J. Dairy Sci. 85:1039–1051
© American Dairy Science Association, 2002.

Species-Specific Identification of Commercial Probiotic Strains

P. S. M. Yeung,* M. E. Sanders,* C. L. Kitts,† R. Cano,† and P. S. Tong*
*Dairy Products Technology Center and
†Environmental Biotechnology Institute
California Polytechnic State University, San Luis Obispo, CA 93407

ABSTRACT

Products containing probiotic bacteria are gaining popularity, increasing the importance of their accurate speciation. Unfortunately, studies have suggested that improper labeling of probiotic species is common in commercial products. Species identification of a bank of commercial probiotic strains was attempted using partial 16S rDNA sequencing, carbohydrate fermentation analysis, and cellular fatty acid methyl ester analysis. Results from partial 16S rDNA sequencing indicated discrepancies between species designations for 26 out of 58 strains tested, including two ATCC *Lactobacillus* strains. When considering only the commercial strains obtained directly from the manufacturers, 14 of 29 strains carried species designations different from those obtained by partial 16S rDNA sequencing. Strains from six commercial products were species not listed on the label. The discrepancies mainly occurred in *Lactobacillus acidophilus* and *Lactobacillus casei* groups. Carbohydrate fermentation analysis was not sensitive enough to identify species within the *L. acidophilus* group. Fatty acid methyl ester analysis was found to be variable and inaccurate and is not recommended to identify probiotic lactobacilli.
(**Key words:** probiotics, *Lactobacillus acidophilus*, *Lactobacillus casei*)

Abbreviation key: FAME = fatty acid methyl esters.

INTRODUCTION

Probiotics are defined as live microorganisms that impart a health benefit to the consumer. Beneficial effects have been achieved through modulation of gut flora populations or activities, through influence on mucosal immunity or through alteration of specific enzymatic activities. Many bacterial genera and species are used commercially for probiotic applications, most commonly, species of *Lactobacillus* and *Bifidobacterium*. Several reports have indicated inaccuracies in labeling of species contained within commercial probiotic products (summarized in Table 1).

Changes over the past decade in the taxonomy of probiotic species (Klein et al., 1998), a failure of some probiotic product manufacturers to apply current methodologies, and perhaps a perceived marketing advantage of labeling for certain species instead of others (e.g., better consumer name recognition) have all likely contributed to inaccurate species labeling on commercial probiotic products. Accurate species labeling is important to responsible quality control efforts, to build consumer confidence in product labeling, and for safety considerations. For example, the presence of significant levels of unlabeled *Enterococcus* populations in commercial probiotic products has been documented (Hamilton-Miller et al., 1996, 1999), even though enterococci with opportunist potential, hemolytic activity, and transferable antibiotic resistance are known (Salminen and von Wright, 1998). While safety may not be compromised if strains of the genus *Lactobacillus* are speciated incorrectly, it is incumbent on manufacturers to accurately represent products to the consumer.

As with bacteria in general, analysis of 16S rDNA sequences has been applied to the speciation of probiotic lactobacilli and bifidobacteria (Tannock, 1999). More rapid DNA-based methods to speciate probiotic species have also been developed, including oligonucleotide probes for three species of the "*L. acidophilus* group" (Pot et al., 1993) and species-specific primers for *Lactobacillus paracasei*, *Lactobacillus rhamnosus*, *Lactobacillus delbrueckii*, *Lactobacillus helveticus* (Tilsala-Timisjarvi and Alatossava, 1997), and *Lactobacillus plantarum* (Quere et al., 1997). Giraffa et al. (1998) succeeded in differentiating between *L. delbrueckii*, *L. helveticus*, and *L. acidophilus*, but not between subspecies lactis and *delbrueckii* of *L. delbrueckii* using amplified rDNA restriction analysis.

Phenotypic methods alone are inadequate for speciation of probiotic lactobacilli and bifidobacteria (Kandler and Weiss, 1986). A polyphasic approach to speciation of lactic acid bacteria was recommended by Vandamme

Received October 16, 2000.
Accepted November 19, 2001.
Corresponding author: M. E. Sanders; e-mail: mesanders@msn.com.

Table 1. Summary of studies reporting discrepancies between product labeling and independent laboratory analysis of species contained in probiotic products.

Strains or products incorrectly labeled[1]/tested	Species not listed but detected in product	Method used	Reference
7/15 pharmaceutical products	*Lactobacillus paracasei* *Lactobacillus leichmannii* *Enterococcus faecium* *Saccaromyces cerevisiae* *Lactobacillus rhamnosus*	Carbohydrate fermentation study	Canganella et al., 1997
Numbers not delineated; indicated "most" products mislabeled	*L. paracasei* *Bifidobacterium animalis* *Lactobacillus johnsonii* *Lactobacillus gasseri*	Protein pattern analysis Carbohydrate fermentation study Pulsed-field gel electrophoresis Randomly amplified polymorphic DNA	Klein et al., 1998
6/19[2] dietary supplement products	*Lactobacillus plantarum* *Lactobacillus delbrueckii* *Pediococcus pentosaceus* *Pediococcus acidilactici* *E. faecium* *L. rhamnosus*	API Rapid ID kits	Hamilton-Miller et al., 1999
3/6 dairy products containing bifidobacteria and labeled with species (10 products tested but labeled with only genus)	*B. animalis*	Carbohydrate fermentation study; colorimetric DNA hybridization	Yaeshima et al., 1996
4/6 *L. acidophilus* strains	*L. gasseri* *L. johnsonii* *L. gallinarum*	Species-specific probes	Sanders et al, 1996
5/13 dietary supplement products	*L. plantarum* *P. pentosaceus* *Lactobacillus fermentum* *L. rhamnosus* *E. faecium* *L. delbreuckii*	API Rapid ID kits	Hamilton-Miller et al., 1996
9/15[3] strains from European mild yogurts	*L. johnsonii* *L. rhamnosus* *L. paracasei* *L. crispatus*	DNA-DNA homology	Schillinger, 1999

[1]Indicates species detected that were not listed on the label.

[2]19 probiotic supplement products tested that specifically indicated species on the label. Other products tested in this report not included in this summary. Products originated from UK and other EU countries.

[3]A total of 26 strains isolated, but only 15 yogurts were labeled with species.

et al. (1996), whereby results of genomic analysis and phenotypic analysis are combined. Phenotypic methods, including analysis of cell wall composition, carbohydrate fermentation (Canganella et al., 1997; Hamilton-Miller, et al., 1999; Chateau et al., 1994), and protein analysis have been used for this purpose (Klein et al., 1998).

In this study, we assessed speciation inaccuracies in a collection of commercial and research probiotic lactobacilli and bifidobacteria using carbohydrate fermentation, partial 16S rDNA sequencing, and cellular fatty acid methyl ester methods, and determined the taxonomic relationship of these probiotic strains using these methods.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

The bacterial strains used in this study are listed along with their sources in Table 2. All *Lactobacillus* and *Bifidobacterium* strains were grown in MRS (Difco Laboratories, Detroit, MI) and MRS supplemented with 0.05% L-cysteine-HCl (Fisher Scientific, Tustin, CA) media, respectively. All plates inoculated with cells were incubated anaerobically in GasPak System with BBL GasPak Plus disposable H_2 and CO_2 generator envelopes (Becton Dickinson Microbiology Systems, Cockeysville, MD). Upon receipt of the bacterial strains, frozen stocks (with the addition of glycerol, 10% final concentration) were immediately prepared from late log phase cultures. Before every experiment, strains from frozen stocks were subcultured at least once in an appropriate medium. Strains were isolated from probiotic-containing food products by streaking product directly on MRS (for lactobacilli) or MRS supplemented with 0.05% L-cysteine-HCl (for bifidobacteria) agar for single strain isolation and incubated for 48 h at 37°C anaerobically. Gram stain reactions were performed on selected colonies to study

Table 2. Bacterial strains used in this study.

Laboratory designation	Designation by product or supplier	Source
DPTC 023		Snow Yogurt + 2, Snow Brand Milk Products Co., Ltd., Kawagoe, Japan
DPTC 024		Snow Yogurt + 2, Snow Brand
DPTC 031		ACE fermented milk drink, Snow Brand
DPTC 032		ACE fermented milk drink, Snow Brand
ATCC 25527	*Bifidobacterum animalis*	ATCC
ATCC 15700	*Bifidobacterum breve*	ATCC
DPTC 001	*B. breve* R-070	Institut Rosell Inc., Montreal, Quebec, Canada
ATCC 15697	*Bifidobacterum infantis*	ATCC
DPTC 047	*B. infantis* BBI	Chr. Hansen, Milwaukee, WI
DPTC 002	*Bifidobacterum lactis* BB12	Chr. Hansen
ATCC 15708	*Bifidobacterum longum*	ATCC
DPTC 004	*B. longum* BB46	Chr. Hansen
DPTC 003	*B. longum* BBL	Chr. Hansen
DPTC 036	*Bifidobacterium* spp.	Rolly fermented milk, Snow Brand
ATCC 4356	*Lactobacillus acidophilus*	ATCC
ATCC 700396	*L. acidophilus*	ATCC
DPTC 025	*L. acidophilus*	Mil Mil fermented milk, Yakult, Tokyo, Japan
DPTC 049	*L. acidophilus*	Mil Mil fermented milk, Yakult
DPTC 046	*L. acidophilus* AS-1	Quest International, Rochester, MN
DPTC 027	*L. acidophilus* DDS-1	Capsule supplement, Natren Inc., Westlake Village, CA
DPTC 010	*L. acidophilus* HP10	Northeast Nutraceuticals, S. Boston, MA
DPTC 011	*L. acidophilus* HP100	Northeast Nutraceuticals
DPTC 012	*L. acidophilus* HP101	Northeast Nutraceuticals
DPTC 013	*L. acidophilus* HP102	Northeast Nutraceuticals
DPTC 014	*L. acidophilus* HP103	Northeast Nutraceuticals
DPTC 015	*L. acidophilus* HP104	Northeast Nutraceuticals
DPTC 048	*L. acidophilus* HP15	Northeast Nutraceuticals
DPTC 005	*L. acidophilus* NCFM	Rhodia Inc., Madison, WI
DPTC 006	*L. acidophilus* NCFM	North Carolina State University (NCSU), Raleigh, NC
DPTC 007	*L. acidophilus* PIM703	Chr. Hansen
DPTC 008	*L. acidophilus* SBT2062	Snow Yogurt + 2, Snow Brand
ATCC 33620	*Lactobacillus amylovorus*	ATCC
ATCC 393	*Lactobacillus casei*	ATCC
DPTC 051	*L. casei* DN-114 001	Actimel Original fermented milk drink, Danone, Paris, France
DPTC 034	*L. casei* LC10	Rhodia
DPTC 035	*L. casei* PIM661	Chr. Hansen
DPTC 033	*L. casei* Shirota	Joie fermented milk drink, Yakult
DPTC 030	*L. casei* Shirota	Health drink produced by Yakult
ATCC 33820	*Lactobacillus crispatus*	ATCC
DPTC 009	*L. crispatus* BG2FO4	NCSU
ATCC 11842	*Lactobacillus delbrueckii* ssp. *bulgaricus*	ATCC
DPTC 020	*L. delbrueckii ssp. bulgaricus* 2038	Yogurt, Meiji Milk Products Co. Ltd., Tokyo, Japan
DPTC 021	*L. delbrueckii ssp. bulgaricus* 2038	Yogurt, Meiji
DPTC 019	*L. delbrueckii* ssp. *bulgaricus* MR120	Rhodia
DPTC 022	*L. delbrueckii* ssp. *bulgaricus* PIM695	Chr. Hansen
DPTC 045	*Lactobacillus rhamnosus* MX1	University of Western Ontario, London, Ontario, Canada
ATCC 33199	*Lactobacillus gallinarum*	ATCC
ATCC 33233	*L. gasseri*	ATCC
DPTC 026	*L. gasseri* ADH	NCSU
DPTC 016	*Lactobacillus helveticus* MR220	Rhodia
DPTC 017	*L. helveticus* NCK388	NCSU
ATCC 33200	*Lactobacillus johnsonii*	ATCC
DPTC 028	*L. johnsonii* 11088 (NCK 088)	NCSU
DPTC 029	*L. johnsonii* La-1	Nestlé, Lausanne, Switzerland
DPTC 018	*Lactobacillus lactis* San	Chr. Hansen
ATCC 25302	*Lactobacillus paracasei*	ATCC
ATCC 23272	*Lactobacillus reuteri*	ATCC
DPTC 037	*L. reuteri* 1063-S	Biogaia Biologics, Stockholm, Sweden
DPTC 038	*L. reuteri* 11284	Biogaia Biologics
DPTC 039	*L. reuteri* SD2112	Biogaia Biologics
DPTC 040	*L. reuteri T-1*	Biogaia Biologics
ATCC 7469	*Lactobacillus rhamnosus*	ATCC
DPTC 042	*L. rhamnosus* GR-1	University of Western Ontario
DPTC 043	*L. rhamnosus* R-011	Institut Rosell
DPTC 044	*L. rhamnosus* R-049	Institut Rosell
ATCC 53103	*Lactobacillus* GG	ATCC
ATCC 10556	*Streptococcus sanguis*	ATCC

their morphologies. Once purified, frozen seeds were prepared as indicated above.

Polymerase Chain Reaction

DNA extraction was conducted by using the commercial FastDNA Kit (Bio 101, Inc., Vista, CA) with the manufacturer's protocol. Extracted DNA was electrophoresed in a 1.5% agarose gel (Fisher Scientific) and was subsequently visualized with UV illumination after ethidium bromide staining. Based on the intensity of the DNA band, dilutions of DNA were prepared and used as templates in PCR.

The oligonucleotide primers used in this study were purchased from Genosys (The Woodlands, TX). Primer PAF [5′ AGA GTT TGA TCC TGG CTC AG 3′] position 8-27 (using the *Escherichia coli* numbering system) and 536R [5′ GTA TTA CCG CGG CTG CTG 3′] position 519-536 were used to amplify the 5′ region of the 16S rDNA gene. PCR was performed in a GeneAmp PCR System 2400 (Perkin-Elmer, Foster City, CA). For each reaction, a 50-μl reaction mixture was prepared. It consisted of 1× buffer without $MgCl_2$ (Promega Corp., Madison WI), 1.5 m*M* $MgCl_2$, 20 μM dNTP, 0.1 μM primers PAF and 536R, 1.5 U Taq Polymerase (Promega Corp.), and 3 μl of template. The amplification was programmed as follows: preincubation at 94°C for 2 min, followed by 40 cycles at: 94°C for 45 s, 55°C for 45 s, and 72°C for 60 s. After these cycles, the reaction was maintained at 72°C for 7 min and then cooled to 4°C. Five microliters of the PCR products were visualized after electrophoresis in a 1.5% agarose gel and were subsequently visualized by UV illumination after ethidium bromide staining. The PCR products were purified from primers and nucleotides using the Microcon YM-100 purification kit (Millipore Corp., Bedford, MA).

Partial 16S rDNA Sequencing

For each sample, two sequencing mixtures were prepared. One contained 4 μl of purified PCR product, 4 μl of BigDyeTerminator Reaction Mix (Perkin-Elmer/Applied Biosystems Division), 1.6 μl of primer PAF (1 μM) and 0.4 μl of dI H_2O. Another mixture was identical to the first one, except primer 536R was used instead. The sequencing reactions were performed in a GeneAmp PCR System 9600 (Perkin-Elmer) with 30 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. The first cycle was preceded by an incubation period for 2 min at 96°C. The temperature was lowered to 4°C after the last cycle. The sequencing products were purified through a column comprised of G-50 Sephadex (Sigma, St. Louis, MO), dried in a Speed-Vac SVC100 (Savant Instruments Inc, Farmingdale, NY) and resuspended in a loading buffer (five parts deionized formamide and 1 part 25 m*M* EDTA, pH 8.0, with 50 mg/ml of blue dextran); 2.2 μl of this mixture was loaded on a polyacrylamide gel, which was made from 42 g of urea, 10 ml of 10× TBE buffer, 46.5 ml of dI H_2O, 11.5 ml of Long Ranger Solution (FMC BioProducts, Rockland, ME) and solidified by adding 500 μl of 10% ammonium persulfate and 69 μl of N,N,N′,N′-tetramethylethylenediamine. The sequence of the 16S rDNA was determined on a 373 automated DNA sequencer (Perkin-Elmer/Applied Biosystems Division) according to the manufacturer's instructions.

Sequence Analysis

Sequences determined by the automated sequencer were edited by Factura (Perkin-Elmer/Applied Biosystems Division). The sequences of about the first 500 base pairs of the 16S rDNA molecules obtained from both directions by primers PAF and 536 R were assembled by Autoassembler (Perkin-Elmer/Applied Biosystems Division). Unresolved bases were treated as partial observations, giving partial weight during the calculations. Base-calling and sequence assembly were confirmed manually. The assembled sequences were used to search the GenBank (National Center of Biotechnology Information, www.ncbi.nlm.nih.gov) and the Ribosomal Database Project (Center for Microbial Ecology at Michigan State University, www.cme.msu.edu/RDP) databases for homologous sequences. The ends of all sequences were trimmed to the same length and aligned by Clustal W (European Molecular Biology Laboratory, Heidelberg, Germany). The relationships of these bacteria based on partial 16S rDNA sequences were determined by Phylip: Phylogeny Inference Package (Felsenstein, 1989) using a maximum likelihood method. Dendrograms were created by TreeView (Page, 1996).

Carbohydrate Fermentation

Miniaturized biochemical test kits API 50 CH (bioMérieux Vitek, Hazelwood, MO) were used to study the carbohydrate fermentation profiles of probiotic lactobacilli. To obtain bacterial cultures for experimentation, MRS broth was inoculated with frozen seed culture and grown overnight. Cultures were transferred into MRS broth, grown to stationary phase, and used as inoculum for streaking onto MRS agar plates. The test procedures were carried out following the manufacturer's guidelines. Duplication was performed on 22 strains. After obtaining the carbohydrate fermentation profile of a strain, species identification was determined by comparison with the database provided by the manufacturer. Furthermore, all profiles were compared and analyzed for studying the relationship among probiotic strains.

Cluster analysis of the API 50 CH results based on a squared Euclidean distance matrix and average linkage method was carried out using Minitab version 12.0 (Minitab Inc., State College, PA).

Fatty Acid Methyl Ester Analysis

Fatty acid methyl ester (**FAME**) analysis was performed according to the MIDI Manual (MIDI, Newark, NJ) for the analysis of anaerobe cultures. Cultures were streaked onto MRS agar plates using a four-quadrant streak pattern. They were incubated at 37 ± 2°C anaerobically for 48 ± 1 h. Cells (50 to 60 mg wet weight) from the third and fourth quadrant were harvested and extracted according to MIDI standard operating procedures. Ten microliters of each fatty acid methyl ester sample was separated on a 6890 Series Gas Chromatograph equipped with a split/splitless injector, flame-ionization detector, a 25-m × 0.2-mm Ultra 2 capillary column (Hewlett Packard, Palo Alto, CA), automatic sampler and computer with the Sherlock software (MIDI). Peaks were integrated automatically, and fatty acid identities and percentages were calculated by microbial identification system (MIDI). The reproducibility of the chromatographic technique was determined by repeated analyses of a standard quantitative FAME mixture (MIDI), and the presence of contamination was detected by using two negative controls in each trial. Replication was performed on 40 strains.

Peak area values for each fatty acid were converted as percentages of the total peak area to eliminate the effect of inoculum size variation.

RESULTS AND DISCUSSION

Table 3 shows the speciation results from the partial 16S rDNA sequencing and the carbohydrate fermentation study and the FAME analysis. Discrepancies between previous species designations and species inferred from 16S rDNA sequence homology were apparent for 26 out of 58 strains tested, including two ATCC *Lactobacillus* strains. When considering only the commercial strains obtained directly from the manufacturers, 14 of 29 strains carried species designations different from those obtained by the partial 16S rDNA sequencing. Strains from six commercial products were from species not listed on the label. In most cases, the *L. acidophilus* strains were found to be *L. crispatus*, one of the species in the *L. acidophilus* group. The *L. acidophilus* group is made up of two DNA-homology groups according to Johnson et al. (1980). Homology group A consists of *L. acidophilus* sensu stricto (A1), *L. crispatus* (A2), *L. amylovorus* (A3), and *L. gallinarum* (A4). Group B consists of *L. gasseri* (B1) and *L. johnsonii* (B2). The relationship among species in the *L. acidophilus* group is indicated in Figure 1. The sum of horizontal distances between any two species within the *L. acidophilus* group (especially among group A) is relatively short, implying that they have a somewhat close relationship. Species with such a close relationship may be difficult to differentiate since they likely have similar phenotypic characteristics. As described by Kandler and Weiss (1986), *L. acidophilus* and *L. gasseri* are found in similar habitats and cannot be distinguished by simple phenotypic criteria. Because phenotypic methods are still widely used today, the poorer differentiation ability of these phenotypic methods may explain why most commercial *L. acidophilus* strains in fact belong to other *Lactobacillus* species. On the other hand, manufacturers may favor using *L. acidophilus* on the label as it is generally more recognized by consumers, at least in the United States.

All *L. casei* strains in this study were speciated as *L. paracasei* by the partial 16S rDNA sequencing and carbohydrate fermentation study. The discrepancy may due to recent changes in taxonomy. Collins et al. (1989) proposed members of *L. casei* ssp. *alactosus*, *L. casei* ssp. *pseudoplantarum*, and *L. casei* ssp. *tolerans*, and the majority of *L. casei* ssp. *casei* strains be granted separate species level, and hence, they suggested the names *L. paracasei* sp. nov., *L. paracasei* ssp. *paracasei*, and *L. paracasei* ssp. *tolerans*. Although some have proposed rejecting the species name *L. paracasei* (Dellaglio et al., 1991; Dicks et al., 1996), it is still being used as the most current nomenclature.

A notable discrepancy also occurred in the taxonomic classification of strain DPTC 046, where 16S rDNA results suggested it was a different genera than indicated by the commercial supplier. DPTC 046 was speciated as *Streptococcus sanguis*, not *L. acidophilus*, as labeled. Although carbohydrate fermentation study of DPTC 046 suggested it was *L. acidophilus*, microscopic observation (cocoid cell morphology) was consistent with the sequencing results. This situation may have occurred due to contamination during the process of culture preparation or improper identity by the strain supplier. After communicating with the supplier, the supplier acknowledged that the strain demonstrated morphology uncommon for *L. acidophilus*.

Carbohydrate fermentation analysis was conducted on lactobacilli. Consistency among replicates of the carbohydrate fermentation study was very good. Only one (DPTC 018) out of 22 strains yielded a different result upon duplication. Speciation by the carbohydrate fermentation study exhibited some discrepancies compared with those by the partial 16S rDNA sequencing. All *L. johnsonii* strains were identified as *L. acidophilus* with profile status ranging from "very good to genus" to "very good." (Profile status is a measure of the reliability of

Table 3. Speciation results from partial 16S rDNA sequencing, carbohydrate fermentation study and fatty acid methyl ester (FAME) analysis.[1]

ID	Species designated by supplier or deduced from label	Partial 16S rDNA sequencing (GenBank)	Carbohydrate fermentation	FAME
ATCC 15697	*Bifidobacterium infantis*	*Bifidobacterium suis*	NT[2]	NT
ATCC 15700	*Bifidobacterium breve*	*B. breve*	NT	NT
ATCC 15708	*Bifidobacterium longum-*	*B. longum*	NT	NT
ATCC 25527	*Bifidobacterium animalis*	*B. lactis*	NT	NT
DPTC 002	*B. lactis*	*B. lactis*	NT	NT
DPTC 003	*B. longum*	*B. lactis*	NT	NT
DPTC 004	*B. longum*	*B. longum*	NT	NT
DPTC 047	*B. infantis*	*B. lactis*	NT	NT
ATCC33199	*Lactobacillus gallinarum*	*Lactobacillus crispatus*	*L. acidophilus*[3]	*Lactobacillus lactis/ Lactobacillus paracasei*
ATCC33200	*Lactobacillus johnsonii*	*L. johnsonii*	*L. acidophilus*[3]	*Lactobacillus coryniformis/ L. paracasei/Lactobacillus fermentum*
ATCC33323	*Lactobacillus gasseri*	*Lactobacillus gasseri*	*L. acidophilus*	*L. lactis/Pediococcus parvulus*[3]
ATCC33620	*Lactobacillus amylovorus*	*L. gallinarum*	*L. crispatus*[3]	*Lactobacillus vaccinostercus*[3]/ No match
ATCC33820	*L. crispatus*	*L. crispatus*	*L. crispatus*	*L. lactis*
ATCC4356	*L. acidophilus*	*L. acidophilus*	*L. acidophilus*	*L. coryniformis/L. lactis*
ATCC53103	*Lactobacillus* ssp.	*Lactobacillus rhamnosus*	*L. paracasei*[3]	*L. fermentum/Lactobacillus parabuchneri*
ATCC700396	*L. acidophilus*	*L. acidophilus*	*L. acidophilus*	*L. coryniformis*
DPTC 005	*L. acidophilus*	*L. acidophilus*	*L. acidophilus*[3]	*L. coryniformis/L. lactis*
DPTC 006	*L. acidophilus*	*L. acidophilus*	*L. acidophilus*	*L. coryniformis/ Lactobacillus catenaformis*
DPTC 007	*L. acidophilus*	*L. acidophilus*	*L. acidophilus*	*L. coryniformis/L. catenaformis/ P. parvulus*
DPTC 008	*L. acidophilus*	*L. acidophilus*	*L. acidophilus*	*L. lactis/L. catenaformis*
DPTC 009	*L. crispatus*	*L. crispatus*	*L. crispatus*[3]	*L. lactis/L. helveticus*
DPTC 010	*L. acidophilus*	*L. crispatus*	*L. helveticus*[3]	No match
DPTC 011	*L. acidophilus*	*L. crispatus*	*L. helveticus*[3]	No match/*Lactobacillus confusa*
DPTC 012	*L. acidophilus*	*L. crispatus*	*L. helveticus*	No match/*L. confusa*
DPTC 013	*L. acidophilus*	*L. crispatus*	*Lactococcus cremoris*	No match/*Lactobacillus mali/ L. confusa*
DPTC 014	*L. acidophilus*	*L. crispatus*	*L. helveticus*	No match/*Lactobacillus bifermentans/ L. confusa*
DPTC 015	*L. acidophilus*	*L. crispatus*	*L. helveticus*[3]	*L. confusa*
DPTC 016	*L. helveticus*	*L. crispatus*	*Lactococcus cremoris*[3]	*L. coryniformis/Leuconostoc pseudomesenteroides*
DPTC 017	*L. helveticus*	*L. crispatus*	*L. helveticus*[3]	*L. coryniformis/ P. parvulus*
DPTC 018	*L. lactis*	*Lactobacillus delbrueckii* ssp. *bulgaricus*	*L. lactis/ L. helveticus*[3]	No match

continued

Table 3 (continued). Speciation results from partial 16S rDNA sequencing, carbohydrate fermentation study and fatty acid methyl ester (FAME) analysis.[1]

ID	Species designated by supplier or deduced from label	Partial 16S rDNA sequencing (GenBank)	Carbohydrate fermentation	FAME
DPTC 019	*L. delbrueckii* ssp. *bulgaricus*	*L. delbrueckii* ssp. *bulgaricus*	*L. lactis*[3]	*Corynebacterium diphtheriae*/ No match
DPTC 020	*L. delbrueckii* ssp. *bulgaricus*	*L. delbrueckii* ssp. *bulgaricus*	*L. delbrueckii* ssp. *bulgaricus*[3]	No match
DPTC 021	*L. delbrueckii* ssp. *bulgaricus*	*L. delbrueckii* ssp. *bulgaricus*	*L. delbrueckii* ssp. *bulgaricus*	No match
DPTC 022	*L. delbrueckii* ssp. *bulgaricus*	*L. delbrueckii* ssp. *bulgaricus*	*L. delbrueckii* ssp. *bulgaricus*	No match[3]/*L. lactis*
DPTC 023[4]	*L. acidophilus*	*L. delbrueckii* ssp. *bulgaricus*	*L. delbrueckii* ssp. *bulgaricus*	No match
DPTC 024[4]	*L. acidophilus*	*L. delbrueckii* ssp. *bulgaricus*	*L. delbrueckii* ssp. *bulgaricus*	No match
DPTC 025	*L. acidophilus*	*L. gasseri*	*L. acidophilus*	*L. catenaformis*
DPTC 026	*L. gasseri*	*L. gasseri*	*L. acidophilus*	*L. coryniformis*/*L. fermentum*
DPTC 027	*L. acidophilus*	*L. gasseri*	*L. acidophilus*[3]	*L. coryniformis*/*L. paracasei*[3]
DPTC 028	*L. johnsonii*	*L. johnsonii*	*L. acidophilus*[3]	*Lactobacillus brevis*/*L. paracasei*[3]
DPTC 029	*L. johnsonii*	*L. johnsonii*	*L. acidophilus*	*Lactobacillus sake*/*L. paracasei*
DPTC 030	*Lactobacillus casei*	*L. paracasei*	*L. paracasei*	*L. fermentum*/*L. confusa*
DPTC 031[4]	*Lactobacillus jugurti*	*L. paracasei*	*L. paracasei*	*Lactobacillus mesenterorides*/*L. confusa*
DPTC 032[4]	*L. jugurti*	*L. paracasei*	*L. paracasei*[3]	*L. fermentum*/*L. parabuchneri*
DPTC 033	*L. casei*	*L. paracasei*	*L. paracasei*[3]	*L. fermentum*/*L. confusa*
DPTC 034	*L. casei*	*L. paracasei*	*L. paracasei*	*L. buchneri*[3]
DPTC 035	*L. casei*	*L. paracasei*	*L. paracasei*	*L. coryniformis*/*Lactobacillus buchneri*
DPTC 036	*Bifidobacterium* species	*L. paracasei*	*L. paracasei*[3]	*L. fermentum*
DPTC 037	*Lactobacillus reuteri*	*L. reuteri*	*L. fermentum*[3]	*L. vaccinostercus*
DPTC 038	*L. reuteri*	*L. reuteri*	*L. fermentum*	*L. parabuchneri*[5]
DPTC 039	*L. reuteri*	*L. reuteri*	*L. fermentum*	*L. confusa*/*L. bifermentans*
DPTC 040	*L. reuteri*	*L. reuteri*	*L. fermentum*	*L. fermentum*/*L. parabuchneri*
DPTC 042*	*L. rhamnosus*	*L. rhamnosus*	*L. rhamnosus*	*L. confusa*/*L. parabuchneri*
DPTC 043	*L. rhamnosus*	*L. rhamnosus*	*L. rhamnosus*[3]	*L. confusa*/*L. parabuchneri*
DPTC 044	*L. rhamnosus*	*L. rhamnosus*	*L. rhamnosus*	*L. fermentum*[3]/*L. parabuchneri*
DPTC 045	*L. rhamnosus*	*L. rhamnosus*	*L. rhamnosus*	*L. parabuchneri*
DPTC 046	*L. acidophilus*	*S. sanguis*	*L. acidophilus*	*L. fermentum*/*Streptococcus mitis*[3]
DPTC 048	*L. acidophilus*	*L. crispatus*	*L. helveticus*[3]	No match/*L. lactis*

[1]Where multiple species are listed, this indicates different speciation results were obtained in replicate trials. Underlined strain IDs indicates strains were isolated from commercial products by our laboratory. Bold-faced strain IDs indicate commercial probiotic strain obtained directly from manufacturer. *Strain being evaluated for commercialization but not yet commercialized.

[2]Not tested.

[3]Conducted in duplicate.

[4]DPTC 023 and DPTC 024 were isolated from the same product as were DPTC 031 and 032. Hence, when calculating the number of discrepancies, each pair was counted as one.

[5]Conducted in triplicate.

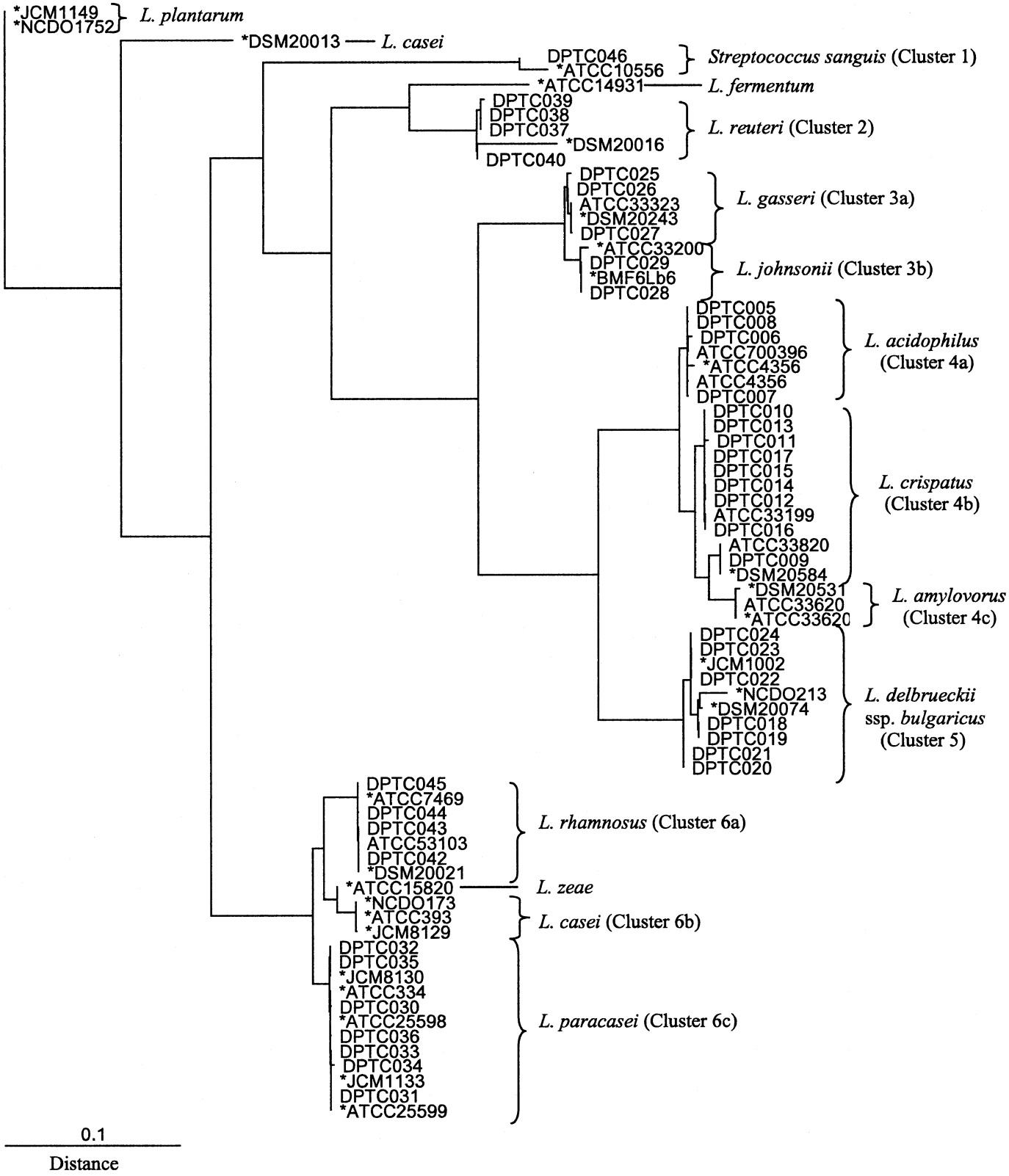

Figure 1. Unrooted tree derived from partial 16S rDNA sequencing shows the relationships of *Lactobacillus* strains tested in this study. Sequences from database GenBank are indicated by an asterisk followed by the strain ID.

the speciation. It was given when the result was compared with the API 50 CH database.) In addition, all *L. reuteri* strains were identified as *L. fermentum* with "good" to "very good" profile status. This identification method, therefore, lacks the ability to differentiate some closely related microorganisms. On the other hand, three commercial *L. gasseri* strains were identified as *L. acidophilus*, but with "low discrimination" profile status. This implied these commercial strains exhibited carbohydrate fermentation profiles rather dissimilar to neotype *L. acidophilus*. This method might, then, be useful to distinguish these strains if the database were more comprehensive.

Variability among replicates of the FAME analysis was so high that it was concluded that this approach was not useful for speciation of probiotic lactobacilli. Problems with limitations in the MIDI database as well as obtaining consistent extraction of fatty acids likely contributed to these difficulties. Gas chromatography of bacterial cellular fatty acid methyl esters is primarily used in clinical microbiology as a means of identifying many medically important gram-negative bacteria such as *Pseudomonas* (Mukwaya and Welch, 1989) and *Campylobacter* (Lambert et al., 1987). It has also been applied to *Lactobacillus* (Rizzo et al., 1987; Gilarova et al., 1994). However, this method was not optimized for the probiotic *Lactobacillus* species in this study. Of 50 strains tested by FAME, only one speciation result agreed with the carbohydrate fermentation study and none with the sequencing results. Moreover, the testing of many strains resulted in a "no match" result, indicating the inadequate nature of the MIDI database for lactobacilli. Slight variations in cultivation temperature, pH, NaCl, and growth state can profoundly affect the cellular fatty acid contents of lactic acid bacteria (Gilarova et al., 1994). Consistent speciation results are therefore difficult to achieve.

The genetic relationships of *Lactobacillus* strains used in this study were visualized as a dendrogram based on the results of the partial 16S rDNA sequencing (Figure 1). This cluster analysis is an important component of 16SrDNA sequence analysis to determine the relationship of unknown strains to control strains. In addition to the probiotic strains used in this study, some database sequences were also used for reference. *Bifidobacterium breve* ATCC 15700 and *Lactobacillus johnsonii* ATCC 33200 were two "outliers" that did not cluster with any strains (data not shown). In Figure 1, six major clusters could be identified. Cluster 1 contained two *Streptococcus* strains, ATCC 10556 and DPTC 046. Cluster 2 consisted of all *L. reuteri* strains in this study together with the reference *L. reuteri* DSM 20016. Cluster 3 was divided into two subclusters, one of which contained *L. gasseri* strains, from homology group B of the *L. acidophilus* group. The *L. johnsonii* strains, also considered the homology group B of *L. acidophilus* group, were found in another subcluster. Cluster 4 is the largest cluster containing 22 strains, in which *L. acidophilus*, *L. crispatus*, and *L. gallinarum* could be found. They are considered as homology group A in the *L. acidophilus* group. Cluster 5 contained *L. delbrueckii* ssp. *bulgaricus* and the reference *L. delbrueckii* ssp. *bulgaricus* JCM 1002. *L. casei*, *L. paracasei*, and *L. rhamnosus* formed another distinct cluster 6. Some reference strains did not cluster with the strains tested in this study. *L. plantarum* JCM 1149 and NCDO 1752 formed a separate group.

The relatedness among clusters can be depicted from the sum of horizontal lengths between them. *Lactobacillus acidophilus* homology group A is more closely related to *L. delbrueckii* ssp. *bulgaricus* than homology group B. In cluster 4b, *L. crispatus* DPTC 009 and ATCC 33820 are separated from other *L. crispatus* strains. This result is consistent with the carbohydrate fermentation study (Figure 2). *Lactobacillus paracasei*, *L. casei*, and *L. rhamnosus* have high similarity in their 16S rDNA sequences. Figure 1 provides another piece of evidence suggesting that *L. casei* ATCC 334 is more closely related to *L. paracasei* than other *L. casei*, even though Dicks et al. (1996) suggested ATCC 334 should be designated the neotype strain of *L. casei.*

The genetic relationships of the study's bifidobacteria strains are represented in Figure 3. All *B. lactis* strains grouped together in a distinct cluster, along with two *Bifidobacterium animalis* strains. However, other *Bifidobacterium* strains (for example, *Bifidobacterium infantis*, *Bifidobacterium longum*) do not form a distinct cluster.

Other than the relatedness of probiotic strains, the dendrogram in Figure 1 may also act to suggest species identity. When submitting a sequence to a database, the speciation is determined by the similarity (expressed as percentage) between the submitted sequence and the database. In the construction of the dendrogram, nucleotide substitution is also considered during the calculation. If reference strain sequences are included in the dendrogram calculation, this approach may be a more accurate way to identify bacteria.

For bifidobacteria, 16S rDNA sequencing was not always regarded as the best approach for speciation (Kullen et al., 1997; Leblond-Bourget et al., 1996), perhaps due to the high 16S rDNA sequence similarity among *Bifidobacterium* species. However, Matsuki et al. (1999) uses the technique successfully for speciation of bifidobacteria from human fecal samples.

Cluster analysis (Figure 2) based on carbohydrate fermentation study was in agreement with traditional classification of lactobacilli (Stiles and Holzapfel, 1997).

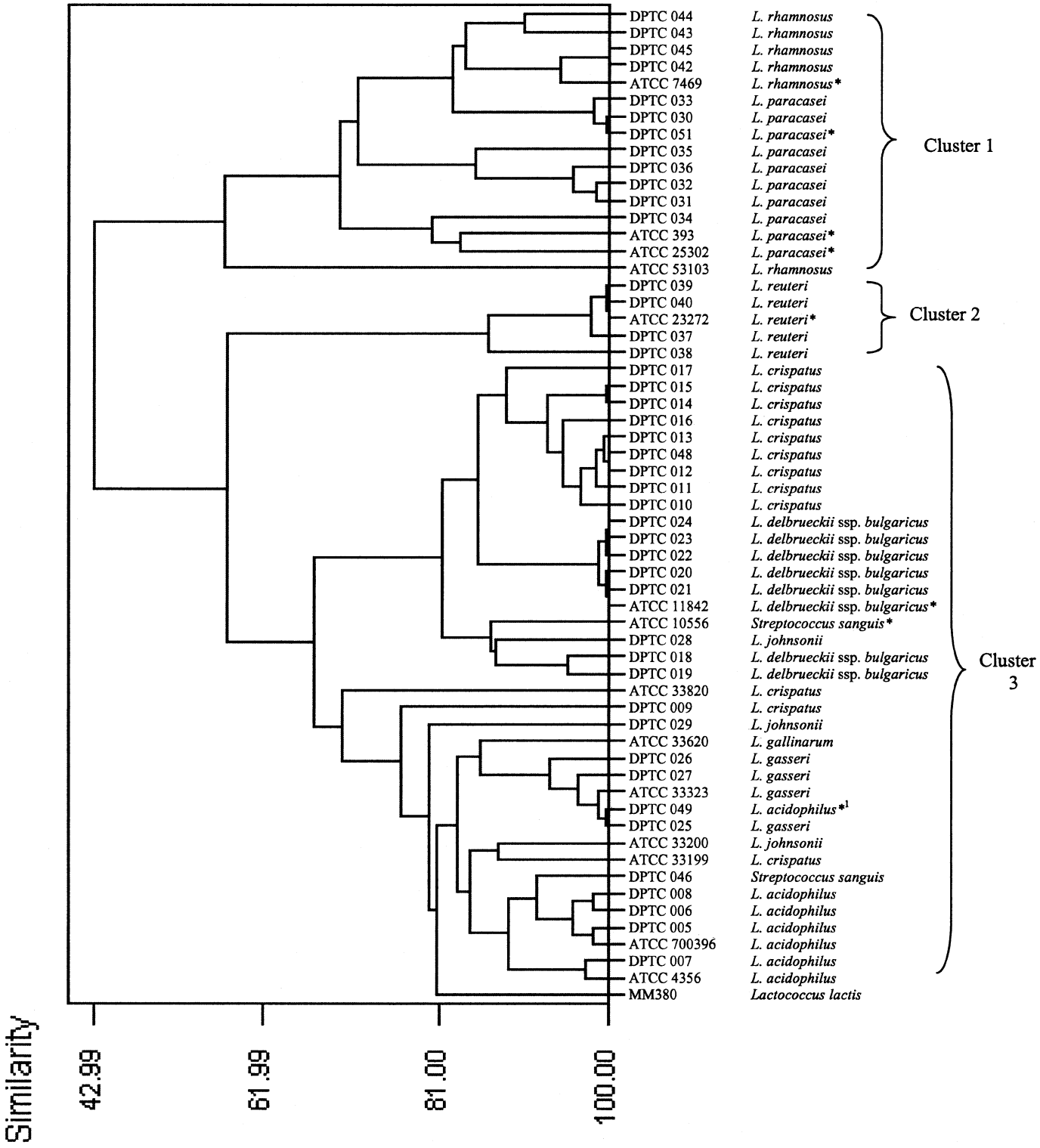

Figure 2. Dendrogram derived from carbohydrate fermentation study. Clustering of strains is based on square Euclidean distance and average linkage method. Three major clusters can be identified: facultative heterofermentators (cluster 1), obligate heterofermentators (cluster 2) and obligate homofermentators (cluster 3). Species determined by partial 16S rDNA sequencing. *Species not determined by sequencing. [1]Pulse-field gel electrophoresis pattern has high similarity to *Lactobacillus gasseri* DPTC 025, indicating these two strains are likely the same (data not shown).

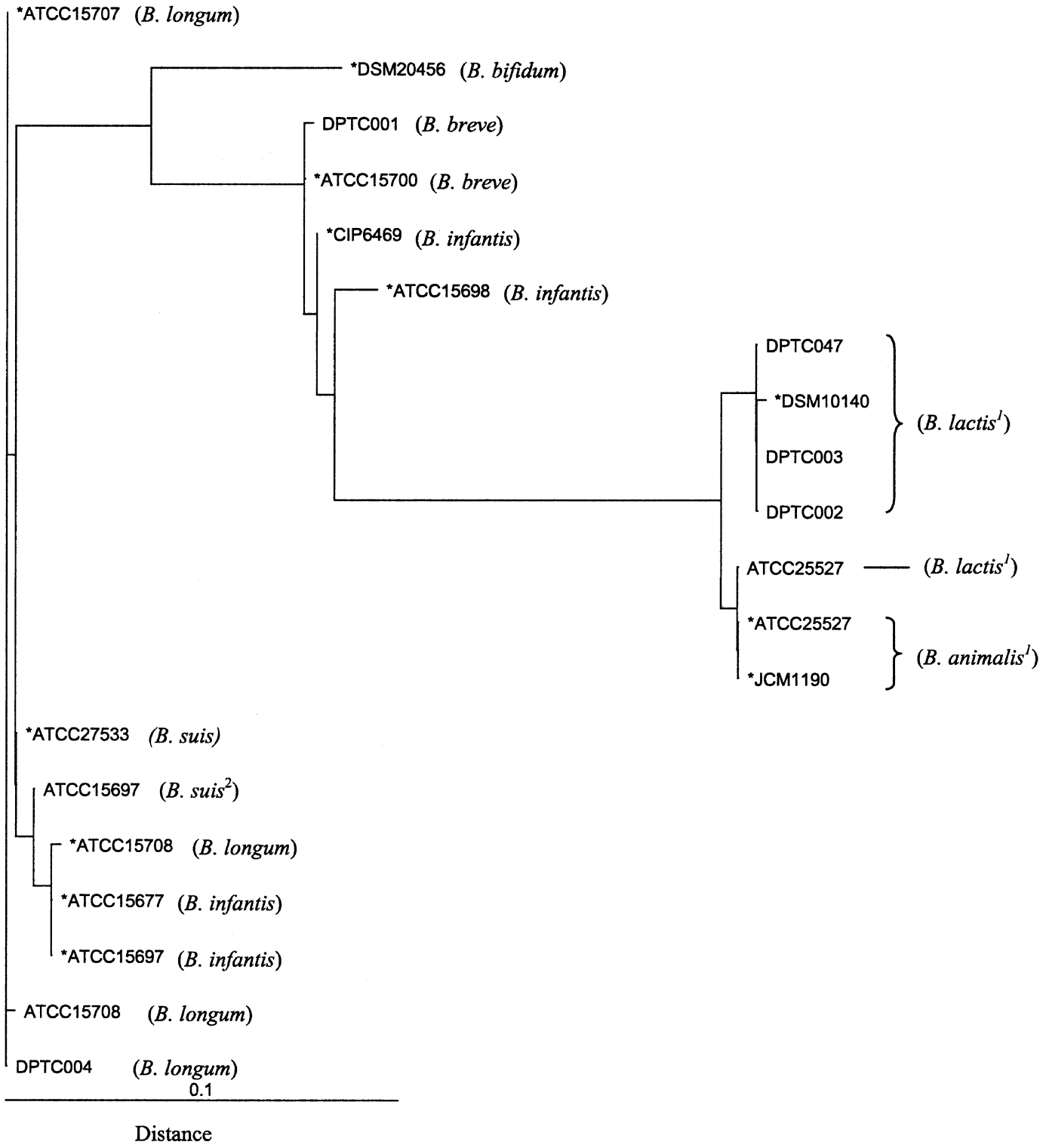

Figure 3. Unrooted tree derived from partial 16S rDNA sequencing shows the relationships of *Bifidobacterium* strains tested in this study. Sequences from database GenBank are indicated by an asterisk followed by the strain ID. [1]*Bifidobacterium animalis* and *Bifidobacterium lactis* are considered subjective synonyms (Cai et al., 2000).

Three distinct clusters can be identified. Cluster 1 consisted of facultative heterofermentators, *L. paracasei* and *L. rhamnosus*. Cluster 2 is the obligatory heterofermentative group that consisted of *L. reuteri*. Cluster 3 is the obligatory homofermentative group that included *L. acidophilus*, *L. gallinarum*, *L. crispatus*, *L. johnsonii*, *L. gasseri* and *L. delbrueckii* ssp. *bulgaricus*. DPTC 046 was speciated as *L. acidophilus* by API 50 CH, but our sequencing result indicated it is *Streptococcus sanguis*. Similar carbohydrate fermentation profiles shared by DPTC 046 and other *L. acidophilus* strains may mistakenly lead to inaccurate speciation. Unlike the 16S rDNA sequence results shown in Figure 1, *L. johnsonii* and *L. gasseri* did not form a separate cluster from *L. acidophilus* homology group A. In addition, there were some variations in the fermentation profiles of *L. johnsonii* strains. Similarly, fermentation profiles of some *L. crispatus* strains (DPTC 009, ATCC 33820, and ATCC 33199) were quite different to other *L. crispatus* strains. This resulted in their positions in different subclusters. However, *L. gasseri* strains appeared to separate from other *L. acidophilus* strains, even though they were unanimously identified as *L. acidophilus* by API 50 CH.

16S rDNA sequences can be used for speciation by homology to sequences from known bacteria in databases (Schleifer et al., 1995). The usefulness of this technique, however, is dependent on the completeness and accuracy of the databases used for comparison. GenBank and the Ribosomal Database Project (RDP) are the most complete 16S rDNA sequence databases. Upon the comparison of speciation results using these two databases (data not shown), some problems were revealed. First, different databases sometimes gave different speciation results. For instance, all strains speciated as *L. crispatus* by GenBank were identified as *L. acidophilus* by the RDP. The low similarity scores (<0.9) indicated RDP database sequences were insufficient to differentiate these two species. Second, some database sequences did not represent the most current nomenclatures. Third, the large influx of submitted sequences by different scientific communities makes control and maintenance of the database difficult. For example, ATCC 33199 was submitted as *L. crispatus* and *L. gallinarum* under two different records in GenBank. As the databases are improved (for example, by rejection of sequences that contain numerous ambiguities as indicated by "N" in the sequence), the reliability of the 16S rDNA speciation will be improved.

The reliability of partial 16S rDNA sequencing was tested by comparing the reference (ATCC) strains against the GenBank database. Except *L. gallinarum* ATCC 33199 and *L. amylovorus* ATCC 33620, all *Lactobacillus* reference strains were speciated correctly. This suggests that the use of the first ~500 bp of the 16S rDNA is effective for species identification. However, *L. gallinarum* and *L. amylovorus* are closely related species and they have high homology in the 16S rDNA sequence. Apparently, the variable region with the first 500 bp is inadequate to differentiate these two species.

Regarding the bifidobacteria, Vincent et al. (1998) noted that *B. animalis* and *B. lactis* exhibited high homology in their 16S rDNA sequencing. In this study, *B. animalis* ATCC 25527 and *B. infantis* ATCC 15697 were speciated as *B. lactis* and *B. suis*, respectively. This suggests variable regions in the partial 16S rDNA sequence we obtained might not be sensitive enough to differentiate these *Bifidobacterium* species. Other identification techniques such as the sequencing of recA (Kullen et al., 1997) and 16S to 23S internal transcribed spacer (Leblond-Bourget et al., 1996) were recommended. As the databases containing sequences of both recA and internal transcribed spacer build, speciation using these regions will be useful for bifidobacteria.

In conclusion, species identification of probiotics remains a challenge for the industry. On the one hand, manufacturers must be compelled to accurately represent the content of their probiotic products to the consumer and government regulatory agencies. On the other hand, consumer familiarity with certain names and the evolving nature of bacterial nomenclature can cause industry to hesitate to label products in a manner consistent with current valid nomenclature. However, the implications of intentional mislabeling of a product should be considered. Mislabeling closely related species of lactobacilli poses no safety risk, but may raise concerns about a company's credibility, both in the eyes of the consumer and regulatory agencies. This is especially true since advances in recent years in bacterial taxonomy and the availability of commercial laboratories performing fee-for-service speciation make accurate species determination of commercial strains a straightforward task. Mislabeling that results in incorrect representation of the genus of a bacterium, such as is the case for products labeling *Bacillus coagulans* as "Lactobacillus sporogenes", or failure to list bacterial contents such as *Enterococcus*, are more grievous offenses. The perpetuation of intentional mislabeling in the long run will serve to erode consumer confidence and undermine the credibility of the probiotic industry.

ACKNOWLEDGMENTS

This research was supported by California Dairy Research Foundation. Strains for this study were kindly provided by T. Klaenhammer, North Carolina State University; G. Reid, University of Western Ontario; L. Peterson, Chr. Hansen, Inc.; S. Bush, Rhodia Inc.; S. Arnaldo, Institute Rosell; R. Zink, Nestlé; W. Sandine, Ore-

gon State University; I. Casas, Biogaia Biologics; and H. Parsekian, Northeast Nutraceuticals. Technical advice provided by R. Jiménez-Flores is greatly appreciated.

REFERENCES

Cai, Y., M. Matsumoto, and Y. Benno. 2000. *Bifidobacterium lactis* Meile et al. 1997 is a subjective synonym of *Bifidobacterium animalis* (Mitsuoka 1969) Scardovi and Trovatelli 1974. Microbiol. Immunol. 44:815–820.

Canganella, F., S. Paganini, M. Ovidi, A. M. Vettraino, L. Bevilacqua, S. Massa, and L. D. Trovatelli. 1997. A microbiological investigator on probiotic pharmaceutical products used for human health. Microb. Res. 152:171–179.

Chateau, N., A. M. Deschamps, and A. H. Sassi. 1994. Heterogeneity of bile salts resistance in the *Lactobacillus* isolates of a probiotic consortium. Lett. Appl. Microbiol. 18:42–44.

Collins, M. D., B. A. Phillips, and P. Zanoni. 1989. Deoxyribonucleic acid homology studies of *Lactobacillus casei*, *Lactobacillus paracasei* sp. nov., ssp. *paracasei* and ssp. *tolerans*, and *Lactobacillus rhamnosus* sp. nov., comb. nov. Int. J. Syst. Bacteriol. 39:105–108.

Dellaglio, F., L. M. T. Dicks, M. Du Toit, and S. Torriani. 1991. Designation of ATCC 334 in place of ATCC 393 (NCDO 161) as the neotype strain of *Lactobacillus casei* ssp. *casei* and rejection of the name *Lactobacillus paracasei* (Collins et al., 1989) request for opinion. Int. J. Syst. Bacteriol. 41:340–342.

Dicks, L. M. T., E. M. Du Plessis, F. Dellaglio, and E. Lauer. 1996. Reclassification of *Lactobacillus casei* subsp. *casei* ATCC 393 and *Lactobacillus rhamnosus* ATCC 15820 as *Lactobacillus zeae* nom. rev., designation of ATCC 334 as the neotype of *L. casei* subsp. *casei*, and rejection of the name *Lactobacillus paracasei*. Int. J. Syst. Bacteriol 46:337–340.

Felsenstein, J. 1989. PHYLIP—Phylogeny Inference Package (version 3.2). Cladistics 5:164–166.

Giraffa, G., P. de Vecchi, and L. Rossetti. 1998. Identification of *Lactobacillus delbrueckii* subspecies *lactis* dairy isolates by amplified rDNA restriction analysis. J. Appl. Microbiol. 85:918–924.

Gilarova, R., M. Voldrich, K. Demnerova, K. Cerovsky, and J. Dobias. 1994. Cellular fatty acids analysis in the identification of lactic acid bacteria. Int. J. Food Microbiol. 24:315–319.

Hamilton-Miller, J. M. T., S. Shah, and J. T. Winkler. 1999. Public health issues arising from microbiological and labeling quality of foods and supplements containing probiotic microorganisms. Pub. Health Nutr. 2:223–229.

Hamilton-Miller, J. M. T., S. Shah, and C. T. Smith. 1996. "Probiotic" remedies are not what they seem. Br. Med. J. 312:55–56.

Johnson, J. L., C. F. Pehlps, C. S. Cummins, J. London, and F. Gasser. 1980. Taxonomy of the *Lactobacillus acidophilus* group. Intern. J. Syst. Bacteriol. 30:53–68

Kandler, O., and N. Weiss. 1986. Genus *Lactobacillus* Beijerinck 1901, 212[AL]. Pages 1209–1234 in Bergey's Manual of Systematic Bacteriology, Vol. 2. P. E. Sneath, N. S. Mair, and M. E. Sharpe, ed. Williams & Wilkins, Baltimore, MD.

Klein, G., A. Pack, C. Bonaparte, and G. Reuter. 1998. Taxonomy and physiology of probiotic lactic acid bacteria. Int. J. Food Microbiol. 41:103–125.

Kullen, M. J., L. J. Brady, and D. J. O'Sullivan. 1997. Evaluation of using a short region of the *recA* gene for rapid and sensitive speciation of dominant bifidobacteria in the human large intestine. FEMS Microbiol. Lett. 154: 377–383.

Lambert, M. A., C. M. Patton, T. J. Barrett, and C. W. Moss. 1987. Differentiation of *Campylobacter* and *Campylobacter*-like organisms by cellular fatty acid composition. J. Clin. Microbiol. 25:706–713.

Leblond-Bourget, N., H. Philippe, I. Mangin, and B. Decaris. 1996. 16S rRNA and 16S to 23S internal transcribed spacer sequence analyses reveal inter- and intraspecific *Bifidobacterium* phylogeny. Int. J. System. Bacteriol. 46:102–111.

Matsuki, T., K. Watanabe, R. Tanaka, M. Fukuda, and H. Oyaizu. 1999. Distribution of bifidobacterial species in human intestinal microflora examined with 16S rRNA-gene-targeted species-specfiic primers. Appl. Environ. Microbiol. 65:4506–4512.

Mukwaya, G. M., and D. F. Welch. 1989. Subgrouping of *Pseudomonas cepacia* by cellular fatty acid composition. J. Clin. Microbiol. 27:2640–2646.

Page, R. D. M. 1996. Treeview: an application to display phylogenetic trees on personal computers. Comp. Appl. Biosci. 12:357–358.

Pot, B., C. Hertel, W. Ludwig, P. Descheemaeker, K. Kersters, and K. H. Scheleifer. 1993. Identification and classification of *Lactobacillus acidophilus*, *L. gasseri* and *L. johnsonii* strains by SDS-PAGE and rRNA-targeted oligonucleotide probe hybridization. J. Gen. Microbiol. 139:513–517.

Quere, F., A. Deschamps, and M. C. Urdaci. 1997. DNA probe and PCR-specific reaction for Lactobacillus plantarum. J. Appl. Microbiol. 82:783–790.

Rizzo, A. F., H. Korkeala, and I. Mononen. 1987. Gas chromatography analysis of cellular fatty acids and neutral monsaccharides in the identification of lactobacilli. Appl. Environ. Microbiol. 53:2883–2888.

Salminen, S., and A. von Wright. 1998. Current probiotics—safety assured? Microbial Ecol. Health Dis. 10:68–77.

Sanders, M. E., D. C. Walker, K. M. Walker, K. Aoyama, and T. R. Klaenhammer. 1996. Performance of commercial cultures in fluid milk applications. J. Dairy Sci. 79: 943–955.

Schillinger, U. 1999. Isolation and identification of lactobacilli from novel-type probiotic and mild yoghurts and their stability during refrigerated storage. Int. J. Food Microbiol. 47:79–87.

Schleifer, K.-H., M. Ehrmann, C. Beimfohr, E. Brockmann, W. Ludwig, and R. Amann. 1995. Application of molecular methods for the classification and identification of lactic acid bacteria. Int. Dairy J. 5:1081–1094.

Stiles, M. E. and W. H. Holzapfel. 1997. Lactic acid bacteria of foods and their current taxonomy. Int. J. Food Microbiol. 36:1–29.

Tannock, G. 1999. Identification of lactobacilli and bifidobacteria. Pages 45–56 in Probiotics. A Critical Review. G. Tannock, ed. Horizon Scientific Press, Norfolk, England.

Tilsala-Timisjarvi, A., and T. Alatossava. 1997. Development of oligonucleotide primers from the 16S-23S intergenic sequences for identifying different dairy and probiotic lactic acid bacteria by PCR. Int. J. Food Microbiol. 35:49–56.

Vandamme, P., B. Pot, M. Gillis, P. de Vos, K. Kersters, and J. Swings. 1996. Polyphasic taxonomy, a consensus approach to bacterial systematics. Microbiol. Rev. 60:407–438.

Vincent, D., D. Roy, F. Mondou, and C. Dery. 1998. Characterization of bifidobacteria by random DNA amplification. Int. J. Food Microbiol. 43:185–193.

Yaeshima, T., S. Takahashi, N. Ishibashi, and S. Shimamura. 1996. Identification of bifidobacteria from dairy products and evaluation of microplate hybridization method. Int. J. Food Microbiol. 30:303–313.