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REGULAR ARTICLE

Characteristics of South Korean traditional fermented barley bran (*Sigumjang*): and isolation of its useful strains

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ABSTRACT

Sigumjang made from barley bran is a traditional fermented food found only in the Gyungsang-do area of South Korea. The appearance, taste, and flavor of *sigumjang* differ from those of *Doenjang* (well-known Korean traditional fermented soybean paste). We investigated the characteristics of *sigumjang* and isolated microbial strains with high enzymatic activity from *sigumjang meju*, which is dried-fermented barley bran that serves as the basis of *sigumjang*. We collected 19 kinds of *sigumjang* and 14 kinds of *sigumjang meju*. Methods for making *sigumjang meju* differ by region involve either directly baking or steaming the barley bran dough. *Sigumjang* showed a lower pH level (4.37 - 5.99) than other Korean traditional fermented *jang* due to lactic acid fermentation. A total of 13 strains showed increased hydrolysis of cellulose, starch, and proteins among the 51 strains collected from the 14 kinds of *sigumjang meju*. The SM-26 stain showed high enzymatic activities for cellulase, amylase, and protease and was identified as *Bacillus amyloliquefaciens subsp.* based on 16S rRNA sequence analysis.

Keywords: Barley bran; Enzymatic activity; Korean traditional-fermented food; Microorganism; *Sigumjang*

INTRODUCTION

Sigumjang, also known as *deunggyeojang*, *gaetteokjang*, is a traditional fermented food made from barley bran and is usually consumed in the Gyongsang-do area of South Korea. Barley bran is mixed with water to make doughnut-shaped *meju*, which is slightly roasted and fermented for 2 months. After the end of fermentation, it is grinded and blended with other ingredients such as boiled barley, soybean, and liquid *yeot* (taffy, *jocheong*) and subsequently fermented at room temperature for several days before being eaten. *Sigumjang* is considered to have a favorable flavor due to its lower level of salt(NaCl) and weaker odor compared to other Korean traditional fermented foods such as *doenjang* (soybean paste), *gochujang* (hot pepper paste), and *ganjang* (fermented soy sauce). To date, however, there have been no studies standardizing methods for production of *sigumjang*. Therefore, *Sigumjang* is traditionally made by farms and is difficult to find in cities.

Barley bran is the main ingredient of *sigumjang* and is a by-product collected from the milling process. Generally, barely bran is used as animal feed. However, a large quantity of β-glucan is found in barley bran, which is expected to be responsible for the functionality of *sigumjang*. β-glucan is water-soluble dietary fiber, which is a non-starch polysaccharide composing the endosperm of grains and cell wall of the aleurone layer. β-glucan has healthy physiological functions, as it regulates responses to blood glucose level and insulin as well as reduces cholesterol level. Furthermore, β-glucan prevents heart disease and inhibits fat storage, making it effective in the prevention of obesity by relieving associated symptoms. Barley bran has been reported to facilitate digestion as well (Lupton and Robinson, 1993). Lupton et al. (1994) and Newman et al. (1992) reported that barley bran has inhibitory effects on cholesterol levels. Although *sigumjang* has been culturally used for centuries to aid digestion and treat diabetes, there has been no study on the functionality of *sigumjang*.

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For *sigumjang*, prior studies have examined traditional recipes and ingredients of *deunggyeojang* in Gyongsang-do of South Korea (Choi, 1991), quality variance depending on fermentation period (Choi et al., 2001), volatile flavor components of traditional *sigumjang meju* (Choi et al., 1999), and changes in aflatoxin and volatile flavor components depending on the traditional fermentation period of *sigumjang* (Son et al., 2000). As most studies have focused on production and flavor component analysis, there have not been many studies comparing *sigumjang* to other traditional fermented foods such as *doenjang*, *gochujang*, and *ganjang*.

Due to increasing popularity, Korean food ingredients have been recently introduced to other countries. Unlike other Korean traditional fermented foods consisting of about 10% NaCl, *sigumjang* has a low NaCl level of only 3% as well as a less distinctive taste or odor. Therefore, *sigumjang* has high applicability to diverse kinds of foods other than Korean food. However, studies are needed to determine the diverse characteristics of *sigumjang* dispersed throughout Gyongsang-do as well as useful strains in order to standardize the manufacturing methods for traditional *sigumjang*.

This study investigated the characteristics of *sigumjang* and *sigumjang meju* collected throughout the Gyongsang-do area of South Korea, and strains with excellent enzyme activity were isolated from *sigumjang meju*. This study provides useful information for commercialization of *sigumjang*.

MATERIALS AND METHODS

Sampling of *sigumjang* and *sigumjang meju*

Nineteen kinds of *sigumjang* and 14 kinds of *sigumjang meju* were collected from Gyungsang-do area of South Korea. *Sigumjang* 1 was collected from Pohang, *sigumjang* 2 was collected from Gyeongju, *Sigumjang* 3-6 was collected from Seongju, *Sigumjang* 7-10 was collected from Goryeong, *Sigumjang* 11 was collected from Daegu, *Sigumjang* 12-13 was collected from Yeongcheon, *Sigumjang* 14 was collected from Changnyeong, *Sigumjang* 15 was collected from Hapcheon, S16 was collected from Daegu and *Sigumjang* 17-19 was collected from Sancheong. *Sigumjang meju* 1 was collected from Seongju, *Sigumjang meju* 2 was collected from Pohang, *Sigumjang meju* 3-5 was collected from Gyeongju, *Sigumjang meju* 6 was collected from Seongju, *Sigumjang meju* 7- 8 was collected from Goryeong, *Sigumjang meju* 9- 11 was collected from Yeongcheon, *Sigumjang meju* 12- 13 was collected from Changnyeong and *Sigumjang meju* 14 was collected from Hapcheon.

Physicochemical analysis of the samples

The pH values were determined using a pH meter (FiveEasy FE20, Mettler Toledo, Switzerland). Surface color values were determined using a color meter. Color of each sample was measured five times based on L (lightness) value, ±a (redness/greenness) value, and ±b (yellowness/blueness) value. Acidity was measured by titration against 0.1 N NaOH to an end point of pH 8.3, monitored with a pH meter. All assays were done in triplicate.

General components analysis of the samples

Moisture, crude ash, crude lipid, and crude protein contents of samples were measured according to AOAC (1995) methods. All assays were done in triplicate.

Total polyphenol contents of the samples

Total phenolic content of each extract was quantified colorimetrically using Folin-Ciocalteu reagent as described in a previous study (Kim D. 2010). *Sigumjang* extract (0.2 mL, 10 mg/mL) was mixed with 1 mL of Folin-Ciocalteu's reagent and then allowed to stand at room temperature for 3 min. Na_2CO_3 (0.8 mL, 10%) was then added to the mixture. After 1 h, the absorbance of the mixture was measured at 765 nm. The results are expressed as gallic acid equivalents (Sigma, USA).

Antioxidant activities of the samples

DPPH radical scavenging activities were measured according to the method of Blois (1958) with some modifications. Sample (0.1 mL) was added 0.1 mL of 0.15 mM DPPH (in MeOH) radical solution. The mixture was shaken and allowed to stand for 30 min at room temperature, after which the absorbance was measured at 518 nm.

$$\text{Scavenging activity (\%)} = [1-(As-Ab)/(Ac-Ab)]\times 100,$$

where As, Ab, and Ac represent the absorbance levels of sample, blank, and control, respectively.

Sensory analysis

Thirteen panelists were female and 5 were male in the range of 20–25 years old of age. They went under training to perform the sensory evaluation of the *sigumjang*. Well trained sensory panel evaluated the color, taste, texture, flavor, and overall acceptability of the samples. Each characteristic was rated based on a 7-point sensory test, with 1 point indicating completely unacceptable and 7 point indicating excellent.

Screening of strains with high enzymatic activity for *sigumjang meju* samples

Isolation of strains with high protease activity was performed by the toothpick method on the surfaces of 10% skim milk agar plates. Each plate was incubated at 37°Cfor 24 h. Colonies surrounded by clear zones were selected, and large clear-zone forming colonies were selected for measurement of protease activity. Isolation of strains with high cellulase and amylase activities was performed by the toothpick method on the surfaces of 1% CMC and 1% starch agar plates, respectively. Each plate was incubated at 37°Cor 24 h. Transparent ring-forming colonies formed after applying Congo red and iodine solution drops were selected, and large transparent ring-forming colonies were selected for measurement of cellulase and amylase activities (Yun et al., 2003).

Analysis of protease activities of the isolated stains

The enzymatic reaction mixture consisted of 200 μL of crude enzyme solution and 500 μL of 1% (w/v) casein in 50 mM glycine-NaOH buffer (pH 9.0). The reaction was initiated by addition of crude enzyme solution and was maintained at 40°C for 10 min. The reaction was stopped by addition of 500 μL of 10% (w/v) trichloroacetic acid (TCA), followed by incubation for 30 min at room temperature and centrifugation (15,000 rpm, 10 min). Enzymatically hydrolyzed casein was measured by the modified Folin-Ciocalteu method. Supernatant (500 μL), 25 mL of 500 mM Na_2CO_3, and 500 μL of 0.4 N Folin reagent were reacted for 10 min at 40°C. Protease activity was then determined by measuring the absorbance at 660 nm using a UV spectrophotometer. One unit of protease activity was defined as the amount of enzyme liberating 1 μg of tyrosine per min (Ghose, 1987).

Analysis of cellulase activities of the isolated stains

Cellulase activity was measured by the DNS (3,5-dinitrosalicylic acid) method. The enzymatic reaction mixture consisted of 250 μL of crude enzyme solution, 500 μL of 1% CMC, and 250 μL of 200 mM sodium phosphate buffer (pH 7.0). The reaction was initiated by addition of crude enzyme solution was maintained at 50°C for 15 min. Cellulase activity was determined based on a calibration curve for glucose. One unit of enzyme activity was defined as the amount of enzyme releasing 1 μg of glucose per minute (Miller, 1959).

Analysis of amylase activities of the isolated stains

Amylase activity was measured by the DNS (3,5-dinitrosalicylic acid) method. The enzymatic reaction mixture consisted of 100 μL of crude enzyme solution and 100 μL of 1% soluble starch solution. This mixture was incubated for 10 min at 37°C, and the reaction was stopped by 200 μL addition of DNS solution. After the treated samples were boiled for 5 min and cooled in water, the optical density was measured at 546 nm. Amylase activity was determined based on a calibration curve for glucose. One unit of enzyme activity was defined as the amount of enzyme releasing 1 μg of glucose per minute (Kunitz, 1947).

Identification of selected stains by 16S rRNA sequence analysis

Identification of the SM-26 strain was carried out by 16S rRNA gene sequencing performed by SolGent Co. (Daejeon, Korea). The 16S rRNA sequence data were compared with available sequence data in the GenBank and EZ-taxon databases using the BLAST algorithm.

Statistical analysis

Values are expressed as mean±standard deviation. One-way analysis of variance (ANOVA) and T-test were used for multiple comparisons. Treatment effects were analyzed using Duncan's multiple range tests by SPSS 19.0 software. Differences are considered significant with P values<0.05.

RESULTS AND DISCUSSION

Characteristics of collected *sigumjang* and *sigumjang meju*

Nineteen kinds of *sigumjang* collected from nine different traditional markets in the Gyonsang-do area of South Korea were analyzed, and differences in the ingredients of *sigumjang* were detected according to area. The processing methods of *sigumjang* were also shown to be diverse. In most regions, boiled barley, liquid *yeot* (taffy, *jocheong*), and *sigumjang meju* were identified as the main ingredients, and various vegetables grown in each region were included as additional ingredients. *Sigumjang* was found to have high popularity in North Gyongsang-do compared to South Gyongsang-do of South Korea. The appearance, taste, and texture of *sigumjang* were found to be slightly different between the two regions. *Sigumjang* from South Gyongsang-do has less moisture and is more reddish in color, as this region uses almost no additional ingredients compared to North Gyongsang-do (Fig. 1a). Fig. 1b shows the characteristics of the 14 kinds of *sigumjang* that were collected. For making *Sigumjang meju*, barley bran is generally mixed with water and kneaded into dough. Then, it is roasted or baked after steaming. *Sigumjang meju* is mostly circular, except for SM1, and the size of *sigumjang meju* can be diverse ranging from 11-16.7 cm. Traditionally, *sigumjang meju* has a hole in the center, which makes it easier to handle and dry and increases air circulation to aid fermentation.

Physicochemical analysis of the samples

The color of *sigumjang* appears differently according to the added ingredients, fermentation period, and condition of *sigumjang meju*. Among the collected samples, S1 and S2 samples showed the lightest colors, whereas S4 and S17 showed the darkest colors. Except for the S4, S9, and S11 samples, which were comparatively darker, the color of

Fig 1. Appearance of *sigumjang* (a) and *sigumjang meju* (b). S1: *sigumjang* collected from Pohang, S2: *sigumjang* collected from Gyeongju, S3-S6: *sigumjang* collected from Seongju, S7-S10: *sigumjang* collected from Goryeong, S11: *sigumjang* collected from Daegu, S12-S13: *sigumjang* collected from Yeongcheon, S14: *sigumjang* collected from Changnyeong, S15: *sigumjang* collected from Hapcheon, S16: *sigumjang* collected from Daegu, S17-S19: *sigumjang* collected from Sancheong. SM1: *sigumjang meju* collected from Seongju, SM2: *sigumjang meju* collected from Pohang, SM3-SM5: *sigumjang meju* collected from Gyeongju, SM6: *sigumjang meju* collected from Seongju, SM7- SM 8: *sigumjang meju* collected from Goryeong, SM9- SM 11: *sigumjang meju* collected from Yeongcheon, SM12- SM 13: *sigumjang meju* collected from Changnyeong, SM14: *sigumjang meju* collected from Hapcheon. SM1's size is 12.3 cm, SM2's size is 12.3 cm, SM3's size is 16.5 cm, SM4's size is 11.9 cm, SM5's size is 16.7 cm, SM6's size is 10.5 cm, SM7's size is 13.8 cm, SM8's size is 11 cm, SM9's size is 17 cm, SM10's size is 16.5 cm, SM11's size is 15.5 cm, SM12's size is 15 cm, SM13's size is 14.2 cm, SM14's size is 12 cm.

sigumjang was similar to *doenjang* (fermented soybean paste), which is the representative traditional fermented food of Korea. The pH of *sigumjang* was shown to be within the range of 4.37-5.99; S13 sample showed the lowest pH while S5 sample showed the highest pH. Among the collected samples, eight were within the pH level of 4, whereas S11 were within the pH level of 5. No *sigumjang* sample had a pH less than 4 or greater than 6. The pH scale of *sigumjang* usually ranged from 4-5, as *sigumjang* undergoes active lactic acid fermentation unlike other Korean fermented *jang* foods such as *doenjang* (soybean paste) and *ganjang* (fermented soy sauce) (Table 1).

Regarding the color of *sigumjang meju*, most had similar dark orange to brown color tones, which was different from the surface. The brightness of *sigumjang meju* ranged from

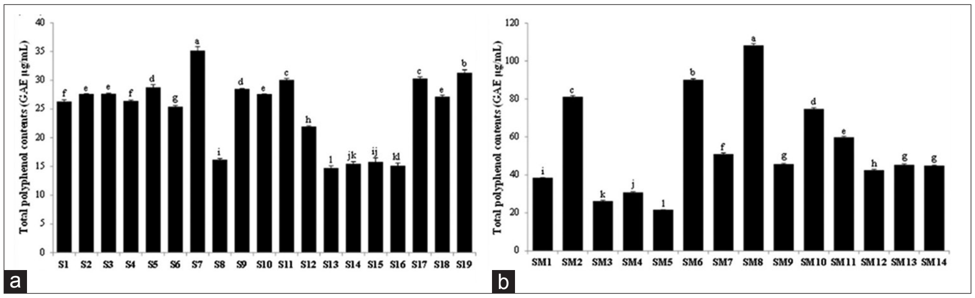

Fig 2. Total polyphenol contents of *sigumjang* (a) and *sigumjang meju* (b). S1-S19 and SM1-SM14: See the legend of Table 2. All values are expressed as mean±SD. Means with different letters in a row are significantly different at α=0.05 by Duncan's multiple range test.

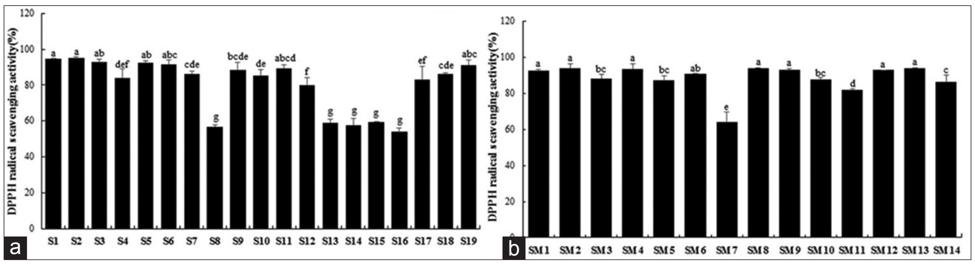

Fig 3. DPPH radical scavenging activities of *sigumjang* (a) and *sigumjang meju* (b). All values are expressed as mean±SD. Means with different letters in a row are significantly different at α=0.05 by Duncan's multiple range test.

Table 1: CIELAB, pH and acidity of *sigumjang*

Group[1)]	CIELAB			pH	Acidity (%)
	L	a	b		
S1	35.70±0.48$^{2)a3)}$	5.68±0.05^{f}	11.65±0.05^{b}	4.43	1.0
S2	30.92±0.53^{e}	4.42±0.06^{kl}	8.82±0.05^{f}	4.67	0.8
S3	30.13±0.26^{f}	5.42±0.02^{g}	8.44±0.03^{g}	5.76	0.8
S4	25.84±0.55^{k}	4.30±0.12^{l}	5.81±0.11^{k}	4.99	1.1
S5	29.70±0.39^{fgh}	5.04±0.05^{i}	7.85±0.09^{i}	5.99	0.8
S6	29.46±0.24^{fgh}	5.19±0.04^{h}	7.55±0.06^{j}	5.86	0.8
S7	29.96±1.35^{fg}	4.77±0.25^{j}	7.40±0.39^{j}	5.76	1.0
S8	34.14±0.29^{b}	5.74±0.07^{f}	9.52±0.10^{e}	5.80	0.7
S9	27.00±0.25^{j}	4.06±0.03^{m}	5.71±0.07^{k}	5.66	0.9
S10	29.19±0.43^{ghi}	5.75±0.13^{f}	8.42±0.13^{g}	4.74	1.1
S11	31.78±0.79^{d}	3.47±0.07^{n}	5.32±0.03^{l}	4.56	1.6
S12	35.77±0.33^{a}	5.42±0.10^{g}	10.45±0.22^{d}	4.48	1.0
S13	31.45±0.15^{de}	6.16±0.03^{e}	10.42±0.01^{d}	4.37	0.8
S14	31.17±0.16^{de}	4.50±0.03^{k}	8.76±0.01^{f}	4.62	1.0
S15	35.68±0.69^{a}	9.41±0.06^{a}	13.55±0.12^{a}	5.47	0.6
S16	32.59±0.95^{c}	7.27±0.18^{b}	11.04±0.19^{c}	5.10	0.8
S17	26.66±0.40^{j}	7.24±0.05^{b}	8.15±0.05^{h}	5.47	1.0
S18	28.46±0.59^{i}	7.09±0.10^{c}	8.19±0.10^{h}	5.46	0.8
S19	29.11±0.86^{hi}	6.64±0.18^{d}	7.58±0.18^{j}	5.50	1.1
F-value	126.774***	900.967***	1156.688***		

[1)]See the legend of Fig. 1. [2)]All values are expressed as mean±SD. [3)]Means with different letters in a column are significantly different at α=0.05 by Duncan's multiple range test

Table 2: CIELAB, pH and acidity of *sigumjang meju*

Group[1)]	CIELAB			pH	Acidity (%)
	L	a	b		
SM1	56.21±0.90$^{2)a3)}$	7.06±0.05^{c}	10.70±0.30^{b}	6.07	0.7
SM2	51.43±0.90^{c}	6.99±0.04^{cd}	9.83±0.22^{c}	6.48	0.7
SM3	55.68±0.34^{a}	7.00±0.10^{cd}	10.64±0.32^{b}	5.53	0.8
SM4	53.61±0.89^{b}	7.90±0.15^{b}	11.51±0.32^{a}	6.05	0.3
SM5	53.74±0.83^{b}	5.52±0.16^{i}	9.55±0.20^{cd}	5.93	0.4
SM6	49.64±1.34^{d}	6.88±0.10^{de}	9.48±0.34^{cd}	5.74	1.5
SM7	51.70±1.82^{c}	6.02±0.06^{h}	7.66±0.16^{f}	6.23	0.2
SM8	56.82±0.60^{a}	6.78±0.07^{ef}	10.58±0.22^{b}	5.85	1.8
SM9	44.46±0.86^{e}	6.65±0.24^{f}	7.36±0.48^{f}	5.71	0.5
SM10	48.74±1.02^{d}	6.44±0.19^{g}	8.65±0.80^{e}	5.52	2.1
SM11	49.82±0.67^{d}	6.89±0.04^{de}	9.21±0.19^{d}	5.86	2.1
SM12	53.22±1.07^{b}	5.90±0.07^{h}	9.47±0.34^{cd}	6.12	0.8
SM13	54.19±0.77^{b}	5.98±0.10^{h}	9.59±0.21^{cd}	5.82	1.7
SM14	57.02±1.22^{a}	8.70±0.15^{a}	11.51±0.35^{a}	5.64	2.0
F-value	62.606***	224.486***	61.513***		

[1)]See the legend of Fig. 1. [2)]All values are expressed as mean±SD. [3)]Means with different letters in a column are significantly different at α=0.05 by Duncan's multiple range test

44.46-57.02 and differed between *meju* samples. Yellowness values was lowest in the darkest sample, SM9 but highest in SM14. Lightness and yellowness values were proportional. The pH of *sigumjang meju* ranged from 5.52-6.48; SM10 had the lowest pH while SM2 had the highest. These values were higher than the pH of *sigumjang* as shown above due to lactic acid fermentation of *sigumjang* (Table 2).

General components analysis of the samples

The moisture contents of *sigumjang* and *sigumjang meju* were 41.30-72.19% and 7.75-19.30%, respectively. Ingredients with high moisture levels such as liquid *yeot*, boiled barley, and boiled beans are included in *sigumjang*. The crude ash contents of *sigumjang* and *sigumjang meju* were 2.30-9.42% and 3.01-8.01%, respectively. Crude fat and crude protein

Table 3: Moisture, crude ash, crude fat and crude protein of *sigumjang* and *sigumjang meju*

Group[1)]	Moisture (%)	Crude ash (%)	Group[1)]	Moisture (%)	Crude protein (%)	Crude fat (%)	Crude ash (%)
S1	72.19	2.30	SM1	9.72	38.00	0.00	4.37
S2	70.90	2.84	SM2	11.87	47.00	5.00	5.95
S3	63.97	4.70	SM3	8.76	27.00	5.00	4.46
S4	54.57	3.71	SM4	7.75	20.00	8.00	4.73
S5	58.98	3.62	SM5	11.51	29.00	5.00	3.01
S6	59.71	3.79	SM6	16.79	36.00	10.00	6.53
S7	57.71	6.66	SM7	11.65	38.00	7.00	4.69
S8	54.91	4.14	SM8	9.66	56.00	10.00	8.01
S9	53.58	3.60	SM9	17.03	35.00	9.00	7.48
S10	58.57	4.29	SM10	19.30	35.00	12.00	4.19
S11	57.09	4.61	SM11	11.20	40.00	8.00	6.15
S12	60.12	2.48	SM12	16.06	33.00	5.00	3.75
S13	60.82	4.00	SM13	14.39	24.00	6.00	4.65
S14	53.33	9.42	SM14	14.76	40.00	8.00	5.23
S15	51.62	8.94	Average	12.89	35.57	7.00	5.23
S16	41.30	5.92					
S17	58.48	5.29					
S18	57.89	4.40					
S19	58.93	5.36					
Average	58.14	4.74					

[1)]See the legend of Fig. 1

contents of *sigumjang* were 0-12% and 20-56%, respectively (Table 3).

Polyphenol content and antioxidant activity

As shown in Figs. 2 and 3, polyphenol content and antioxidant activity of each *sigumjang* and *sigumjang meju* sample were slightly different according to region. The polyphenol contents of *sigumjang* and *sigumjang meju* ranged from 17-37 μg/mL and 20-105 μg/mL, respectively. Higher polyphenol content was observed in *sigumjang meju* compared to *sigumjang* due to high water content. For free radical scavenging activity (Fig. 3), *sigumjang* and *sigumjang meju* showed approximately 90% activities at a concentration of 10 mg/mL, and *sigumjang meju* showed higher activity than *sigumjang*.

Sensory characteristics

The results of the sensory evaluation of *sigumjang* are shown in Table 4. For evaluation of color, S15 and S16 scored the most favorable. Since both S15 and S16 had a reddish color, it can be determined that the evaluators favored lighter reddish-colored *sigumjang* than darker-colored *sigumjang*. For taste, S10 and S18 scored the most favorable, whereas S9 was the least favored. For odor, S1, S10, and S16 scored the most favorable, whereas S9 was shown to be the least favored. For overall acceptability, S10, S17, and S18 received the highest scores, whereas S9 received the lowest score. Summarizing these sensory evaluation results, S10 *sigumjang* collected from Goryeong in the Gyongsang-do area and S18 *sigumjang* collected from Sanchung scored excellent. Furthermore, S9 *sigumjang* showed the lowest evaluation

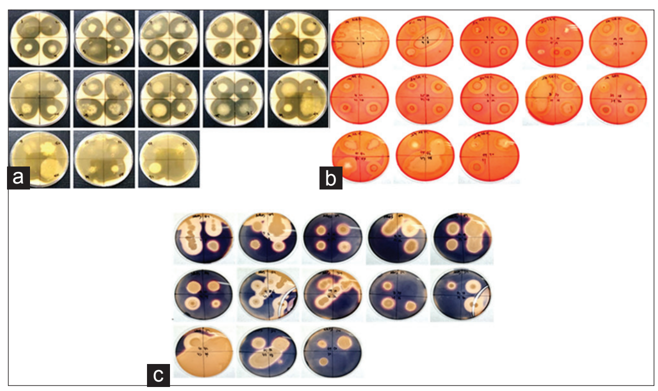

Fig 4. Hydrolysis of proteins (a), cellulose (b), and starch (c) by strains collected from *sigumjang meju*. SM-1 to SM-51: Strains isolated from *sigumjang meju.*

results and was collected from a Goryeong traditional market. The two *sigumjang* samples collected from the same region received the highest evaluation and lowest evaluation, respectively, due to the production method and not regional characteristics.

Hydrolysis analysis of proteins, cellulose, and starch in the isolated stains

In order to select excellent strains among the 51 strains isolated from *sigumjang meju*, dissociations of proteins, cellulose, and starch, which influence the taste of *sigumjang*, were examined by using a selection badge. The results are shown in Fig. 4. The strains with excellent protein dissociation were as follows: SM-8, SM-9, SM-14, SM-26, SM-39, and SM-40. The strains with excellent cellulose

Table 4: Sensory evaluation of *sigumjang*

Group [1)]	Sensory parameter				
	Color	Taste	Flavor	Sourness	Overall acceptability
S1	3.39±1.09$^{2)cdefg3)}$	3.17±1.20^{abcde}	4.12±1.50^{ab}	3.39±1.29^{abcd}	3.22±1.06^{abc}
S2	3.11±1.02^{defgh}	3.22±1.40^{abcde}	3.35±1.17^{abc}	3.17±1.47^{abcde}	3.22±1.26^{abc}
S3	2.83±1.19^{gh}	2.83±1.62^{bcde}	3.82±0.95^{abc}	2.94±1.26^{bcde}	2.78±1.31^{c}
S4	2.89±1.08^{fgh}	2.78±1.40^{cde}	3.35±1.27^{abc}	2.94±1.47^{bcde}	2.61±1.20^{cd}
S5	3.89±1.37^{bcd}	2.39±1.42^{ef}	2.88±1.41^{c}	3.22±1.40^{abcde}	2.56±1.46^{cd}
S6	3.83±1.25^{bcde}	2.78±1.35^{cde}	3.71±0.92^{abc}	3.28±1.13^{abcde}	2.89±0.68^{bc}
S7	3.78±0.88^{cdef}	3.78±1.48^{abc}	3.88±1.17^{abc}	3.89±1.64^{abcd}	3.50±1.38^{abc}
S8	4.17±1.58^{bc}	2.67±1.61^{de}	3.12±1.45^{bc}	2.78±1.48^{de}	2.78±1.52^{c}
S9	2.33±1.19^{h}	1.61±0.78^{f}	1.88±1.22^{d}	2.22±1.00^{e}	1.78±1.06^{d}
S10	4.17±1.20^{bc}	3.94±1.26^{a}	4.18±0.81^{ab}	3.89±1.18^{abcd}	4.06±0.87^{a}
S11	3.06±1.00^{defgh}	2.39±1.14^{ef}	3.41±1.18^{abc}	3.00±2.11^{abcde}	2.72±1.32^{c}
S12	3.72±1.45^{cdefg}	3.22±1.56^{abcde}	3.53±1.77^{abc}	3.33±1.78^{abcde}	3.17±1.15^{abc}
S13	3.78±1.35^{cdef}	3.50±1.50^{abcd}	3.41±1.50^{abc}	3.17±1.25^{abcde}	3.44±1.46^{abc}
S14	2.94±1.00^{efgh}	2.89±1.23^{abcde}	3.35±1.37^{abc}	2.89±1.32^{cde}	3.06±1.00^{bc}
S15	5.39±1.14^{a}	2.61±1.14^{de}	3.65±1.27^{abc}	3.00±1.28^{abcde}	3.28±1.02^{abc}
S16	5.06±1.21^{a}	3.78±1.26^{abc}	4.35±1.58^{a}	3.72±1.56^{abcd}	3.78±1.35^{ab}
S17	4.06±1.00^{bc}	3.67±1.24^{abcd}	3.88±1.11^{abc}	3.94±1.47^{abc}	4.06±1.06^{a}
S18	4.72±1.36^{ab}	3.89±1.64^{ab}	3.61±1.42^{abc}	4.11±1.18^{a}	4.06±1.51^{a}
S19	4.22±1.11^{bc}	3.61±1.42^{abcd}	3.94±1.14^{abc}	4.06±1.35^{ab}	3.83±1.34^{ab}
F-value	7.819***	3.759***	3.045***	2.269**	4.334***

[1)]See the legend of Fig. 1. [2)]All values are expressed as mean±SD. [3)]Means with different letters in a column are significantly different at α=0.05

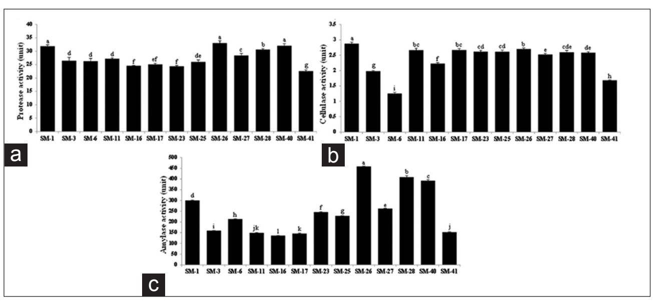

Fig 5. Protease (a), cellulose (b), and amylase (c) activities of strains collected from *sigumjang meju*. SM-1, SM-3, SM-6, SM-11, SM-16, SM-17, SM-23, SM-25, SM-26, SM-27, SM-28, SM-40, and SM-41 in the order of left, top, right, down: Strains isolated from *sigumjang meju*. All values are expressed as mean±SD. Means with different letters in a row are significantly different at α=0.05 by Duncan's multiple range test.

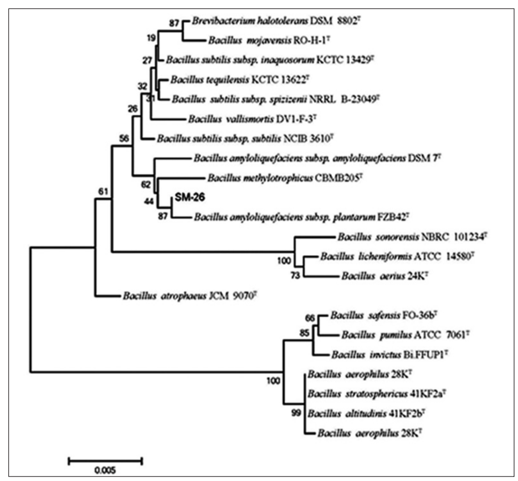

Fig 6. Neighbor-joining phylogenetic tree deduced from the 16S rDNA sequences showing the positions of SM-26 and other related taxa. SM-26: One strain selected by checking enzymatic activity isolated from *sigumjang meju*.

dissociation were as follows: SM-7, SM-19, SM-26, SM-29, SM-37, SM-41, and SM-42. The strains with excellent starch dissociation were as follows: SM-1, SM-2, SM-3, SM-6, SM-26, SM-28, SM-30, SM-31, SM-38, SM-40, and SM-41.

Analysis of enzyme activation of protease, cellulase, and amylase in the isolated stains

The enzyme activities of protease, cellulase, and amylase were measured for the selected strains using selection medium (Fig. 5). The top 10 strains from this result were selected, and the strain containing all three enzyme activities was selected. In the first round of activity measurement, SM-1 and SM-26 activities were shown to be the greatest. In the second round of measurement, S-9, S-23, SM-6, SM-26, and SM-40 activities were shown to be the highest. Combining these two results, SM-26 was shown to have the highest enzyme activities for protease, cellulase, and amylase.

Identification of the isolated strains

Isolated SM-26 was finally selected as the excellent strain. For accurate identification of the isolated microorganism, 16S rDNA sequencing was performed. Comparison was done using EzTaxon Server 2.1 with type strains showing an over 97% match. The 16S rDNA sequences of SM-26 showed a 99.86% match with *Bacillus amyloliquefaciens* subsp. *plantarum* strain FZB42. The 16S rDNA sequences and accession numbers for each type strain were obtained from the NCBI bank, and multiple alignment was performed by using ClustalX 2.0.12 and Bioedit 7.0.5.2. A phylogenetic tree was constructed through the neighbor-joining method using MEGA 5.03 (Fig. 6). As a result, the strain with the highest enzymatic activity isolated from *sigumjang meju* was identified as *Bacillus amyloliquefaciens*.

CONCLUSION

Taken together, our data provide the first report of the appearances, physicochemical characteristics, and manufacturing methods of both *sigumjang* and *sigumjang meju* collected from various regions of the Gyongsang-do area of South Korea. *Sigumjang* showed a lower pH (4.37-5.99) than other Korean traditional fermented *jang* foods such as *doenjang* and *ganjang* due active lactic acid fermentation of *sigumjang*. Among the 51 strains isolated from *sigumjang meju*, strains related to taste and with excellent enzyme activities were isolated and analyzed. SM-26 selected as the best strain was identified as *Bacillus amyloliquefaciens*. These findings provide useful information to understand the status of *sigumjang* for commercialization.

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Authors contributions

Hojeong Jeong conducted the majority of the experiments and prepared the first draft of the manuscript; Sangdon Lee conducted some experiments, and Jiyeong Yoon prepared the samples for experiments; Hyunchae Chung interpreted the results; and Gi Dong Han planned and designed this study and participated in data interpretation and helped to draft the manuscript.

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