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Selection of Starter Cultures and Optimum Conditions for Lactic Acid Fermentation of Onion

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Abstract Lactic acid bacteria (LAB) isolated from various fruits and vegetables were screened in order to determine appropriate fermentation starters for manufacturing functional fermented onion juice. From the initial screening test comprising more than 700 isolated LAB, 16 isolates were selected based on their acid production rate. Among the selected isolates, the fermentation broth of KC-007 exhibited the highest electron donating and nitrite scavenging activities, with values at pH 1.2 of 95.6 and 68.7%, respectively. From the overall results obtained in this study, we finally selected the bacterium KC-007 as a fermentation starter. This bacterium was identified and named as *Pediococcus pentosaceus* based on its morphological and physiological characteristics, carbon-utilization pattern (as assessed using an API 50CHL kit), and molecular genetic characteristics (as assessed using the nucleotide sequence of the 16S rRNA gene). The optimal temperature, pH, and starter inoculation concentration (v/v) required for growth of the isolated strain were 40°C, pH 4.0-6.0, and 2%(v/v), respectively.

Keywords: lactic acid bacteria (LAB), functional fermented onion juice, *Pediococcus pentosaceus*

Introduction

An onion is a term used for many plants in the genus *Allium*. It is a vegetable that commonly used in everyday meals worldwide. Onions have been cultured for very long time and used in various ways, mainly as a spice. Nowadays, however, their health-promoting effects are gaining attention. The study of onion-based products has been established in a variety of fields including a production and treatment of onion snack, dried onion, onion-supplemented bread and jam, and onion-based drinks. Moreover, new fields such as onion fermentation are being developed.

Research into the bioactive components of onions has recently been gaining interest. Onion is already known to have antimicrobial, anticholesterolemic, hypotensive, hypoglycemic, antiasthmatic, anticancer, and antioxidant properties (1). Quercetin (3',3',4',5,7-pentahydroxyflavone), a flavonoid found in onions, is known for its antioxidant and free radical scavenging power and its ability to protect against cardiovascular disease (2). Recent studies have validated the previously reported bioactive effects of onions bioactive, confirming that many folk remedies from onion are indeed effective. It appears that these effects are attributable mainly to sulfur compounds such as *S*-alkenyl thiosulfinate and sulfide (3).

Lactic acid bacteria (LAB) produced by the fermentation of onion have also been shown to have health-promoting properties. It can prevent the growth of harmful bacteria in the intestines and thus prevent diarrhea, prevent constipation by acidifying the intestines, enhance immunity, and reduce the risk of developing cancer. Onion has a sugar content of 8%, a value that suggests that this vegetable can be fermented.

As mentioned earlier, onions possess great potential as a health-promoting food, and fortunately can be produced in great quantities. More than 1 million tons of onions are produced per year in Korea, but 15% of this quantity is disposed of as agricultural waste because onions cannot be stored over long periods. If we are to make the most of the inherent health-promoting properties of this vegetable, it is necessary to develop effective methods of improving its longevity, and in particular considering the use of fermentation.

The use of onion-derived products in the food and drink industry is currently quite poor; they mostly used as flavoring rather than as health-promoting, bioactive substances. The reasons for this lie in the many difficulties associated with handling onions, such as the aforementioned storage problems and controlling the taste, but there are many possibilities with which to overcome these difficulties. Many methods of treating onions are being studied, and some of these are now being used in practice. However, the fermentation method -which has great potentials such as flavor masking and functionality improvement in this context- has not been studied extensively, and certainly not used in practice.

In this study, LAB was screened to determine that is the

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best to use as a fermentation starter in the fermentation of onions, thereby enabling the manufacture of fermented onion juice.

Materials and Methods

Isolation of latic acid bacteria (LAB) LAB were isolated by homogenizing a 10 g sample of various fruit and vegetable products for 2 min in 90 mL of Ringer's solution, and plating onto MRS agar medium (Difco, Detroit, MI, USA) containing 1.5% agar. The plates were incubated overnight at 37°C under aerobic conditions.

Onion juice media preparation Onion juice was prepared as follows. Onions were cut into pieces and pressed in a mechanical juicer. The extract was heated at 105°C for 15 min, centrifuged (8,000×g, 4°C) to remove insoluble materials, and then heat-sterilized at 105°C for 15 min. If so called 'ginseng steamed red' or 'red ginseng' was added to the onion juice, it was done so 1:4 (v/v red ginseng extract/onion juice).

Fermentation Onion juice was fermented by inoculating the isolated LAB cultures to final concentration of 2%(v/v) and incubating anaerobically at 37°C for up to 18 hr.

Analysis The pH and titratable acidity (TA) were monitored periodically. The pH was measured using a pH meter (740p; Istek, Seoul, Korea). TA was determined in 10 mL of the sample by titration with 0.1 N NaOH and expressed as lactic acid.

The Brix values of the samples were measured with the aid of a Brix meter (N-2E; Atago, Tokyo, Japan), and organic acid contents were analyzed with high performance liquid chromatography (HPLC, Alliance 2690 Separation Module; Waters, Milford, MA, USA).

Determination of electron donating ability The electron donating ability (EDA) was measured with some modifications of the Blois method (4). Each sample solution (0.5 mL) was vortex-mixed with 0.8 mL of 4×10^{-4} M 1,1-diphenyl-2-picrylhydrazyl (DPPH) dissolved in 99.9% ethanol. Each mixture was allowed to stand for 10 min at room temperature and the absorbance of testing solution at 525 nm was measured with the aid of a UV spectrophotometer (Nanochem L810; HNBT, Seoul, Korea).

Determination of nitrite scavenging ability The nitrite scavenging ability was measured with modified version of the Gray method (5). Each 1 mL sample and control solution was mixed with 2 mL of 1 mM $NaNO_2$. Each mixture was adjusted to the required pH (pH 1.2, 3.0, 4.2, or 6.0) with hydrochloric acid, citric acid, and NaOH, and made up to a volume of 10 mL. After incubation in a water bath for 1 hr at 37°C, 1 mL of each sample was added 5 mL of 2% acetic acid and 0.4 mL of Griess reagent (1% sulfanilic acid:1% naphthylamine=1:1). The intensity of the color that developed in the samples after 15 min was determined by a spectrophotometer at 520 nm.

Antimicrobial activity The antimicrobial activity of the samples was tested against the following food-poisoning bacteria: *Escherichia coli* O157:H7, *Staphylococcus aureus* (ATCC 14458), *Bacillus cereus* (KCCM 11204), *Enterobacter sakazakii* (ATCC 51329), and *Listeria monocytogenes* (KCCM 40307).

One colony of test bacteria was inoculated into 5 mL of tryptic soy broth (TSB) culture medium for 1 day, after which 0.1 mL of both the sample material and cultured test bacteria fluid were put into test tube containing 5 mL of fresh TSB culture medium. After cultivation at 37°C for 24 hr, the number of live bacteria was measured using the standard agar plate count method. As a control, 0.1 mL cultured test bacteria fluid was cultivated with 0.1 mL of distilled water instead of the sample material; this allowed calculation of the antimicrobial effect as follows:

$$\text{Antimicrobial activity (\%)}=[1-(\log \text{cell number of test group}/\log \text{cell number of test group})]\times100$$

Determination of organic acids and sugars Organic acids were analyzed by HPLC according to the method described by the AOAC (6) as modified by Mugula *et al.* (7). After 24 and 48 hr of fermentation, 5 g of each sample was taken and centrifuged (4°C, 7,000×g) for 15 min.

The supernatant was filtered into an HPLC sample vial through a 0.2-mm filter (MFS-13; Advantec MFS, Dublin, CA, USA). The organic acids were separated with a 250 ×250 mm HPLC column, particle size 5 μm (Prevail organic acid columns; Grace Davison Discovery Sciences, Deerfield, IL, USA) held at 45°C, using 25 mM KH_2PO_4 (pH 2.1 with phosphoric acid) as a mobile phase at a flow rate of 1.0 mL/min. The acids were identified and quantified by comparison of their retention times with those of standard solutions of the following acids: oxalate, tartarate, malate, lactate, acetate, citrate, succinate, and fumarate (Sigma-Aldrich, St. Louis, MO, USA).

Identification of LAB Six bacteria that produce high levels of lactic acid and have superior bioactivity were identified. Their morphological and physiological properties were assessed using Bergey's manual of systematic bacteriology for first identification (8). Morphological properties were examined by Gram staining and the KOH test, assessing motility, and spore staining. The glucose and catalase tests were used to assess the physiological properties. The API 50CHL kit (BioMériux, Marcy l'Etoile, France) was used to determine carbohydrate utilization. Finally, bacterial identification was achieved by sequence analysis of the 16S rRNA gene for each of the bacteria.

Effect on fermentation of initial pH, temperature, and starter inoculation concentration Prepared onion juice was fermented by inoculating *Pediococcus pentosaceus* KC-007 cultures to a final concentration of 2%(v/v) and incubating them anaerobically at 37°C for up to 54 hr. Cell growth was monitored by measuring the optical density of the culture broth at 600 nm with a spectrophotometer (Nanochem L810; HNBT).

The effect of pH was determined by incubating *P. pentosaceus*-inoculated samples of onion juice at an initial pH of 3.0, 4.0, 5.0, 6.0, 7.0, or 8.0. The onion juice was adjusted to the required pH by adding hydrochloric acid,

citric acid, and NaOH, as appropriate. To investigate the effect of temperature on fermentation, the onion juice was fermented at the temperature range of 25-40°C (i.e., 25, 30, 35, or 40°C). The optimum inoculation concentration of the starter culture was determined by inoculating one of 4 different concentrations (0.5, 1, 2, and 3%, v/v) of starter culture were into the onion juice.

Results and Discussion

Screening of LAB We aimed to identify the LAB that exhibit the highest production rate from the fermentation of onion juice, in order to facilitate the production of a highly functional lactic acid fermentation beverage. Onion juice was fermented by inoculating the 702 isolated LAB cultures to a final concentration of 2%(v/v) and incubating them anaerobically at 37°C for up to 48 hr. The pH and TA were measured at 24 and 48 hr of incubation.

The strains of probiotic LAB with the highest acid production were determined; 61 strains were selected that produced 8 times the initial level of lactic acid. The ability of these 61 lactic acid-producing strains to facilitate the fermentation of onion juice was tested with the aid of Brix-extracted ginseng steamed red, as follows. Ten superior onion juice fermenting LAB and 10 superior extracted ginseng steamed red in onion juice fermenting LAB were selected respectively after measuring the TA and pH during fermentation at 37°C every 24 hr for 96 hr. Four of the samples were duplicates, so that the following 16 strains of LAB were tested in total: BU-001, CA-010, CA-024, CA-025, GO-009, GO-014, GO-016, GO-023, GO-029, KC-007, KC-009, KC-015, PA-001, PE-001, PG-011, and RP-011. The bioactivities of these selected bacteria were measured based on their ability to facilitate the lactic acid fermentation of onion juice.

Table 1. Electron donating ability of onion juice fermented with lactic acid bacteria (LAB)

Strain	Electron donating ability (%)	
	24 hr	48 hr
BU-001	91.84	94.85
CA-025	90.03	91.51
CA-010	93.48	92.55
CA-024	92.61	88.83
GO-009	94.58	93.10
GO-014	90.36	89.76
GO-016	83.41	90.85
GO-023	94.96	94.47
GO-029	94.58	89.49
KC-007	95.56	90.85
KC-009	93.37	89.10
KC-015	90.85	90.36
PA-001	86.20	82.42
PE-001	93.15	91.62
PG-011	59.69	61.23
RP-011	95.29	94.20
Onion	85.71	

Electron donating ability (EDA) Since the free radicals generated inside the human body -particularly as a result of the oxidation of fat- promote the aging process in cells, reduce cell immunity, and impede cellular activity, prevention of free radical generation or stabilization of already generated free radicals should thus protect cells from these processes (9). In this regard, antioxidants create a complex with organic active free radicals by providing them with an electron or a proton. In addition, DPPH forms

Table 2. Nitrite scavenging ability of onion juice fermented with LAB

Strain	Nitrite scavenging ability (%)							
	24 hr				48 hr			
	pH 1.2	pH 3.0	pH 4.2	pH 6.0	pH 1.2	pH 3.0	pH 4.2	pH 6.0
BU-001	51.37	21.75	17.00	0.52	33.79	32.11	27.50	0.53
CA-010	65.30	23.78	18.75	3.55	49.77	30.28	15.25	4.50
CA-024	49.32	13.87	18.00	3.51	26.94	27.85	32.50	5.82
CA-025	60.05	32.11	26.50	7.43	34.93	36.59	30.50	9.52
GO-009	66.89	18.29	28.50	2.42	16.21	29.67	19.50	3.21
GO-014	24.89	19.31	21.00	1.24	36.99	37.40	23.25	6.35
GO-016	30.72	26.42	17.75	1.24	37.21	25.41	20.25	1.23
GO-023	70.32	25.61	16.50	1.53	29.91	21.95	23.25	2.32
GO-029	60.05	16.06	26.00	0.35	47.95	30.28	31.50	1.85
KC-007	68.72	33.94	35.00	4.23	49.77	22.97	26.75	7.41
KC-009	56.16	16.67	19.50	0.12	42.47	34.35	20.25	0.26
KC-015	52.74	23.58	19.25	2.02	40.87	42.28	31.25	4.23
PA-001	52.97	18.50	25.25	1.23	58.22	36.38	48.00	3.70
PE-001	51.37	27.85	26.00	0.23	23.74	31.50	20.25	0.53
PG-011	65.30	31.71	22.00	2.42	30.14	27.85	17.25	3.70
RP-011	65.98	23.58	15.25	4.23	35.16	38.62	20.00	7.41
Onion	24.89	23.17	18.50	2.21				

an irreversibly stable molecule by receiving an electron or proton from an antioxidant; thus, antioxidant activity can be presumed from EDA (10). Hudson and others (11,12) reported that the chemical structure of flavonoid types in the onion confers antioxidation activity; polyhydroxy flavonoid can function as an early stage antioxidant at the free radical reaction of unsaturated fatty acids. In addition, the ability of *Lactobacillus* spp., such as SBT-2028, to remove various free radicals via an antioxidation enzyme *in vivo* and to reduce free radical accumulation inside the

Table 3. Antimicrobial activities of onion juice fermented with lactic acid bacteria

Strain	Antimicrobial activity (%)				
	E. coli	*S. aureus*	*B. cereus*	*E. sakazakii*	*L. monocytogenes*
BU-011	11.22	12.43	2.84	6.30	5.46
CA-010	8.30	11.42	11.50	2.38	12.32
CA-024	7.68	9.15	7.54	2.73	11.20
CA-025	13.22	8.22	2.93	0.41	1.33
GO-009	9.81	16.40	5.17	3.04	4.95
GO-014	10.91	15.23	2.61	0.01	0.65
GO-016	13.93	8.57	0.86	3.67	2.46
GO-023	13.02	7.94	4.63	2.02	12.07
GO-029	12.69	11.66	10.83	3.27	2.36
KC-007	11.71	18.44	5.03	0.35	7.08
KC-009	10.84	15.78	5.27	0.41	10.64
KC-015	8.38	11.90	3.53	4.16	1.80
PA-001	8.97	12.87	4.85	4.80	1.61
PE-001	12.80	2.98	4.72	9.37	11.41
PG-011	8.69	18.44	9.46	2.94	2.43
RP-011	10.17	11.78	4.80	3.45	3.94
Onion	8.26	3.34	2.81	0.69	1.04

Table 4. Contents of organic acids in non-fermented and fermented onion juice (mg/100 g)

Organic acid and time	Malic acid	Malonic acid	Lactic acid	Acetic acid	Citric acid	Succinic acid	Fumaric acid
CA010 24 hr	ND[1)]	ND	571.43	ND	16.69	ND	0.21
CA010 48 hr	6.87	118.86	898.50	31.00	22.96	ND	0.26
CA025 24 hr	88.69	ND	389.36	209.87	38.21	ND	0.93
CA025 48 hr	91.35	ND	476.34	268.12	26.37	22.67	ND
GO029 24 hr	116.40	ND	373.31	202.37	48.16	ND	0.72
GO029 48 hr	98.07	ND	522.22	341.19	34.83	ND	0.79
KC007 24 hr	ND	58.87	583.52	ND	38.29	ND	ND
KC007 48 hr	6.65	99.57	921.56	42.17	14.90	7.67	0.27
PG011 24 hr	ND	ND	318.26	225.68	ND	ND	ND
PG011 48 hr	4.98	460.87	587.25	334.22	ND	ND	ND
RP001 24 hr	ND	76.06	975.43	ND	19.03	ND	2.26
RP001 48 hr	4.57	105.90	916.89	50.46	27.55	ND	0.26
Onion	176.94	ND	ND	ND	40.25	11.81	0.63

[1)]Not detected.

Table 5. Morphological and physiological characteristics of isolate

Characteristic	KC-007	CA-010	RP-011	CA-025	GO-029	PG-011
Morphology	Cocci in tetrads	Cocci in tetrads	Cocci in tetrads	Cocci in tetrads	Cocci	Cocci
Gram-stain	+[1)]	+	+	+	+	+
3% KOH test	-	-	-	-	-	-
Motility	-	-	-	-	-	-
Catalase test	-	-	-	-	-	-
Spore stain	-	-	-	-	-	-
Gas production from glucose	-	-	-	+	+	+

[1)]+ and - mean that each characteristic is positive or negative, respectively.

human body has been reported (antioxidation capability of *Lactobacillus*).

The EDA of onion juice was 85.7%; in the case of fermented onion juice, the following strains of bacteria

Table 6. Carbon utilization of isolates as assessed using an API 50CHL kit

Carbohydrate	KC-007	CA-010	RP-011	CA-025	GO-029	PG-011
Glycerol	-[1)]	-	-	-	-	-
Erythritol	-	-	-	-	-	-
D-Arabinose	-	-	-	-	-	-
L-Arabinose	+	+	+	-	+	+
D-Ribose	+	+	+	+	+	-
D-Xylose	-	-	-	-	+	+
L-Xylose	-	-	-	+	-	-
D-Adonitol	-	-	-	-	-	-
Methyl-β-D-xylopyranoside	-	-	-	-	-	-
D-Galactose	-	-	-	-	+	-
D-Glucose	+	+	+	+	+	+
D-Fructose	+	+	+	+	+	+
D-Mannose	+	+	+	+	+	+
L-Sorbose	-	-	-	+	-	-
L-Rhamnose	+	+	+	-	-	-
Dulcitol	-	-	-	-	-	-
Inositol	-	-	-	-	-	-
D-Mannitol	-	-	-	-	-	-
D-Sorbitol	-	-	-	+	-	-
Methyl-α-D-mannopyranoside	-	-	-	-	-	-
Methyl-α-D-glucopyranoside	-	-	-	-	+	-
N-Acetyl glucosamine	+	+	+	+	+	+
Amygdalin	+	+	+	+	+	-
Arbutin	+	+	+	-	+	+
Esculin ferric citrate	-	-	-	-	-	-
Salicin	+	+	+	-	+	+
D-Cellobiose	+	+	+	-	+	-
D-Maltose	+	+	+	+	+	+
D-Lactose (bovine origin)	-	-	-	-	-	-
D-Melibiose	+	+	+	+	+	-
D-Saccharose (sucrose)	+	+	+	+	+	+
D-Trehalose	+	+	+	+	+	+
Inulin	-	-	-	-	-	-
D-Melezitose	-	-	-	-	-	-
D-Raffinose	+	+	+	-	+	-
Amidon (starch)	-	-	-	-	-	-
Glycogen	-	-	-	-	-	-
Xylitol	-	-	-	-	-	-
Gentiobiose	+	+	+	-	+	-
D-Turanose	-	-	-	+	+	+
D-Lyxose	-	-	-	-	-	-
D-Tagatose	+	+	+	-	-	-
D-Fucose	-	-	-	-	-	-
L-Fucose	-	-	-	-	-	-
D-Arabitol	-	-	-	-	-	-
L-Arabitol	-	-	-	-	-	-
Potassium gluconate	-	-	-	-	-	+
Potassium 2-ketogluconate	-	-	-	-	-	-
Potassium 5-ketogluconate	-	-	-	-	-	-

[1)]+ and - mean that carbohydrate utilization occurs or not, respectively.

exhibited a higher EDA (i.e., consistently over 90%) than onion juice and than the other bacteria tested (Table 1): BU-001, CA-010, GO-009, GO-023, KC-007, PE-001, and RP-011. The high EDA of fermented onion extract is thought to be attributable to a combination of the effects of flavonoids and the fermentation of *Lactobacillus*.

Nitrite scavenging activity Nitrate and nitrite are held in high repute as precursors of the carcinogen *N*-nitrosamine (NA). The major sources of these materials for humans are the food additives used on processed meat to settle meat colors and prevent toxic generation due to *Clostridium botulinum*, vegetables, meat and poultry, and water contaminated with nitrate. Nitrate is not in itself toxic, but when it is converted to nitrite due to the action of nitrate reductase or reducing *Bacilli* in saliva or inside the stomach, hemoglobin within the blood is oxidized, forming methemoglobin, causing various toxic effects such as methemoglobinemia. Nitrite is also more reactive than nitrate and can react as a nitrosatable substance by its ready conversion into nitrous acid. Therefore, where nitrite coexists with amines within food, it can induce the production of NA, a reaction that can also easily occur within the human body or other animal stomach. The measurement of nitrite eliminating ability can thus be a gauge of the production of this carcinogen, and therefore preempt carcinogenesis (13-15).

The nitrite scavenging activity of onion juice was highest at pH 1.2 (24.89%), reducing drastically thereafter with increasing pH; the nitrogen scavenging rates at pH 3.0, 4.2, and 6.0 were 23.17, 18.5, and 2.21%, respectively (Table 2). This result is in agreement with those obtained for unripe and fully ripe black raspberry and leaf extract, which exhibit their highest nitrite scavenging rate at pH 1.2; and with those of pine needles, wormwood, and *Cassia tora*, which exhibit a higher nitrite scavenging activity at lower pH. The optimum pH for NA generation was found to be 2.5-3.0, which is also in agreement with the finding of a high nitrite scavenging rate at low pH and a reduced scavenging rate with increasing pH.

In the case of lactic acid-fermented onion extract, most bacteria also exhibited the highest nitrite scavenging activity at pH 1.2, as well as the tendency toward a reduction in scavenging activity with increased pH. Among the separated bacteria, KC-007 and CA-025 exhibited the highest nitrite-scavenging activities (68.72 and 60.05%, respectively, at

Table 7. 16S rRNA sequences of the isolates in the GenBank database

Strain	Genus or species match	Homology (%)
KC-007 RP-011	*P. pentosaceus* strain MY-800 *P. pentosaceus* NRIC 0123 *P. pentosaceus* ATCC 25745	100
CA-010	*P. pentosaceus* strain MY-800 *P. pentosaceus* NRIC 0123 *P. pentosaceus* ATCC 25745	99
CA-025	*L. mesenteroides* NRIC 1517 *L. mesenteroides* NRIC 1513 *L. mesenteroides* subsp. *mesenteroides* ATCC 8293	99
GO-029	*L. mesenteroides* PC13	100
PG-011	*L. citreum* KM20 *L. citreum* IH22	99

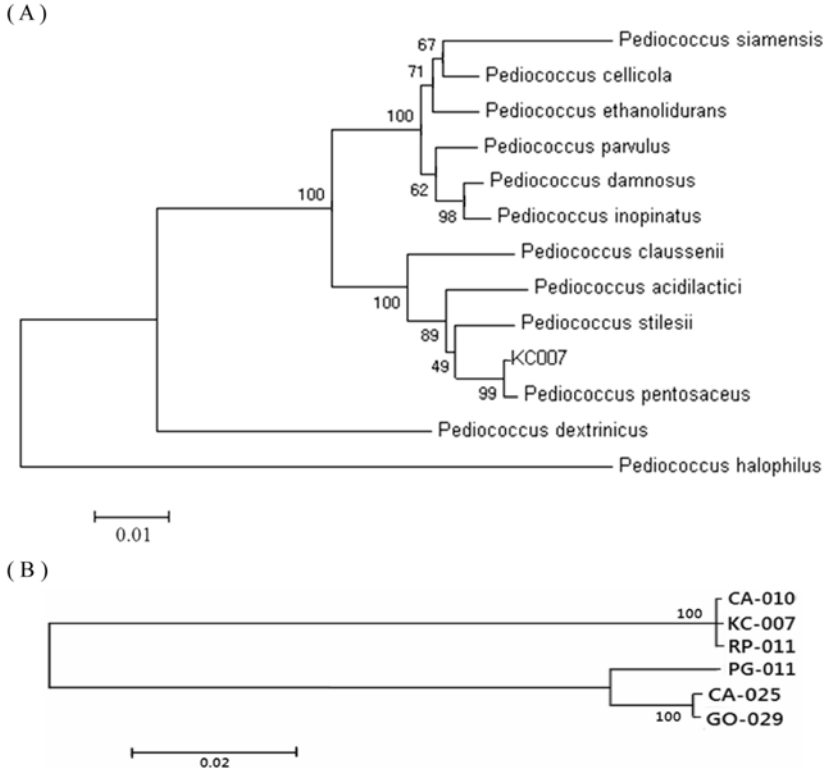

Fig. 1. Dendrogram of the 16S rRNA gene from strain KC-007 (A) and 6 strains (B).

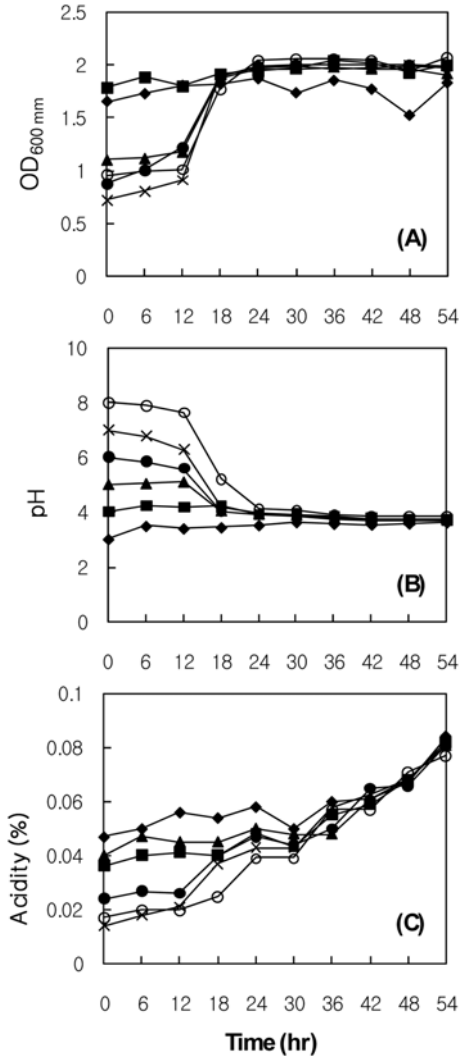

Fig. 2. Changes of growth patterns (A), pH (B), or acidity (C) on fermentation of onion juice by *P. pentosaceus* KC-007 at various pH conditions (pH 3-8). ◆, pH 3; ■, pH 4; ▲, pH 5; ●, pH 6; ×, pH 7; ○, pH 8.

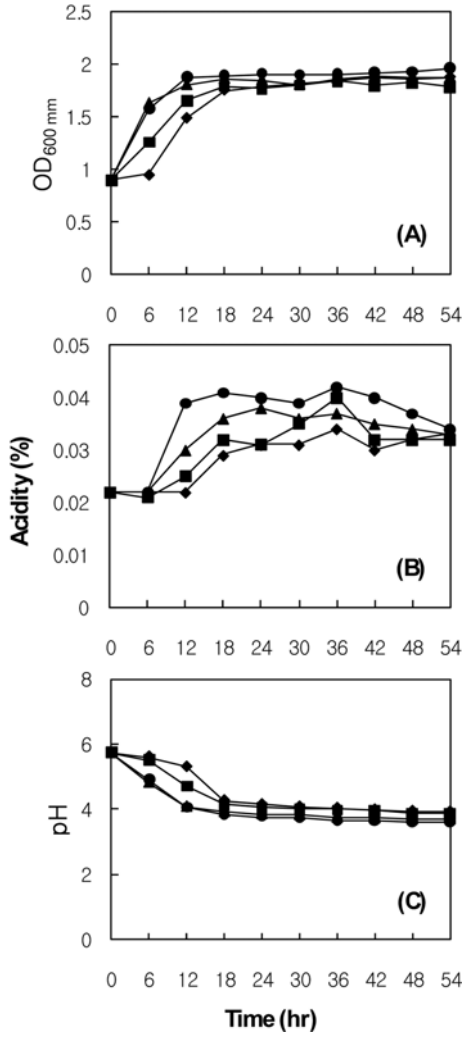

Fig. 3. Changes of growth patterns (A), acidity (B) or pH (C) on fermentation of onion juice by *P. pentosaceus* KC-007 at various temperature conditions. ◆, 25°C; ■, 30°C; ▲, 35°C; ●, 40°C.

pH 1.2; and 33.94 and 32.11%, respectively, at pH 3.0) followed by CA-010, PG-011, and RP-011.

Antimicrobial activity The antimicrobial activity of onion juice and lactic acid-fermented onion juice was assessed by measuring the negative effect on the breeding of various food poisoning bacteria in liquid culture medium. Onion juice reduced the breeding rate of *E. coli* O157:H7 by 8.26%, which was the highest antimicrobial activity of all of the strains of bacteria examined; it impeded the breeding of *S. aureus* and *B. cereus* to similar degrees, by 3.34 and 2.81%, respectively (Table 3). In the case of fermented onion juice, KC-007 and PG-011 both exerted the greatest antimicrobial effect on *S. areus*, reducing its breeding rate by 18.44%; they reduced that of *E. coli* O157:H7 by 11.71 and 8.69%, respectively. CA-010 reduced the breeding rates of *B. cereus* and *L. monocytogenes* by 11.50 and 12.07%, respectively. It has been reported that onion liquid extract inhibits the growth of Gram-positive *Bacilli* and mold; Cho (16) reported the excellent antimicrobial ability of onion skin extract on *S. aureus* and *E. coli*. It was not active against the Gram-negative *Salmonella* spp. (17). Im *et al.* (18) found that the strong antimicrobial ability of onion, of the genus *Allium*, on *Staphylococcus* spp. and *Vibrio parahaemolyticus* was due to the sulfur compound contained within the plant.

Our results thus confirm that both onion juice and lactic acid-fermented onion juice have antioxidation capabilities, such as EDA and nitrite scavenging activity, and that they also exert antimicrobial activities against some of the bacteria that cause food poisoning. Based on these results, 6 of the 16 highly fermentable isolated bacteria (CA-010, CA-025, GO-029, KC-007, PG-011, and RP-011) with proven excellent antioxidation activity and antimicrobial activity were selected and used for identification and final starter selection.

Chemical characteristics The organic acids detected during fermentation include lactic acid, succinic acid, citric acid, acetic acid, and fumaric acid. Neither oxalic acid nor tartaric acid was detected. Levels of lactic acid and acetic

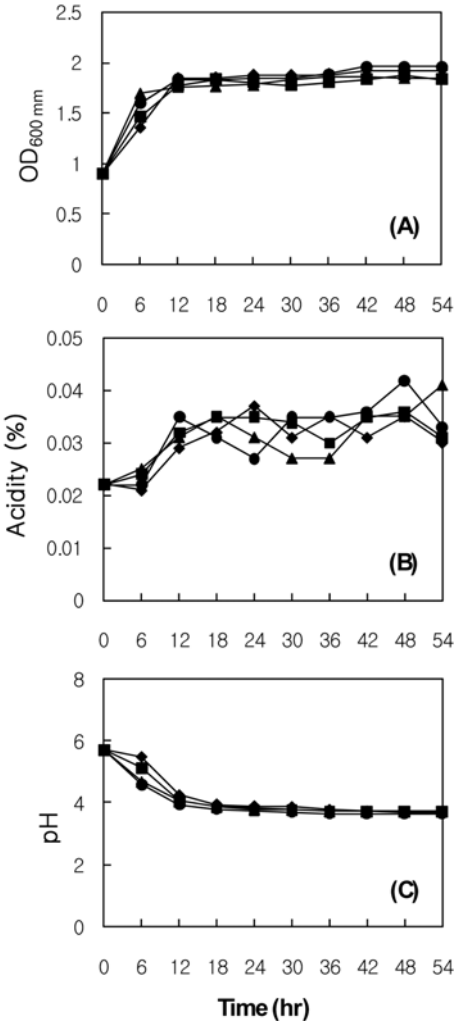

Fig. 4. Changes of growth patterns (A), acidity (B) or pH (C) on fermentation of onion juice by *P. pentosaceus* KC-007 at various starter concentrations. ◆, 0.5%; ■, 1.0%; ▲, 2.0%; ●, 3.0%.

acid increased over the 48 hr fermentation period. A particularly high level of lactic acid was found in KC-007 (Table 4).

Identification of selected LAB The strains were Gram-positive, catalase-negative, nonmotile, and non spore-forming (Table 5). The results of the API 50CH kit test (Table 6) and 16S rRNA sequencing (Table 7) showed that the properties of KC-007, CA-010, and RP-011 were characteristic of *P. pentosaceus*. CA-025 and GO-029, and PG-011 were shown to be *Leuconstoc mesenteroides* and *Leuconstoc citreum*, respectively (Fig. 1A, 1B). From the overall results obtained from this study, we finally selected KC-007 as a fermentation starter.

Effect of initial pH The effect of pH on the LAB is well known, and culture conditions, such as pH, can have a large impact on bacteriocin production (19). The present study evaluated the effect of pH on cell growth by measuring the effects of initial pH on the cell growth, pH, and acidity of onion juice fermented with *P. pentosaceus* KC-007 under various pH conditions. Fermentation was carried out at an initial pH of 3.0-8.0. Cell growth was unaffected by an initial pH of 5-8 (Fig. 2A). The pH of fermented onion juice was lowest at an initial pH of 3-7 (Fig. 2B) and the acidity was highest at an initial pH of 5.0 and 6.0 for 30 hr after fermentation (Fig. 2C). Because the pH of fermented onion juice was 5.7, its pH remained unchanged. It was found that the optimum pH for *P. pentosaceus* growth was 6.0-7.0 (20).

Effect of the fermentation temperature The effects of fermentation temperature on the cell growth, pH, and acidity of onion juice fermented with *P. pentosaceus* KC-007 were examined under various temperature conditions. Cell growth was best at 35 and 40°C, increasing until 12 hr of fermentation (Fig. 3A). Increasing the temperature resulted in an increase in acidity (Fig. 3B) and a decrease in pH (Fig. 3C). This finding confirms a previous report that growth of *P. pentosaceus* growth increases with temperature (21).

Effect of starter inoculation concentration The effects of starter inoculation concentration on the cell growth, pH, and acidity of onion juice fermented with *P. pentosaceus* KC-007 were examined under various temperature conditions. The highest cell growth and acidity were obtained at the inoculation concentration of 2%(v/v) (Fig. 4A) and 1-2% (Fig. 4B), respectively. The pH of the fermented onion juice was lowest at the starter inoculation concentration of 2-3% until 12 hr of fermentation (Fig. 4C). The results of this study indicate that the optimum pH, fermentation temperature, fermentation time, and inoculation concentration were 5.7, 40°C, 12 hr, and 2%(v/v), respectively.

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