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Preparation of *Kimchi* Containing *Bifidobacterim animalis* DY-64

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Abstract Aero-tolerant microorganisms were isolated from healthy Koreans over the age of 95 years. The microorganisms were then identified based on their morphological and biochemical characteristics and 16S rDNA sequences. The growth properties of the isolated strains were investigated in *kimchi*. The characteristics of *kimchi* containing the microorganisms were studied microscopically, physicochemically, and organoleptically. Among 7 aero-tolerant strains, a strain with a 16S rDNA sequence exhibiting 99% homology with *Bifidobacterim animalis* strain B83 was selected and named *B. animalis* DY-64. The new strain showed a better acid resistance and salt resistance ($p<0.05$) than *B. animalis* ATCC 25527. After 15 days of fermentation in *kimchi*, the viability of *B. animalis* DY-64 was about 10%, and the *kimchi* had a better overall edible quality than conventional *kimchi*. Thus, it was found that the application of *B. animalis* DY-64 to *kimchi* preparation produced a good overall edible quality.

Key words: *Bifidobacterium animalis* DY-64, *kimchi*

The genus *Bifidobacterium* is comprised of strictly anaerobic, Gram-positive, nonmotile, and nonsporulating fermentative rods, which are often club-shaped or spatulated extremities. The genus degrades glucose exclusively and characteristically via the fructose-6-phosphate shunt [20]. *Bifidobacteria* also produce acetate and lactate (3:2) as their major end products [20]. The mole percent G+C of the DNA of bifidobacteria is high and ranges between 55 and 64%. On the basis of DNA-DNA hybridization measurements and sugar fermentation patterns, 32 species have already been distinguished within the genus *Bifidobacterium* according to the present classification [2]. These species have also exhibited health-promoting properties, including maintaining an improved intestinal bacterial composition, reducing serum cholesterol, inhibiting the growth of potential pathogens, stimulating the immune response, and possible anticarcinogenic activity [2, 4, 6–8, 14, 16–18].

Kimchi is a traditional Korean fermented vegetable food. Various kinds of lactic acid bacteria such as *Lactobacillus*, *Leuconostoc*, and *Pediococcus* are involved during the fermentation of *kimchi*, and the viable lactic acid bacteria usually reaches 10^9 cells per gram-*kimchi*. As such, *kimchi* is a major dietary component that provides probiotics to Koreans. It has already been established that the lactic acid bacterial count in the human gut increases when ingesting *kimchi* [12]. Several studies have also reported on the application of functional bifidobacteria to *kimchi* preparation, and such bacteria need to have the following properties: aerotolerance, acid resistance, and salt resistance. Accordingly, this report describes the isolation of a novel bifidobacteria, named *Bifidobacterium animalis* DY-64, from healthy elderly individuals and its application to *kimchi* preparation.

Materials and Methods

Cultivation of Fecal Microorganism

Fecal samples were collected from healthy individuals living in Dam-yang, a Korean village renowned for longevity [9]. The fresh feces (0.1–0.2 g) were then decimally diluted with a sterile anaerobic diluting solution (0.45% KH_2PO_4, 0.6% Na_2HPO_4, 0.05% L-cysteine·HCl, 0.05% Tween 80, 0.1% Bacto agar). The microbial samples were inoculated into bifidobacteria selective (BS) medium and incubated at 37°C for 2 days in a Gaspak anaerobic jar (Gas Pak, BBL, U.S.A.).

Physiological and Biochemical Traits of Bifidobacteria

The shapes of the microorganisms were identified using scanning electron microscopy (S-4100, Hitachi, Japan). The cells were fixed in 2% (v/v) glutaraldehyde + 2%

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paraformaldehyde in a 0.05 M cacodylate buffer (pH 7.2), washed in a cacodylate buffer, dehydrated though a graded series of ethanol concentrations, freeze-dried, and finally embedded in gold. The Gram staining was performed using a conventional method. The F6PPK assay was performed essentially as described by Grill *et al.* [5], but scaled down to a volume suitable for 1.5-ml Eppendorf tubes. To identify the aero tolerance of the isolated microorganisms, the bacteria were cultivated on a BS agar medium under aerobic conditions for 3 days. The colony-forming microorganisms were regarded as aero-resistant strains. The sugar fermentation experiments were conducted using TPY media containing 0.5% sugar (arabinose, raffinose, starch, lactose, cellobiose, melezitose, sorbitol, maltose, or mannose) and 0.003% bromocresol purple as the pH indicator. The microorganisms were inoculated and incubated at 37°C for 72 h, and the production of acid identified based on the appearance of a yellow color. The acid resistance of the bifidobacteria was tested using 0.05% sodium phosphate buffers (pH 2.0, pH 2.5, pH 3.0).

As such, the bifidobacteria (10^8 cfu/ml) were incubated in the solution for 2 h at 37°C, and then surviving cells were counted. Meanwhile, to determine the salt tolerance of the isolated bifidobacteria, the microorganisms (3.8×10^9 cfu/ml) were incubated in an MRS (0.05% L-cysteine added) broth containing 1%, 1.5%, 2%, 2.5%, or 3% NaCl and subsequently incubated at 37°C for 3 days. The viable counting was then performed using BS agar media.

16S rDNA Analysis

The 16S rDNA full sequencing of the isolated bifidobacteria was conducted by the Korean Culture Center of Microorganisms, Seoul, Korea using synthetic PCR primers (Bif164 GGGTGGTAATGCCGGATG, Bif662 CCACCG-TTACACCGGGAA, 1100R GGGTTGCGCTCGTTG, 1492R TACGGYCCTTGTTACGACTT).

Preparation of *Kimchi* with Bifidobacteria

To make the cabbage *kimchi*, oriental Chinese cabbages, cut into 4 sections, were dipped in a 12% salt solution for 6 h. The pieces of cabbage were then washed twice with tap water, drained, and mixed with the following spices and additives: sliced radish 10 g, pepper powder 3 g, garlic 1.4 g, onion 1.5 g, leek 2 g, ginger 0.6 g, fermented anchovy sauce 2 g, fermented shrimp 1 g, boiled starch 0.5 g, sugar 0.5 g, and 100 g of the brined cabbage. The isolated bifidobacterium, grown for 3 days at 37°C in an MRS broth (Difco, Detroit, MI, U.S.A.) supplemented with 0.05% L-cysteine·HCl (w/w) (Sigma Chemical Co., St. Louis, MO, U.S.A.) was harvested by centrifugation at 10,000 rpm for 15 min. The cells were then added to the *kimchi* at a concentration of 10^8 cfu/g and the *kimchi* left to ferment at 4°C.

Viability of Bifidobacteria in *Kimchi*

The colonies were analyzed by a PCR. The *bifidobacteria* in the *kimchi* sample were cultured on BS agar plates by incubating for 72 h in an anaerobic system. The colonies were then analyzed by a PCR using genus-specific PCR primers, Bif164 and Bif 662 (Bioneer Science, Korea). The amplification was carried out in a thermal cycler (Perkin-Elimer, Boston, MA, U.S.A.). The reaction mixture contained the following chemicals: colony, approximately 25 pmol of each primer, 2 μl of premix (Bioneer Science, Korea), and 5 μl of SDDW. The DNA fragment was amplified as previously describer by Venema and Mathuirs [22]. Aliquots (5 μl) of the amplified products were then subjected to electrophoresis in 2% agarose gels in a TAE buffer (40 mM Tris acetate, 1 mM EDTA, pH 8.2). The gels were stained with ethidium bromide (5 μg/ml) and visualized under UV light. A 1 kbp DNA ladder (Elpis, Seoul, Korea) was used as the molecular mass marker.

Physicochemical Analysis of *Kimchi*

About 200 g of the *kimchi* was homogenized using a Stomach (Interscience, France) for 60 s, and then the broth was collected, diluted 10-fold with 0.85% saline, and smeared on LBS agar plates for a total count of the *Lactobacillus* spp. The plates were then incubated at 37°C for 48 h and the colony-forming units per ml counted. During the fermentation of the *kimchi*, the pH was measured using a Corning pH meter. Homogenized, centrifuged (12,000 rpm, 10 min), and 1/10 diluted samples were used to determine the organic acid and vitamin C levels during the fermentation. The organic acid analysis was performed using HPLC with an Aminex HPX-87H column. The operation conditions were as follows: mobile phase, 4 mM sulfuric acid; flow rate, 0.6 ml/min; detector, UV detector at 206 nm; temperature, 37°C. Meanwhile, the Vit C was measured using the 2,4-dinitrophenylhydrazine method with L-ascorbic acid as the standard.

Sensory Evaluations

Sensory tests were applied to the *kimchi* during the 30 days of fermentation. Fifteen trained panel members evaluated the *kimchi* using a 9-point method based on color, smell, taste, and texture. The results were analyzed using a significant-independent t-test from the Statistical Analysis System (SAS) [19].

RESULTS AND DISCUSSION

Isolation and Identification of Microorganisms

To apply bifidobacteria as probiotics, bifidobacteria-selective media were used to isolate bifidobacteria from the feces of healthy elderly individuals over the age of 95

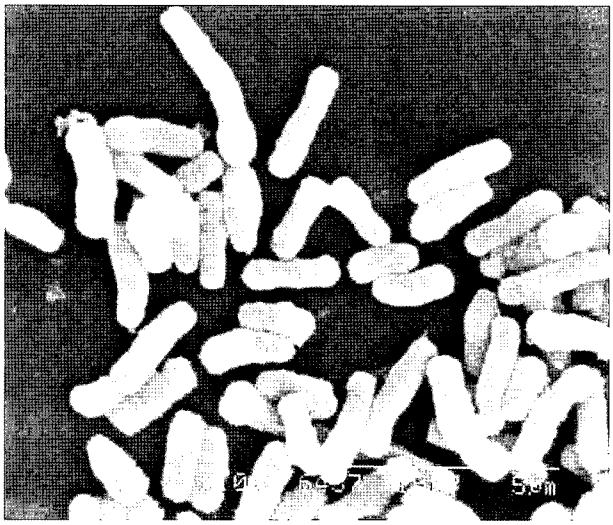

Fig. 1. Scanning electron micrograph of isolated strain (×10,000).

years in the Dam-yang area of Jeollanam-do, a region renowned for longevity in Korea.

Morphological Characteristics

The strains isolated from the feces samples were identified as *Bifidobacteria* spp. based on a fructose-6-phosphate phosphoketolase assay. The strains were then grown in an MRS broth at 37°C for 2 days and their shapes observed by scanning electron microscopy. As shown in Fig. 1, the shapes of the strains were spatula- or club-like rods, typical of bifidobacteria, and their sizes ranged from 1.4–2.5×0.5–0.8 μm. Shin *et al.* [21] also reported that the shapes of bifidobacteria from Korean were V-like or club-like rods.

16S rDNA Sequence of the Isolate

One strain was further identified using 16S rDNA sequencing. The length of the full sequence was 1,432 bp, and the sequence was compared with other reported sequences in the GenBank using the BLAST program. Figure 2 shows that the strain exhibited a 99% homology with *B. animalis* strain B83 (AY166508.1); thus, the newly isolated strain was named *B. animalis* DY-64 and deposited in the Korean Culture Center of Microorganisms in July 2, 2004 under the number KCCM-10583.

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B. animalis      ----------TGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGG  50
B. lactis        AGTTTGATCATGGCTCAGGATGAACGCTGGCGGCGTGCTTAACACATGCAAGTCGAACGG  60
isolated strain  ----------------------------------------TAACACATGCAAGTCGAACGG  21
                                                          **********************

B. animalis      GATCCCTGGCAGCTTGCTGTCGGGGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCAA  110
B. lactis        GATCCCTGGCAGCTTGCTGTCGGGGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCAA  120
isolated strain  GATCCCTGGCAGCTTGCTGTCGGGGGTGAGAGTGGCGAACGGGTGAGTAATGCGTGACCAA  81
                 ************************************************************

B. animalis      CCTGCCCTGTGCACCGGAATAGCTCCTGGAAACGGGTGGT---------AATACCGGATG  161
B. lactis        CCTGCCCTGTGCACCGGAATAGCTCCTGGAAACGGGTGGT---------AATACCGGATG  171
isolated strain  CCTGCCCTGTGCACCGGAATAGCTCCTGGAAATGGGTGGTGGGTGGTAAAAATCCGGATG  141
                 ********************************* *******        **  *******

B. animalis      GACCCTTGTGGGGGGAGCCGTCTAAGGTGAGACTCGTGATTGGGACTAAGTCGTAACAAG 1481
B. lactis        GACCCTTGTGGGGGGAGCCGTCTAAGGTGAGACTCGTGATTGGGACTAAGTCGTAACAAG 1491
isolated strain  GACCCTTGTGGGGGGAGCC-TCTAAG---TAACTC---------------------------- 1432
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Fig. 2. Alignments of 16S rDNA sequences from the isolated strain *Bifidobacterium animalis* (AY166508.1) and *Bifidobacterium lactis* (AY700230.1). Conserved DNA is depicted by (*).

Physiological Characteristics

According to Bergey's manual, the central portion of *B. animalis* is described as being slightly enlarged or distally inflated [20]. The shapes of *B. animalis* DY-64 were also similar, although the shapes of bifidobacteria can differ depending on the growth media or growth conditions. Table 1 shows the phenotypic characteristics of *B. animalis* DY-64. The Gram reaction of *B. animalis* DY-64 was Gram-positive and the optimum growth temperature was 37°C. *B. animalis* DY-64 did not ferment starch, contrary to the type strain *B. animalis* ATCC25527.

Probiotic Characteristics

Aero Tolerance. The criterion of aero tolerance is whether the bifidobacterium forms colonies on BS media incubated

Table 1. Comparison of phenotypic characteristics of *B. animalis*

Characteristics	*B. animalis* ATCC25527	*B. animalis* DY-64	Characteristics		*B. animalis* ATCC25527	*B. animalis* DY-64
Shape	rod	rod	Acid from	Arabinose	+[a]	+
Gram staining	+	+		Raffinose	+	+
Spore	–	–		Starch	+	–
Motility	–	–		Lactose	+	+
Anaerobic growth	+	+		Cellobiose	d[b]	–
Catalase	–	–		Melezitose	d	d
Opt. temp	37–41°C	37°C		Sorbitol	–	–
Growth at 15°C	–	–		Maltose	+	+
Growth at 45°C	–	–		Mannose	–	–

[a] +, positive; –, negative.
[b] d, delayed.

aerobically in an incubator at 37°C for 3 days. *B. animalis* DY-64 was found to be aero tolerant. However, the growth of the microorganism was faster under anaerobic conditions. Therefore, *B. animalis* DY-64 was considered to have excellent aero tolerance.

Generally, bifidobacteria cannot grow aerobically, as they are strictly anaerobic. However, Kim *et al.* [10] previously reported that *B. lactis* DSM 10140 was able to grow aerobically. According to their observation, the bacterium became aero tolerant when the bifidobacterium was repeatedly exposed to aerobic conditions. Thus, since an aero-tolerant bifidobacterium is required for industrial application, a mutation study is currently underway to obtain an aero-tolerant mutant [1]. Accordingly, it would appear that *B. animalis* DY-64 can be used as probiotics in foods in contact with oxygen during their preparation, such as *kimchi*.

Acid Tolerance. A strong acid tolerance is required for a probiotics to be used in acid foods and survive the gastric juices. As such, the acid tolerance of *B. animalis* DY-64 was tested at pH 2.0 for 2 h, as shown in Fig. 3. The initial concentration of *B. animalis* DY-64 was 9.6×10^8 cfu/ml at pH 3.0, pH 2.5, and pH 2.0. *B. animalis* ATCC25527 was used as the control microorganism. After 2 h of incubation, the number of viable cells was over 10^8 cfu/ml in the 3 experimental preparations. At pH 2.0 and pH 2.5, *B. animalis* ATCC25527 was more rapidly destroyed than *B. animalis* DY-64, with a significant difference ($p<0.05$) (Fig. 3), indicating that *B. animalis* DY-64 has a superior acid-tolerant property. Thus, it would appear that *B. animalis* DY-64 can be used in fully fermented *kimchi* without any significant decrease in viable cells, as the pH of fermented *kimchi* is only about pH 4.0 [3]. Furthermore, *B. animalis* DY-64 can also survive in the gastrointestinal tract, as the pH in the stomach is between 2.0 and 2.5 [2]. Kim *et al.* [10] previously reported that *B. lactis* was 50% viable at pH 2.5, whereas Shin *et al.* [21] reported that the isolated bifidobacterium survived at a concentration of 10^4 cfu/ml for 2 h at pH 2.3, when the initial inoculum was 10^8 cfu/ml.

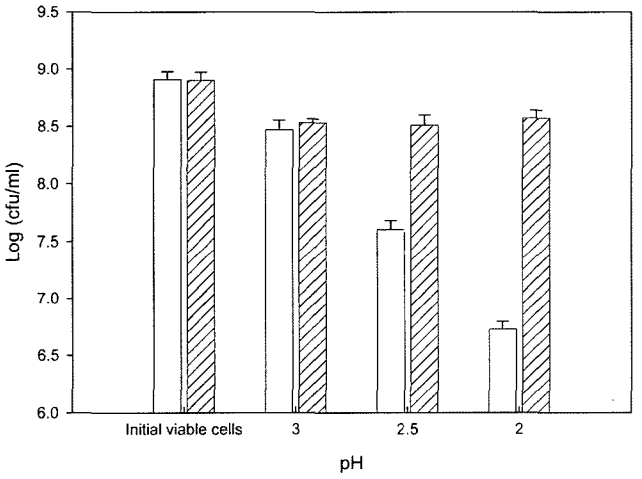

Fig. 3. Viable count (cfu/ml) of *B. animalis* under various pH conditions.

The cells were treated under various pH conditions for 2 h and the viable cells counted on a BS agar. The bars represent the mean values of triplicate determinations with standard deviations. The symbol (*) means that the values are significantly different at $P<0.05$, as determined by an independent t-test using the Statistical Analysis System. □: *B. animalis* ATCC25527; ▨: *B. animalis* DY- 64.

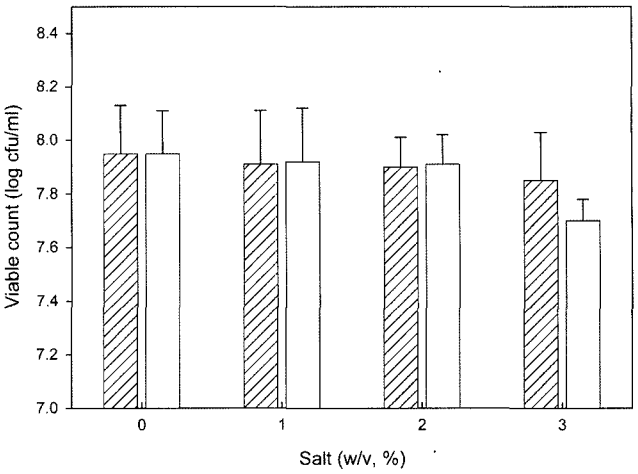

Fig. 4. Viable count (cfu/ml) of *B. animalis* DY- 64 under various salt conditions.

The bars represent the mean values of triplicate determinations, and the standard deviations are shown (Initial concentration: 4×10^8 cfu/ml, ▨: after 2 days, □: after 3 days).

Salt Tolerance. To investigate the salt tolerance of *B. animalis* DY-64, a viable count was conducted after inoculating the microorganism into MRS media containing 0–3% (w/v) sodium chloride. The initial viable count was 4×10^8 cfu/ml. After 3 days of incubation at 4°C, the viable count was 1.5×10^8 cfu/ml in the media containing 1% NaCl. However, the numbers decreased to 8.2×10^7 cfu/ml in the MRS media containing 2 to 3% NaCl (Fig. 4). Therefore, these results indicate that, as probiotics, *B. animalis* DY-64 can survive the cold chain of *kimchi*, as the viability of the microorganism did not decrease much at 4°C for 3 days. Kim *et al.* [10] also reported on the salt tolerance of *B. lactis* in an MRS broth containing 2% NaCl and found that the viable count was 10^8 cfu/ml after 24 h at 4°C, when the initial concentration was 1.5×10^9 cfu/ml. Therefore, it would appear that the *B. animalis* isolated in this study is more salt tolerant than *B. lactis*.

Application to *Kimchi* Preparation

Survivability of *B. animalis* DY-64 in *kimchi*. To investigate whether *B. animalis* DY-64 could be applied to *kimchi* as probiotics, the survivability of the microorganism was first examined during the fermentation of *kimchi* at 4°C for 25 days, when the initial viable count of the microorganism was 1.6×10^8 cfu/g-*kimchi*. Figure 5 shows

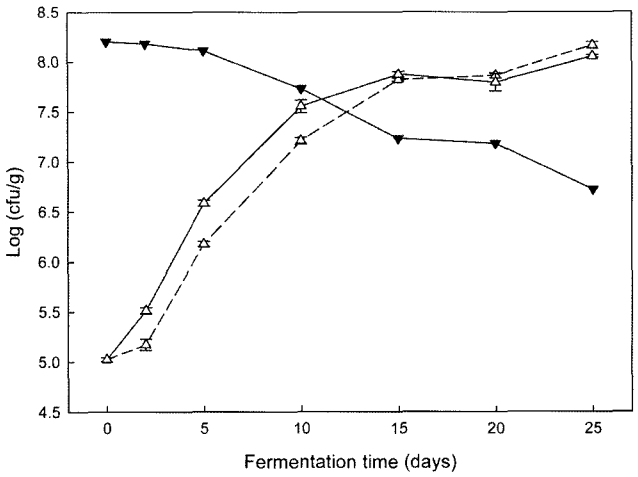

Fig. 5. Changes in viable microbial counts for *kimchi* during fermentation at 4°C. The dotted and solid lines indicate the conventional *kimchi* and *kimchi* containing *Bifidobacterium animalis* DY-64, respectively.
Symbols: △, lactobacillus; ▼, bifidobacteria.

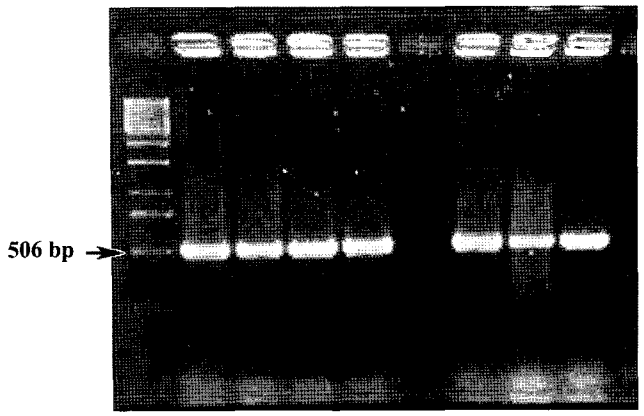

Fig. 6. Viability of *B. animalis* DY-64 in *kimchi* during fermentation, using PCR.
Lane 1: 1 Kb DNA ladder marker; lanes 2–5 and 7–9: *Bifidobacterium animalis* DY-64; lane 6: *Lactobacillus* spp.; lane 10: negative control.

the number of lactobacillus and bifidobacteria during the fermentation of conventional *kimchi* and *kimchi* containing bifidobacteria for 25 days at 4°C. The number of viable bifidobacteria was found to slowly decrease during the *kimchi* fermentation. The growth of lactobacillus was also found to be higher in the *kimchi* containing *B. animalis* DY-64. Murti *et al.* [15] previously reported that the presence of bifidobacteria stimulated the growth of lactic acid bacteria during the fermentation of soymilk. Wang *et al.* [23] also reported similar results. The viable *B. animalis* was identified using a colony PCR technique (Fig. 6) after spreading an inoculation of *kimchi* on BS media and subsequently incubating at 37°C for 3 days. No colonies were formed on the BS medium when the conventional *kimchi* sample was inoculated. The size of the typical PCR product was about 506 bp when bifidobacteria-specific primers were used against the 16S rDNA sequence. Meanwhile, the theoretical size of the amplified DNA was 506 bp when the *B. animalis* DY-64 gene was used as the template. The excellent survivability of *B. animalis* DY-64 in *kimchi* at 4°C is represented in Fig. 6. After 15 days of fermentation, the time generally known as the optimum fermentation period for *kimchi*, the viable count of *B. animalis* was 5.4×10^7 cfu/g-*kimchi* (one-tenth of the original number of viable cells). After 25 days of fermentation, the cells remained at a level of 5.3×10^6 cfu/g-*kimchi*. Kim *et al.* [10] previously reported on the survivability of *B. lactis* after 6 days in fermented *kimchi* at 4°C using a PCR technique. However, they did not provide a quantitative analysis of the numbers of bifidobacteria. Lee *et al.* [13] also showed that *B. longum* could survive in Mul-*kimchi* containing 2% (w/w) sodium chloride, decreasing from 10^8 cfu/ml-*kimchi* to 10^7 cfu/ml-*kimchi* after 10 days of fermentation at 4°C [13]. Thus, when taken together, the present study found that *B. animalis* DY-64 can be effectively used as probiotics in *kimchi*.

pH and Organic Acids in *kimchi* Containing Bifidobacteria. The changes in the pH and organic acid concentrations were analyzed during *kimchi* fermentation. Based on a sensory evaluation, the optimum fermentation period was 15 to 20 days (Table 2). Lactic acid, the major organic acid in fermented *kimchi*, increased to twofold in the conventional *kimchi* after 20 days of fermentation, whereas acetic and ascorbic acid increased about 50% from the initial amount. As shown in Fig. 7, the bifidobacteria-included *kimchi* had a much higher amount of organic acids than the conventional *kimchi* as the fermentation proceeded. In particular, with a fermentation time of 15 to 20 days, a significant difference in the organic acid amount was found between the two types of *kimchi*. A significant difference was also found in the acetic acid amount in both types *kimchi* after 10 days of fermentation. Bifidobacteria are known to produce lactic and acetic acid from carbohydrates in a molar ratio of 2 to 3 [20]. The fact that the amount of acetic acid was higher in the bifidobacteria-containing *kimchi* can be explained by 1) a continuous acetic acid production metabolism in *kimchi* at 4°C despite the cessation of bifidobacterial growth, or 2) the growth promotion of heterofermentative lactic acid bacteria by bifidobacteria [15]. The ascorbic acid concentration always decreases during the initial period of *kimchi* fermentation, and the bifidobacteria-containing *kimchi* was no exception, as shown in Fig. 7. However, the ascorbic acid concentration in the well-ripened *kimchi* (after 15 days of fermentation) was 25 to 30 mg%. Similar results were obtained in previous studies [11]. It was also observed that the pH of the 5-day fermented conventional *kimchi* decreased significantly from pH 5.5 to 4.5. Yet, the pH of the bifidobacteria *kimchi* did not decrease as much as that of the conventional *kimchi*.

Table 2. Sensory score for conventional Kimchi and Kimchi containing *B. animalis* DY-64 during fermentation at 4°C

Attributes	*Kimchi*	Fermentation time (days)					
		2	5	10	15	20	25
Color	A	4.9±1.9[a]	3.9±1.6	4.5±1.3	6.0±0.9	5.6±1.5	4.7±1.9
	B	3.9±1.8	4.4±2.0	5.1±1.4	6.3±1.1	5.7±1.2	6.0±1.6
Sour flavor	A	4.8±1.6	5.3±1.9	5.4±1.2	5.5±1.6	6.1±1.6	5.6±1.2
	B	4.6±1.4	5.8±1.7	5.9±0.9	5.6±1.4	6.2±1.5	6.4±1.5
Taste							
Hot taste	A	5.1±1.4	5.4±1.9	4.6±1.5	5.2±0.9[b]	5.7±1.3	5.4±1.6
	B	4.4±1.9	4.8±1.6	4.5±1.2	6.3±1.0[a]	5.9±1.6	6.6±1.0
Sourness	A[c]	4.1±2.1	5.2±2.3	4.9±1.6	4.4±1.2[b]	5.4±1.3[b]	5.3±1.7[b]
	B	4.1±1.7	4.5±1.7	4.2±1.8	6.6±0.8[a]	6.6±0.8[a]	6.5±1.5[a]
Texture							
Softness	A	5.3±1.7	5.5±1.6	5.4±1.2	5.6±1.1[b]	5.8±1.2	5.4±1.6
	B	5.2±1.4	5.4±1.3	5.1±1.2	6.6±1.0[a]	6.1±1.7	7.3±0.8
Crispness	A	6.2±1.8	5.9±1.5	5.9±1.7	5.6±1.0[b]	5.8±0.8[b]	5.9±1.3[b]
	B	5.9±1.7	5.9±1.8	6.4±1.5	7.0±1.0[a]	6.7±0.7[a]	7.2±1.4[a]
Overall acceptability	A	5.2±1.4	4.6±1.4	4.8±1.5	5.6±1.0[b]	5.5±1.1[b]	5.4±1.7[b]
	B	4.8±1.5	5.3±2.0	5.4±1.0	6.6±0.8[a]	6.5±0.8[b]	6.6±1.2[a]

[a]Mean±SD, Data were analyzed by independent t-test.

[b]Different superscripts within a column indicate a significant difference ($p<0.05$).

[c]A, Conventional *kimchi*; B, *kimchi* containing *B. animalis* DY-64.

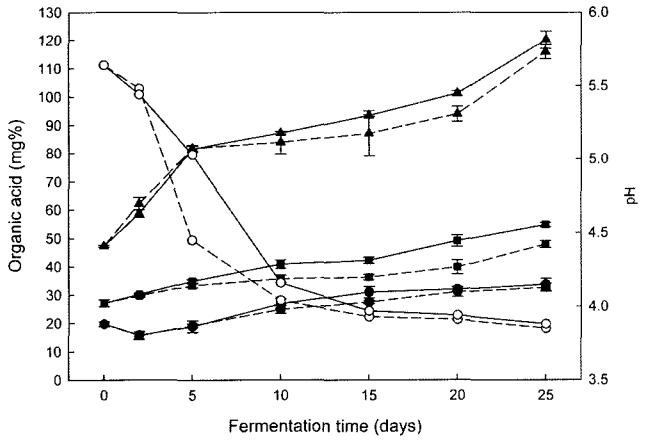

Fig. 7. Changes in organic acid contents and pH of *kimchi* during fermentation at 4°C. The dotted and solid lines indicate the conventional *kimchi* and *kimchi* containing *Bifidobacterium animalis* DY-64, respectively.
Symbols: ○, pH; ●, ascorbic acid; ■, acetic acid; ▼, lactic acid.

During the fermentation time, the number of lactobacillus increased 10-fold, while the amount of lactic acid increased significantly from 47 mg% to 80 mg%. Nonetheless, the pH of the bifidobacteria-containing *kimchi* was still higher, probably due to the buffering capacity of the bifidobacterial cell materials.

Organoleptic Analysis of *Kimchi* Containing Bifidobacteria. To compare the two types of *kimchi* with and without *B. animalis* DY-64, a sensory evaluation was performed during the fermentation, as shown in Table 2. No significant difference was found between the sensory scores for the two types *kimchi* as regards the color and sour flavor during fermentation. Thus, it would seem that the bifidobacteria did not effect the color or volatile acid production in the *kimchi*. Yet, the hot taste and softness were significantly different between the two *kimchi* types after 15 days of fermentation, and the *kimchi* with bifidobacteria was apparently more acceptable after the optimal ripening period. After 15 days of fermentation, the *kimchi* containing *B. animalis* DY-64 was superior in sourness, crispness, and overall acceptability than the conventional *kimchi* ($p<0.05$). This can be explained by the production of nonvolatile organic acids that increased the texture preference and overall acceptability. Consequently, since the bifidobacteria-containing *kimchi* had a better acceptability than the conventional *kimchi*, it would appear that bifidobacteria can improve the quality of *kimchi*. Kim *et al.* [10] previously reported that *B. lactis* did not influence the quality of *kimchi*. Therefore, bifidobacteria may specifically control the quality species in *kimchi*.

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