Phylogenetic Analysis of *Butyrivibrio* Strains Reveals Three Distinct Groups of Species within the *Clostridium* Subphylum of the Gram-Positive Bacteria

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The phylogenetic positions of 40 *Butyrivibrio* strains were determined by performing a comparative sequence analysis of the 16S rRNA genes of these organisms. We found that all of the strains which we studied belong to cluster XIVa (M. D. Collins, P. A. Lawson, A. Willems, J. J. Cordoba, J. Fernandez-Garayzabal, P. Garcia, J. Cai, H. Hippe, and J. A. E. Farrow, Int. J. Syst. Bacteriol. 44:812–826, 1994) of the *Clostridium* subphylum of the gram-positive bacteria, which also includes several *Clostridium*, *Coprococcus*, *Eubacterium*, and *Ruminococcus* species. We also found that the *Butyrivibrio* strains which we examined were genotypically heterogeneous and exhibited 12 distinct rRNA sequence types. The 12 rRNA sequence types formed three distinct lineages in cluster XIVa, which were separate from each other and from all other species belonging to this cluster. One lineage consisted of strains which exhibited a single rRNA type and corresponded to the species *Butyrivibrio crossotus*. The second lineage consisted of 12 strains designated *Butyrivibrio fibrisolvens* which exhibited seven distinct rRNA sequence types. The type strain of *B. fibrisolvens* was a member of this lineage, but its position was peripheral. The third lineage comprised 26 *B. fibrisolvens* strains which exhibited four distinct rRNA sequence types. Tree topology and sequence divergence considerations indicated that the three lineages correspond to three separate genera and that the genus *Butyrivibrio* should be restricted to the group that contains the type strain of *B. fibrisolvens*.

The genus *Butyrivibrio* as currently defined (1) contains curved, non-spore-forming, strictly anaerobic, rod-shaped bacteria that are motile by means of one or more polar to subpolar flagella. *Butyrivibrio* strains are gram negative, but some strains (including the type strain) have been shown to possess an atypical gram-positive cell wall ultrastructure (4, 7, 12). These organisms have a fermentative metabolism, use mainly carbohydrates, and characteristically produce butyrate from the fermentation of glucose or mannose (1, 11). The butyrivibrios are a major component of the ruminal microflora and have been isolated from the gastrointestinal tracts of various animals, as well as from human, rabbit, and horse feces (1, 11). They contribute to fiber digestion in both animals (11, 18, 21) and humans (24) through degradation of cellulose and xylan (10, 18, 19, 27). The following two *Butyrivibrio* species are currently recognized: *Butyrivibrio fibrisolvens* (3), the type species of the genus, which includes monotrichous isolates obtained from animals, and *Butyrivibrio crossotus* (20), which includes lophotrichous isolates obtained from human feces. Because of the considerable morphological (3, 7), metabolic (2, 23, 25), and serological (9, 17) diversity displayed by *Butyrivibrio* strains, it is generally accepted that this genus contains several more species (1, 3, 11, 25). Early attempts to subdivide the genus on the basis of the phenotypic criteria mentioned above were not very successful because of the extreme heterogeneity of the strains (9, 25). More recently, large numbers of *Butyrivibrio* strains have been studied by chromosomal DNA-DNA hybridization and extracellular polysaccharide analyses. The results of these analyses also revealed the substantial heterogeneity in the genus and identified several groups of strains which may represent new species (15, 16, 26). The phylogenetic diversity

of the various groups identified by DNA-DNA hybridization is not clear at this time. It is not known whether these genotypically distinct organisms form a monophyletic group or constitute phenotypically similar but phylogenetically remotely related groups of species, as is the case, for example, with the genus *Ruminococcus* (22). To investigate the phylogenetic diversity of the genus *Butyrivibrio*, we compared partial 16S rRNA gene sequences of 40 *Butyrivibrio* strains. Our results indicate that the genus *Butyrivibrio*, as presently defined, is phylogenetically heterogeneous and encompasses three remotely related species groups.

MATERIALS AND METHODS

Strains used and determination of 16S rRNA gene sequences. The *Butyrivibrio* strains which we used are listed in Table 1. Lyophilized cells were resuspended in 500 µl of TES buffer (0.05 M Tris-HCl, 0.005 M EDTA, 0.05 M NaCl; pH 8.0), and total DNA was extracted by the method of Lawson et al. (14). The almost complete 16S rRNA gene was amplified by PCR by using conserved primers close to the 3' and 5' ends of the gene. The amplified fragment corresponded to positions 30 to 1521 of the *Escherichia coli* 16S rRNA gene. The PCR products were purified by using a Prep-A-Gene kit (Bio-Rad, Hercules, Calif.) according to the manufacturer's instructions and were sequenced directly by using a *Taq* Dye-Deoxy terminator cycle sequencing kit (Applied Biosystems, Inc., Foster City, Calif.) and a model 373A automatic DNA sequencer (Applied Biosystems, Inc.).

Phylogenetic analysis of the sequence data. Phylogenetic analyses were performed by using the Genetics Computer Group package (6) and the PHYLIP phylogeny inference package (8). To determine the closest known relatives of the *Butyrivibrio* strains, we performed preliminary searches in the EMBL data library with the program FASTA (6). The resulting sequences and the sequences of other strains known to belong to the same phylogenetic group were retrieved from the EMBL, GenBank, and Ribosomal Database Project data libraries and aligned with the sequences of the *Butyrivibrio* strains by using the program PILEUP (6). The rRNA alignment was corrected manually, and approximately 100 bases at the 5' end of the molecule were omitted from further analyses because of alignment uncertainties. A distance matrix was obtained by using the programs PRETTY (6) and DNADIST (8) and the Kimura-2 parameter, and a phylogenetic tree based on the neighbor-joining method was constructed by using the program NEIGHBOR (8). The stability of the groups was determined by performing a bootstrap analysis (500 replications) by using the programs DNABOOT, DNADIST, NEIGHBOR, and CONSENSE (8). For comparison,

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TABLE 1. Strains used

Strain ^a	Other designation(s)	Source ^b	Origin
<i>B. fibrisolvens</i>			
rRNA type 1 strain NCDO 2221 ^{Tc}	D1, ATCC 19171	Bryant	Bovine rumen
rRNA type 2 strains			
NCDO 2222 ^c	A38, ATCC 27208	Bryant	Bovine rumen
Bu 25	K202-21-07-6D	van Gylswyk	
rRNA type 3 strains			
NCDO 2398 ^c	B835	Mann	Heifer rumen
NCDO 2417	B834	Hobson	
Bu 22	K27BF1, 1	van Gylswyk	
rRNA type 4 strain NCDO 2432 ^c	Ce65	van Gylswyk	Sheep rumen
rRNA type 5 strain NCDO 2434 ^c	Bu 42, K13-26-037-A	van Gylswyk	
rRNA type 6 strains			
NCDO 2435 ^c	Bu 24, K10-02-04-6B	van Gylswyk	
NCDO 2436	Bu 44, Ce78	van Gylswyk	Sheep rumen
Bu 36	Ce47	van Gylswyk	Sheep rumen
rRNA type 7 strain Bu 43 ^c	Ce64	van Gylswyk	Sheep rumen
rRNA type 8 strains			
NCDO 2223 ^c	Bu 49	Bryant	Bovine rumen
NCDO 2418	Bu 27, 19	van Gylswyk	Sheep rumen
NCDO 2419	Bu 31, CXS 18	van Gylswyk	Sheep rumen
NCDO 2421	Bu 41, Ce58	van Gylswyk	Sheep rumen
Bu 12	K29CF, 12	van Gylswyk	Sheep rumen
Bu 15	K29HF, 15	van Gylswyk	Sheep rumen
Bu 17	K13KF, 17	van Gylswyk	Sheep rumen
Bu 26	18	van Gylswyk	Sheep rumen
Bu 28	20	van Gylswyk	Sheep rumen
Bu 29	21	van Gylswyk	Sheep rumen
Bu 30	24	van Gylswyk	Sheep rumen
Bu 32	CXS13	van Gylswyk	Sheep rumen
Bu 33	22	van Gylswyk	Sheep rumen
Bu 34	Ce36	van Gylswyk	Sheep rumen
Bu 35	Ce46	van Gylswyk	Sheep rumen
Bu 37	Ce51	van Gylswyk	Sheep rumen
Bu 38	Ce52	van Gylswyk	Sheep rumen
Bu 39	Ce53	van Gylswyk	Sheep rumen
Bu 40	Ce56	van Gylswyk	Sheep rumen
Ce52	Bu 38	van Gylswyk	Sheep rumen
rRNA type 9 strains			
NCDO 2249 ^c	Nor37	NIRD	Bovine rumen
NCDO 2400	1L 6-31	NIRD	Bovine rumen
NCDO 2438	Ce74	van Gylswyk	Sheep rumen
rRNA type 10 strain NCDO 2397 ^c	JL	Leatherwood	
rRNA type 11 strains			
NCDO 2399 ^c	Bu 21, RO3-21-08-7A	van Gylswyk	
Bu 21	NCDO 2399, RO3-21-08-7A	van Gylswyk	
<i>B. crossotus</i> rRNA type 12 strains			
NCDO 2416 ^{Tc}	Bu 46, T940A	VPI	Human feces
NCDO 2415	Bu 45, C3824	VPI	Human feces

^a NCDO, National Collection of Dairy Organisms, Reading, United Kingdom.

^b Bryant, M. P. Bryant, University of Illinois, Urbana; Hobson, P. N. Hobson, Rowett Research Institute, Aberdeen, United Kingdom; Leatherwood, J. M. Leatherwood, North Carolina State University, Raleigh; Mann, S. O. Mann, Rowett Research Institute, Aberdeen, United Kingdom; van Gylswyk, N. O. van Gylswyk, Veterinary Research Institute, Onderstepoort, South Africa; NIRD, National Institute for Research in Dairying, Shinfield, United Kingdom; VPI, Virginia Polytechnic Institute and State University, Blacksburg.

^c Strain whose nearly complete 16S rRNA gene sequence was determined as a representative of the rRNA type. Partial 16S rRNA gene sequences (approximately 600 bases proximal to the 5' end of the rRNA, including diagnostic variable regions V1 to V3) were determined for the other strains. The sequences determined for all members of each rRNA type were identical.

we also constructed a pairwise similarity matrix by repeatedly using the program GAP (6). The similarity values were converted to nucleotide substitution (K_{nuc}) values (13), and a phylogenetic tree was drawn with the program NEIGHBOR.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences of *B. fibrisolvens* NCDO 2221^T (T = type strain), NCDO 2222, NCDO 2398, NCDO 2432, NCDO 2434, NCDO 2435, Bu 43, NCDO 2223, NCDO 2249, NCDO 2397, and NCDO 2399 and *B. crossotus* NCFB 2416^T have been deposited in the EMBL data library under accession numbers X89970 to X89981.

RESULTS AND DISCUSSION

To shed light on the phylogenetic diversity of the genus *Butyrivibrio*, we compared the partial 16S rRNA gene se-

quences of 40 *Butyrivibrio* strains. Our initial sequence characterization and comparison of a stretch of approximately 600 to 700 nucleotides encoding the 5' end of the molecule revealed that there were 12 rRNA types or groups among the strains which we studied (Table 1). Consequently, the complete 16S rRNA gene sequence of one representative strain of each group was determined. The derived 16S rRNA primary sequences consisted of between 1,478 and 1,496 nucleotides and represented approximately 97% of the rRNA molecule. One of the 12 rRNA types which we found consisted of strains of *B.*

FIG. 1. Unrooted phylogenetic tree obtained by performing a neighbor-joining analysis of a distance matrix obtained from a multiple-sequence alignment, showing the positions of *Butyrivibrio* strains within cluster XIVa (5) of the *Clostridium* subphylum of the gram-positive bacteria. Bootstrap values (expressed as percentages of 500 replications) are shown at the branch points; values of 90% or more were considered significant.

crossotus, while the remaining 11 rRNA types included only strains designated *B. fibrisolvens*. rRNA type 8 was the largest group, consisting of 20 of the 40 strains studied. rRNA types 3, 6, and 9 each contained three strains; rRNA type 2 contained two strains; and rRNA types 1, 4, 5, 7, 10, and 11 were each represented by a single strain. rRNA type 1 contained the type strain of *B. fibrisolvens*. The levels of sequence similarity for strains belonging to the different *Butyrivibrio* rRNA types are shown in Table 2. Although there is no precise or absolute level of sequence similarity for delineating species, the levels of sequence divergence found for representatives of the various *Butyrivibrio* rRNA groups indicate that these taxa are at least separate species. This view is supported by the results of studies in which extracellular polysaccharide analysis and DNA-DNA hybridization were used to classify *Butyrivibrio* strains (15, 16, 26). These results confirmed the separateness of strains D1 (corresponding to rRNA type 1) and A38 (rRNA type 2) and placed strains Bu 49, Ce51, Ce52, and 12 in the same DNA group (rRNA type 8). However, one difference between the results of the DNA-DNA hybridization study of Mannarelli (15) and the results of our investigation was apparent. Mannarelli (15) reported that strains 1L6-31, Nor37, and B835 belong to a single DNA homology group. We found that while

strains 1L6-31 and Nor37 constitute a distinct rRNA group (rRNA type 9), strain B835 belonged to an rRNA subline that was quite separate (rRNA type 3). The level of 16S rRNA sequence divergence between the strains belonging to these rRNA types (approximately 13%) is clearly incompatible with the hypothesis that these organisms belong to the same species.

Comparative sequence searches of the EMBL and GenBank data libraries revealed that all of the *Butyrivibrio* strains which we examined were most closely related to species belonging to cluster XIVa of the *Clostridium* subphylum of the gram-positive bacteria as defined by Collins et al. (5). To determine the phylogenetic positions of the *Butyrivibrio* rRNA types within cluster XIVa, we performed a comprehensive phylogenetic analysis in which we included all known members of cluster XIVa and *Clostridium butyricum* (the type species of the genus *Clostridium*) as an outgroup. The phylogenetic tree that resulted from our analysis based on multiple sequence alignments is shown in Fig. 1. This tree was very similar to the tree obtained after pairwise alignment of the sequences (data not shown) in that all significant groups were present on both trees. The treeing analyses revealed that the 12 *Butyrivibrio* rRNA types formed three separate lineages within cluster XIVa (Fig. 1). The *B. crossotus* (rRNA type 12) strains formed a distinct

TABLE 2. Similarity values for the 16S rRNA genes of representative strains of the different *Butyrivibrio* rRNA types

Strain	% Similarity with:										
	NCDO 2221 ^T	NCDO 2222	NCDO 2398	NCDO 2432	NCDO 2434	NCDO 2435	Bu 43	NCDO 2223	NCDO 2249	NCDO 2397	NCDO 2399
<i>B. fibrisolvens</i> NCDO 2222 (rRNA type 2)	94.1										
<i>B. fibrisolvens</i> NCDO 2398 (rRNA type 3)	93.6	97.1									
<i>B. fibrisolvens</i> NCDO 2432 (rRNA type 4)	93.6	96.6	97.1								
<i>B. fibrisolvens</i> NCDO 2434 (rRNA type 5)	93.6	98.5	97.6	96.2							
<i>B. fibrisolvens</i> NCDO 2435 (rRNA type 6)	93.1	96.8	97.0	97.3	97.1						
<i>B. fibrisolvens</i> Bu 43 (rRNA type 7)	93.1	95.9	96.5	97.2	96.8	96.8					
<i>B. fibrisolvens</i> NCDO 2223 (rRNA type 8)	88.2	86.9	86.9	87.1	87.1	87.0	86.6				
<i>B. fibrisolvens</i> NCDO 2249 (rRNA type 9)	88.2	86.8	86.8	87.2	87.3	87.1	86.8	98.0			
<i>B. fibrisolvens</i> NCDO 2397 (rRNA type 10)	88.3	87.0	87.1	87.4	87.4	87.3	87.1	98.4	99.0		
<i>B. fibrisolvens</i> NCDO 2399 (rRNA type 11)	88.0	86.7	86.8	87.1	87.2	87.2	86.9	97.7	98.7	98.8	
<i>B. crossotus</i> NCDO 2416 ^T (rRNA type 12)	89.2	88.4	88.3	88.1	87.9	88.1	87.8	90.8	90.3	90.5	90.3

lineage that was only remotely related to the other *Butyrivibrio* rRNA types (levels of sequence similarity, 87.8 to 90.8%) (Table 2) and all members of cluster XIVa (levels of sequence similarity, 86.2 to 91.4%). The *B. fibrisolvens* rRNA type 1 to 7 strains formed a second lineage within cluster XIVa and exhibited levels of sequence similarity of 93.1 to 98.5% with each other (Table 2), but significantly lower levels of sequence similarity (85.5 to 92.1%) with other members of cluster XIVa. The rRNA type 1 strain, which is the type strain of the type species of the genus *Butyrivibrio*, was the most peripheral member of this lineage and exhibited levels of sequence similarity of 93.1 to 94.1% with rRNA type 2 to 7 strains (Table 2). The rRNA type 2 to 7 strains were more closely related to each other and exhibited intragroup levels of sequence similarity of 95.9 to 98.5% (Table 2). The third *Butyrivibrio* lineage was made up of rRNA type 8 to 11 strains, which exhibited levels of sequence similarity of 97.7 to 99.0% with each other (Table 2) and significantly lower levels of relatedness (levels of sequence similarity, 84.3 to 93.5%) with members of other species belonging to cluster XIVa. The three major phylogenetic lineages identified by the comparative rRNA gene sequence analysis were not consistent with the previous phenotypic groups based on cellular morphology and metabolism. For example, the two metabolic groups defined by Shane et al. (25) in the genus *Butyrivibrio* did not correlate with the two major phylogenetic groups (Table 2) within the species; group 1 of Shane et al. included nine rRNA type 8 strains and one rRNA type 6 strain (strain Ce47), while group 2 of Shane et al. contained rRNA type 4 strain Ce65, rRNA type 6 strain Ce78, rRNA type 7 strain Ce64, and rRNA type 9 strain Ce74. On the basis of cell wall structure Dibbayan et al. (7) placed *B. fibrisolvens* B835, Bu 49, and Nor37 in a single group that was separate from *B. fibrisolvens* D1. Phylogenetically, these four strains belong to four different rRNA types, with B835 belonging to the same major lineage as the type strain of *B. fibrisol-*

vens and Bu 49 and Nor37 belonging to a different lineage (Table 2).

From the genotypic data presented here and elsewhere (16), it is clear that the different *B. fibrisolvens* rRNA types correspond to separate species. Furthermore, 16S rRNA sequence divergence values and tree topology considerations (supported by bootstrap data [Fig. 1]) indicate that *B. fibrisolvens* rRNA types 1 to 7, *B. fibrisolvens* rRNA types 8 to 11, and *B. crossotus* constitute three remotely related lineages within *Clostridium* cluster XIVa, each meriting status as a separate genus. The numbers of species in these lineages are likely to increase, and/or other major lineages may be identified as additional *Butyrivibrio* strains, including members of other genotypically distinct groups described by Mannarelli et al. (16), are included in future phylogenetic studies. However, phenotypic data that support division of the genus *Butyrivibrio* along phylogenetic lines are not available at this time and may not be readily forthcoming. Most *Butyrivibrio* strains closely resemble one another; for example, they ferment similar carbohydrates, produce the same fermentation products, and share many other traits, including cell wall structure. The emerging picture of a phylogenetically dispersed genus *Butyrivibrio* also raises serious questions concerning the taxonomy of the other members of *Clostridium* cluster XIVa. At present this group encompasses a broad spectrum of species belonging to various genera (viz., the genera "*Acetitomaculum*," *Butyrivibrio*, *Clostridium*, *Coprococcus*, *Eubacterium*, *Roseburia*, and *Ruminococcus*) having diverse phenotypes (e.g., cocci and rods, spore forming and non-spore forming). The phylogenetic intermixing of many of these taxa and the general complexity of the branching within cluster XIVa (Fig. 1) indicate that it may be difficult to divide this group into genera that are phenotypically coherent and distinguishable. It is clear that several major lines within cluster XIVa are currently represented by a single species or strain. As PCR-based rRNA sequence analysis is routinely used to

phylogenetically characterize anaerobic isolates and study diversity, it may be shown that many of these isolated lines represent the nuclei of new genera, as is the case with butyrvibrios. The problem of phenotypic circumscription and differentiation which is currently preventing comprehensive taxonomic reorganization in this and other clostridial clusters may then prove to be even more complex.

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REFERENCES

- Bryant, M. P. 1984. Genus IV. *Butyrivibrio* Bryant and Small 1956, 18, emend. Moore, Johnson and Holdeman 1976, p. 641–643. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*, vol. 1. The Williams and Wilkins Co., Baltimore.
- Bryant, M. P., and I. M. Robinson. 1962. Some nutritional characteristics of predominant culturable ruminal bacteria. *J. Bacteriol.* **84**:605–614.
- 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.
- Cheng, K.-J., and J. W. Costerton. 1977. Ultrastructure of *Butyrivibrio fibrisolvens*: a gram-positive bacterium? *J. Bacteriol.* **129**:1506–1512.
- Collins, M. D., P. A. Lawson, A. Willems, J. J. Cordoba, J. Fernandez-Garayzabal, P. Garcia, J. Cai, H. Hippe, and J. A. E. Farrow. 1994. The phylogeny of the genus *Clostridium*: proposal of five new genera and eleven new species combinations. *Int. J. Syst. Bacteriol.* **44**:812–826.
- Devereux, J., P. Haeberli, and D. Smithies. 1984. A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res.* **12**:387–395.
- Dibbayawan, T., G. Cox, K. Y. Cho, and D. M. Dwart. 1985. Cell wall and plasma membrane architecture of *Butyrivibrio* spp. *J. Ultrastruct. Res.* **90**: 286–293.
- Felsenstein, J. 1989. PHYLIP—phylogeny inference package (version 3.2). *Cladistics* **5**:164–166.
- Hazlewood, G. P., M. K. Theodorou, A. Hutchings, D. J. Jordan, and G. Galfre. 1986. Preparation and characterization of monoclonal antibodies to a *Butyrivibrio* sp. and their potential use in the identification of rumen butyrvibrios, using an enzyme-linked immunosorbent assay. *J. Gen. Microbiol.* **132**:43–52.
- Hespell, R. B. 1992. Fermentation of xylans by *Butyrivibrio fibrisolvens* and *Thermoanaerobacter* strain B6A: utilization of uronic acids and xylanolytic activities. *Curr. Microbiol.* **25**:189–195.
- Hespell, R. B. 1992. The genera *Butyrivibrio*, *Lachnospira*, and *Roseburia*, p. 2022–2033. In A. Balows, H. G. Trüper, M. Dworkin, W. Harder, and K.-H. Schleifer (ed.), *The prokaryotes. A handbook on the biology of bacteria: ecophysiology, isolation, identification, application*, 2nd ed., vol. II. Springer-Verlag, New York.
- Hespell, R. B., K. Kato, and J. W. Costerton. 1993. Characterization of the cell wall of *Butyrivibrio* species. *Can. J. Microbiol.* **39**:912–921.
- Hori, H., and S. Osawa. 1979. Evolutionary change in 5S rRNA secondary structure and phylogenetic tree of 54 5S rRNA species. *Proc. Natl. Acad. Sci. USA* **76**:381–385.
- Lawson, P. A., S. E. Gharbia, H. N. Shah, and D. R. Clark. 1989. Recognition of *Fusobacterium nucleatum* subgroups Fn-1, Fn-2 and Fn-3 by ribosomal RNA gene restriction patterns. *FEMS Microbiol. Lett.* **65**:41–46.
- Mannarelli, B. M. 1988. Deoxyribonucleic acid relatedness among strains of the species *Butyrivibrio fibrisolvens*. *Int. J. Syst. Bacteriol.* **38**:340–347.
- Mannarelli, B. M., R. J. Stack, D. Lee, and L. Ericsson. 1990. Taxonomic relatedness of *Butyrivibrio*, *Lachnospira*, *Roseburia*, and *Eubacterium* species as determined by DNA hybridization and extracellular-polysaccharide analysis. *Int. J. Syst. Bacteriol.* **40**:370–378.
- Margherita, S. S., and R. E. Hungate. 1963. Serological analysis of *Butyrivibrio* from the bovine rumen. *J. Bacteriol.* **86**:855–860.
- Miron, J. 1991. The hydrolysis of lucerne cell-wall monosaccharide components by monocultures or pair combinations of defined ruminal bacteria. *J. Appl. Bacteriol.* **70**:245–252.
- Miron, J., and D. Benghedahlia. 1993. Digestion of cell-wall monosaccharides of ryegrass and alfalfa hays by the ruminal bacteria *Fibrobacter succinogenes* and *Butyrivibrio fibrisolvens*. *Can. J. Microbiol.* **39**:780–786.
- Moore, W. E. C., J. L. Johnson, and L. V. Holdeman. 1976. Emendation of *Bacteroidaceae* and *Butyrivibrio* and description of *Desulfomonas* gen. nov. and ten new species in the genera *Desulfomonas*, *Butyrivibrio*, *Eubacterium*, *Clostridium*, and *Ruminococcus*. *Int. J. Syst. Bacteriol.* **26**:238–252.
- Orpin, C. G., S. D. Mathiesen, Y. Greenwood, and A. S. Blix. 1985. Seasonal changes in the ruminal microflora of the high-arctic Svalbard reindeer (*Rangifer tarandus platyrhynchus*). *Appl. Environ. Microbiol.* **50**:144–151.
- Rainey, F. A., and P. H. Janssen. 1995. Phylogenetic analysis by 16S ribosomal DNA sequence comparison reveals two unrelated groups of species within the genus *Ruminococcus*. *FEMS Microbiol. Lett.* **129**:69–74.
- Roché, C., H. Albertyn, N. O. van Gylswyk, and A. Kistner. 1973. The growth response of cellulolytic acetate-utilizing and acetate-producing butyrvibrios to volatile fatty acids and other nutrients. *J. Gen. Microbiol.* **78**:253–260.
- Rumney, C. J., S. H. Duncan, C. Henderson, and C. S. Stewart. 1995. Isolation and characteristics of a wheatbran-degrading *Butyrivibrio* from human feces. *Lett. Appl. Microbiol.* **20**:232–236.
- 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.
- Stack, R. J. 1988. Neutral sugar composition of extracellular polysaccharides produced by strains of *Butyrivibrio fibrisolvens*. *Appl. Environ. Microbiol.* **54**:878–883.
- Williams, A. G., and S. E. Withers. 1992. Induction of xylan degrading enzymes in *Butyrivibrio fibrisolvens*. *Curr. Microbiol.* **25**:297–303.