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Isolation and Identification of Superior *Bifidobacterium* strains from Korean Feces

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Abstracts

For isolating and identifying *Bifidobacterium* spp. originating from Korea, feces were sampled from healthy Korean infants nursery school and postpartum care center. Through the use of gram staining and microscopic examination for cell morphology, 87 bacterial strains presumed to be the *Bifidobacterium* strains were isolated from 59 Koreans. To identify the *Bifidobacterium* strains at the genus level, these bacteria were then analyzed using the TLC method. As a result, 29 of the isolated strains were confirmed as members of the genus *Bifidobacterium*. 29 *Bifidobacterium* strains were tested acid, bile salts and oxygen tolerance and investigated antioxidative effect specially. And determined the superiority of 5 strains out of 29 *Bifidobacterium* strains. Finally, the selected bifidobacteria was identified with using designed 16S-ITS rDNA primer.

Introduction

Bifidobacteria which has beneficial physiological effects on human bodies are affected by oxygen in processing and circulation periods of fermented milk and probiotics. And in case of fermented milk, acidity of product is rapidly decreased by lactose fermentation, so, survivability of strain is affected badly. Also, when intake dairy products including bifidobacteria, bifidobacteria in products lose their viability because of low pH(1-3) of stomach and secreted bile salts to small intestine. Therefore, we need to screen the strains have acids, bile salts, and oxygen tolerance. Recently, it has been interested in the correlation between oxidation and its damage. So, we investigated the antioxidative activity of isolated bifidobacteria.

Purpose of this works are to isolate the superior strains by test of viability in various conditions and antioxidative effect and to identify the isolated superior strains.

Materials and Methods

Feces were sampled from healthy Korean infants in a nursery school where under two years of age and postpartum care center. The stool samples were plated on a TPY agar gel and incubated in an anaerobic system for 72 h at 37℃. Selected colonies from every morphological type were picked from the TPY agar plates. These colonies were then Gram-stained and examined under a microscope for the presence of the predominant strains of *Bifidobacterium*. A identification that the isolated bacterial stains were from the genus *Bifidobacterium* was made on the basis of a thin layer chromatographic (TLC). To test the acid, bile and oxygen tolerance of bifidobacteria, 4 ㎖ TPY broth was adjusted to pH 5.0, 4.0, 3.0 and 2.0 by the addition of the solution that prepared by mixing acetic acid and lactic acid(3:2)(M), 4 ㎖ TPY broth with and without 0.6% of Oxgall(Difco, U.S.A.) and 4 ㎖ TPY broth with and without 1,000 ppm of oxygen peroxide(H_2O_2) was prepared. Both 1 ㎖ culture of strains were inoculated and incubated at 37℃ for 60 min. Inoculated 200 ㎕ cultured strains in new 10 ㎖(pH 6.5) TPY broth and incubated 37℃ for 18h. Bacterial growth was measured spectrometrically at 600 ㎚.

The measurement of antioxidative activity of Bifidobacteria was performed by the thiobarbituric acid (TBA) method, based on the monitoring of inhibition of linoleic acid peroxidation and on the scavenging ability of α,α-diphenyl-β-picrylhydrazyl(DPPH) free radicals. After direct amplification and recovery of gene fraction which is coding 16S rRNA from genomic DNA was performed, the sequence was identified. First, prepare the chromosomal DNA, PCR amplification was carried out using designed 16S-ITS rDNA primer. The thermal cycle was set for 35 cycles consisting of 95℃(5 min) for initial denaturation, 94℃ (1 min) for annealing, and 72℃(2 min) for an extension. And final extension was carries out at 72℃ 3min. The resulting amplified 16S-ITS rRNA gene was blotted on 1% agarose gel by electrophoresis.

Result and Discussion

In order to isolate and identify *Bifidobacterium* spp. originating from Korea, through the use of gram staining and microscopic examination, 87 bacterial strains presumed to be the *Bifidobacterium* strains were isolated from the feces 43 out of 59 infants. After the incubation and centrifugation of these isolated strains, the resulting supernatants were analyzed by Thin Layer Chromatography

(TLC). In 43 of the strains, two separate spots representing lactic acid and acetic acid were detected. In contrast. These results indicate that 29 strains were bifidobacteria. In addition, an F6PPK test, which can identify whether a strain includes fructose-6-phosphokinase (F6PPK), also produced results that corresponded with the results determined by a TLC analysis(data not shown). Accordingly, 29 isolates were determined as members of the genus *Bifidobacterium*.

Selected 29 *bifidobacterium* strains and 5 type *bifidobacterium* strains of human origin, *B. longum* KCTC 3128, *B. breve* KCTC 3220, *B. bifidum* KCTC 3202, *B. infantis* KCTC 3127, and *B. adolescentis* KCTC 3216. tested bacterial growth. 5 type *bifidobacterium* strains of human origin used as control strain. Therefore, in these isolated strains, 20 strains which O.D was over 1.70 were selected the first superior strains. And these strains tested acid, bile salts, hydrogen peroxide tolerance and storage stability with 5 control strains. To determine the most superior bifidobacteria in first selected strains, the grade of strains was determined as follow based on the results of microbial characteristic test. The result was shown at Table 1. Incase of bacterial growth test, the O.D value over 2.1, 2.0-2.1, 1.9-2.0, 1.8-1.9, 1.7-1.8 and below 1.7 determined the grade from 1 to 6, in good order. The grade of acid tolerance were determined against control. After reaction at pH 3, bacterial growth was divide into a hundred parts, over 70% , 50-70%, 30-50%, 10-30%, and below 10% decided from 1 to 5 grade. Like these the grade of bile salt tolerance and oxygen tolerance were decided 5 grades, from over 80% to below 50% and from over 90% to below 30%. And antioxidative ability were determined with 6 grades which were over 40%, 35-40%, 30-35%, 25-30%, 20-25% and below 20% on TBA methods and over 30%, 25-30%, 20-25%, 15-20%, 10-15% and blow 10% on DPPH methods.

In these strains, the best grade strain was HJL 7511, the grade was 1.40 , the next was 1.53 grade HJL 7501, and HJL 7519 was the third and the grade was 1.70. So, HLC 7511 and HLC 7519 were selected to superior *Bifidobacterium* strain.

Finally the selected 2 superior bifidobacteria were identified with using designed 16S-ITS rDNA primer. These 2 strains were identified *B. infantis*(Data not shown)

On the basis of these results, we concluded that *B. infantis* HJL 7511 and *B. infantis* HJL 7519 isolated from Korean feces were characteristic superior *B.*

longum of Korean. And these strains will be useful for functional fermented milk and probiotis.

Table 1. Effect of acid, bile salt and oxygen tolerance and antioxidative ability of bifidobacteria and 5 types KCTC strains

Strains	control	Acid Tolerance	Bile Slat Tolerance	Oxygen Tolerance	average
HJL 7501	1.39[a]	56.8[b]	81.8	80.0	1.53
HJL 7509	1.21	45.5	66.9	53.4	4.40
HJL 7511	1.73	83.3	69.6	82.7	1.40
HJL 7519	1.51	38.4	69.0	94.4	1.73
HJL 7521	1.09	43.7	75.7	92.4	2.47
HJL 7523	1.33	25.0	75.4	25.9	4.80
HJL 7526	1.42	15.6	57.8	33.6	4.33
HJL 7527	1.28	39.1	67.5	63.3	2.73
HJL 7528	1.32	21.2	77.1	40.0	2.73
HJL 7529	1.43	54.6	69.6	90.4	2.20
HJL 7530	1.54	59.7	46.7	88.5	2.20
HJL 7532	1.36	38.2	65.3	53.6	2.73
HJL 7533	1.49	7.4	75.8	95.2	3.00
HJL 7534	1.31	6.6	49.6	11.2	3.73
HJL 7537	1.41	39.7	89.9	54.2	4.46
HJL 7539	1.27	63.8	37.4	42.6	4.26
HJL 7540	1.05	14.3	67.2	103.6	2.40
HJL 7541	1.60	56.3	42.1	88.7	3.33
HJL 7542	1.54	18.8	74.5	98.1	2.40
HJL 7543	1.55	4.5	46.7	107.3	2.47
B. longum	1.46	61.0	73.8	79.9	4.33
B. bifidum	1.52	21.1	60.5	51.5	3.60
B. adolescentis	0.61	4.9	66.7	52.6	4.67
B. breve	0.42	28.6	52.1	42.9	4.73
B. infantis	0.81	22.2	62.8	76.7	4.73

[a]Optical density of bifidobacteria broth after incubation at 37℃ for 18 h

[b]Growth percentage compared with initial growth

Fig. 1. Antioxidative activity of bifidobacteria on TBA method

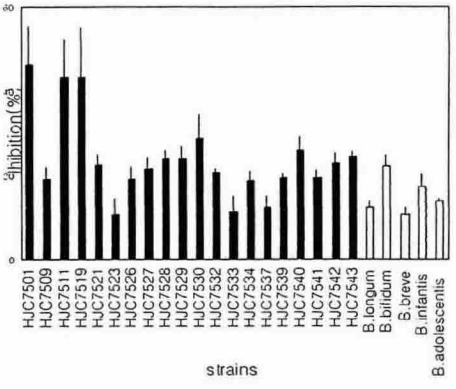
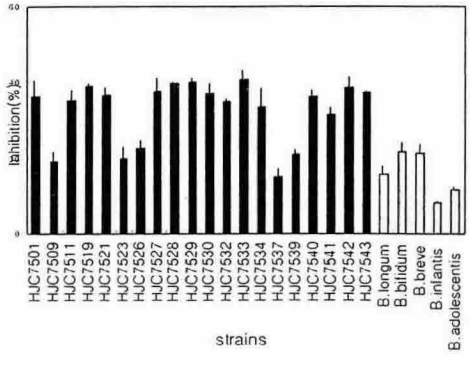


Fig. 2. Scavenging effect of bifidobacteria on DPPH free radical



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1. Lee, K.Y., and T.R. Heo, Identification of *Bifidobacterium* strains at the genus level by thin layer chromatographic determination of organic acids with culture broth of isolated bacteria strain from human feces(1998), Food Sci. Biotechnol. Vol(7), pp.95-99

2. Bezkorovainy, A., M.C. Robin, Morphology of bifidobacteria: In biochemistry and physiology of bifidobacteria(1998), pp.73-92. CRC Press, Floride

3. Shimamura, S., F. Abe, N. Ishibashi, and H. Miakawa, Relationship between oxygen sensitivity and oxygen metabolism of *Bifidobacteriuim* species(1992), J. Dairy Sci. Vol(75), pp.3296-3306.

4. MEEI-YN LIN, Phd and FEN-JUAN CHANG, Antioxidative effect of intestinal Bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356(2000), *Digestive Diseases and sciences*, Vol(45), pp.1617-1622

5. Takahiro Matsuki, Koichi Watanabe, Ryuichiro Tanaka and Hiroshi Oyaizu, Rapid identification of human intestinal bifidobacteria by 16S rRNA-targeted species- and group-specific primers(1998), FEMS Microbiology Letters, Vol(167), pp.13-121