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Comparison of Cellular Fatty Acid Composition and Genotypic Analysis of *Bifidobacterium longum* MK-G7 with Commercial Bifidobacteria Strains

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Abstract This study was conducted to compare the cellular fatty acid composition and genotypic analysis of *Bifidobacterium longum* MK-G7 originated from Koreans with other commercial type strains of bifidobacteria. The cellular fatty acid of *Bif. longum* MK-G7 was shown to be composed of $C_{16:0\ FAME}$, $C_{18:1\ cis\ DMA}$, $C_{18:1\ cis\ 9\ FAME}$, $C_{14:0\ FAME}$, $C_{19:0\ cyc\ 9,10\ DMA}$, Feature 7($C_{17:2\ FAME}$), and Feature 10($C_{18:1\ t11/t9/t6\ FAME}$). *Bif. longum* MK-G7 showed 99.9% homology and the highest relatedness with *Bif. longum* ATCC 15707 type strain. Both *Bif. longum* MK-G7 and *Bif. longum* ATCC 15707 showed 153 bp products on RAPD (randomly amplified polymorphic DNA) analysis, however, they showed quite different band patterns on PFGE (pulsed-field gel electrophoresis) analysis. Consequently, our present study showed that *Bif. longum* MK-G7 was different from any commercial type strains of *Bif. longum* tested.

Key words: *Bifidobacterium longum* MK-G7, cellular fatty acid composition, 16S rRNA sequence, DNA homology, phylogeny, genomic DNA polymorphisms, Korean origin

Bifidobacteria were first isolated from the feces of breast-fed infants, and well known to have typical pleomorphisms such as branching, bifurcated Y and V forms, and spatulate or club-shaped morphological characteristics [18]. Bifidobacteria are nonpathogenic, Gram-positive, and strictly anaerobic bacteria which inhabit intestinal tracts of humans and animals. In breast-fed infants, bifidobacteria comprise more than 90% of the bacterial population [1, 16]; however, their numbers gradually decrease over the life time of the host. Bifidobacteria are used for commercial fermented dairy products and have been suggested to exert health promoting effects on the host by maintaining balance of intestinal microflora, improving lactose intolerance, reducing serum cholesterol levels, increasing the synthesis of vitamins, and aiding anticarcinogenic activity [2, 4, 5, 9]. Identification of bifidobacteria isolated from stools or other materials may be made by tentative morphological appearance and biochemical tests such as carbohydrate fermentation patterns. Besides traditional methods, gas chromatography was introduced to analyze cellular fatty acid composition of the microorganisms. Microbial cellular fatty acid composition, due to its great variations, may be employed in microbiological taxonomic studies. Subsequently, it was considered that cellular fatty acid compositions of bifidobacteria depended upon the strains and was regarded as very useful tools for the identification of bifidobacteria. In general, genotypic analysis methods are more sensitive and accurate than phenotypic analysis methods for the identification of microorganisms. Recently, PCR-based DNA fingerprinting methods using arbitrary primers have been developed for studying genomic DNA polymorphisms. PCR-based molecular typing has been widely used for identifying bifidobacteria strains [7]. Furthermore, a rapid identification system for bifidobacteria isolated from foods and feces has been developed, using bifidobacteria-specific oligonucleotide probes. PFGE (pulsed-field gel electrophoresis) analysis is also a very useful method for identifying bifidobacteria. Herein, we show that a newly developed *Bif. longum* MK-G7, which has superior viability during growth in milk and survival rate during storage, was identified as a *Bif. longum* strain by means of cellular fatty acid composition and genotypic analyses.

MATERIALS AND METHODS

Test Cultures

The isolation and description of *Bif. longum* MK-G7 were previously reported [6]. Test culture strains used for this

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Table 1. Bifidobacteria strains used in this work.

Bifidobacteria strains tested	Original sources
Bif. infantis Y-2	Commercial
Bif. longum Y-3	Commercial
Bif. lactis Y-4	Commercial
Bif. longum Y-5	Commercial
Bif. longum Y-6	Commercial
Bif. adolescentis Y-8	ATCC 15706
Bif. infantis Y-11	ATCC 15697
Bif. longum Y-12	ATCC 15707
Bif. longum MK-G7	Maeil Dairy

study are listed in Table 1. For preservation and storage, test cultures were streaked and incubated on BS agar medium. Typically pure colonies were taken from the plates and serially transferred three times into MRS soft agar.

Cellular Fatty Acid Composition

Analysis of extracted fatty acids was performed by automated gas chromatography (Hewlett Packard series II model 6890, Microbial ID Inc., U.S.A.) according to its operating manual [17] and the HP 19091B-102 separation column was used. Microbial Identification System Software was used for FAMEs profile and the identification of the peak, retention time, peak area, and area ratio were measured, and compared with standard calibration solution.

16S rRNA Sequencing Analysis

Chromosomal DNA was extracted from *Bif. longum* MK-G7 and universal primers, 9F(5'-GAGTTTGATCCTGGC-TCAG-3', positions 9 to 27, *E. coli* 16S rRNA numbering) and 1542R(5'-AGAAAGGAGGTGATCCAGCC-3', positions 1542 to 1525, *E. coli* 16S rRNA numbering) were used for 16S rRNA gene amplification. The 5'-terminal of each primer was phosphorylated by T4 polynucleotide kinase. After PCR amplification, PCR products were precipitated using isopropanol using a Strandase ssDNA preparation kit (Novagen Inc., Madison, U.S.A.), and suspended into 10 μl of sterilized distilled water. The strand which contained the 5'-phosphorylated primer of PCR products was removed by exonuclease, and the remaining single strand was used as the template for the sequencing. Sequencing was performed using ^{35}S-labeled dATP and DNA sequencing kit (Amersham, Piscataway, U.S.A.), including previously known primers. Determined sequences of the 16S rRNA gene of *Bif. longum* MK-G7 were aligned with sequences from some lactobacilli and other species, based on Clustal W Software, and homology similarity was assessed.

RAPD Analysis

Bif. longum MK-G7, *Bif. longum* ATCC 15707, and *Bif. lactis* Y-4 were incubated in MRS broth at 37°C. BIL-1(GTTCCCGACGGTCGTAGAG) and BIL-2(GTGAGT-TCCCGGCATAATCC) were used as PCR primers. The size of the PCR amplified fragment was 153 bp [20]. For the extraction of genomic DNA, 3 ml of overnight culture was treated according to the modified Marmur's method [15]. Extracted DNA was suspended in 50 μl TE buffer. As for cell lysate for PCR, overnight culture was washed twice with PBS buffer and with distilled water once. Two-and-a-half-μl of suspension was added to 47.5 μl Triton-X 100 solution (1%) and heated at 100°C for 5 min. Thereafter, it was cooled on ice. Isolated DNA or cell lysate were used as template. A total 20 μl working volume was used in the test. PCR (Bio-Rad Gene Cycler) was done for 30 cycles consisting of 95°C for 5 min, 95°C for 30 sec, 55°C for 30 sec, and 72°C for 1 min, and 10 μl of final PCR product was analyzed on 1% agarose gel electrophoresis.

PFGE (Pulsed-Field Gel Electrophoresis) Analysis

Eight bifidobacteria strains were grown and inoculated (2%) in 15 ml of MRS broth containing 0.05% L-cysteine · HCl and 1% glycine. When the OD_{600} value reached 0.5, a 3 ml aliquot was taken and centrifuged at 12,000 rpm for 10 min. The cell pellet was washed with 1.5 ml TE buffer (10 mM Tris-HCl, 1 mM EDTA), resuspended in 100 ml TE buffer, and then kept in a water bath at 50°C. Two % low-melting point agarose was mixed with an equal volume of suspension at 50°C. The suspension was blocked in a sample mold at 4°C for 10 min. The block was transferred into an Eppendorf tube containing 1 ml lysis solution (1 M NaCl, 10 mM Tris-HCl, 0.5% Sarkosyl, 0.2% sodium deoxycholate, 0.25 M EDTA, 1 mg lysozyme). After standing at 37°C for 24 h, it was transferred into 1 ml ES solution which contained 0.25 M EDTA, 1% Sarkosyl, and proteinase K (1 mg/ml) and stored at 50°C for 24 h. The sample was washed with 1 ml TE buffer containing 1 mM PMSF (phenyl methyl sulfonyl fluoride) at room temperature for 30 min and washed with 1 ml of 10 mM Tris-HCl for 1 h. Following storage with 200 μl restriction enzyme buffer at 37°C for 1 h, the remaining buffer in the Eppendorf tube was dried completely with tissue paper, and the agarose block was digested with *Xba*I (30 unit, 37°C for 24 h). The restriction enzyme-treated block was loaded into the wells of 1% agarose gel containing 0.5× TBE buffer. PFGE was done using a Bio-Rad Chef DR II system according to Roy *et al.* [19].

Results and Discussion

Cellular Fatty Acid Composition

Bif. longum MK-G7 showed typical morphological characteristics such as branching, bifurcated Y and V forms, and spatulate or club shapes under scanning electron microscopy (data not shown). Cellular fatty acid compositions of bifidobacteria depended upon the species and was a very useful tool for the identification of bifidobacteria. The cellular fatty acid of *Bif. longum* MK-G7 was shown to

Table 2. Cellular fatty acid composition of *Bif. longum* MK-G7 and other bifidobacteria type strains.

Index	Feature names	Mean % of cellular fatty acid				
		Bif. longum MK-G7	*Bif. longum*	*Bif. adolescentis*	*Bif. breve*	*Bif. suis*
4	10:0 FAME[a]	0.27	-	0.60	1.63	0.81
11	12:0 FAME	1.07	0.67	1.41	1.77	1.04
12	11:0 DMA[b]	0.65	-	-	-	0.47
19	14:0 FAME	6.74	6.93	5.43	12.30	7.67
22	14:0 DMA	4.12	3.22	2.77	3.21	4.66
32	16:1 cis 7 FAME	1.30	1.05	2.08	2.27	1.20
33	16:1 cis 9 FAME	0.74	0.73	1.41	1.12	1.09
35	16:0 FAME	30.76	34.02	26.92	29.67	32.74
38	16:1 cis 9 DMA	0.48	-	-	-	0.56
39	16:0 DMA	0.85	0.88	0.60	0.47	0.84
52	17:0 cyc DMA	-	-	1.00	-	-
57	18:1 cis 9 FAME	11.97	17.94	22.35	21.72	12.70
60	18:0 FAME	5.12	7.53	5.48	4.21	6.33
62	18:1 cis 9 DMA	15.10	12.71	18.72	10.59	12.35
64	18:1 cis 11 DMA	0.80	-	-	-	0.85
65	18:0 DMA	0.63	0.57	0.44	-	0.72
70	19 cyc 9.10/:1 FAME	4.70	3.79	-	2.26	4.90
73	19:0 cyc 9,10 DMA	6.35	3.21	-	1.87	5.64
78	18:0 12OH	0.46	-	1.08	-	0.54
	18:2 cis 9,12 FAME	0.44	-	-	-	-
80	Summed Feature #1	0.68	1.18	0.89	1.06	1.13
86	Summed Feature #7	2.06	2.77	3.84	2.16	1.81
89	Summed Feature #10	4.70	1.65	3.42	2.03	1.74
91	Summed Feature #12	-	-	-	0.41	-

[a]Fatty acid methyl esters.
[b]Dimethyl acetal

be composed of $C_{16\,0\,FAME}$, $C_{18\,1\,cis\,DMA}$, $C_{18\,1\,cis\,9\,FAME}$, $C_{14\,0\,FAME}$, $C_{19\,0\,cyc\,9,10\,DMA}$, Feature 7($C_{17\,2\,FAME}$), and Feature 10($C_{18\,1\,cis11/9/t6\,FAME}$). Therefore, the cellular fatty acid composition of *Bif. longum* MK-G7 was quite similar to that of *Bif. longum* type strain, but different from those of *Bif. adolescentis*, *Bif. breve*, and *Bif. suis* (Table 2).

Genotypic Analysis

The 5'- and 3'-terminal sequences of 16S rRNA genes are quite similar in most bacteria. Many researchers [3, 8, 10, 11, 12, 13, 14, 21] have developed a rapid and easy-to-handle identification system for the members of genus *Bifidobacterium* isolated from foods and feces, and designed an oligonucleotide probe which is specific for the genus *Bifidobacterium*. Taxonomic and phylogenetic experimental results on *Bif. longum* MK-G7 by 16S rRNA sequencing analysis showed 99.9% homology with *Bif. longum* ATCC 15707 type strain (data not shown). Recently, PCR-based molecular typing has been widely used for the purpose of identifying bifidobacteria strains [7]. PCR analytical results using genomic DNA and cell lysate of test cultures are shown in Fig. 1. Although *Bif. longum* MK-G7 and *Bif. longum* ATCC 15707 showed 153 bp PCR products, *Bif. lactis* Y-4 did not produce any band at all, both in genomic DNA and in cell lysate. As an identification method for lactic acid bacteria, PFGE of genomic DNA digested with various restriction enzymes is very useful. The band patterns of 8 bifidobacteria strains were confirmed by the PFGE analysis method. *Bif. infantis* Y-2, *Bif. longum* Y-3, *Bif. longum* Y-6, and *Bif. adolescentis* ATCC 15706 showed similar band patterns (lane 1, 2, 4, 5, respectively). However, *Bif. longum* MK-G7 (lane 8) and *Bif. longum* ATCC 15707 (lane 7) showed somewhat different band

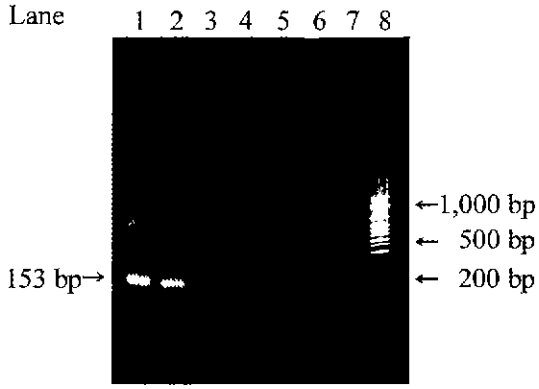

Fig. 1. PCR products in 1% agarose gel for bifidobacteria strains tested with BIL-1 and BIL-2 primers.
Genomic DNA lane 1: *Bif. longum* ATCC 15707; 2: *Bif longum* MK-G7; 3: *Bif. lactis* Y-4; Cell lysate lane 4: *Bif. longum* ATCC 15707, 5: *Bif longum* MK-G7; 6· *Bif lactis* Y-4; Lane 7. No DNA; Lane 8. DNA size marker.

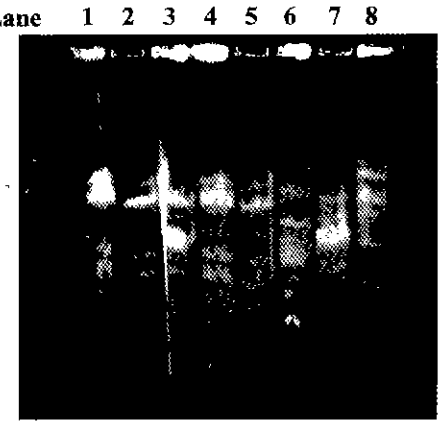

Fig. 2. Genomic DNA band patterns of bifidobacteria strains after digestion with *Xba*I by PFGE.
Lane 1. *Bif. infantis* Y-2· 2: *Bif longum* Y-3; 3: *Bif. longum* Y-5, 4: *Bif longum* Y-6; 5: *Bif. adolescentis* ATCC 15706; 6. *Bif. infantis* ATCC 15697, 7. *Bif. longum* ATCC 15707: 8: *Bif. longum* MK-G7.

patterns (Fig. 2). Consequently, based on cellular fatty acid composition and genotypic analysis, it is clear that *Bif. longum* MK-G7 is not identical to the commercial and type strains of *Bif. longum* tested. In conclusion, the cellular fatty acid composition and genotypic analysis employed in this study were found to be very useful means for the identification of *Bifidobacterium* strains.

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