Korean J. Microbiol. Biotechnol. (2014), 42(4), 367–376
http://dx.doi.org/10.4014/kjmb.1409.09002
pISSN 1598-642X eISSN 2234-7305

KJMB
Korean Journal of Microbiology and Biotechnology

Fermentation and Quality Evaluation of *makgeolli*, Korean Rice Wine Supplemented with Alcohol-tolerant *Pediococcus acidilactici* K3

Danbie Jang[1], Hyunjoo Lee[1], Sangeun Pyo[1], Seong Woon Roh[2], Jin-Kyu Rhee[3]*, and Han-Seung Lee[1,4]*

[1]*Department of Bio-Food Materials, College of Medical and Life Sciences, Silla University, Busan 617-736, Republic of Korea*
[2]*Jeju Center, Korea Basic Science Institute, Jeju 690-140, Republic of Korea*
[3]*Western Seoul Center, Korea Basic Science Institute, Seoul 120-140, Republic of Korea*
[4]*Research Center for Extremophiles and Marine Microbiology, Silla University, Busan 617-736, Republic of Korea*

Received: September 12, 2014 / Revised: September 25, 2014 / Accepted: September 26, 2014

This study's purpose was to investigate the characteristics of a traditional Korean rice wine containing lactic acid bacteria (LAB), called *makgeolli*. The *makgeolli* was brewed with the alcohol-tolerant *Pediococcus acidilactici* strain K3, and was analyzed for LAB cell counts, alcoholic content, turbidity, pH, total acidity, amino nitrogen, total sugars, reducing sugars, solid contents, and organic acids. The physicochemical properties of the *makgeolli* were mostly maintained during fermentation (9 d) and storage (15 d). We also monitored the properties of LAB-supplemented commercial *makgeollies*, after adding *P. acidilactici* K3 at a concentration of 10^7 CFU/ml *makgeolli*, for one month. Most of their properties, such as alcoholic content, turbidity, pH, total acidity, amino nitrogen, total sugars, reducing sugars, solid contents, and organic acids, were preserved during storage at 10°C, suggesting that *makgeolli* supplemented with live LAB can be produced. These results suggest that alcohol-tolerant *P. acidilactici* K3 can be used for *makgeolli* brewing either as a starter or as a supplement.

Keywords: *Pediococcus acidilactici*, lactic acid bacteria, *makgeolli*, fermentation, alcohol-tolerance

Introduction

Makgeolli is a traditional Korean rice wine with an alcohol content of more than 3% (v/v). *Makgeolli* is brewed with rice and *nuruk* (Korean fermentation starter). *Makgeolli* is also called *takju,* for its turbidity, or *nongju,* because it is a popular lunchtime and thirst-quenching drink among farmers [15]. *Makgeolli* is one of Korea's most famous drinks, and it has a distinctive taste, which comes from the combination of the sweet-sour taste of lactic acid, the taste of amino acids (a protein breakdown product), the bitter taste of alcohol, and the sweet taste of saccharides, which are a starch breakdown product [7, 8]. In addition, *jubak* (rice wine residue) formed during the process of filtration during *makgeolli* production contains many nutrients, not only starches and proteins, but fiber, minerals, vitamins, alcoholic and organic acids, enzymes, and yeast. Several studies of the health benefits of consuming *jubak*-containing noodles have been performed, and they have reported various biological functions, including anti-diabetes, anti-cancer, anti-hypertension, and anti-cardiovascular disorder activities. In addition, several studies aimed at applications for patents have investigated the application of *makgeolli* concentrate as an antioxidant and whitening agent, *makgeolli* soap, and natural cosmetics made from grain fermentation [18].

***Corresponding authors**
H.-S. L.
Tel: +82-51-999-6308, Fax: +82-51-999-5458
E-mail: hanslee@silla.ac.kr
J.-K. R.
Tel: +82-2-6908-6224, Fax: +82-2-6908-6215
E-mail: jkrhee@kbsi.re.kr
© 2014, The Korean Society for Microbiology and Biotechnology

Lactic acid bacteria (LAB) in *makgeolli* form acids at the initial stage of fermentation and lower the pH, preventing contamination with various other microbes and producing various organic acids that improve the taste of fermented wine; however, overgrowth of LAB can increase acidity, impart a sour taste, and cause acidification that negatively affect stable fermentation of *makgeolli* [12, 16]. Therefore, application of LAB as an additive in *makgeolli* needs optimization to balance between advantages and disadvantages. Since alcohol concentration of *makgeolli* brewed usually reaches above 12% (v/v), LAB cannot survive actively in such an environment with more than 5% of alcohol. When *makgeolli* is fermented using the traditional yeast, LAB are rapidly reduced as the alcohol concentration surpasses 10% [2, 19].

Recently we isolated and reported alcohol-tolerant *Pediococcus acidilactici* K3 for *makgeolli* brewing [6]. In this study, we added the strains to *makgeolli* before fermentation (starter addition) and after fermentation (post-fermentation supplementation) for the application of the alcohol-tolerant strains to *makgeolli*. We measured the physicochemical properties of the *makgeollies* and evaluated the feasibility for production of LAB contained *makgeolli*.

Materials and Methods

Ethanol, acid, and bile tolerance

To measure the viability of alcohol-tolerant *P. acidilactici* K3, its growth at each alcohol concentration was compared to that of the standard neotype strain *P. acidilactici* DSM 20284. Bacteria were cultivated at 37°C on MRS liquid medium for 12 h and then cells were precipitated by centrifugation (6,000 rpm, 10 min). The precipitated bacteria were suspended in a tenth volume of 0.1 M phosphoric acid buffer (pH 7.0). The 1% (v/v) of suspended bacteria solution was inoculated into 5 ml of 0.1 M phosphoric acid buffer containing various concentrations of alcohol (0, 6, 12, and 18% v/v). After incubation with shaking for 4 h at 37°C, the bacteria were diluted using a serial dilution method, stained, and the number of colonies was counted. To measure the resistance to low pH, bacteria were cultivated in MRS liquid medium for 12 h and precipitated by centrifugation (7,000 rpm, 10 min). The precipitated bacteria were washed twice with phosphate-buffered saline (PBS) solution, and the diluted bacteria solution, at 1%, was inoculated into 5 ml of MRS liquid medium at various pH values (pH 2.0, 2.5, and 3.0). The bacteria were incubated at 37°C for 2 h, sampled every hour, diluted using a serial dilution method, stained, and the number of colonies was counted. To measure bacterial resistance to bile acid, bacteria were cultivated in MRS liquid medium for 12 h and precipitated by centrifugation (7,000 rpm, 10 min). The precipitated bacteria were washed twice with phosphate-buffered saline (PBS) solution, and the diluted bacterial solution, at 1%, was inoculated into 5 ml of MRS liquid medium containing 0.3% ox gall. The bacteria were incubated at 37°C for 4 h, and sampled every 2 h, diluted using a serial dilution method, stained, and the number of colonies was counted.

Pre- and post supplementation of LAB to *makgeolli*

To produce LAB-containing *makgeolli*, 0.3% white-*koji* mold (Choongmu fermentation Inc.) was added to 880 g of hard-cooked rice and incubated at 30°C for 2 h to produce the starting culture. Eighty grams of this mixture and 150 ml of water were mixed and 3% pre-cultivated yeast (*Saccharomyces cerevisiae*) was injected to produce the mix; the resulting mixture was incubated at 25°C for 2 d. Eight hundred grams of the mixture and 1350 ml of water were added to a fermentation container and were subjected to an initial soaking. After 2 d, 3.2 kg of hard-cooked rice, 4.8 L of water, and 1.5 g of *P. acidilactici* K3 was added to the initial soaking container, and were subject to primary soaking. This mixture was fermented for 9 d and was sampled at 24-h intervals for chemical analysis and viable cells counts (LAB). After filtering, the *makgeolli* was stored in a refrigerator at 10°C for 15 d, and sampled at 24-h intervals for the first 2 d, and every 5 d from the third day for chemical quality analysis and viable LAB cell counts. For post-fermentation supplementation experiments, 2.5 g of *P. acidilactici* K3 was added to 2 L of commercial *makgeollies* from Company K. For the first two days, the *makgeolli* was sampled every day and the number of viable LAB cells was counted. Afterwards, it was sampled every 5 d for chemical quality analysis and to count the number of viable LAB cells.

Viable cell counts

One milliliter of sample was diluted using a serial dilution method and then smeared on MRS solid medium containing BCP-sample. After 24 h of incubation at 37°C, the number of bacterial colonies was counted.

http://dx.doi.org/10.4014/kjmb.1409.09002

Alcohol content and turbidity

The *makgeolli* was sampled at 24-h intervals, and 10 ml of sample was quantified in a mass cylinder, and it was then transferred to a distillation flask. The cylinder used to quantify the sample was washed twice with distilled water, and the rinsing water was added to the distillation flask. Then, the sample-containing flask was heated and the liquid was distilled by connecting the flask to the cooler. When 70 ml of distilled solution was obtained, the distillation was stopped and distilled water was added to raise the volume to 100 ml. The alcohol content was read using an alcoholmeter (Deakwang, Inc., Korea), and the temperature was adjusted according to Gay-Lussak's law. The alcohol content was expressed as % (v/v). Turbidity was measured by obtaining a fixed quantity of sample, diluting it to the appropriate concentration, and measuring the absorbance at 660 nm with a UV spectrophotometer (Genequant 1300, GE Healthcare Co., UK).

Saccharide contents

Saccharide content was measured using a refractive saccharometer (Master-M, ATAGO, Japan). The reduced-saccharide content was measured by adding 0.3 ml of appropriately diluted sample to a tube, applying 0.9 ml of dinitrosalicylic acid (DNS) reagent, thoroughly mixing, heating in boiling water for 15 min and cooling on ice. The absorbance was measured at 550 nm with a UV spectrophotometer (Genequant 1300, GE Healthcare Co., UK). The reduced-saccharide content (%) was determined using a glucose standard curve. To measure the total saccharide content, 0.5 ml of the diluted sample, 0.25 ml of 5% phenol, and 1.25 ml of 95% sulfuric acid solution were added to a tube, and allowed to react for 30 min at room temperature. Afterwards, the absorbance at 492 nm was measured using a UV spectrophotometer (Genequant 1300, GE Healthcare Co.). The total saccharide content (%) was determined using a glucose standard curve.

pH, Acidity and amino nitrogen

The pH was measured using a pH meter (Model GmbH8603, Mettler-Toledo Co., Switzerland). For acidity measurement, 3-5 drops of 1% phenolphthalein was added to 10 ml of sample, and optimized by addition of 0.1 N NaOH. Then, optimal acidity (%) was calculated by inserting the amount of NaOH added into the following equation:

$$\text{Acidity (\%)} = (\text{NaOH Added} \times \text{NaOH Strength} \times 0.006) / \text{Sample Volume} \times 100$$

To measure the optimum amount of amino nitrogen, 1 ml of sample was added to a 50-ml volumetric flask, and diluted with distilled water. Afterwards, it was separated into a 25-ml conical flask and mixed with 20 ml of formalin solution (Sigma, USA) and 20 ml of water. Then, 3-5 drops of 1% phenolphthalein was added, and optimized by addition of 0.05 N NaOH. The amino nitrogen content was determined by inserting the amount of NaOH added into the following equation:

$$\text{Amino Nitrogen (\%)} = \{(\text{NaOH Added to Sample} - \text{NaOH Added to Blank}) \times \text{NaOH Potency} \times 10 \times 0.007 / \text{Sample Volume} \times 100$$

Organic acid contents

Ten milliliters of *makgeolli* was obtained and precipitated by centrifugation (8,000 rpm, 10 min). The supernatant was filtered using a 0.2-μm membrane filter (Minisart® syringe filter, Sartorius Stedim, Germany), and analyzed using high-performance liquid chromatography (HPLC) on a ZORBAX SB-Aq (4.6 mm × 150 mm × 5 μm film thickness, Agilent J & W Scientific, Folsom, USA) column to analyze the organic acids present.

Results and Discussion

To investigate the change in the viable LAB count in *mak-*

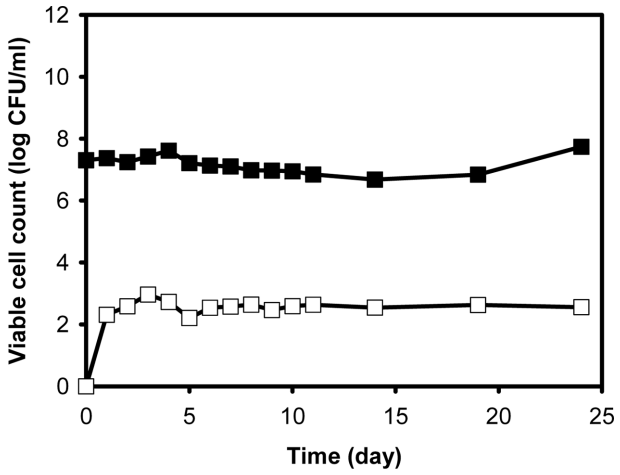

Fig. 1. Growth of lactic acid bacteria of *makgeollies* brewed with *P. acidilactici* K3. □, Without *P. acidilactici* K3; ■, with *P. acidilactici* K3.

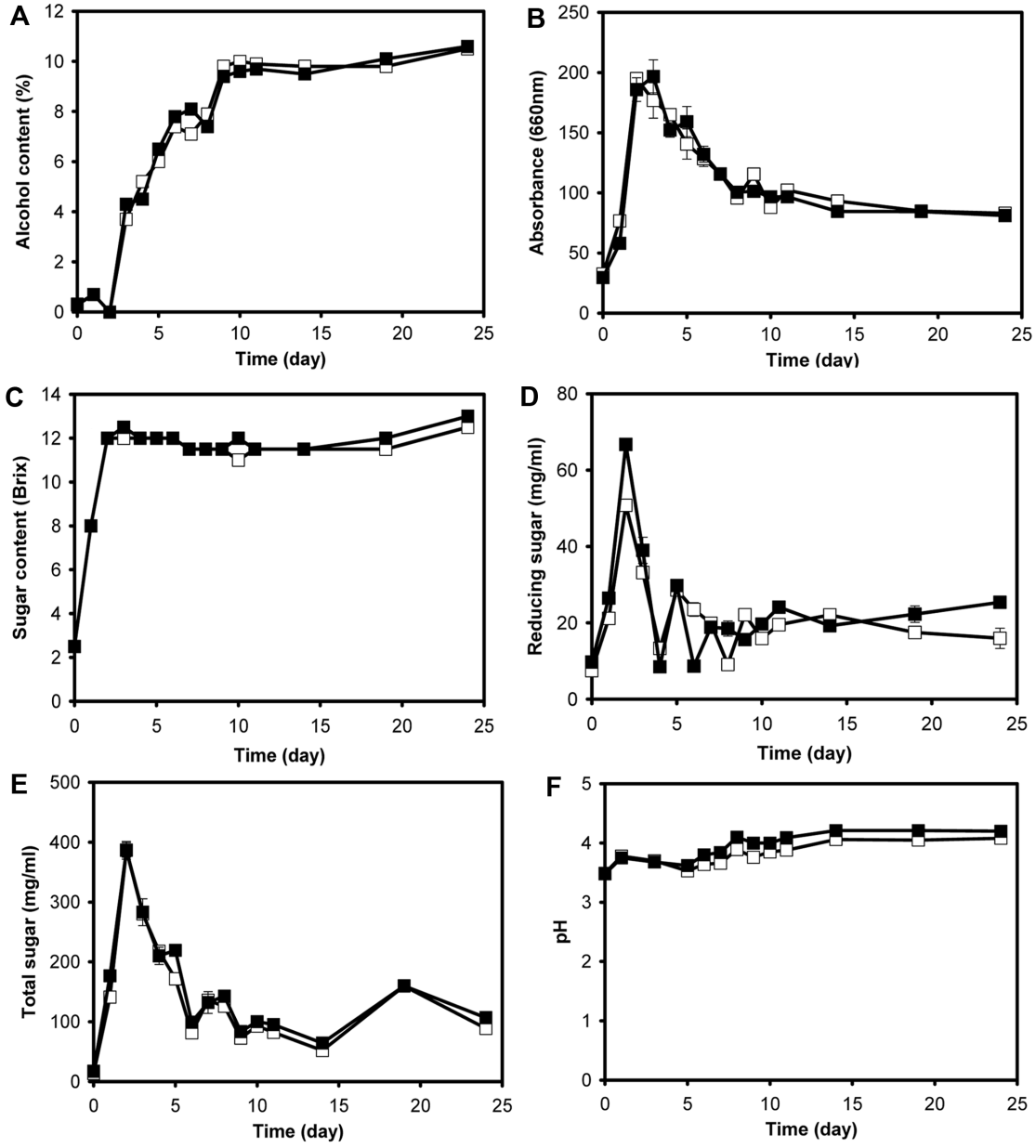

Fig. 2. (A) Alcohol content, (B) turbidity, (C) sugar content, (D) reducing sugar, (E) total sugar, (F) pH, (G) titratable acidity, and (H) amino nitrogen of *makgeollies* brewed without (white square) or with (black square) *P. acidilactici* K3. All measurements were performed in triplicate, and values are means of 3 replicates. 0.3% white-koji mold (Choongmu fermentation Inc.) was added to 880 g of hard-cooked rice and incubated at 30°C for 2 h to produce the starting culture. Eighty grams of this mixture and 150 ml of water were mixed and 3% pre-cultivated yeast (*Saccharomyces cerevisiae*) was injected to produce the mix; the resulting mixture was incubated at 25°C for 2 d. Eight hundred grams of the mixture and 1350 ml of water were added to a fermentation container and were subjected to an initial soaking. After 2 d, 3.2 kg of hard-cooked rice, 4.8 L of water, and 1.5 g of *P. acidilactici* K3 was added to the initial soaking container, and were subject to primary soaking. This mixture was fermented for 9 d and was sampled at 24-h intervals for chemical analysis and viable cells counts (LAB). After filtering, the *makgeolli* was stored in a refrigerator at 10°C for 15 d, and sampled at 24-h intervals for the first 2 d, and every 5 d from the third day for chemical quality analysis and viable LAB cell counts. For post-fermentation supplementation experiments, 2.5 g of *P. acidilactici* K3 was added to 2 L of Commercial *makgeollies* from Company K. For the first two days, the m*akgeolli* was sampled every day and the number of viable LAB cells was counted. Afterwards, it was sampled every 5 d for chemical quality analysis and to count the number of viable LAB cells.

http://dx.doi.org/10.4014/kjmb.1409.09002

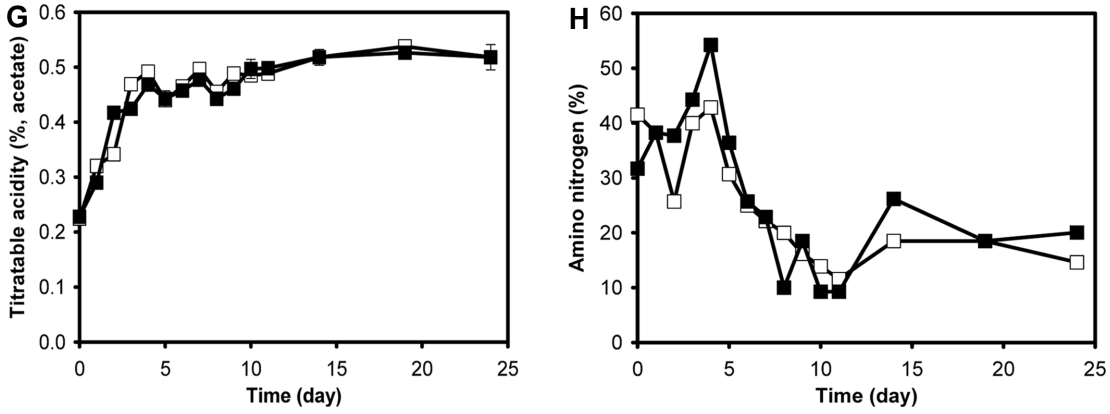

Fig. 2. Continued.

geolli when LAB were added during the fermentation and storage period, the viable bacteria were counted, and the result (Fig. 1) confirmed that *makgeolli* to which LAB had been added contained an approximately 3-log higher viable bacterial count than *makgeolli* without addition of LAB. The changes in the quality of *makgeolli* to which LAB was added before fermentation, during the fermentation period and storage period, are illustrated in Fig. 2. First, alcohol concentration is an essential quality of *makgeolli*. According to Korea's liquor laws, the standard alcohol concentration of *takju* is >3% (v/v). In the present study, the alcohol concentration of *makgeolli* started to increase 3 d after fermentation, and by the 9th day, the concentration had reached 9.4% (v/v) in LAB-supplemented *makgeolli*, and 9.8% (v/v) in non-LAB-supplemented *makgeolli*. During the storage period, the alcohol content was maintained at 9.4-10.6% (v/v) (Fig. 2A). The turbidity increased until day 3 and then declined until day 9. Turbidity was maintained during the storage period, but the turbidity may have been high initially because water was initially absorbed into the hard-cooked rice and as the yeast converted starches into sugars for use as energy, they produced carbon dioxide and alcohol, lowering the turbidity (Fig. 2B). Saccharide content (Fig. 2C) rapidly increased from the initial 2.5 degrees Brix (day 0) to 12°Bx (day 2), regardless of LAB addition, which demonstrated active starch breakdown. After fermentation, the saccharide content was maintained at 11.5-13°Bx during the storage period. The saccharide content of the *makgeolli* was high, unlike the saccharide content (2.7-4.6°Bx) measured in *makgeolli*-sake, which uses a different rice product [10], or the saccharide content (2.9-4.7°Bx) in commercially sold *makgeolli* [15]. Next, reducing saccharide is an important substance that, in harmony with the sour and savory flavors, contributes to the unique taste and sweetness of *takju* [15]. In the present study, the initial (day 0) reducing saccharide contents of LAB-supplemented *makgeolli* and non-LAB-supplemented *makgeolli* were 7.5 mg/ml and 9.7 mg/ml, respectively. On day 3, it rapidly increased to 50.8 mg/ml and 6.9 mg/ml, respectively, showing a similar pattern to saccharide content; after fermentation, it was reduced to and then maintained at 9.1 mg/ml and 18.5 mg/ml, respectively, during the storage period (Fig. 2D). Total saccharide content displayed the same pattern of rapid increase until day 3, regardless of the addition of LAB, followed by a decline until the end of fermentation, reaching a level that was maintained during the storage period (Fig. 2E). Such changes are thought to be due to the fact that reducing saccharide is used as substrate for alcohol fermentation, and after the yeast consumes the saccharide, it decreases the reducing saccharide and the total saccharide contents. The greatest problem caused by addition of LAB is the lowering of pH, caused by the acid produced by LAB, and the resulting increase in acidity. To confirm this, pH change was monitored. It shows a gradual pH increase ranging from 3.5 to 4, regardless of LAB addition (Fig. 2F). This may have been caused by the presence of amino acids and the buffering effect of peptides, a product of protein breakdown [9] . The acidity increased from the initial value (day 0) of 0.2% to 0.5% on day 5 (Fig. 2G). Han *et al.* [5] reported that the total saccharide content increased due to the production of different acids by various microbes, including yeast and LAB, during fermentation, but observed no major differences between *makgeolli* with and without LAB addition. Free amino nitrogen indicates the amount of nitrogen avail-

Table 1. Organic acid composition of *makgeollies* brewed with *P. acidilactici* K3.

Day	Lactic acid		Acetic acid		Citric acid		Succinic acid	
	N.C	K3	N.C	K3	N.C	K3	N.C	K3
0	2.23 ± 0.08	1.68 ± 0.35	3.03 ± 0.06	2.91 ± 0.16	0.53 ± 0.04	0.42 ± 0.03	0.84 ± 0.05	0.84 ± 0.05
1	3.47 ± 0.14	3.76 ± 0.18	4.94 ± 0.48	3.62 ± 0.29	1.08 ± 0.05	1.14 ± 0.17	1.66 ± 0.05	2.27 ± 0.08
2	10.03 ± 0.3	10.21 ± 0.37	4.22 ± 0.45	4.65 ± 0.59	1.68 ± 0.30	1.92 ± 0.18	2.89 ± 0.49	2.53 ± 0.11
3	13.58 ± 0.4	14.47 ± 0.18	3.80 ± 0.73	3.11 ± 0.35	1.40 ± 0.06	1.33 ± 0.10	6.97 ± 0.49	7.55 ± 1.01
4	16.98 ± 1.17	15.65 ± 0.67	4.81 ± 0.43	2.90 ± 0.09	1.66 ± 0.18	1.98 ± 0.15	2.21 ± 0.09	4.52 ± 0.94
5	18.74 ± 0.91	18.63 ± 1.24	5.01 ± 1.61	3.52 ± 0.67	2.28 ± 0.21	1.40 ± 0.31	5.81 ± 0.71	4.70 ± 0.79
6	22.34 ± 2.46	19.55 ± 0.77	3.04 ± 0.25	3.87 ± 0.35	1.57 ± 0.16	1.59 ± 0.16	5.24 ± 1.78	2.38 ± 0.34
7	18.29 ± 0.16	17.55 ± 0.21	2.48 ± 0.32	2.06 ± 0.20	0.57 ± 0.00	1.79 ± 0.04	5.20 ± 0.45	3.87 ± 0.83
8	19.73 ± 0.68	16.02 ± 0.41	2.38 ± 0.18	5.74 ± 0.40	1.95 ± 0.00	1.72 ± 0.27	4.16 ± 0.48	4.91 ± 0.78
9	16.38 ± 1.18	18.23 ± 2.96	5.26 ± 0.62	4.67 ± 0.52	1.01 ± 0.16	1.73 ± 0.46	3.27 ± 0.28	4.92 ± 0.68
10	21.46 ± 1.68	22.81 ± 2.01	7.20 ± 0.23	5.94 ± 0.94	1.54 ± 0.16	1.75 ± 0.12	5.50 ± 0.40	6.00 ± 0.38
11	22.45 ± 1.75	23.09 ± 2.23	5.72 ± 0.62	6.13 ± 0.18	1.75 ± 0.11	1.47 ± 0.02	6.08 ± 0.39	6.32 ± 0.31
14	25.68 ± 0.8	26.57 ± 1.29	6.71 ± 0.32	6.46 ± 0.32	2.39 ± 0.09	2.04 ± 0.04	6.53 ± 0.49	7.44 ± 0.15
19	27.59 ± 0.42	25.03 ± 1.45	5.93 ± 0.09	8.02 ± 0.79	1.57 ± 0.31	1.79 ± 0.12	6.66 ± 1.14	4.26 ± 0.40
25	31.84 ± 0.86	31.14 ± 1.10	6.75 ± 0.73	8.79 ± 0.82	1.52 ± 0.16	1.30 ± 0.17	6.91 ± 1.21	2.81 ± 0.75

able to microbes in solution, and it indicates the quantity of protein breakdown because it is produced when protein is broken down into amino acids. In the present study, the amino nitrogen content was maintained at 40% during the initial fermentation period, but it rapidly declined, and then was maintained at 20% during the storage period (Fig. 2H). The organic acid content was measured using HPLC analysis, and the results are shown in Table 1. The supernatant of makgeolli was analyzed at a detection wavelength of 210 nm. The oven temperature was 35°C, the solvent used was 1% ACN/99% 20 mM NaHPO4, pH 2.0, and the flow rate was 1.0 ml/min. Lactic, acetic, citric, and succinic acids were used as standards. On day 0, the lactic acid content of *makgeolli* with and without LAB-supplementation was 2.26 mg% and 1.68 mg%, respectively, and at the end of fermentation, it had increased to 16.38 mg% and 18.23 mg%, respectively. On the 15th day of storage, it had reached 31.84 mg% and 31.14 mg%; this result confirmed that it is one of the major organic acids present in *makgeolli*. This corresponds to a report by Woo *et al.* [21] that found that most of the organic acid in *makgeolli* brewed from brown rice is lactic acid. However, it does not correspond with results reported by Park *et al.* [14], which found that succinic acid is the major organic acid formed during fermentation. In the present study, citric acid was also detected, which corresponds to a report that citric acid is produced during the manufacturing process [20].

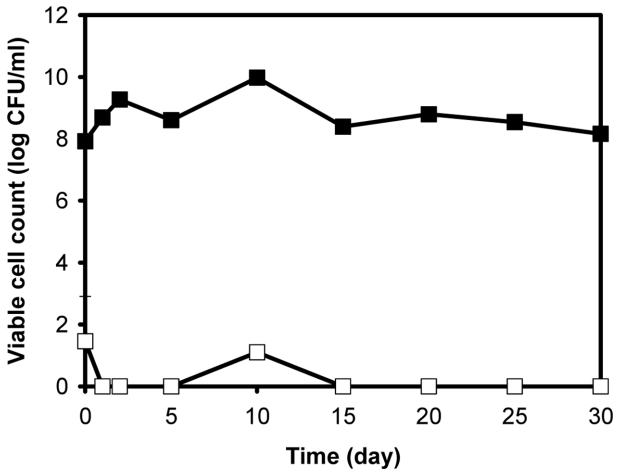

Fig. 3. Growth of lactic acid bacteria of *makgeollies* stored with *P. acidilactici* K3. □, Without *P. acidilactici* K3; ■, with *P. acidilactici* K3 at 10°C.

LAB was added to commercially available *makgeolli,* and the stability of the LAB during the storage period was measured (Fig. 3). In *makgeolli*-K, to which no LAB was added, almost no LAB was detected. Then, 10^7 CFU/ml of LAB added in *makgeolli*-K was increased up to 10^{10} CFU/ml, then maintained ~10^8 CFU/ml during the storage period. Chemical changes in LAB-supplemented *makgeolli* during the storage period were observed (Fig. 4). Alcohol concen-

http://dx.doi.org/10.4014/kjmb.1409.09002

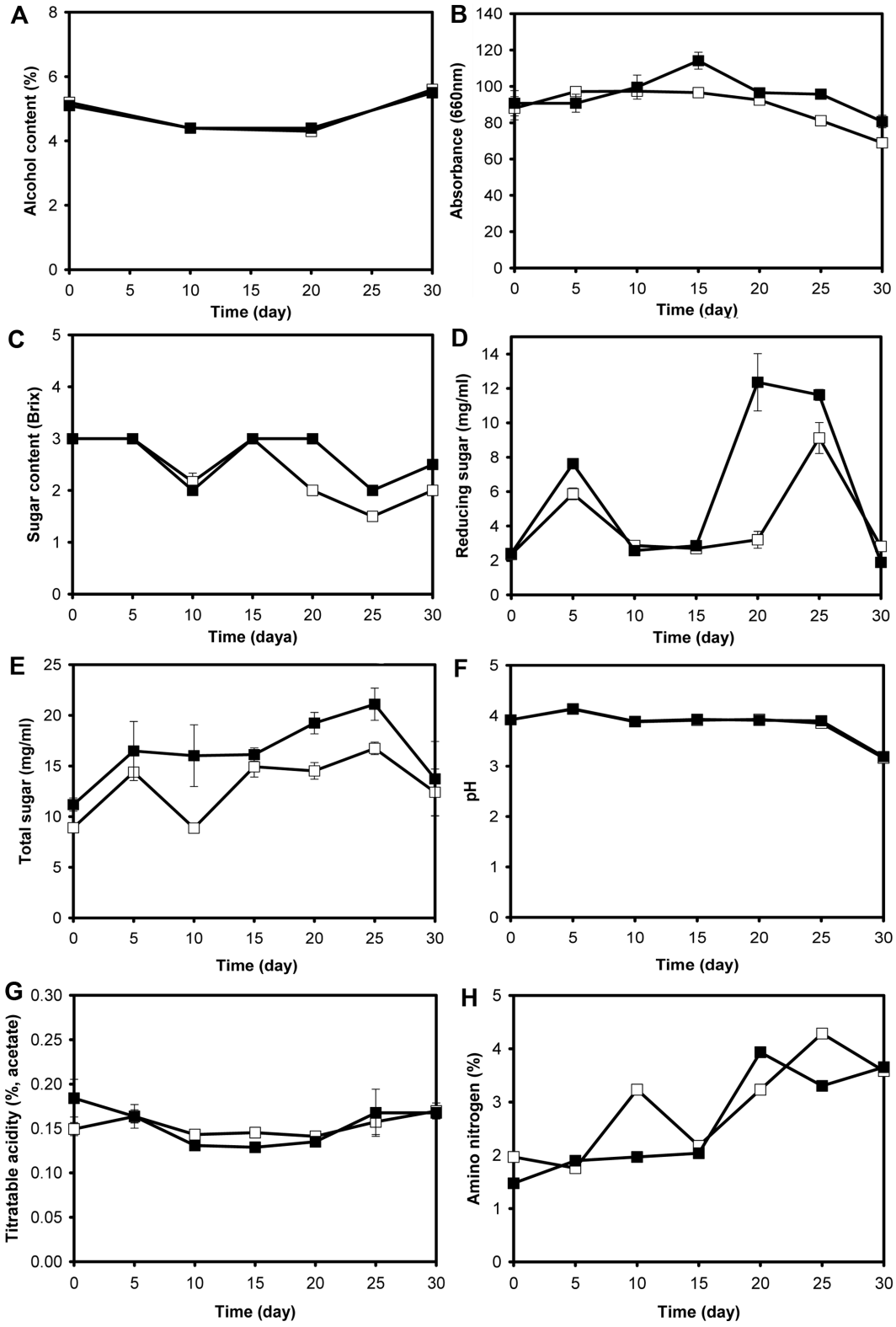

Fig. 4. (A) Alcohol content, (B) turbidity, (C) sugar content, (D) reducing sugar, (E) total sugar, (F) pH, (G) titratable acidity, and (H) amino nitrogen of *makgeollies* stored without (white square) or with (black square) *P. acidilactici* K3 at 10°C. All measurements were performed in triplicate, and values are means of 3 replicates.

tration of *makgeolli*-K was maintained below 6%, regardless of the addition of LAB (Fig. 4A). This is within the range of alcohol concentrations for commercially available *makgeolli* in Korea (5.7-7.5% (v/v)) previously reported by Park *et al.* [15], and the results are similar to the alcohol concentrations obtained by Park *et al*. [17], 4.8-7.5% (v/v) in commercial *takju.* Turbidity appeared to decline with time, and no major difference was observed between the *makgeolli*-K with and without addition of LAB (Fig. 4B). Saccharide contents (Fig. 4C) declined by approximately 0.5-1°Bx, regardless of LAB addition, and the saccharide content observed was within the range previously reported for commercial *makgeolli* of 2.9-4.7°Bx [15]. The total saccharide content (Fig. 4E) was 8.9 mg/ml and 11.2 mg/ml in *makgeolli*-K without and with LAB supplementation, respectively, on day 0 of the storage period; it was increased by approximately 2.5-3.5-fold, to 12.4 mg/ml and 13.7 mg/ml, respectively, on day 30. This increase is thought to be due to breakdown of starch by fungi. Reducing saccharide (Fig. 4D) showed a tendency to increase beginning on day 15, regardless of the addition of LAB; reducing saccharide content is thought to increase due to a decrease in yeast activity. No major differences were observed between *makgeolli* with and without LAB supplementation. Regardless of the addition of LAB to *makgeolli*, the pH was maintained at pH 4, and the pH decreased on day 25-30 (Fig. 4F), that suits the acceptable pH range by regulation, 3.8-4.7 [15]. No detectable heterofermentation was observed, then acidity was maintained with no major changes (Fig. 4G) to fits the acceptable acidity range by regulation, <0.5% [15]. The amino nitrogen content of LAB-supplemented and unsupplemented *makgeolli*-K was 1.97% and 1.48%, respectively, on day 0 of the storage period; after 30 days of storage, it increased to 3.58% and 3.65%, respectively (Fig. 4H). The results of HPLC analysis of organic acids is shown on Table 2. Regardless of the addition of LAB and the type of *makgeolli*, lactic acid rapidly increased, from 0.24-0.3 mg% on day 0 of the storage period, to 4.81-6.29 mg% on day 10 of the storage period. Levels of lactic acid were higher than levels of other organic acids; therefore, lactic acid is the major organic acid, and it was confirmed that there was no change in the lactic acid content with the addition of LAB. Organic acid is an important component that imparts a sour taste in alcoholic drinks; when present in small amounts, it improves the taste and aroma of *takju,* but in excess, it impedes the fermentation process, and it lowers the quality of *takju* by initiating acidic fermentation from alcohol acidification [21]. In the present study, the amount of acetic acid observed was smaller than the amount of other organic acids in two types of *makgeolli*, regardless of the presence of LAB.

Gram-positive, non-motile, non-spore-forming bacteria, LAB are reported to have an outstanding ability to produce bioactive substances, and their use is growing more common, in applications such as functional foods, health supplements, medicines, animal probiotics and animal foods [13]. For a strain of bacteria, including LAB, to be recognized as probiotic [1] it should withstand stomach acids and bile acids, and reach the small intestine, [2] it should settle and proliferate in the intestine, [3] it should show useful effects in the intestinal canal, and [4] it should be non-toxic and non-pathogenic. Bacteria that are used as probiotics include *Lactobacillus* spp., *Streptococcus* spp., *Bifidobacterium* spp., and *Bacillus* spp., which is an ascospore LAB [6]. The currently reported LAB from the domestic yeast include *Leuconostoc mesenteroides, Pediococcus acidilactici, P. damnosus, Lactobacillus plantarum, L. casei, L. brevis, Lactococcus lactis,* and *Enterococcus facium* [2]. In addition, as a study in the development of LAB seed, it showed a reduction in wine, and *L. paracasei* subsp. *parapcasei, L.*

Table 2. Organic acid composition of *makgeolli* stored with *P. acidilactici* K3 at 10°C.

Day	Lactic acid		Acetic acid		Citric acid		Succinic acid	
	N.C	K3	N.C	K3	N.C	K3	N.C	K3
0	0.26 ± 0.01	0.28 ± 0.01	0.23 ± 0.01	0.23 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.22 ± 0.01	0.11 ± 0.01
5	0.25 ± 0.00	0.20 ± 0.00	0.22 ± 0.00	0.33 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.14 ± 0.00	0.29 ± 0.00
10	4.87 ± 0.17	4.81 ± 0.17	0.89 ± 0.17	0.81 ± 0.17	0.13 ± 0.17	0.13 ± 0.17	1.02 ± 0.17	4.34 ± 0.17
15	5.20 ± 0.24	5.57 ± 0.24	0.70 ± 0.24	0.57 ± 0.24	0.64 ± 0.24	0.46 ± 0.24	0.72 ± 0.24	3.85 ± 0.24
20	6.18 ± 0.07	5.21 ± 0.07	1.02 ± 0.07	0.96 ± 0.07	1.43 ± 0.07	0.91 ± 0.07	2.41 ± 0.07	5.36 ± 0.04
25	5.24 ± 0.26	7.00 ± 0.26	1.11 ± 0.26	2.76 ± 0.26	0.79 ± 0.26	0.40 ± 0.26	1.88 ± 0.26	3.15 ± 0.26
30	8.34 ± 1.70	6.74 ± 1.70	0.80 ± 1.70	1.01 ± 1.70	0.43 ± 1.70	0.65 ± 1.70	2.73 ± 1.70	3.83 ± 1.7

http://dx.doi.org/10.4014/kjmb.1409.09002

sake, L. plantarum, and *Oenococcus oeni* were reported to prevent acidification; *L. sakei* L5 promotes the breakdown of α-rice by production of autolysate, *Saccharomyces sake* 7-2 resists to antiyeast substance F 16-2 and does not easily break down, and *L. sake* does not affect the fermentation of *S. sake* 7-2 [11].

In Korea, *makegeolli* has been received great attention for LAB, however commercially brewed *makgeolli* showed severe deviation for number of LAB because LAB are not well controlled during fermentation. In this study, alcohol-tolerant *P. acidilactici* K3 was added to *makgeolli* before fermentation and after fermentation. The number of the strain were maintained consistently in both ways and physicochemical properties of the two type of makgeollies were not greatly changed. These results suggested that alcohol-tolerant *P. acidilactici* K3 could be used for makgeolli brewing as a starter or supplementation on either way.

Acknowledgments

This research was supported in part by a Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (Grant No. 2010-0013133), by a project fund (C34703) to J.S. Choi from the Center for Analytical Research of Disaster Science of Korea Basic Science Institute, and by KBSI grant (T34525) to J.-K. Rhee from Korea Basic Science Institute Western Seoul Center.

References

1. Albano H, Todorov SD, van Reenen CA, Hogg T, Dicks LM, Teixeira P. 2007. Characterization of two bacteriocins produced by *Pediococcus acidilactici* isolated from "Alheira", a fermented sausage traditionally produced in Portugal. *Int. J. Food Microbiol.* **116**: 239-247.
2. Altuntas EG, Cosansu S, Ayhan K. 2010. Some growth parameters and antimicrobial activity of a bacteriocin-producing strain *Pediococcus acidilactici* 13. *Int. J. Food Microbiol.* **141**: 28-31.
3. Bae K, Shin KS, Ryu H, Kwon C, Sohn H. 2007. Identification and fermentation characteristics of lactic acid bacteria isolated from the fermentation broth of Korean traditional liquor, Andong-Soju. *Korean J. Microbiol. Biotechnol.* **35**: 310-315.
4. Baek H, Choi M, Oh K. 2012. Characterization and antibacterial activity of *Lactobacillus casei* HK-9 isolated from Korean rice wine, *makgeolli*. *Korean Soc. Biotechnol. Bioeng.* **27**: 161-166.
5. Han E, Lee T, Noh B, Lee D. 1997. Quality characteristics in mash of *takju* prepared by using different *nuruk* during fermentation. *Korean J. Food Sci. Technonol.* **29**: 555-562.
6. Jang D, Park S, Lee H, Pyo S, Lee HS. 2013. Isolation of the alcohol-tolerant lactic acid bacteria *Pediococcus acidilactici* K3 and S1 and their physiological characterization. *Korean J. Microbiol. Biotechnol.* **41**: 442-448.
7. Jo KY, Ha DM. 1995. Isolation and identification of the lactic acid bacteria from *nuruk*. *Agric. Chem. Biotechnol.* **38**: 95-55.
8. Kwon S, Sohn J. 2012. Analysis of microbial diversity in *nuruk* using PCR-DGGE. *J. Life Sci.* **22**: 110-116.
9. Kwon Y, Lee A, Kim H, Kim J, Ahn B. 2013. Quality properties of *makgeolli* brewed with various rice and Koji. *Korean J. Food Sci. Technonol.* **45**: 70-76.
10. Kwon Y, Lee A, Kim J, Kim H, Ahn B. 2012. Changes of physicochemical properties and microbial during storage of commercial *makgeolli*. *Korean J. Mycol.* **40**: 210-214.
11. Lee H, Lee J, Chang Y. 2013. Quality characteristics of *makgeolli* supplemented with cranberries. *J. East Asian Soc. Dietary Life.* **23**: 85-91.
12. Min J, Baek S, Lee J, Kim H. 2011. Changes of yeasts and bacterial flora during the storage of Korean traditional *makgeolli*. *Korean J. Mycol.* **39**: 151-153.
13. Park C, Jang S, ParK E, Yeo S, Kim O, Jeong Y. 2011. Comparison of the quality characteristics of commercial *makgeolli* type in South Korea. *Korean J. Food Preserv.* **18**: 884-890.
14. Park C, Jung H, Park H, Hong J. 2007. Identification and fermentation characteristics of lactic acid bacteria isolated from Hahyangju *nuruk*. *Korean J. Food Preserv.* **14**: 188-193.
15. Park C, Lee T. 2007. Quality characteristics of *takju* prepared by wheat flour *nuruks*. *Korean J. Food Sci. Technonol.* **34**: 296-302.
16. Park M, Jeong E, Oh S, Song M, Doo J, Jeong Y, *et al.* 2013. Rapid *in vivo* colonization screening of probiotic bacteria isolated from human infants using *caenorhabditis elegans* surrogate host. *Korean J. Food Sci. An.* **33**: 522-530.
17. Park S, Lee SG, Jin H. 2012. Isolation and indentification of acid-forming bacteria form a fresh wheat *makgeolli* in Jeonju. *Korean J. Food Nutr.* **25**: 951-956.
18. Seo G, Choi S, Kim T, Rys S, Park JY, Lee S. 2005. Functional activities of *makgeolli* by-products as cosmetic materials. *J. Korean Soc. Food Sci Nutr.* **42**: 505-511.
19. Seo M, Lee J, Ahn B, Cha S. 2005. The changes of microfloar during the fermentation of *takju* and *yakju*. *Korean J. Food Sci. Technonol.* **37**: 61-66.
20. So M, Lee Y, Noh W. 1999. Changes in microorganisms and main components during *takju* brewing by a modified *nuruk*. *Korean J. Food Nutr.* **12**: 226-232.
21. Woo S, Shin J, Seong J, Yeo S, Choi J, Kim T, *et al.* 2010. Quality characteristics of brown rice *takju* by different *nuruks*. *J. Korean Soc. Food Sci. Nutr.* **39**: 301-307.

국문초록

알코올 내성 젖산균 *P. acidilactici* K3와 혼합 발효한 막걸리의 품질 연구

장단비[1], 이현주[1], 표상은[1], 노성운[2], 이진규[3], 이한승[1,4*]

[1]*신라대학교 의생명과학대학 바이오식품소재학과*

[2]*한국기초과학지원연구원 제주센터 분석연구부*

[3]*한국기초과학지원연구원 서울서부센터 분석연구부*

[4]*신라대학교 해양극한미생물연구소*

막걸리내 유산균의 특성을 연구하기 위해 알코올 내성이 있는 *P. acidilactici* K3를 첨가하여 막걸리를 담갔을 때와 시중에 판매되고 있는 생막걸리에 *P. acidilactici* K3를 첨가하여 한 달간 저장했을 때의 이화학적 변화와 젖산균수의 변화를 조사하였다. 그 결과, *P. acidilactici* K3를 첨가하여 발효하였을 때의 젖산균 생균수는 이를 첨가하지 않은 막걸리보다 3배 이상 많았으나 알코올 함량은 큰 차이를 보이지 않았으며 당도, 환원당, 총당, 탁도, pH, 산도, 아미노태 질소함량은 큰 차이를 보이지 않았다. 또한 시판되는 생막걸리에 *P. acidilactici* K3를 첨가하여 저장기간 동안 이화학적 변화를 관찰한 결과, 젖산균 생균수는 *P. acidilactici* K3를 첨가한 생막걸리는 10^7 CFU/ml을 유지하였으며, 알코올함량, 탁도, 당도, 환원당, 총당, pH, 총산, 아미노태 질소함량은 큰 차이가 없었다. 본 실험결과, 알코올 내성 젖산균 *P. acidilactici* K3가 막걸리의 이화학적 성질에 큰 영향을 미치지 않으며, 막걸리 발효균주 및 첨가제로의 응용이 가능함을 확인하였다.

http://dx.doi.org/10.4014/kjmb.1409.09002