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Isolation and Characterization of Lactic Acid Bacteria from Feces of Newborn Baby and from *Dongchimi*

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Lactic acid bacteria were screened from feces of newborn baby and from *dongchimi*. Selection criteria employed included the ability of strains to withstand environmental conditions such as low pH, high bile concentration, and oxygen. The isolates were applied to the juice of various vegetables, and fermentabilities of isolates were compared. Strains F20-3, F35-3, and F35-6 showed high stability compared to the other strains at pH 3.0 and 2.3. Strains D1 and D2 showed the highest survival at pH 3.0 and survived at 1% high bile concentration. The selected strains were able to survive at low pH and relatively high bile concentration and were not affected by oxygen. The growth of isolates was $>10^7$ cfu/mL in natural media, and strains were not affected by the pH values of the vegetables. Therefore, isolated strains are thought to survive through the intestinal ecosystem and are considered to be suitable for application of the fermented product using various vegetables for their functionality. The isolates were identified as *Lactobacillus plantarum* and *Lactobacillus fermentum*.

KEYWORDS: Probiotic; lactic acid bacteria; bile tolerance; acid tolerance; fermented products

INTRODUCTION

In recent years, the probiotic activity of lactic acid bacteria (LAB) has been emphasized. Probiotics are live microbial feed supplements, which beneficially affect the host animal by improving its intestinal microbial balance (*1*). The most widely used probiotic LAB are *Lactobacillus* and *Bifidobacterium* strains, and extensive studies on beneficial effects for human health of these species have been reported (*2*). The health benefits (for customers) attributed to probiotic bacteria in the literature can be categorized as either nutritional benefits or therapeutic benefits. Nutritional benefits include their role in enhancing the bioavailability of calcium, zinc, iron, manganese, copper, and phosphorus (*3*); and an increase of the digestibility of protein in yogurt and synthesis of vitamins in yogurt (*4*). The therapeutic benefits of probiotics reported include treatments of conditions including gastrointestinal disorders, hypercholesterolemia, and lactose intolerance; suppression of procarcinogenic enzyme; inhibitory effects on Ehrlich ascites tumor cells; immunomodulation; and treatment of food-related allergies (*5*). Adhesion to intestinal surface is a necessary property for probiotic strains, because intestinal attachment is important for colonization on the gastrointestinal tract for many bacteria species. In addition, tolerances to low pH, bile salt, and oxygen are important properties for the survival of bacteria under the conditions of the stomach and intestine (*6*, *7*).

Selection criteria for probiotic microorganisms include the following (*8*): (1) A probiotic strain must be safe (Generally Recognized As Safe). (2) It must be capable of being prepared in a viable manner and on a large scale. (3) It should remain viable and stable during use and under storage. (4) It should be able to survive in the intestinal ecosystem.

Probiotic strains have long been studied because of their functionality, but one problem with commercial preparation is stability of the probiotic during storage and fermentation. Many products contain low numbers of viable microorganisms, and there may be some taxonomic discrepancy regarding probiotic strains in some products (*9*).

We screened 16 strains from newborn baby meconium (a dark greenish mass that accumulates in the bowel during fetal life and is discharged shortly after birth), feces, and *dongchimi* (vegetables fermented in brine by lactic acid bacteria). After preliminary selection of the 16 strains, those isolates were further screened for their ability to survive at low pH, at high bile concentration, and under exposure to oxygen.

A synergistic effect of the dietary fiber and LAB for the improvement of the health of the large intestine of the host may be achieved by providing fermented fiber-rich natural plants to the host (*10*−*12*).

Therefore, in this study we compared the growth of isolated strains in various vegetable juices to the application of the development of new fermented vegetable products and to the study their viability.

MATERIALS AND METHODS

Isolation and Selection of Strains from Fecal Sample and Dongchimi. The newborn baby meconium and feces from 61 clinically healthy Korean newborn babies (31 males; 30 females) were obtained from Hana Obstetrics and Gynecology Hospital in Jeonju, Korea. The

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fecal specimens were collected in a sterile bottle and immediately transported to the laboratory, and three samples of dongchimi were purchased from a local retail market. The stool and dongchimi samples were thoroughly mixed with anaerobic solution prepared following the method of Mitsuoka (*13*). Approximately 1 mL of each mixed sample [sample/anaerobic solution, 1:1 or 1:2 (w/v)] was incubated on LBS agar (BBL, Cockeysville, MD). Screening for stability to bile and for stability to somewhat acidic environment was accomplished according to the method of Gorbach et al. (*14*). The bile salt (1.5 g) was added into 1 L of dissolved LBS agar, and the pH was adjusted to 5.5 using 0.1 N HCl. LBS agar plates are stored in an anaerobic chamber for 1−3 days prior to use and then used without autoclaving.

Isolated microorganisms from fecal specimens of healthy newborn babies and dongchimi were inoculated one more time into *Lactobacilli* MRS broth (Difco, Sparks, MD). The pH was adjusted to 3.0 using 0.1 N HCl to screen microorganisms for stability to more acidic condition. After incubation under anaerobic conditions for 24 h using Anaero Pack Helico (Mitsubishi Gas Chemical Co., Tokyo, Japan) (APH), which is a disposable O_2-absorbing and CO_2-generating agent, strains that showed a growth $>10^7$ cfu/mL were selected for further study. Isolated strains were kept at −60 °C in a deep freezer after freeze-drying.

Determination of Acid Tolerance. Isolated strains were grown in *Lactobacilli* MRS broth at 37 °C under anaerobic conditions using APH and then subcultured two or three times in MRS broth. Cultures was inoculated into 9 mL of 0.05 M sodium phosphate buffer solution adjusted to different pH values (7.0, 3.0, and 2.3) with 0.1 N HCl and NaOH. The initial bacterial concentration was 10^7-10^8 cfu/mL and was checked by viable cell population determination on MRS agar.

Samples were incubated at 37 °C for 2 h. Cells were serially diluted 10-fold in anaerobic solution to neutralize the medium acidity and keep the anaerobic condition. The viable cell population was determined by dilution and plate counting on MRS agar after 24−48 h of incubation. The survival rate was calculated as the percentage of colonies grown on MRS agar compared to the initial bacterial concentration.

$$\% \text{ survival} = \frac{\log \text{number of viable cells survived (cfu/mL)}}{\log \text{number of initial viable cells inoculated (cfu/mL)}} \times 100 \quad (1)$$

Determination of Bile Tolerance. Each test culture subcultured two or three times in MRS broth was supplemented in fresh MRS broth containing 0.25, 0.50, 0.75, or 1% (w/v) bile salts (Sigma Chemical Co., St. Louis, MO; catalog no. B-8756) composed of 50% sodium cholate and 50% sodium deoxycholate to determine the bile tolerance at different bile concentrations. The initial inoculum concentration was 10^6-10^8 cfu/mL, and all samples were incubated at 37 °C for 24 h under anaerobic conditions using APH.

Growth in test cultures containing bile was monitored by viable cell population determination on MRS agar medium after 24−48 h of incubation. The survival rate of test cultures was expressed as the percentage of colonies grown on MRS agar compared to the initial bacterial concentration after 24 h of incubation (eq 1).

Determination of Oxygen Tolerance. Test cultures were subcultured two or three times for determination of oxygen tolerance using an MRS broth. Then, test cultures were serially diluted 10-fold in anaerobic solution and 0.1 mL of each was transferred to three MRS agar plates using the spread plate method. Plates were incubated aerobically at 37 °C for 0 and 20 h. Thereafter, plates exposed to oxygen for 20 h were recultivated in an anaerobic jar (Oxoid, Hampshire, U.K.) for 48 h using APH.

Oxygen tolerance was regarded as the ratio of viable cell population on aerobically cultivated cultures to viable cell population on anaerobically cultivated cultures.

Vegetable Juice Medium Preparation and Fermentation by Isolated Strains. The fresh vegetables were purchased from a local market in Jeonju, Korea, and stored at 4 °C in a refrigerator until used. The vegetables were crushed individually using an Angel juice extractor (Angel Life Co., Ltd., Seoul, Korea) and filtered with a 180 μm (80 mesh) sieve (Endecotts Ltd., London, U.K.) to separate pulp from the juice. The vegetable juice was stored in a deep freezer (−60 °C) for future use.

Table 1. Origin of Selected Strains after Screening for Stability to Bile and Acidic Environment

strains	origin
D1, D2	dongchimi
F20-1, F20-2, F20-3, F20-4, F26-2, F42-1, F42-2, F42-3, F42-4	newborn baby feces
F35-1, F35-2, F35-3, F35-4, F35-6	newborn baby meconium
K1	Korean Food Research Institute

The vegetable juice media (VJM) was sterilized in a water bath at 90 °C for 30 min and then cooled to room temperature. The test culture subcultured was inoculated into VJM and incubated anaerobically to determine the fermentation characteristics of isolated strains at 37 °C for 24 h. Viable cell populations of initial and final growths were counted using MRS agar, and then the pH of each VJM was measured to compare the change of initial and final pH values.

Identification of Selected Strains. The chromosomal DNAs of strains selected in this study were isolated according to the method described by Yoon et al. (*15*). The oligonucleotide primers used for amplification of 16S rDNA were synthesized, and primers annealing at the 5′ and 3′ ends of the 16S rRNA genes were 5′-GAGTT TGAT CTGGC TCAG-3′ and 5′-AGAAA GGAGG TGATC CAGCC-3′, respectively. Then, PCR amplification was performed. Purification with a Qiaquick PCR purification kit (Qiagen Co., Germany) was carried out by following the manufacturers' instructions. Purified DNA was used for analysis of DNA sequence with a 377 Genetic Analyzer (Perkin-Elmer Co., Norwalk, CT).

RESULTS AND DISCUSSION

Isolation and Selection of Strains for Probiotics. Our first strategy in this experiment was to select strains of human origin and Korean fermented food that have acid and bile resistance, and oxygen tolerance, so that the cells isolated from different origins would be more likely to colonize in the human intestine. The species and subspecies of bacteria detected in individual fecal samples and dongchimi are shown in **Table 1**.

In this study, we screened strains K1, K29, K30, K32, K34, K35, and K40 obtained from the Korean Food Research Institute (KFRI) and strains *Bifidobacterium bifidum* ATCC 29521, *Bifidobacterium longum* Reuter ATCC 15707, *Bifidobacterium adolescentis* ATCC 15700, *Bifidobacterium infantis* Reuter ATCC 15697, and *Bifidobacterium animalis* ATCC 25527 received from the Korean Culture Center of Microorganisms (KCCM). After two or three subculturings, all strains were inoculated into MRS broth wih the pH adjusted to 3.0 using 0.1 N HCl. Strain K1 showed a high acid resistance, but the other strains were sensitive to acidic conditions, so we selected strain K1 for further study.

The isolated strains from newborn baby fecal and dongchimi samples were transferred to MRS agar containing 0.004−0.006% (w/v) bromocresol purple (Sigma Chemical Co.) to examine lactic acid production and confirmed by Gram staining. Finally, two strains were selected from dongchimi samples, five strains from newborn baby meconium, nine strains from newborn baby fecal samples, and one strain from strains tested previously (**Table 1**). Selected bacteria preferably can proliferate at pH below 3.0 and high bile concentration because they grew in LBS agar with 0.15% (w/v) bile salts and MRS broth with pH adjusted to 3.0.

Determination of Acid Tolerance. To develop or select new probiotic organisms, their stability on acid, bile, and oxygen

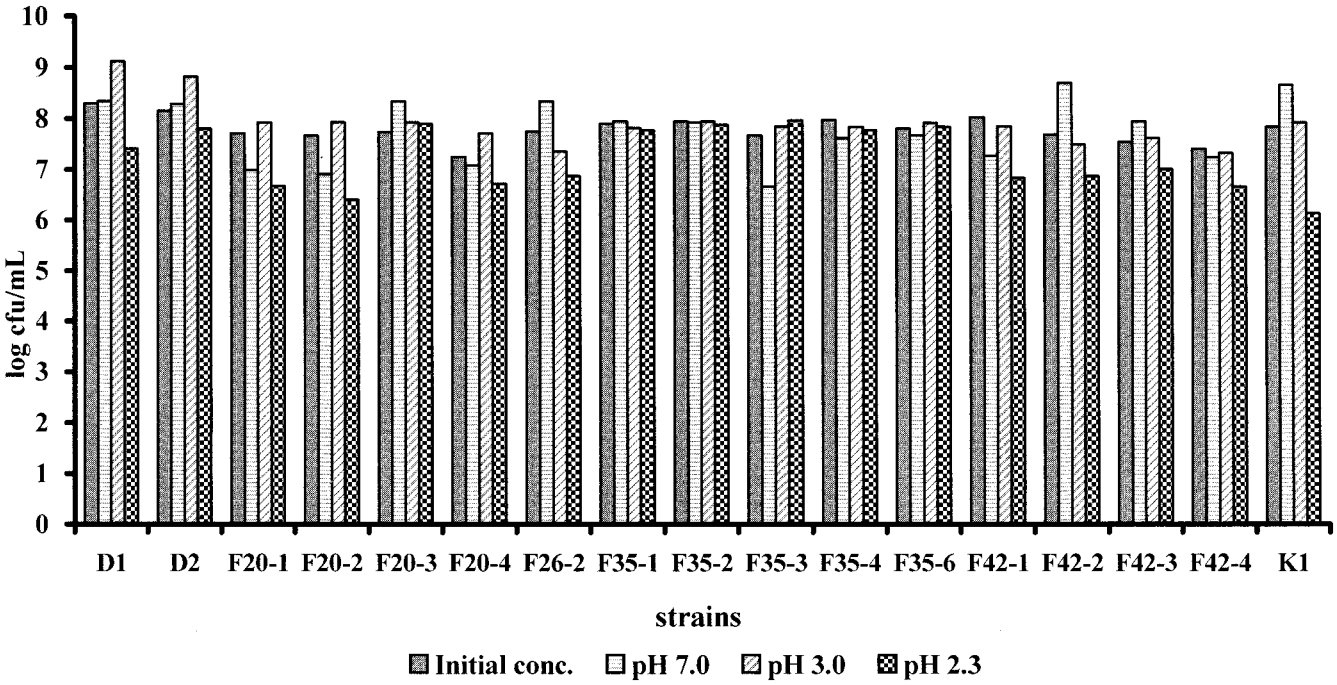

Figure 1. Survivability of isolated strains in sodium phosphate buffer solution adjusted to pH 7.0, 3.0, or 2.3 at 37 °C for 2 h.

should first be assessed (*16*). Therefore, we tested here three effective properties as prerequisites for probiotics, that is, tolerance to low pH, high bile concentration, and oxygen.

Survival of all strains was examined at pH between 2.3 and 7.0 (**Figure 1**). Resistant strains showed little or no decrease in viable cell numbers even after 2 h of incubation at pH 3.0 and 2.3. Survival rates of strains F20-3, F35-3, and F35-6 among selected strains were found to be 102.6/102.1%, 102.3/103.8%, and 100.3/100.1% at pH 3.0 and 2.3, respectively. These three strains were more stable than other strains at pH 3.0 and 2.3. Also, D1 (109.9%) and D2 (108.1%) strains isolated from dongchimi showed the highest survivability at pH 3.0. On the other hand, strain K1 reached <80% survival rate compared to the other strains at pH 2.3.

Gupta et al. (*17*) observed that only two of seven *L. acidophilus* strains tested exhibited growth at pH 3.0, whereas the results of Suscovic et al. (*18*) on an *L. acidophilus* strain suggested a high acid tolerance at pH 3.0. Xanthopoulous et al. (*19*) also observed that three *L. paracasei*. subsp. *paracasei* (strains DC411, DC412, and DC416) and one *L. rhamnosus* strain (DC425) remained almost unaffected by the low pH after 2 h, but most of the tested microorganisms were sensitive to acid.

As seen from **Figure 1**, 16 strains of tested organisms indicated a benchmark of survival rate >95% after 2 h of incubation at pH 3.0, and 7 strains showed the survival rates >95% after 2 h of incubation at pH 2.3 (D2, 95.8%; F35-1, 98.3; F35-2, 99.1; F35-4, 97.5%).

In general, most of the strains tested in this study showed a better resistance to low pH than the strains tested previously (*7*, *20*). Therefore, it has been assumed that isolated strains may survive passage through the digestive system that has specific condition such as the low pH of the stomach.

Determination of Bile Tolerance. Bile tolerance was known to be one of the essential properties required for LAB to survive in the small intestine and the role it plays in physiological functions (*20*, *21*). Among the tested strains, strains D1, D2, F20-1, F20-2, F20-3, F35-3, and F35-6 showed a high resistance to 0.25% bile concentration (**Figure 2**). Eleven strains of tested organisms showed survival rates >75% in broth containing 0.25% bile salt. In addition, strain F35-3 showed the highest survival rate (87.8%) compared to the other strains at 0.25% concentration. However, the survival rates of most strains tested were rapidly decreased at 0.5% bile concentration after 24 h of incubation. Comparison of the ability of isolated strains to grow in the MRS broth with and without bile salts revealed considerable variation among strains. When compared with the control broth, 0.25, 0.50, 0.75, and 1% bile salts exert inhibitory effects on all strains, but strains D1 and D2 were the most resistant strains at 0.75 and 1% bile concentration.

Shin et al. (*22*) reported that the growth of LAB in modified EG medium containing 0.3% bile salt under anaerobic conditions decreased 1.62−2.26 log cycles/mL compared to the control group. In this study, although the growth of isolated strains was slightly suppressed by bile salt, they are thought to show normal growth curves.

Klaenhammer (*23*) reported that probiotic bacteria vary considerably in their level of bile tolerance. Also, they explained the mechanism of tolerance is not understood and the minimum acceptable level of bile tolerance for a candidate probiotic remains unknown. Until now, the lethality of bile on microorganisms in the human small bowel was thought to be low, even negligible, because of in vitro data that showed conjugated bile salts, which constitute the majority of bile salts present in the small bowel, were less bactericidal than deconjugated bile salts (*24*, *25*).

In the present study, bile exerted a strong influence on the survival of the bacterial species tested (**Figure 2**), and the survival rate varied within a small range of bile concentration.

These findings support the importance of investing the sensitivity of microorganisms to bile as a selection step for potential probiotic. Marteau et al. (*25*) reported that although probiotic microorganisms are generally thought to survive the transit through the gastrointestinal tract for their functionality, the damaging effect of bile salts on yogurt bacteria also seemed to have a positive consequence. For these reasons, isolated strains are thought to survive the transit through the gastrointes-

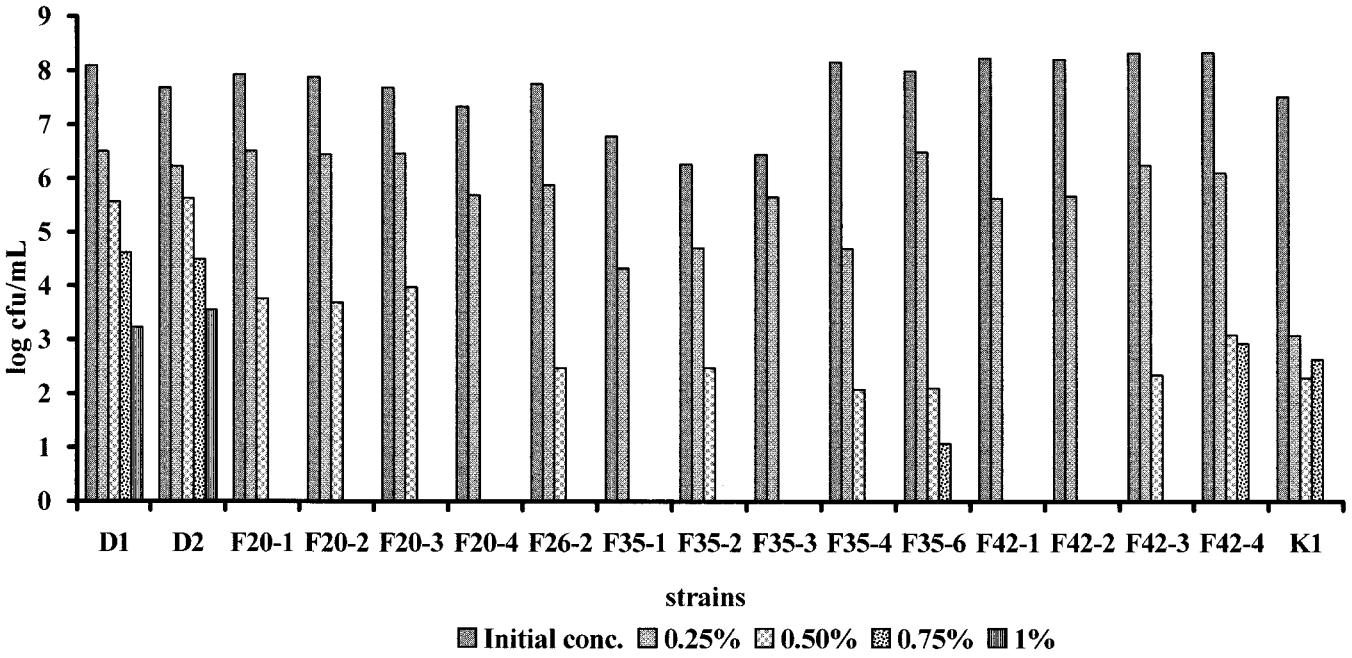

Figure 2. Effect of high bile concentration on growth of isolated strains at 37 °C for 24 h.

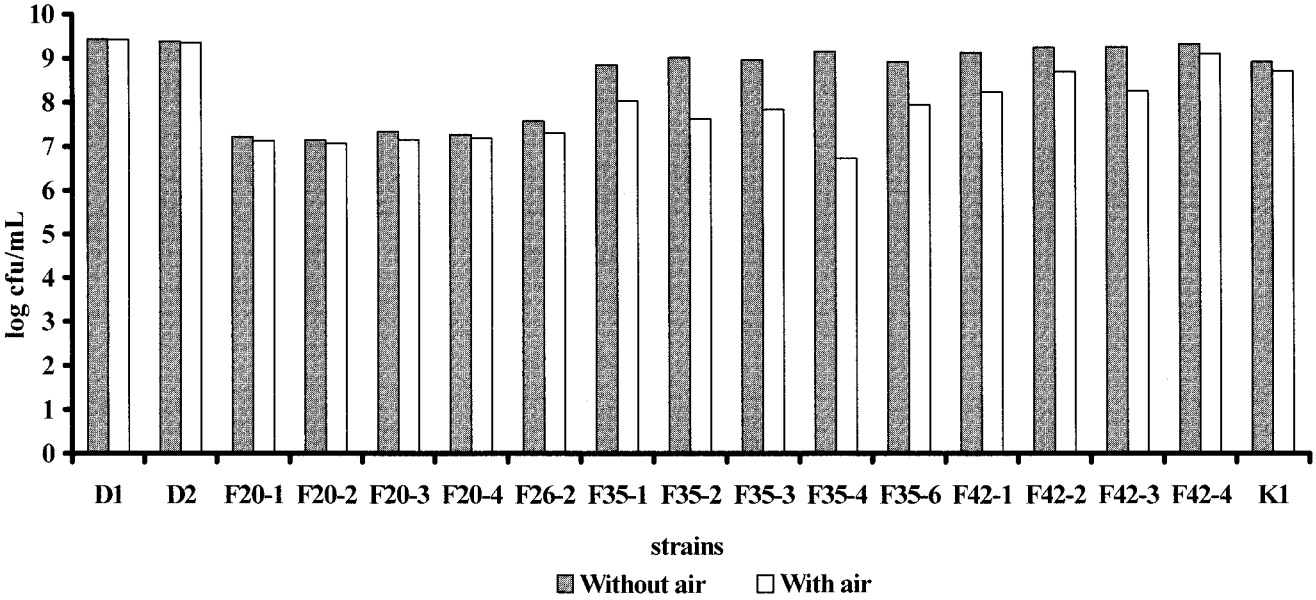

Figure 3. Survivability of aerobically incubated strains at 37 °C for 0 and 20 h.

tinal tract for the functionality, because most of the strains tested survived 0.5% bile concentration.

Determination of Oxygen Tolerance. Figure 3 shows the survival rate of isolated strains under oxygen. The survival rates of strains D1 and D2 were 99.9 and 99.7%, respectively, and survival rate decreased in the order F20-4 (99.0%), F20-2 (98.9%), F20-1 (98.5%), and F42-4 (97.6%). Among tested organisms, nine strains showed survival rates >95% after 24 h of aerobic incubation, but F35-4 showed the lowest survival rate (75.4%) compared to the other strains. From the result of this experiment, we conclude that isolated strains are generally not affected by oxygen.

Growth and Characteristics of Isolated Strains in VJM. Vegetable juice was sterilized at 90 °C for 30 min and subcultured cultures transferred into VJM prepared. **Table 2** shows the pH values of the various plant-inducing media. As seen from **Table 2**, significant variation of initial pH values was not found except in tomato and red sweet pepper, which were pH 4.06 and 4.95, respectively. After 24 h of incubation at 37 °C, pH values of VJM were a little different depending on vegetable media. Despite relatively low pH condition, when cultured in tomato and red sweet pepper media, isolated strains grew well and were not affected by pH of the vegetable sources (**Table 3**). Although the data in the literature are difficult to compare because different media and inoculation rates were used, strains F20-1, F20-2, F20-4, and F26-2 reached $>10^8$ cfu/mL in the VJM. Kale, *Angelica*, mature pumpkin, and red sweet pepper showed desirable color and flavor during fermentation.

Gardner et al. (*12*) reported that *Lactobacillus* strain grew well on the carrot vegetable medium, whereas Mrocek-Delclos (*26*) reported the *Lactobacillus* did not grow well on this substrate. Kim et al. (*10*) reported leek, Chinese cabbage, cabbage, green onion, and spinach produced unfavorable flavors during fermentation.

Table 2. pH of Natural Media Using Vegetables

	vegetable juice media								
strain	tomato (4.06[a])	radish (6.95)	kale (6.41)	cabbage (6.25)	native lettuce (6.05)	lettuce (6.3)	*Angelica keiskei* Koidz (6.06)	mature pumpkin (6.26)	red sweet pepper (4.95)
D1	3.39[b]	3.53	3.96	3.43	4.36	3.26	3.68	3.57	3.56
D2	3.41	3.53	3.94	3.47	4.36	3.27	3.67	3.57	3.57
K20-1	3.54	3.83	5.93	4.33	5.24	3.75	4.33	3.86	3.61
K20-2	3.54	3.84	5.96	4.37	5.15	3.79	4.27	3.70	3.60
K20-3	3.54	3.68	6.32	3.84	5.29	3.56	4.22	3.70	3.62
K20-4	3.56	3.89	5.95	4.33	5.20	3.74	4.32	3.78	3.62
F26-2	3.56	3.79	5.14	4.29	5.23	3.77	4.34	3.77	3.61
F35-1	3.67	3.83	5.14	3.90	4.40	3.30	3.70	3.56	3.55
F35-2	3.65	3.73	4.91	3.93	4.39	3.29	3.69	3.57	3.60
F35-3	3.63	3.79	4.93	3.97	5.18	3.60	4.21	3.76	3.82
F35-4	3.67	3.82	4.95	4.04	4.38	3.28	3.68	3.59	3.58
F35-6	3.65	3.83	4.85	4.06	5.16	3.60	4.21	3.75	3.79
F42-1	3.65	3.79	4.87	4.00	5.16	3.60	4.22	3.73	3.79
F42-2	3.67	3.94	4.93	3.95	5.17	3.60	4.22	3.73	3.83
F42-3	3.63	3.78	4.85	3.95	4.78	3.55	4.12	3.69	3.59
F42-4	3.66	3.91	4.88	3.97	5.17	3.60	4.22	3.83	3.82
K1	4.13	4.46	4.47	4.43	4.35	3.26	3.67	3.57	3.57

[a] Initial pH of vegetable juice media sterilized at 90 °C for 30 min. [b] pH of culture medium incubated anaerobically at 37 °C for 24 h.

Table 3. Growth of Isolated Strains in Natural Media Using Vegetables at 37 °C for 24 h[a]

	vegetable juice media																	
	tomato		radish		kale		cabbage		native lettuce		lettuce		*Angelica keiskei* Koidz		mature pumpkin		red sweet pepper	
strain	0 h[b]	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
D1	8.38[c]	8.44	8.28	9.06	7.36	8.68	8.37	8.88	8.19	8.25	8.26	8.60	8.31	9.08	8.25	8.91	8.34	8.86
D2	8.40	8.58	8.28	8.79	7.35	8.71	8.30	8.90	8.17	8.22	8.26	8.38	8.27	9.06	8.26	8.76	8.16	9.36
K20-1	7.32	7.14	6.72	8.94	7.46	9.28	7.32	8.98	6.96	8.72	6.92	8.56	7.05	8.75	7.37	8.49	7.90	8.60
K20-2	7.31	8.30	6.59	8.94	7.41	9.29	7.72	9.08	7.07	9.38	7.05	8.64	6.97	8.21	8.26	8.77	6.77	8.91
K20-3	7.07	8.38	6.52	8.92	7.34	8.86	7.51	8.67	7.64	8.68	6.65	8.61	7.31	8.53	8.43	8.79	6.51	8.85
K20-4	6.61	7.25	6.34	9.09	7.26	9.31	6.79	9.11	6.93	8.85	6.97	9.37	7.19	8.52	7.08	8.58	6.57	8.83
F26-2	6.85	7.54	6.70	8.95	7.34	9.39	8.10	9.21	6.94	8.59	6.99	8.40	7.07	8.79	7.41	8.29	7.48	8.97
F35-1	7.37	8.35	6.73	7.01	7.80	8.61	7.66	7.96	8.19	9.09	8.29	8.64	8.33	9.08	8.25	8.91	8.24	9.06
F35-2	7.00	8.16	6.15	7.93	7.72	8.61	7.81	8.05	8.12	8.30	8.28	8.58	8.36	9.10	8.30	8.89	7.85	9.31
F35-3	7.21	8.53	6.51	6.00	7.81	8.72	7.58	8.10	6.82	7.89	6.95	7.06	7.15	8.73	7.40	8.02	6.83	7.26
F35-4	8.09	7.92	5.90	6.73	7.89	8.79	7.51	8.15	7.20	8.73	8.21	8.72	8.29	9.17	8.32	9.07	8.21	8.97
F35-6	7.66	8.78	6.30	6.70	7.81	8.61	7.09	7.94	7.13	8.13	6.56	6.90	6.81	8.78	7.30	7.85	6.75	7.60
F42-1	7.78	8.16	7.15	7.30	8.34	8.57	8.02	8.06	7.94	8.46	7.18	7.62	7.41	8.79	7.90	8.12	7.63	7.83
F42-2	7.20	7.52	6.41	7.16	8.10	8.70	7.88	8.03	7.79	8.29	7.65	8.18	7.53	8.70	7.91	7.74	7.65	7.95
F42-3	7.54	8.05	6.66	7.03	7.85	8.72	7.16	7.79	7.33	7.99	6.62	7.58	7.69	8.29	7.86	8.96	6.78	8.41
F42-4	7.20	7.69	6.54	6.52	8.13	8.67	7.91	7.88	7.66	8.24	7.21	7.43	7.16	8.89	7.83	8.22	7.28	8.11
K1	7.69	7.25	7.48	8.11	8.00	8.54	7.64	7.73	8.30	8.81	8.31	8.65	8.30	9.09	8.29	8.83	8.23	9.11

[a] Strains were incubated anaerobically at 37 °C. [b] Time incubated in vegetable juice medium. [c] Viable cell population (log cfu/mL).

Some of the natural media tested in this study were considered to be suitable for the application of the new fermented product using various vegetables.

Identification of Strains. We selected some strains having acid and bile tolerance and oxygen resistance. In a preliminary study we examined antimicrobial effects of food-borne disease microorganisms on isolated strains (unpublished data). Thereafter, some selected strains (D2, F20-3, and F35-2) were phylogenetically identified by using 16S rDNA gene sequence. As a result of the 16S rDNA similarity level for strains analyzed by following the method described by Yoon et al. (*15*), D2 and F35-2 were identified as *Lactobacillus plantarum* JCM 1149 (99.9%). Also, strain F20-3 was identified as *Lactobacillus fermentum* ATCC 14931 (99.6%).

Adawi et al. *(27)* reported *L. plantarum* reduced bacterial translocation and hepatocellular damage in an acute liver injury rat model. Also, administration of *L. plantarum* enhances gut immune function and stimulates gastrointestinal epithelial proliferation and barrier function (*28*). Cangemi de Gutierrez et al. (*29*) reported that the number of pathogens in non-*L. fermentum*-treated mice was higher in all organs (between 10^1 and 10^5 cfu per organ), whereas *L. fermentum*-treated mice showed only a small number of the pathogen (10^1 cfu per organ).

Further investigation on the probiotic property of isolated strains is needed, but those strains are thought to be available as probiotic microorganisms for the fermented food industry and functional food development in the future.

Conclusion. We isolated some strains from meconium and feces of newborn baby and from dongchimi (vegetables fermented in brine by lactic acid bacteria) to select strains of human origin and fermented product that have acid and bile resistance. Resistant strains showed little or no decrease in viable cell populations even after 2 h of incubation in 0.05 M sodium phosphate buffer solution adjusted to pH 2.3 and 3.0. However, tolerance to high bile concentration (0.25 and 0.5%, w/v) was observed with some isolated strains, and they varied among strains.

Although strains isolated grew well in the vegetable juices, a detailed study would be necessary on mixed vegetable juices for the advantages such as the production of acid taste and unique flavor, control of pathogenic bacterial infection, and improvement of storage quality.

The results from this study can be used as fundamental data for the development of vegetable-based fermented product. However, much more further research is needed for new applications of isolated lactic acid bacteria in the processing or preservation of fermented vegetable product.

ABBREVIATIONS USED

APH, Anaero Pack Helico; LAB, lactic acid bacteria; VJM, vegetable juice media; cfu, colony-forming units.

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