Food Sci. Biotechnol. Vol. 17, No. 4, pp. 857 ~ 860 (2008)

RESEARCH NOTE

Food Science and Biotechnology

S-Adenosyl-L-methionine (SAM) Production by Lactic Acid Bacteria Strains Isolated from Different Fermented *Kimchi* Products

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Abstract *S*-Adenosyl-L-methionine (SAM) is a bioactive material used in the treatment of depression, osteoarthritis, and liver disease. To obtain lactic acid bacteria (LAB) producing high concentrations of SAM, LAB were isolated from commercial *kimchi* and from prepared *kimchi* products that contained shrimp *jeotgal* (fermented salty seafood) or sand lance *jeotgal* or that were fermented at 5 or 10°C, respectively, when pH was 4.2 to 4.8 and titratable acidity 0.6 to 0.9. Among the 179 LAB strains isolated from the fermented *kimchi* products, the genus *Leuconostoc* produced the highest intracellular level of SAM (1.58 mM) and *Lactobacillus* produced the second highest level (up to 1.47 mM) in the strain culture. This is the first study to quantify SAM in LAB isolated from fermented *kimchi* prepared by a general *kimchi* recipe. Ultimately, the selected strains (*Leuconostoc mesentroides* subsp. *mesenteroides/dextranicum* KSK417, *L. mesentroides* subsp. *mesenteroides/dextranicum* KJM401, and *Lactobacillus bifermentans* QMW327) could be useful as starters to manufacture fermented foods containing high levels of SAM.

Keywords: *S*-adenosyl-L-methionine, lactic acid bacteria, *kimchi*, fermentation temperature, *jeotgal*

Introduction

Kimchi is a traditional Korean food containing naturally fermented vegetables that is manufactured by combining such ingredients as Chinese cabbage, radish, red pepper, garlic, ginger, green onion, a fish source, and salt. Fermented *kimchi* contains diverse lactic acid bacteria (LAB) such as *Lactobacilli* spp., *Leuconostoc* spp., *Lactococcus* spp., and *Pediococcus* spp. (1-4). *Kimchi* also contains high amounts of vitamins and fiber, has a unique taste, is low in calories, and has probiotic effects from its LAB; it can also elevate metabolism due to its spice ingredients (5,6). *Kimchi*'s antimutagenic and immunostimulating effects are widely reported (7). In a previous report, the immuno-stimulating properties of *kimchi* were improved by adding diverse herbal components to products (8). It is known that LAB has functional properties, including antioxidant and anticarcinogenic effects, improvements of gastro-intestinal disorders, and serum cholesterol reduction (9,10).

S-Adenosyl-L-methionine (SAM) is a major methyl donor in all living organisms, and therefore, plays an important role in metabolism. SAM is highly recognized as a pharmaceutical material and has been applied in treating depression, osteoarthritis, and liver disease (11). Furthermore, methyl donors relate to decreases in colon cancer risk (12). Although there are many reports pertaining to the production of SAM by *Streptomyces* spp. and yeast (13), reports on SAM production by LAB are limited (14). In this study, as a source of SAM, superior LAB capable of producing high levels of SAM was isolated from fermented *kimchi*. The overall aim of this work was to determine how much SAM could be produced by such LAB as a basic approach to manufacturing functional *kimchi* products containing high levels of SAM.

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Received October 8, 2007; Revised December 20, 2007;
Accepted December 20, 2007

Materials and Methods

***Kimchi* preparation** Six types of *kimchi* were examined in this study: 2 prepared using different fermentation temperatures, 3 prepared with different types of added *jeotgal*, and 1 commercial type. To prepare the *kimchi*, salt and water (5 times the weight of the salt) were added to Chinese cabbage until the salt content reached 16.7%. The salted water was then removed and the brined Chinese cabbage was washed. The ratio of red pepper powder: garlic:ginger:Chinese cabbage:radish:shrimp (or sand lance) *jeotgal*:table salt was 3:2:0.5:75:14:2:1. The *kimchi* product manufactured to test different fermentation temperatures was fermented at 5 or 10°C until the pH reached 4.2-4.8. The *jeotgal kimchi* was fermented at 5°C until the pH reached 4.2-4.8. The commercial *kimchi* was purchased from a supermarket and kept at 4°C until the pH reached 4.2-4.8. A 100 g sample of each *kimchi* product was blended for 90 sec under sterile conditions using a sterile Waring blender (Model 31BL9; Waring Commercial®, New Hartford, CT, USA). The blended contents were filtered using sterile gauze and the filtered *kimchi* solutions were then used for subsequent experiments.

Measurements of pH and titratable acidity The changes in *kimchi* pH during fermentation were monitored using a pH meter, and titratable acidity was calculated by the method of Hong and Park (15). Titration was performed with 0.1 N NaOH to a pH of 8.30 and the titratable acidity was expressed as the percentage of lactic acid.

Enumeration of LAB in the *kimchi* The enumeration of LAB in the *kimchi* products followed the method of Miyao and Ogawa (16). For *Lactobacillus* selection, a *Lactobacillus* selective medium with added acetic acid and sodium acetate [modified LBS agar medium: 74.5 g of Rogosa agar (Difco, Becton Dickinson Co., Sparks, MD, USA), 0.3 mL of acetic acid, and 15 g of sodium acetate in 1 L of distilled water] was used for culture at 30°C for 3 days. The *Leuconostoc* genus was selected by using a phenyl ethyl alcohol sucrose agar medium (PES) [5 g of tryptone (Difco); 0.5 g of yeast extract; 20 g of sucrose; 2 g of $(NH_4)_2SO_4$, $MgSO_4 \cdot 7H_2O$; 1 g of KH_2PO_4; 15 g of agar; and 2.5 mL of phenylethyl alcohol in 1 L of distilled water], was cultured at 20°C for 5 days in an anaerobic jar. For selecting *Enterococcus* spp., samples were cultured in KF *Streptococcus* agar (Difco) at 37°C for 4 days, and then red colonies were counted as *Enterococcus* and white colonies as *Pediococcus*.

Isolation of LAB The colonies were allowed to grow at 30°C for 2-5 days in MRS agar (Difco) and were then isolated. Initially, the strains were categorized according to morphological characteristics such as shape, size, thickness, surface color, transparency, and mucosity. Then, Gram-staining, catalase tests, and an API 50 CHL kit (Biomérieux, Marcy L'Etoile, France) were employed for strain identification.

Measurement of SAM produced by LAB The SAM production levels of the LAB were determined by reversed-phase high performance liquid chromatography (HPLC, Jasco, Tokyo, Japan) (17). The strains were grown on MRS agar covered with cellophane. At the times indicated (72 hr after inoculation), which were used to maximize population size, the cellophane sheets containing the strains were pealed from the plates and immediately transferred to petri dishes containing 10 mL of 1 M formic acid, followed by standing at 4°C for 1 hr. The formic acid was collected, filtered through a 0.45-μm pore-size filter, and then lyophilized. The lyophilized samples were dissolved in 1 mL of water and analyzed with HPLC.

HPLC apparatus and conditions HPLC was performed with a Jasco system that consisted of a model PU-980 pump, a ultraviolet (UV)/VIS detector (model UV-975), and an AS-2057plus autosampler. Seperation was carried out with a Capcell-Pak C_{18} column (4.6×250 mm, 5 μm) (Shiseido, Tokyo, Japan). The mobile phase consisted of 2 solvents: 100% methanol (solvent A) and 0.25 M ammonium acetate (solvent B). Before use, the solvent was filtered through a 0.45-μm membrane filter, and the HPLC column was equilibrated with 0% solvent A. The sample was then injected and separation was obtained using a step gradient. The gradient first consisted of solvent A at 0-40% for 20 min. The column was then washed with 100% solvent A for 5 min. The flow rate was 1.5 mL/min and detection was monitored at 254 nm; HPLC was performed at 40°C. SAM was identified according to retention time and co-chromatography with a SAM standard. Quantification was based on peak area integration and compared to the standard calibration curves of SAM (18).

Results and Discussion

We investigated the SAM-producing capabilities of LAB strains acquired from 6 fermented *kimchi* products: 2 fermented at 5 and 10°C, respectively; 3 prepared with different types of added seafood *jeotgal* such as sand lance and shrimp; and 1 commercial *kimchi*. The LAB was isolated from the *kimchi* when pH reached 4.2 to 4.8. The titratable acidity of the tested *kimchi* products ranged from 0.6 to 0.9 (Table 1). According to the morphological categorization of the LAB colonies on selective media, the total combined populations of *Lactobacillus* spp., *Enterococococcus* spp., *Pediococcus* spp., and *Leuconostoc* spp. in the products were 6-8 log CFU/g (data not shown). Overall, 179 strains were isolated from the various *kimchi* products and were identified using an API 50 CHL kit (Table 2). When SAM was quantified by HPLC analysis, *Lactobacillus* spp. and *Leuconostoc* spp. showed the highest levels of production (Table 3). In the *kimchi* fermented at 5°C, *Lactobacillus bifermentans* produced up to 1.47 mM SAM, while within the LAB isolated from the *kimchi* fermented at 10°C, a maximal amount of 1.22 mM SAM was produce by 1 isolated strain (Table 3). Table 4 summarizes the strains that produced the most SAM in each type of *kimchi*. The genera that produced the most SAM were *Lactobacillus* and *Leuconostoc* (Table 3 and 4), regardless of the *jeotgal* addition or fermentation temperature.

There have been many reports identifying SAM or SAM synthetases in Gram-positive bacteria, yeast, and archaea (13,19,20). Creason *et al.* (21) identified SAM from food sources such as soybeans and radish leaves. However, no reports have identified and quantified the SAM-producing capabilities of LAB strains isolated from fermented foods.

This work is focused on the functional properties of fermented *kimchi* products according to changes in fermentation conditions, including different additions of *jeotgal* and fermentation temperatures, within a general *kimchi* recipe, to carry out fermentation by natural flora. To include strains isolated from a diverse number of sources, a commercial *kimchi* was also analyzed. It is known that SAM accumulates in microorganism cells (17). In our preliminary work, the SAM content of *kimchi* increased not only by *jeotgal* concentration, but also by fermentation time. However, adding 2% *jeotgal* had much less effect on

Table 1. The pH, titratable acidity, and fermentation times of *kimchi* products used in this study

Experimental condition	Fermentation temp.		*Jeotgal*			Commercial *kimchi*
	5°C	10°C	Shrimp *jeotgal*	Sand lance *jeotgal*	*Jeotgal* free	
pH	4.2	4.2	4.4	4.4	4.7	4.8
Acidity(%)	0.6	0.6	0.9	0.8	0.8	0.7
Fermentation time (day)	15	20	25	25	25	25

Table 2. Numbers of identified strains isolated from the different fermented *kimchi* products

Species		Fermentation temp.		*Joetgal*			Commercial *kimchi*
		5°C	10°C	Shrimp *jeotgal*	Sand lance *jeotgal*	*Jeotgal* free	
Lactobacillus	*bifermentans*	1	-	-	-	1	1
	brevis	15	12	4	4	13	5
	collinoides	3	2	3	3	-	1
	curvatus	-	1	-	-	-	-
	fermentum	-	1	3	3	-	-
	pentosus	1	1	-	-	2	-
	salivarius	-	1	-	-		-
Lactococcus	*lactis* subsp. *lactis*	1	-	2	2	-	2
	plantarum	-	-	1	1		2
Leuconostoc	*citreum*	1	-	-	-	1	1
	mesenteroides subsp. *mesenteroides*	18	13	14	14	10	14
Pediococcus	*pentosaceus*						1
	Total	40	31	27	27	27	27

Table 3. The average intracellular *S*-adenosyl-L-methionine (SAM) contents (mM) of lactic acid bacteria strains isolated from different fermented *kimchi* products

Species		Fermentation temp.		*Jeotgal*			Commercial *kimchi*
		5°C	10°C	Shrimp *jeotgal*	Sand lance *jeotgal*	*Jeotgal* free	
Lactobacillus	*bifermentans*	1.43±0.05	-	-	-	1.16±0.08	1.18±0.04
	brevis	1.10±0.04	1.01±0.25	0.90±0.41	1.12±0.14	0.91±0.38	0.82±0.25
	collinoides	1.10±0.03	1.10±0.01	0.93±0.06	1.19±0.02	-	0.51±0.03
	curvatus	-	1.22±0.01	-	-	-	-
	fermentum	-	0.90±0.02	0.80±0.66	0.96±0.32	-	-
	pentosus	1.02±0.01	1.02±0.01	-	-	0.66±0.33	-
	salivarius	-	1.16±0.01	-	-	-	-
Lactococcus	*lactis* subsp. *lactis*	0.47±0.01	-	1.20±0.14	0.66±0.16	-	0.55±0.05
	plantarum	-	-	1.40±0.01	1.42±0.01	-	1.40±0.14
Leuconostoc	*citreum*	1.06±0.06	-	-	-	1.36±0.01	0.59±0.01
	mesenteroides subsp. *mesenteroides*	1.05±0.28	1.14±0.20	1.37±0.23	0.90±0.27	1.11±0.37	1.38±0.25
Pediococcus	*pentosaceus*	-	-	-	-	-	1.11±0.08

Table 4. Selected strains that produced the highest *S*-adenosyl-L-methionine (SAM) levels after 72 hr of incubation at 30°C

Kimchi	Strain	SAM (mM)
5°C	*Lactobacillus bifermentans* QMW327	1.47±0.05
10°C	*Lactobacillus curvatus* SKP313	1.22±0.01
Jeotgal-free	*Leuconostoc mes mes/dext* KFM402	1.48±0.06
Sand lance *jeotgal*	*Lactococcus plantarum* KKM408	1.42±0.01
Shrimp *jeotgal*	*Leuconostoc mes mes/dext* KSK417	1.58±0.05
Commercial *kimchi*	*Leuconostoc mes mes/dext* KJM401	1.46±0.08

the SAM production of the LAB than the fermentation time (data not shown). The increases in extracellular SAM that occur during *kimchi* fermentation (data not shown) may relate to the dominant microorganisms present in the fermented *kimchi*, which include *Lactobacillus* spp. and *Leuconostoc* spp. (1). During *kimchi* fermentation, SAM production increased up to 4-fold as compared to the levels in unfermented *kimchi* (data not shown). Previously, Shiozaki *et al.* (14) compared intracellular levels of SAM among yeasts, molds, basidomycetes, bacteria, actinomycetes, and LAB, showing that 2 strains of tested LAB produced only <0.04 mg/mL, while there were some yeast that produced more than 0.4 mg/mL.

Even though the absolute amount of SAM that can be produced by LAB is not high as compared to yeast, the results of this study are meaningful as basic data for further experiments that may investigate the optimal *kimchi* fermentation conditions for producing high levels of SAM;

especially since Koreans consumed high amounts of *kimchi* throughout their lives. Selected LAB that is capable of producing high SAM concentrations will prove to be important sources for food applications, including utilization as fermentation starters to manufacture *kimchi* with high levels of SAM.

Acknowledgment

This work was supported by the Biogreen 21 Project of the Rural Development Administration, Korea.

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