.** 1977. Ultrastructure of *Butyrivibrio fibrisolvens*: a gram-positive bacterium? J. Bacteriol. **129**:1506–1512.
2. **Cotta, M. A., and R. B. Hespell.** 1986. Proteolytic activity of the rumen bacterium *Butyrivibrio fibrisolvens*. Appl. Environ. Microbiol. **52**:51–58.
3. **Dehority, B. A., and J. A. Grubb.** 1981. Bacterial population adherent to the epithelium on the roof of the dorsal rumen of sheep. Appl. Environ. Microbiol. **41**:1424–1427.
4. **Dinsdale, D., E. J. Morris, and J. S. D. Bacon.** 1978. Electron microscopy of the microbial populations present and their modes of attach on various cellulosic substrates undergoing digestion in the sheep rumen. Appl. Environ. Microbiol. **36**:160–168.
5. **Garcia, W. J., C. W. Blessin, and G. E. Inglett.** 1972. Mineral constituents in corn and wheat germ by atomic absorption spectroscopy. Cereal Chem. **49**:158–167.
6. **Glickman, M.** 1982. Food applications of gums, p. 270–295. *In* D. R. Lineback and G. E. Inglett (ed.), Food carbohydrates. AVI Publishing Co., Westport, Conn.
7. **Greene, R. V., S. N. Freer, and S. H. Gordon.** 1988. Determination of solid-state fungal growth by fourier transform infrared-photoacoustic spectroscopy. FEMS Microbiol. Lett. **52**:73–78.
8. **Ha, Y. W., R. L. Thomas, L. A. Dyck, and M. E. Kunkel.** 1989. Calcium binding of two microalgal polysaccharides and selected industrial hydrocolloids. J. Food Sci. **54**:1336–1340.
9. **Hespell, R. B., and M. P. Bryant.** 1981. The genera *Butyrivibrio*, *Succinivibrio*, *Succinimonas*, *Lachnospira*, *Selenomonas*, p. 1479–1494. *In* M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes. A handbook on habitats, isolation and identification of bacteria. Springer-Verlag, Berlin.
10. **Hespell, R. B., and P. J. O'Bryan-Shah.** 1988. Esterase activities in *Butyrivibrio fibrisolvens* strains. Appl. Environ. Microbiol. **54**:1917–1922.
11. **Hespell, R. B., R. Wolf, and R. J. Bothast.** 1987. Fermentation of xylans by *Butyrivibrio fibrisolvens* and other ruminal bacteria. Appl. Environ. Microbiol. **53**:2849–2853.
12. **Lewis, S. M., and B. A. Dehority.** 1985. Microbiology and ration digestibility in the hindgut of the ovine. Appl. Environ. Microbiol. **50**:356–363.
13. **Mannarelli, B. M.** 1988. Deoxyribonucleic acid relatedness among strains of the species *Butyrivibrio fibrisolvens*. Int. J. Syst. Bacteriol. **38**:340–347.
14. **Rasmussen, M. A., B. A. White, and R. B. Hespell.** 1989.

Improved assay for quantitating adherence of ruminal bacteria to cellulose. Appl. Environ. Microbiol. **55**:2089–2091.
15. **Rendleman, J. A.** 1978. Metal-polysaccharide complexes. Part 2. Food Chem. **3**:127–162.
16. **Sanderson, G. R.** 1981. Polysaccharides in foods. Food Technol. **7**:50–57, 83.
17. **Sandford, P. A., and J. Baird.** 1983. Industrial utilization of polysaccharides, p. 411–490. *In* G. O. Aspinall (ed.), The polysaccharides. Academic Press, Inc., New York.
18. **Silverstein, R. M., G. C. Bassler, and T. C. Morrill.** 1974. Spectroscopic identification of organic compounds. John Wiley & Sons, New York.
19. **Stack, R. J.** 1987. Identification of L-altrose in the extracellular polysaccharide from *Butyrivibrio fibrisolvens* strain CF3. FEMS Microbiol. Lett. **48**:83–87.
20. **Stack, R. J.** 1988. Neutral sugar composition of extracellular polysaccharides produced by strains of *Butyrivibrio fibrisolvens*. Appl. Environ. Microbiol. **54**:878–883.
21. **Stack, R. J., and L. D. Ericsson.** 1988. Methods for the identification and analysis of L-altrose in bacterial polysaccharides. FEMS Microbiol. Lett. **56**:1–6.
22. **Stack, R. J., R. D. Plattner, and G. L. Cote.** 1988. Identification of L-iduronic acid as a constituent of the major extracellular polysaccharide produced by *Butyrivibrio fibrisolvens* strain X6C61. FEMS Microbiol. Lett. **51**:1–6.
23. **Stack, R. J., T. M. Stein, and R. D. Plattner.** 1988. 4-O-(1-Carboxyethyl)-D-galactose: a new acidic sugar from the extracellular polysaccharide produced by *Butyrivibrio fibrisolvens* strain 49. Biochem. J. **256**:769–773.
24. **Stack, R. J., and D. Weisleder.** 1990. 4-O-(1-Carboxyethyl)-L-rhamnose, a second unique acidic sugar found in an extracellular polysaccharide from *Butyrivibrio fibrisolvens* strain 49. Biochem. J. **268**:281–285.
25. **Wachenheim, D. E., and J. A. Patterson.** 1988. Potential for industrial polysaccharides from anaerobes. Enzyme Microb. Technol. **10**:56–57.
26. **Wachenheim, D. E., and J. A. Patterson.** 1990. Effects of nitrogen nutrition on the anaerobic production of extracellular polysaccharide by *Butyrivibrio fibrisolvens* strain nyx, abstr. O-48, p. 271. Abstr. 90th Annu. Meet. Am. Soc. Microbiol. 1990. American Society for Microbiology, Washington, D.C.