APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Nov. 1999, p. 5173–5176 Vol. 65, No. 11
0099-2240/99/$04.00+0

A New Selective Medium for *Bifidobacterium* spp.

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Received 10 February 1999/Accepted 7 August 1999

A new selective antibiotic-free medium for *Bifidobacterium* spp. is defined. This medium has lactulose as the main carbon source and includes methylene blue, propionic acid, and lithium chloride as inhibitors of some related bacterial species. The low pH of the medium contributes to the inhibition of the growth of *Enterobacteriaceae*. This new selective medium has a simple composition, and the level of recovery it yields is similar to those yielded by nonselective media for *Bifidobacterium* strains. It could thus be used for routine analysis in environmental or food microbiology.

The genus *Bifidobacterium* is the third most numerous bacterial population in the human intestine after the genera *Bacteroides* and *Eubacterium* (4). It is also the predominant population during childhood (9). Some species have been observed in high numbers in human feces (1). Because of these characteristics, it has been proposed that *Bifidobacterium* be used as an indicator of fecal contamination (8, 14), although this proposal is controversial. Several studies pointed out species-specific relationships between certain *Bifidobacterium* species and their hosts which suggest the use of these species to determine the origin of fecal contamination (3, 12, 17, 19). Moreover, *Bifidobacterium* spp. are unlikely to grow in water because of their obligate anaerobic physiology and complex growth requirements. Such characteristics support the possible use of *Bifidobacterium* as a fecal indicator. Several *Bifidobacterium* species have been incorporated in yogurts, as they are considered to have health-promoting properties (9). The availability of easy and inexpensive methods for detection, identification, and enumeration of *Bifidobacterium* is consequently important in environmental and food microbiology. Several media for selective or differential isolation have been described (2, 11, 13, 15–17). Most of them have complex compositions that include antibiotics as growth inhibitors, require long incubation times, or show low bacterial recovery levels. These factors hinder their routine use for monitoring when *Bifidobacterium* is used as a fecal indicator or for the enumeration of bifidobacterial populations in dairy products. In this study, a new selective medium for *Bifidobacterium* spp. is described. It has been evaluated for the enumeration of bifidobacteria in yogurts.

Forty-two bacterial strains were used in this study. The media used to cultivate the different strains are listed in Table 1, and the strains are listed in Tables 2 and 3. *Bifidobacterium* strains were grown at 37°C for 48 h in anaerobic jars (GasPak; BBL). Strains other than *Bifidobacterium* spp. were grown at 37°C for 24 h. *Bacteroides fragilis* was also cultured in anaerobic jars. The enumeration of bacteria in yogurts was carried out with the differential LS-Cennan medium (ADSA, Barcelona, Spain) (7) and incubation of plates at 37°C for 48 h in anaerobic jars (GasPak; BBL). Bacterial strains in yogurts were identified by colonial morphology on the differential medium according to the method described by Eloy in 1976 (7), Gram staining, and cell morphology.

An initial list of the potential components of the new medium was based on the components of previously described media (16) and the physiological characteristics of the genus (10). The significance of the effects these components have on growth and selectivity for *Bifidobacterium* were studied by the statistical model 2^k (5, 6), which analyzes the importance of k factors in a determinate event. Fifteen factors ($k = 15$) were selected for evaluation at two levels or concentrations each, as follows: meat extract, 2 and 5 g/liter; yeast extract, 3 and 7 g/liter; starch, 0.5 and 2 g/liter; lactulose, 1 and 5 g/liter; L-cysteine, 0.5 and 2 g/liter; sodium chloride, 1 and 5 g/liter; casein, 1 and 5 g/liter; maltose, 1 and 5 g/liter; peptone, 2 and 5 g/liter; tryptone, 2 and 5 g/liter; riboflavin, 0.25 and 1 mg/liter; thiamine, 0.25 and 1 mg/liter; pantothenic acid, 0.5 and 2 mg/liter; propionic acid, 1 and 5 ml/liter; and pH levels of 5 and 6.5. According to these parameters, 2^{15} media could be defined. The number of potential media was reduced to 40 combinations (statistical design, $2^{k-p} = 40$) in order to minimize analyses. The bacterial strains used to evaluate the media was *Bifidobacterium pseudolongum* DSM 20099^T due to its high growth rate. A McFarland no. 3 cell suspension in 0.25× Ringer solution supplemented with 0.5 g of L-cysteine/liter was prepared by growing *B. pseudolongum* on Columbia blood agar (CBA) supplemented with 5 g of glucose per liter and 0.5 g of L-cysteine per liter for 48 h. Ten-fold dilutions were made from these suspensions in 0.25× Ringer solution supplemented with 0.5 g of L-cysteine/liter. Four replicates of plating for each experimental medium were performed. Plates were inoculated with aliquots of 10 μl of each dilution and incubated at 37°C for 48 h in anaerobic jars. The media were compared by counting the *B. pseudolongum* colonies after 48 h of culture. This permitted a comparison of the levels of recovery on the different media tested to establish the basic composition of the new selective medium by using the components with a positive effect on the growth of *Bifidobacterium*. The use of some inhibitors for bacterial species physiologically or ecologically related to *Bifidobacterium* (*Enterobacteriaceae*, *Enterococcus* spp., and *Lactobacillus* spp.) was also studied. The inhibitors and their concentrations were determined according to previous studies (11, 20) and were as follows: methylene blue, 0.130 g/liter; lithium chloride, 2 g/liter; propionic acid, 5 ml/liter; crystal violet, 0.4 mg/liter; dodecyl sulfate sodium salt, 0.1 g/liter; propionic acid sodium salt, 3 g/liter; sodium sulfite, 2 g/liter; fuchsin basic, 0.5 g/liter; and a pH of 5.5. Each inhibitor was tested by including it in the basic composition of the

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TABLE 1. Media used for the culture of the different studied strains

Bacteria	Solid and broth media[a]
Bifidobacterium spp.	CBA, MRS
Lactobacillus spp.	MRS agar, MRS
A. hydrophila	TSA, TSB
S. typhimurium	TSA, TSB
E. coli	TSA, TSB
Vibrio spp.	TSA2, TSB2
B. fragilis	BPRM agar, BPRM
P. penneri	NA, NB
E. tarda	NA, NB
Y. enterocolitica	NA, NB
Enterococcus spp.	BHIA, BHI
S. thermophilus	BHIA, BHI

[a] CBA, Columbia blood agar (Difco) supplemented with 5 g of glucose/liter and 0.5 g of L-cysteine/liter; MRS, Man, Rogosa, and Sharpe medium (Scharlau); TSA, tryptone soy agar (ADSA); TSB, tryptone soy broth (Scharlau); TSA2, tryptone soy agar supplemented with 1.5 g of sodium chloride/liter; TSB2, tryptone soy broth supplemented with 1.5 g of sodium chloride/liter; BPRM, *Bacteroides* Phage Recovery Medium (ADSA); NA, nutrient agar (ADSA); NB, nutrient broth (Biokar); BHIA, brain heart infusion agar (Difco); BHI, brain heart infusion broth (Difco).

medium established in the previous study. The colony counts of the media with or without inhibitors were compared by using *B. pseudolongum* DSM 20099, *Lactobacillus bulgaricus* CECT 4005, and *Escherichia coli* CN13. A McFarland no. 3 cell suspension for each strain was made in 0.25× Ringer solution supplemented with 0.5 g of L-cysteine/liter after 48 h of growth on CBA. Ten-fold dilutions were performed in 0.25× Ringer solution supplemented with L-cysteine. Plates of each experimental medium with inhibitor were inoculated in the same way as plates in the 2^{k-p} experiment described earlier. Plates were incubated at 37°C for 48 h in anaerobic jars. Those inhibitors that reduced the colony counts for *L. bulgaricus* and *E. coli* but not for *B. pseudolongum* were added to the final composition of the new *Bifidobacterium* medium (BFM).

The selectivity of BFM was determined by comparing the growth of 26 strains belonging to 24 *Bifidobacterium* species (Table 2) and 16 strains from other bacterial genera (Table 3). In order to measure the recovery on BFM agar, two media were used for comparison: CBA supplemented with glucose and L-cysteine, an enriched medium, and a previously described *Bifidobacterium* medium, BL medium (21). Bifidobacterial strains were grown in Reinforced Clostridial Medium (RCM) for 48 h at 37°C in 25-ml glass tubes. An aliquot of 0.1 ml from this culture was again inoculated in RCM and incubated for 24 h at 37°C. Ten-fold dilutions of this bacterial culture were performed in 0.25× Ringer solution supplemented with 0.5 g of L-cysteine/liter. Aliquots (10 μl) of 10-fold dilutions were plated in duplicate on BFM, CBA supplemented with glucose and L-cysteine, and BL medium. Plates were incubated anaerobically for 48 h at 37°C, and colonies were counted. The recovery of strains other than *Bifidobacterium* spp. was also evaluated by colony counting on BFM and on the appropriate medium (Table 1) after incubation at 37°C for 24 h.

The enumeration of *Bifidobacterium* spp. in three different commercial yogurts was performed twice to evaluate the use of BFM. Ten-fold dilutions from each yogurt were prepared in 0.25× Ringer solution supplemented with 0.5 g of L-cysteine/liter and plated on BFM and LS-Cennan medium (ADSA) in duplicate. Colonies of different morphology were counted after 48 h of incubation at 37°C in anaerobic jars. Morphologically

TABLE 2. Recovery levels for *Bifidobacterium* strains grown on the three media studied[a]

Strain[b]	Avg log CFU/ml (SD)			BFM:BL medium recovery ratio
	CBA	BL medium	BFM	
Bifidobacterium adolescentis DSM 20083^T	8.96 (0.01)	9.02 (0.09)	7.15 (0)	0.79
Bifidobacterium angulatum DSM 20098^T	8.61 (0.01)	8.69 (0.09)	8.59 (0.03)	0.99
Bifidobacterium animalis DSM 20104^T	8.17 (0.18)	8.19 (0.02)	8.16 (0.17)	0.99
Bifidobacterium asteroides DSM 20089^T	7.50 (0.04)	7.51 (0.09)	7.50 (0.03)	0.99
Bifidobacterium bifidum DSM 20082^T	8.93 (0.12)	9.09 (0.05)	8.90 (0.02)	0.98
Bifidobacterium bifidum DSM 20239^T	7.44 (0.23)	7.44 (0.01)	7.36 (0.05)	0.99
Bifidobacterium bifidum DSM 20456^T	7.77 (0.16)	7.33 (0.21)	0 (0)	0
Bifidobacterium boum DSM 20432^T	8.89 (0.03)	8.99 (0.01)	9.04 (0)	1
Bifidobacterium breve DSM 20213^T	8.55 (0.06)	7.16 (0.02)	7.80 (0.10)	1.09
Bifidobacterium catenulatum DSM 20103^T	8.26 (0)	8.29 (0.20)	7.00 (0.01)	0.84
Bifidobacterium choerinum DSM 20434^T	8.71 (0.06)	8.73 (0.02)	8.72 (0.02)	0.99
Bifidobacterium coryneforme DSM 20216^T	9.45 (0)	9.41 (0.04)	0 (0)	0
Bifidobacterium cuniculi DSM 20435^T	8.50 (0.03)	8.51 (0.05)	8.51 (0.11)	1
Bifidobacterium dentium DSM 20084	8.67 (0.01)	8.63 (0.02)	8.65 (0.05)	1
Bifidobacterium globosum DSM 20092^T	8.02 (0.13)	8.07 (0.10)	8.16 (0.02)	1.01
Bifidobacterium indicum DSM 20214^T	9.37 (0.09)	8.84 (0.85)	9.45 (0.10)	1.07
Bifidobacterium infantis DSM 20088^T	8.29 (0.02)	5.79 (0.07)	6.35 (0.07)	1.09
Bifidobacterium longum DSM 20219^T	8.87 (0.02)	8.90 (0.02)	6.81 (0.08)	0.76
Bifidobacterium magnum DSM 20222^T	8.15 (0.11)	8.39 (0.12)	8.04 (0.06)	0.96
Bifidobacterium minimum DSM 20102^T	8.79 (0.01)	8.83 (0.17)	8.73 (0.24)	0.98
Bifidobacterium pseudocatenulatum DSM 20438^T	8.33 (0.04)	8.42 (0.03)	8.18 (0.14)	0.97
Bifidobacterium pseudolongum DSM 20099^T	8.55 (0.03)	8.52 (0.01)	8.44 (0.13)	0.99
Bifidobacterium pullorum DSM 20433^T	7.43 (0.12)	7.57 (0.02)	0 (0)	0
Bifidobacterium subtile DSM 20096^T	9.31 (0.01)	9.19 (0.02)	9.24 (0.02)	1
Bifidobacterium suis DSM 20211^T	9.42 (0.01)	9.42 (0.03)	9.36 (0)	0.99
Bifidobacterium thermophilum DSM 20210^T	9.96 (0.62)	9.55 (0.05)	9.45 (0.12)	0.99

[a] CBA, Columbia blood agar supplemented with 5 g of glucose/liter and 0.5 g of L-cysteine/liter. For a definition of BL medium, see reference 17. BFM, *Bifidobacterium* medium.
[b] DSM, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany.

TABLE 3. Recovery levels for strains other than *Bifidobacterium* spp. on a rich medium, on BL medium, and on BFM agar

Strain[i]	Avg log CFU/ml (SD)			BFM:BL medium recovery ratio
	Rich medium	BL medium	BFM agar	
Lactobacillus bulgaricus CECT 4005^T	7.93 (0.03)[a]	7.89 (0)	3.40 (0.14)	0.43
Lactobacillus acidophilus CECT 903^T	8.34 (0)[a]	8.27 (0)	0 (0)	0
Lactobacillus plantarum CECT 748^T	8.08 (0.05)[a]	8.15 (0.04)	8.07 (0.10)[g]	0.93
Lactobacillus reuteri CECT 925^T	8.38 (0.29)[a]	8.69 (0.03)	ND[h]	
Vibrio furnissii LMG 7910	7.22 (0.15)[b]	7.09 (0.07)	0 (0)	0
Vibrio cholerae CECT 658	7.51 (0.01)[b]	7.55 (0.03)	0 (0)	0
Aeromonas hydrophila CECT 398	6 (0)[c]	6.28 (0.11)	0 (0)	0
Salmonella typhimurium WG49	8.16 (0.02)[c]	8.08 (0.05)	0 (0)	0
Escherichia coli WG5	7.28 (0.19)[c]	7.18 (0.04)	0 (0)	0
Bacteroides fragilis HSP40	8.97 (0.02)[d]	8.87 (0.18)[g]	ND	
Proteus penneri CECT 864^T	5.79 (0.04)[e]	7.69 (0.04)	0 (0)	0
Edwardsiella tarda CECT 849^T	8.19 (0.06)[e]	8.04 (0)	0 (0)	0
Yersinia enterocolitica CECT 754	7.91 (0.03)[e]	7.60 (0.07)	0 (0)	0
Enterococcus faecalis CECT 481^T	8.66 (0.01)[f]	8.72 (0.01)	8.91 (0.12)[g]	1.02
Enterococcus faecium CECT 410^T	9.01 (0.05)[f]	8.96 (0)	8.99 (0.07)[g]	1
Streptococcus thermophilus CECT 986^T	9.02 (0.03)[f]	9.03 (0.07)	0 (0)	0

[a] Mann, Rogosa, and Sharpe medium was used.
[b] Tryptone soy agar supplemented with 1.5 g of sodium chloride/liter was used.
[c] Tryptone soy agar was used.
[d] *Bacteroides* Phage Recovery Medium was used.
[e] Nutrient agar was used.
[f] Brain heart infusion agar was used.
[g] Colony diameters were smaller than 0.5 mm.
[h] ND, not determined (colonies were too small to be counted).
[i] CECT, Colección Española de Cultivos Tipo, Universidad de Valencia, Burjasot, Spain; LMG, Laboratorium voor Microbiologie, Rijksuniversiteit, Ghent, Belgium.

different colonies on BFM agar and LS-Cennan medium were characterized by Gram staining and the fructose-6-phosphate phosphoketolase test (7, 10).

Eleven of the 15 factors tested enhanced the growth of *B. pseudolongum* DSM 20099 and constitute the composition of BFM. Low concentrations of meat extract and tryptone had a greater effect on the colony numbers than did high concentrations. In contrast, high concentrations of yeast extract, starch, lactulose, L-cysteine, sodium chloride, peptone, riboflavin, and thiamine yielded colony counts higher than those yielded with low concentrations. No effect on the colony enumeration for *B. pseudolongum* DSM 20099 was observed with casein, maltose, or pantothenic acid. Methylene blue (16 mg/liter), propionic acid (99%; 5 ml/liter), lithium chloride (2 g/liter), and a pH of 5.5 did not inhibit colony growth of *B. pseudolongum* DSM 20099, although they did inhibit colony growth of strains of other genera, such as *Lactobacillus* spp. and *E. coli*. The new medium (BFM) was established with the following components: meat extract (2 g/liter; Merck, Darmstadt, Germany), yeast extract (7 g/liter; Scharlau, Barcelona, Spain), starch (2 g/liter; Panreac, Barcelona, Spain), L-cysteine hydrochloride (0.5 g/liter; Sigma, St. Louis, Mo.), sodium chloride (5 g/liter; Pancreac), peptone 5 g/liter; Difco, Detroit, Mich.), tryptone (2 g/liter; Scharlau), lactulose (5 g/liter; Merck), riboflavin (1 mg/liter; Merck), thiamine chloride hydrochloride (1 mg/liter; Merck), methylene blue (16 mg/liter; Merck), lithium chloride (2 g/liter; Merck), and propionic acid (99%; 5 ml/liter; Sigma). All components except propionic acid were mixed and resuspended in distilled water. The medium was sterilized by autoclaving at 121°C for 20 min and then allowed to cool to 55°C. Propionic acid was then added, and the pH was adjusted to 5.5 with sterile 10 N NaOH. When BFM agar plates were made, agar-agar (ADSA) was added at a concentration of 15 g/liter. The principal carbon source in this medium is lactulose. The growth stimulation of this oligofructose for *Bifidobacterium* observed in this study was consistent with that of other studies (9, 18). Two vitamins (riboflavin and thiamine) acted as growth factor promoters. Propionic acid, lithium chloride, and methylene blue inhibited the growth of some related bacterial species. The low pH (5.5) of BFM inhibited the growth of *Enterobacteriaceae*.

The selectivity of BFM did not affect the growth of 23 of the 26 *Bifidobacterium* strains tested. Most of them showed round, blue colonies approximately 2 mm in diameter a few minutes after removal from the anaerobic jar. There was no growth of colonies of three strains (*Bifidobacterium bifidum* DSM 20456, *Bifidobacterium coryneforme* DSM 20216, and *Bifidobacterium pullorum* DSM 20433) on BFM, but two others strains of *B. bifidum* (DSM 20082 and DSM 20239) grew on BFM. No colony growth was observed on BFM for the following strains: *Lactobacillus acidophilus* CECT 903, *Vibrio furnissii* LMG 7910, *Vibrio cholerae* CECT 658, *Aeromonas hydrophila* CECT 398, *Salmonella typhimurium* WG49, *E. coli* WG5, *Proteus penneri* CECT 864, *Edwardsiella tarda* CECT 849, *Yersinia enterocolitica* CECT 754, and *Streptococcus thermophilus* CECT 986. Four tested isolates that do not belong to the *Bifidobacterium* genus grew on both media (Table 3). However, these isolates were easily differentiated from *Bifidobacterium* spp. because of their small diameters (<0.5 mm) and differential colonial morphologies.

For most of the 26 *Bifidobacterium* strains, similar colony counts were obtained on BFM, CBA, and BL medium (Table 2). However, lower colony counts on BFM agar with respect to CBA and BL medium were observed for *Bifidobacterium adolescentis* DSM 20083, *B. bifidum* DSM 20456, *Bifidobacterium catenulatum* DSM 20103, *B. coryneforme* DSM 20216, *Bifidobacterium longum* DSM 20219, and *B. pullorum* DSM 20433 (Table 2). No colony growth on BFM agar was observed for most of the studied strains other than *Bifidobacterium* spp. The colony counts on BL agar for these strains were similar to these obtained on rich media. BL medium has been described as an optimal medium for *Bifidobacterium* (16). However, BFM is easier to prepare and proved to be more selective than BL medium (Table 3). A lower level of recovery from BFM

TABLE 4. Enumeration of bacteria with the three morphologies present in the commercial yogurts

Yogurt	Avg log CFU/ml (SD) for colonial type:					
	L[a]		S[b]		B[c]	
	LS-Cennan	BFM	LS-Cennan	BFM	LS-Cennan	BFM
A	6.42 (0.06)	NG[d]	9.02 (0.08)	NG	6.75 (0.21)	6.30 (0.06)
B	6.29 (0.02)	NG	8.91 (0.03)	NG	7.88 (0.05)	6.17 (0)
C	7.14 (0.09)	NG	7.82 (0.06)	NG	7.06 (0.03)	6.29 (0.02)

[a] Colonies showing the usual colonial morphology for *Lactobacillus* on LS-Cennan medium, having gram-positive rods and being fructose-6-phosphate phosphoketolase negative.
[b] Colonies showing the usual colonial morphology for *Streptococcus* on LS-Cennan medium, having gram-positive cocci and being fructose-6-phosphate phosphoketolase negative.
[c] Colonies showing colonial morphology for *Bifidobacterium* on BFM and other than the usual colonial morphology for *Lactobacillus* or *Streptococcus* on LS-Cennan medium, having gram-positive irregular rods and being fructose-6-phosphate phosphoketolase positive.
[d] NG, no growth.

agar was observed for *L. bulgaricus* CECT 4005. Only three strains other than *Bifidobacterium* (*Lactobacillus plantarum* CECT 748, *Enterococcus faecalis* CECT 481, and *Enterococcus faecium* CECT 410) showed colony counts on BFM agar similar to those obtained on rich media, although their colony sizes were smaller on BFM (Table 3) as described earlier.

Three colony morphologies were observed on LS-Cennan medium inoculated with the yogurt tested. Gram staining and cell morphology observed via optical microscopy confirmed the presumptive relationship between genus and colony morphology for the usual bacterial components of this type of yogurt (*Streptococcus*, *Lactobacillus*, and *Bifidobacterium*) (7). Large, irregular, red colonies were gram-positive rods (*Lactobacillus* spp.); large, round, red colonies were gram-positive cocci (*Streptococcus* spp.); and small, round colonies were gram-positive polymorphic cells (*Bifidobacterium* spp.). The fructose-6-phosphate phosphoketolase test confirmed that the latter isolates belonged to the genus *Bifidobacterium* (10). Two colonial morphologies, small and large, were observed on BFM agar. Both belonged to *Bifidobacterium* spp. according to the results with Gram stain and the fructose-6-phosphate phosphoketolase test (10). Large colonies presented both morphological colony types when they were again streaked on BFM. No differences in colony counts of bifidobacteria on BFM agar and LS-Cennan medium were observed for the three yogurts. The colonial morphologies associated with *Lactobacillus* spp. and *Streptococcus* spp. on LS-Cennan medium (7) were not found on BFM agar (Table 4). BFM is suitable for the bifidobacteria present in yogurt. It inhibits the growth of *Lactobacillus* and *Streptococcus* and allows a differential growth of bifidobacteria.

In conclusion, the simple composition of BFM makes it a feasible medium for routine monitoring purposes. Its selectivity could provide a differential tool for the recovery of bifidobacteria in environmental and food microbiology. The recovery on BFM agar suggests the practical use of BFM for enumeration of *Bifidobacterium* in routine monitoring of fermented dairy products such as yogurts.

This research was supported by the program 1997SGR00069 of the Generalitat de Catalunya. Y. Nebra has a fellowship from the Universitat de Barcelona.

REFERENCES

1. **Bahaka, D., C. Neut, A. Khattabi, D. Monget, and F. Gavini.** 1993. Phenotypic and genomic analysis of human strains belonging or related to *Bifidobacterium longum*, *Bifidobacterium infantis*, and *Bifidobacterium breve*. Int. J. Syst. Bacteriol. **43:**565–573.
2. **Beerens, H.** 1990. An elective and selective isolation medium for *Bifidobacterium* spp. Lett. Appl. Microbiol. **11:**155–157.
3. **Cabelli, V. J.** 1979. Evaluation of recreational water quality, the EPA approach, p. 1–23. *In* A. James and L. Evison (ed.), Biological indicators of water quality. John Wiley & Sons, Chichester, England.
4. **Charteris, W. P., P. M. Kelly, L. Morelli, and J. K. Collins.** 1997. Selective detection, enumeration and identification of potentially probiotic *Lactobacillus* and *Bifidobacterium* species in mixed bacterial populations. Int. J. Food Microbiol. **35:**1–27.
5. **Christen, P., and M. Raimbault.** 1991. Optimization of culture medium for aroma production by *Ceratocystis fimbriata*. Biotechnol. Lett. **13:**521–526.
6. **Davies, K. D.** 1993. Design of experiments for predictive microbial modeling. J. Ind. Microbiol. **12:**295–300.
7. **Eloy, C. R. L.** 1976. Milleux différentiel pour le depistage des microorganismes du yogourt. Bull. Rech. Agron. Gembloux **11:**83–86.
8. **Evison, L. M., and A. James.** 1974. *Bifidobacterium* as an indicator of faecal pollution in water, p. 107–116. *In* Proceedings of the 7th International Conference on Water Pollution Research. Pergamon Press Ltd., Oxford, England.
9. **Gibson, G. R., and M. B. Roberfroid.** 1995. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J. Nutr. **125:**1401–1402.
10. **Jones, D., and M. D. Collins.** 1986. Irregular, nonsporing Gram-positive rods, p. 1261–1434. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore, Md.
11. **Lapierre, L., P. Undeland, and L. J. Cox.** 1992. Lithium chloride–sodium propionate agar for the enumeration of bifidobacteria in fermented dairy products. J. Dairy Sci. **75:**1192–1196.
12. **Levin, M. A.** 1977. *Bifidobacterium* as water quality indicators, p. 131–138. *In* A. W. Hadley and B. J. Dutka (ed.), Bacterial indicators—health hazards associated with water. ASTM Publications, Philadelphia, Pa.
13. **Lim, K. S., C. S. Huh, and Y. J. Baek.** 1995. A selective enumeration medium for bifidobacteria in fermented dairy products. J. Dairy Sci. **78:**2108–2112.
14. **Mossel, D. A. A.** 1958. The suitability of bifidobacteria as part of a more extended bacterial association indicating faecal contamination of foods, p. 440–441. *In* 7th International Congress of Microbiology abstracts of papers. Almquist & Wikesells, Uppsala, Sweden.
15. **Muñoa, F. J., and R. Pares.** 1988. Selective medium for isolation and enumeration of *Bifidobacterium* spp. Appl. Environ. Microbiol. **54:**1715–1718.
16. **Pacher, B., and W. Kneifel.** 1996. Development of a culture medium for the detection and enumeration of bifidobacteria in fermented milk products. Int. Dairy J. **6:**43–64.
17. **Resnick, I. G., and M. A. Levin.** 1981. Assessment of bifidobacteria as indicators of human fecal pollution. Appl. Environ. Microbiol. **42:**433–438.
18. **Sahota, S. S., P. M. Bramley, and I. A. Menzies.** 1982. The fermentation of lactulose by colonic bacteria. J. Gen. Microbiol. **128:**319–325.
19. **Scardovi, V., L. D. Trovatelli, G. Zani, F. Crociani, and D. Matteuzzi.** 1971. Deoxyribonucleic acid homology relationships among species of the genus *Bifidobacterium*. Int. J. Syst. Bacteriol. **21:**276–294.
20. **Shimada, K., and K. Shimahara.** 1987. Effect of alternating current exposure on the resistivity of resting *Escherichia coli* B. J. Appl. Bacteriol. **62:**261–268.
21. **Teraguchi, S., M. Uehara, K. Ogasa, and T. Mitsuoka.** 1978. Enumeration of bifidobacteria in dairy products. Jpn. J. Bacteriol. **33:**753–761.