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Selection and Characterization of *Cheonggukjang* (Fast Fermented Soybean Paste)-Originated Bacterial Strains with a High Level of S-adenosyl-L-methionine Production and Probiotics Efficacy

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ABSTRACT This study was executed to develop probiotics producing S-adenosyl-L-methionine (SAMe), a methyl group donor in the 5-methyltetrahydrofolate methylation reaction in animal cells. SAMe is an essential substance in the synthesis, activation, and metabolism of hormones, neurotransmitters, nucleic acids, phospholipids, and cell membranes of animals. SAMe is also known as a nutritional supplement for improving human brain function. In this study, SAMe-producing strains were identified in six kinds of *Cheonggukjang*, and strains with excellent SAMe production were identified, with one strain in the *Enterococcus* genus and six strains in the *Bacillus* genus. Strains with a large amount of SAMe production included lactic acid bacteria, such as *Enterococcus faecium*, *Enterococcus durans*, and *Enterococcus sanguinicola*, as well as various strains in the *Bacillus* genus. The SAMe-overproducing strains showed antibacterial activity against some harmful microbes, in addition to weak acid resistance and strong bile resistance, indicating characteristics of probiotics. *Cheonggukjang*-originated beneficial bacterial strains overproducing SAMe may be commercially useful for manufacturing SAMe-rich foods.

KEY WORDS: • **Cheonggukjang** • ***probiotics*** • ***S-adenosyl-L-methionine***

INTRODUCTION

S-ADENOSYL-L-METHIONINE (SAMe) was first discovered by Cantoni in 1952. SAMe is a substance primarily present in tissues and body fluids of animals, and it plays an important role in the 5-methyltetrahydrofloate (5-MTHF) methylation reaction as a methyl group donor.[1] SAMe is an important bioregulator made from essential amino acids, L-methionine, and ATP by methionine adenosyltransferase.[2] It is mostly synthesized in the liver at a rate of about 8 g a day and is involved in many biochemical metabolism processes.[3]

Many studies have confirmed that SAMe content varies depending on the content of L-methionine in foods.[4] Its functionality has been recognized in western societies, including Europe and the USA, and SAMe has been reported to be an essential substance in processes, including in polyamine synthesis in cells and the synthesis, activation, and metabolism of hormones, neurotransmitters, nucleic acids, phospholipids, and cell membranes.[5]

Also, it is required in the production of important brain compounds such as neurotransmitters and phospholipids, including phosphatidylcholine and phosphatidylserine, and is known to be effective in improving brain functions.[6] The metabolic reactions of SAMe in the body include transmethylation, transsulfuration, and polyamine.[7] In the methylation reaction, the methyl group of SAMe is donated to various acceptor substrates such as DNA, phospholipids, and proteins; in the transsulfuration reaction, SAMe is converted to taurine, a major antioxidant in cells, and cysteine, a precursor of glutathione, through several enzymatic reactions.[8]

Finally, SAMe is used in the synthesis of polyamines that are essential to the growth of normal cells.[9] It also donates the methyl group to the propolyamine group in the synthesis of spermine and spermidine.[10] It has been reported that SAMe has pharmacological effects in the restoration of hepatic functions in alcoholic liver disease, in reducing muscle fatigue and rigidity in patients with fibromyalgia syndrome, and in improvement in patients with depression with about a 66% clinical improvement.[11,12] The pharmacological effects of SAMe appear to be due to the metabolic reactions of SAMe in the body, including transmethylation, transsulfuration, and polyamine.[6]

SAMe is also converted to taurine (a major antioxidant in cells) and cysteine (a precursor of glutathione, another well-known antioxidant in cells) through several enzymatic steps of the transsulfuration reaction.[6] This reaction can remove reactive oxygen species that are detrimental to the body. SAMe is used in the synthesis of polyamines that are essential to the growth of normal cells[6]

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Up to now, studies on SAMe primarily reported its effects in the treatment of depression, arthritis, and hepatic cirrhosis, and it has been recognized as a healthful functional food.[13] Foods containing SAMe include *Cheonggukjang*, one of our traditional fermented foods. Thus, this study was performed to isolate *Cheonggukjang*-originated strains that produce physiologically functional material, SAMe, select and identify strains that produce large amounts of SAMe in foods, and find strains producing SAMe with probiotics through antibacterial activity to function against harmful microbes such as food poisoning bacteria.

MATERIALS AND METHODS

Cheonggukjang *sample preparation*

Naturally existing strains in six kinds of *Cheonggukjang* were isolated and their colonies were analyzed to select strains that improve SAMe production. *Cheonggukjang* samples used in the study were purchased in September, 2012. A various type of *Chenoggukjang* samples were purchased and collected to isolation of their bacterial strains, which were mixed in a different ratio of ingredients as C1 (soybean 95%, salt 5%; Sunchang-gun, Korea), C2 (soybean 70%, water 28%, salt 2%; Sunchang-gun, Korea), C3 (soybean 98%, salt 2%; Cheongsong-gun, Korea), C4 (soybean 100%; Changwon-si, Korea), C5 (soybean 95%, salt 5%; Gwangju-si, Korea), and C6 (soybean 97%, salt 2%, red pepper powder 1%; Anseong-si, Korea).

Characteristics of selected strains

Cheonggukjang samples were diluted 10 times (0.85% NaCl 225 mL + sample 25 g) under a sterilized environment and then homogenized using a stomacher (speed level 5, 1 min). Then, the homogenate was smeared on each selective medium and incubated to separate total microbes, lactic acid bacteria, anaerobic bacteria, yeast, and mold. Microbial separation and collection were performed depending on the colony types of microbes produced. Microbes grown in different selective media were identified. The separation of microbes used tryptic soy agar (TSA) for analysis of the number of total microbes. In addition, for the analysis of total lactic acid bacteria, MRS agar was adjusted to pH 5.5. For a detailed analysis of the lactic acid bacteria, m-LBS (for *Lactobacillus* species), KF-Streptococcus (for *Enterococcus* and *Pediococcus* species), and phenylethyl alcohol with 2% sucrose agar (PES, for *Leuconostoc* species) were used.[14,15] Also, potato dextrose agar with an adjusted pH using tartaric acid was used for the analysis of the yeast and mold.

Culture of strains isolated from Cheonggukjang

To examine SAMe production ability, strains were inoculated onto a nutrient medium TSB, incubated at 35°C for 24–48 h to maintain 10^8–10^9 CFU/mL, and centrifuged (3,000 *g*, 4°C, 10 min) to eliminate microbial cells and collect the supernatant for analysis.

SAMe production

The production of SAMe was analyzed using high-performance liquid chromatography (HPLC).[16,17] Prepared samples were filtered using a 0.45 μm syringe filter and stored at −20°C for analysis. The resultant solution was analyzed using the HPLC column (C18-4.6 × 205 nm, 5 μm; Shiseido, Tokyo, Japan). The mobile phases were 100% methanol and 0.25 M ammonium acetate (pH 5.5) with acetic acid at a flow rate of 1.5 mL/min, an oven temperature of 40°C. An injection volume was 20 μL with UV detection (UV-2075; Jasco, Tokyo, Japan) at 210 nm. The concentration of SAMe was measured based on peak areas. The standard for SAMe was purchased from Sigma-Aldrich (St. Louis, MO, USA), and the ammonium acetate and methanol used in the analysis and all other reagents were purchased for HPLC use.

Analysis of sugar fermentation by isolated strains

Among the isolated strains, the *Enterococcus* genus strains used an API 20 Strep system kit (bioMérieux, Marcy l'Etoile, France) and the *Bacillus* genus strains used an API 50 CHB system kit (bioMérieux) for the analysis of the sugar fermentation of the strains. According to the API kit manual, diluted strains from *Cheonggukjang* samples were transferred to each selective medium and incubated at 37°C for 24–48 h to identify the presence/absence of various sugar fermentations.

Acid resistance and bile resistance of strains

For the resistance to pH, strains were activated in TSB (10^8–10^9 CFU/mL), washed with PBS or 0.85% NaCl, and then centrifuged. After TSA medium preparation with pH levels of 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5 (control pH 7.0—medium pH), strain samples were spotted on TSA, and changes of the colony after 18–24 h were measured. Similarly, for the bile resistance of strains, the resistance of each strain was measured in the medium with or without Oxgall. After strain activation on TSB (10^8–10^9 CFU/mL), samples were washed with PBS or 0.85% NaCl (centrifuge use), 1% of the washed strain was inoculated onto the medium with or without 0.3% Oxgall and spotted on TSA, and the changes of the colony were measured after 18–24 h.

Antibacterial activity of strains

The activity of strains that inhibited five of the indicators, including *Escherichia coli* O157:H7, *Enterococcus faecalis, Salmonella choleraesuis, Staphylococcus aureus,* and *Listeria monocytogenes*, were measured. Similarly, in the soft agar method, strains were activated and spotted on TSA and incubated for 12 h in an incubator. Then, 10 mL of soft agar with 0.75% agar concentration was prepared and indicators were added to the agar at 50°C to reach 10^5 CFU/mL before it was hardened. It was evenly spotted onto TSA with grown strains, and the circle around the colony was observed every 4 h.

Identification of isolated strains by 16S rRNA sequence analysis

Strains with a higher production of SAMe were identified by Macrogen, Inc. (Seoul, Korea). Genomic DNA was

extracted from each strain and was used for the PCR amplification of 16S rRNA gene. We performed PCR using two primers, 27F (forward primer, 5′-AGAGTTTGATCMTGG CTCAG-3′) and 1492R (reverse primer, 5′-TACGGY TACCTTGTTACGACTT-3′), for 16S rRNA sequencing.[18]

RESULTS

Isolation of Cheonggukjang-*originated colonies*

Colonies were analyzed from six kinds of *Cheonggukjang*. Microbes were grouped by shape, color, and characteristics and a total of 36 different colonies were classified. Six strains producing the highest SAMe for each type of *Cheonggukjang* were selected as the experimental group. The *Bacillus* genus, which is known as the major fermentation strain in *Cheonggukjang*, was isolated using a method for all the microbes with TSA medium; although yeast and molds, in addition to lactic acid bacteria, were separated using a selective medium. In the *Cheonggukjang*, most of the dominant microbes were in the *Bacillus* genus and the *Enterococcus* genus. The amount of SAMe production was measured in the 36 strains isolated from the 6 kinds of *Cheonggukjang* as shown in Table 1. As a result, a high level of SAMe-producing strain C4 isolated from *Cheonggukjang* produced 2.23% of SAMe. Two C4-producing stains produced the most SAMe (Table 1). To utilize these strains in future studies, it was considered necessary to examine the characteristics and identification of these 36 strains and thus, these strains were sent to Macrogen Inc. for the determination of 16S rRNA sequencing and identification through NCBI's blast search, as shown in Table 1. Population sizes of strains producing high levels of SAMe were greater than the other strains.

Sugar fermentation of strains

Substrate utilization is one of the important characteristics of microbes. Information regarding sugar utilization can be useful

Table 1. SAMe Production and Identification of Strains Isolated from *Cheonggukjang*

Name	*Isolation medium*	*SAMe production (mM)*	*Population size*	*Identification result*
C1-1	TSA	0.31	2.1×10^3	*Bacillus licheniformis*
C1-2	PES	0.27	2.7×10^2	*Bacillus subtilis*
C1-3	PES	0.46	4.1×10^4	*Bacillus subtilis*
C1-4	mLBS	0.55	1.6×10^2	*Bacillus licheniformis*
C1-5*	**MRS**	**0.63**	$\mathbf{1.7\times10^5}$	*Enterococcus faecium*
C1-6	KF	0.19	2.3×10^2	*Enterococcus faecium*
C2-1	**TSA**	**0.63**	$\mathbf{3.5\times10^6}$	*Bacillus licheniformis*
C2-2	TSA	0.38	1.2×10^4	*Bacillus licheniformis*
C2-3	PES	0.40	1.9×10^3	*Bacillus cytotoxicus*
C2-4	mLBS	0.21	3.2×10^4	*Bacillus subtilis*
C2-5	MRS	0.32	8.1×10^3	*Bacillus subtilis*
C2-6	KF	0.39	7.7×10^4	*Bacillus licheniformis*
C3-1	TSA	0.48	9.6×10^2	*Bacillus licheniformis*
C3-2	**PES**	**0.66**	$\mathbf{4.7\times10^5}$	*Bacillus amyloliquefaciens* subsp. *plantarum*
C3-3	PES	0.59	3.4×10^4	*Bacillus subtilis*
C3-4	mLBS	0.21	5.7×10^3	*Bacillus subtilis*
C3-5	mLBS	0.36	9.7×10^4	*Enterococcus faecium*
C3-6	MRS	0.19	4.2×10^3	*Bacillus subtilis*
C4-1	**TSA**	**1.575**	$\mathbf{1.3\times10^5}$	*Bacillus amyloliquefaciens*
C4-2	TSA	0.48	3.4×10^3	*Bacillus amyloliquefaciens*
C4-3	**PES**	**2.23**	$\mathbf{6.0\times10^4}$	*Bacillus subtilis*
C4-4	mLBS	0.36	1.4×10^4	*Bacillus amyloliquefaciens*
C4-5	KF	0.39	3.7×10^3	*Bacillus* sp.
C4-6	KF	0.34	4.4×10^4	*Pediococcus acidilactici*
C5-1	**TSA**	**0.83**	$\mathbf{2.2\times10^6}$	*Bacillus subtilis*
C5-2	TSA	0.29	8.9×10^4	*Bacillus subtilis*
C5-3	PES	0.49	9.5×10^4	*Enterococcus faecium*
C5-4	MRS	0.35	7.2×10^3	*Bacillus subtilis*
C5-5	MRS	0.49	2.4×10^4	*Bacillus amyloliquefaciens*
C5-6	KF	0.38	2.3×10^3	*Bscillus subtilis*
C6-1	TSA	0.51	2.4×10^2	*Bacillus subtilis*
C6-2	TSA	0.34	4.3×10^4	*Bacillus amyloliquefaciens*
C6-3	PES	0.66	2.5×10^4	*Bacillus licheniformis*
C6-4	**mLBS**	**0.86**	$\mathbf{1.9\times10^6}$	*Bacillus licheniformis*
C6-5	mLBS	0.40	3.0×10^4	*Bacillus subtilis*
C6-6	KF	0.25	2.4×10^4	*Enterococcus faecium*

*Top seven samples shown in bold.

KF, KF-streptococcus agar; mLBS, modify-lactobacillus selective agar; MRS, modify-rogesa agar; PES, phenylethyl alcohol with 2% sucrose agar; SAMe, S-adenosyl-L-methionine; TSA, tryptic soy agar.

when we introduce SAMe-producing microbial strains into food. It is also known that substrate utilization is important for producing antibacterial agents. For the six strains identified as *Bacillus* genera, an experiment was performed using an API 50 CHB system kit and for the C1-5 strain identified as *Enterococcus* genus, the sugar fermentation was measured using an API 20 Strep kit (Table 2). As a result, the *Enterococcus faecium* genus strain exhibited sugar fermentation ability with ribose, lactose, trehalose, and raffinose, among 10 carbohydrates in the API 20 Strep kit. Each of the remaining six *Cheonggukjang*-originated strains in the *Bacillus* genus had different substrate preferences for fermentation depending on the strain, but they also showed strong fermentation in substrates such as glucose, fructose, mannose, glycerol, sucrose, trehalose, maltose, and N-acetyl-glucosamine. Although the difference among fermentable substrates was not great for each strain, it is

Table 2. Sugar Fermentation Characteristics of Strains with Excellent SAMe Production

Carbohydrate	*C1-5*	*C2-1*	*C3-2*	*C4-1*	*C4-3*	*C5-1*	*C6-4*
Control	−	−	−	−	−	−	−
Glycerol	ND	+	+ +	+	+ +	+ +	+ +
Erythritol	ND	−	−	−	−	−	+
D-arabinose	ND	−	−	−	−	−	+
L-arabinose	−	−	−	−	−	+ +	+ +
Ribose	+	−	+	+	−	+	+
D-xylose	ND	−	+	−	−	−	−
L-xylose	ND	−	−	−	−	−	−
Adonitol	ND	−	−	−	−	−	−
Methyl-B-xylopyranoside	ND	−	−	−	−	+	−
Galactose	ND	+	+	+	−	+	+
Glucose	ND	+ +	+ +	+ +	+ +	+ +	+ +
Fructose	ND	+ +	+ +	+ +	+ +	+ +	+ +
Mannose	ND	+ +	+ +	+ +	+ +	+ +	+ +
Sorbose	ND	−	−	−	−	+	−
Rhamnose	ND	−	−	−	−	−	−
Dulcitol	ND	−	−	−	−	−	−
Inocitol	ND	−	+ +	−	+	−	+ +
Mannotol	−	−	+ +	−	−	+ +	+ +
Sorbitol	−	−	+ +	−	−	+ +	+ +
Methyl-α-D-mannopyranoside	ND	+	−	−	−	−	−
Methyl-α-D-glucoside	ND	+	+	−	−	+ +	+
N-acetyl-glucosamine	ND	+ +	+	+	+	+ +	+
Amygdalin	ND	+ +	+	+	+	+ +	−
Arbutin	ND	+ +	−	−	−	+ +	−
Esculin	ND	+ +	+ +	+ +	+ +	+ +	−
Salicin	ND	+ +	+	−	+	+ +	−
Cellobiose	ND	+ +	+ +	+ +	+ +	+ +	−
Maltose	ND	+ +	+	+	+	+ +	+ +
Lactose	+	−	+	−	+	+ +	−
Melibiose	ND	−	−	+	−	−	+
Sucrose	ND	+ +	+ +	+ +	+ +	+ +	+ +
Trehalose	+	+ +	+	+ +	+	+ +	+ +
Inulin	−	−	−	−	−	−	+
Melezitose	ND	−	−	−	−	−	−
Raffinose	+	−	+	−	+	−	−
Starch	−	−	−	−	−	−	−
Glycogen	−	−	−	−	−	−	−
Xylitol	ND	−	−	+	−	−	−
Gentiobiose	ND	−	+ +	−	+	−	−
D-turanose	ND	−	−	−	−	−	+
D-lyxose	ND	−	−	−	−	−	−
D-tagatose	ND	+	−	−	−	+	−
D-fucose	ND	−	−	−	−	−	−
L-fucose	ND	−	−	−	−	−	−
D-arabitol	ND	−	−	−	−	−	−
L-arabitol	ND	−	−	−	−	−	−
Gluconate	ND	−	−	−	−	−	−
2-keto-gluconate	ND	−	−	−	−	+	−
5-keto-gluconate	ND	−	−	−	−	−	−

ND, not detected; + + , strong positive; +, positive; −, negative.

Table 3. Weak Acid and Bile Resistance of SAMe-Producing Strains

Strain	*pH 4.0*	*4.5*	*5.0*	*5.5*	*7.0 (con)*	*Oxgall*
C1-5	−	−	−	+	+	+
C2-1	−	−	−	+	+	+
C3-2	−	−	−	+	+	+
C4-1	−	−	−	+	+	+
C4-3	−	−	−	+	+	+
C5-1	−	−	−	+	+	+
C6-4	−	−	−	+	+	+

considered that these strains seem to have enzymes that use many sugars and can ferment various sugars available in the body through food intake.

Acid resistance and bile resistance

Probiotics need to have strong acid resistance and bile resistance to survive in acidic gastric environments. Among the probiotic characteristics of the seven strains isolated from *Cheonggukjang*, acid resistance and bile resistance were examined. It was difficult to screen strains with strong acid resistance because strains could not grow below pH 5.0, but grew above pH 5.0. Most strains showed resistance to weak acids. All strains could grow at pH 5.5 (Table 3). As for bile resistance, seven strains showed similar growth compared with the control group, suggesting that these strains have strong bile resistance.

Antibacterial activity

The antibacterial activity was measured for five indicators such as *E. coli* O157:H7, *E. faecalis, S. choleraesuis, S. aureus,* and *L. monocytogenes* using *Cheonggukjang*-originated strains, which showed that the antibacterial activity was not observed when using the paper disc method. Instead, the antibacterial activity for the five indicators was partially identified in seven strains with the exception of one strain, C6-4, through a soft agar method (Table 4). There was a strain that inhibited all five indicators, C3-2 and C5-1. C1-5 strains inhibited at least four indicator strains, showing bacteria-inhibiting ability for several indicators. C3-2 and C5-1 could grow at the lowest pH and showed a strong inhibition to indicators, suggesting that these may be considered the most beneficial strains with a strong efficacy of probiotics.

DISCUSSION

Cheonggukjang-originated strains that produce physiologically the functional material, SAMe, were isolated as well as selected strains that could produce large amounts of SAMe in foods. The production of SAME was measured in 36 strains from 6 kinds of *Cheonggukjang* and, after the identification of these strains, mostly belonged to the *Bacillus* genus. Particularly among strains isolated from *Cheonggukjang*, two strains showed over 1.0 mM of SAMe production. The strain with the maximum production of SAMe was a C4-3 strain isolated from the C4 *Cheonggukjang* sample, which produced 2.23 mM. The mechanism of SAMe production by the strains from *Cheonggukjang* was not clear. However, the results were similarly shown in a previous study as SAMe product strains from Kimchi. The strains isolated from the fermented Kimchi products, produced the amount of SAMe 1.22–1.58 mM.[13] To utilize these strains in future studies, it was considered necessary to examine the characteristics and identification of these seven strains.

Among probiotic characteristics, acid resistance and bile resistance were examined in seven strains. The gastric pH in healthy humans can be as low as about 2–2.5[19] or high as pH 6 or above after food intake.[20] Most strains could grow in the weak acidic condition over pH 5.5. On the other hand, for bile resistance, all strains showed similar growth compared with the control group. This result suggested that these strains have bile resistance and, thus, probiotic characteristics. To be utilized as probiotics, lactic acid bacteria are required to have strong resistance against the acidic gastric environment and bile containing various digestive enzymes.[21] It was also reported that the surviving bacteria should colonize on surfaces of the animal gut with the extreme environment to be able to promote immune activity and to show anticancer activity.[21] In line with this, *Lactobacillus acidophilus* RMK567 isolated from raw milk is well-known as probiotics. As compared to this strain, the SAMe-producing strains screened in this study displayed weak acid resistance, but strong bile resistance surviving the medium containing 0.3% Oxgall.[22] Similarly, a screened *E. faecalis* OA18 strain producing functional material ornithine was utilized for a fermented milk drink made with kefir grains.[23]

The results of antibacterial activity for five indicators such as *E. coli* O157:H7, *E. faecalis, S. choleraesuis, S. aureus,* and *L. monocytogenes* in the strains originating from the

Table 4. Antibacterial Activity of Strains with a High Level of SAMe Production

Strains	Escherichia coli *O157:H7*	Enterococcus faecalis	Salmonella choleraesuis	Staphylococcus aureus	Listeria monocytogenes
C1-5	+	+	+	+	−
C2-1	−	+	−	+	−
C3-2	+	+	+	+	+
C4-1	−	−	+	+	−
C4-3	−	−	−	+	−
C5-1	+	+	+	+	+
C6-4	−	−	−	−	−

Cheonggukjang showed the antibacterial activity. These strains are considered to be strains that can be involved in the regulation of the intestines. More importantly, *Bacillus subtilis* MP56 strains showed negligible effect on gram negative bacteria, but showed antibacterial activity against gram-positive bacteria such as *S. aureus.*[24] As compared to the *B. subtilis* MP56 strain, however, the *Bacillus* genus screened in this study, deployed strong antibacterial activity against both gram-negative and gram-positive bacteria. The *Bacillus* species screened in this study can be useful as probiotics having antibacterial activity against both gram-negative and gram-positive bacteria. Similarly, *Doenjang* fermented with a *B. subtilis* strain exhibited antibacterial activity, suppressed harmful bacteria, and promoted the growth of beneficial yeasts and fungi.[25] Therefore, we may utilize the screened *Bacillus* strains for other fermented foods exhibiting antibacterial activity against both gram-negative and gram-positive bacteria.

In terms of fermentation characteristics, the results of the substrate utilization of six strains showed that fermentable substrates vary depending on the strains. Thus, strains seem to have enzymes for using sugars and can ferment various sugars that enter the body through food intake. It has been considered that utilization of carbon and nitrogen sources is an important characteristic for producing antibacterial agents.[26] Rye *et al.* isolated the *B. subtilis* producing antibacterial agent from traditional fermented food, *Doenjang*. Interestingly, the *B. subtilis* strain producing antibacterial agent showed similar sugar utilization to the strains screened in this study, suggesting that the screened strain may be employed for production of antibacterial agents.[26] In addition, sugar utilization information is important when we implement SAMe-producing microbial strains for food. Yu *et al.* utilized *E. faecalis* for fermenting milk. Therefore, we may apply the screened SAMe-producing strains to various foods more efficiently based on the information of substrate utilization. In the previous research, the *Bacillus* spp. strains producing functional materials were used for making *Doenjang* and preparing soy ice cream with fermented soybean powder.[27] The screened strains can be customized as starters in various fermented foods to provide SAMe and probiotics in food based on the substrate utilization.

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REFERENCES

1. Shelly CL: S-adenosyl-L-methionine. *Int J Biochem Cell Biol* 2000;32:391–395.
2. Wang W, Kramer PM, Yang S, Pereira MA, Tao LH: Reversed phase high performance liquid chromatography procedure for the simultaneous determination of S-adenosyl-L-methionine and S-adenosyl-L-homocysteinein mouse liver and the effect of methionine on their concentrations. *J Chromatogr B* 2001;762:59–65.
3. Horikawa JS, Shimizu HO, Tsukada K: Molecular cloning and nucleotide sequence of cDNA encoding the rat kidney S-adenosylmethionine synthetase. *J Biol Chem* 1990;23:13683–13686.
4. Lee MK, Lee JK, Son JA, Kang MH, Koo KH, Suh JW: S-adenosyl-L-methionine (SAM) production by lactic acid bacteria strains isolated from different fermented kimchi products. *Food Sci Biotechnol* 2008;17:857–860.
5. Santi RD, Rodegerdts U: Functional myelography in spondylolisthesis. *Arch Orthop Trauma Surg* 1983;101:75–82.
6. Mato JM, Corrales F, Martin-Duce A, Ortiz P, Pajares MA, Cabrero C: S-adenosyl-L-methionine treatment prevents carbontetra chloride induced S-adenosyl-L-methionine synthetase in activation and attenuates liver injury. *Drugs* 1990;40:58–64.
7. Mato JM, Camara J, Fernandez de Paz J: S-adenosyl-L-methionine in alcoholic liver cirrhosis: a randomized, placebo-controlled, double-blind, multicenter clinical trial. *J Hepatol* 1999;30:1081–1089.
8. Porter CW, Sufrin JR: Interference with polyamine biosynthesis and/or function by analogs of polyamines or methionine as a potential anticancer chemotherapeutic strategy. *Anticancer Res* 1986;6:525–542.
9. Cooney CA: Are somatic cells inherently deficient in methylation metabolism? A proposed mechanism for DNA methylation loss, senescence and aging. *Growth Dev Aging* 1993;57:261–273.
10. Lee NK, Jeon EH, Lee HJ, Cho IJ, Hahm YT: Isolation, identification, and characterization of *Bacillus* spp. from the traditionally fermented Cheonggukjangs in the Gyeonggi and the Gangwon Provinces. *J Korean Soc Appl Biol Chem* 2006; 49:276–280.
11. Koning B: A long-term clinical trial with S-adenosyl-L-methionine for the treatment of osteoarthritis. *Am J Med* 1987;20:89–94.
12. Lieber CS: Role of S-adenosyl-L-methionine in the treatment of liver diseases. *J Hepatol* 1999;30:1155–1159.
13. Lee MK, Lee JK, Son JA, Kang MH, Koo KH, Suh JW: S-adenosyl-L-methionine (SAME) production by lactic acid bacteria strains isolated from different fermented *Kimchi* products. *Food Sci Biotechnol* 2008;17:857–860.
14. Al-Zoreky N, Sandine WE: *Lactococcus* genus: a selective and differential agar medium. *J Food Sci* 1991;56:1729–1730.
15. Lee MK, Park WS, Kang KH: Selective media for isolation and enumeration of lactic acid bacteria from Kimchi. *J Korean Soc Food Sci Nutr* 1996;25:754–760.
16. Guattari B: High-performance liquid chromatographic determination, with ultraviolet detection, of S-adenosyl-L-methionine and of normetanephrine and metanephrine for phenylethanolamine-N-methyltransferase or catechol-O-methyltransferase activity. *J Chromatogr* 1991;567:254–260.
17. Burren KA, Mills K, Copp AJ, Greene ND: Quantitative analysis of S-adenosyl-L-methionine and S-adenosyl-L-homocysteine in neurulation-stage mouse embryos by liquid chromatography tandem mass spectrometry. *J Chromatogr B Analyt Technol Biomed Life Sci* 2006;844:112–118.
18. Weisburg WG, Bams SM, Pellertier DA, Lane DJ: 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 1991;173: 697–703.
19. Fernandez MF, Boris S, Barbes C: Probiotic properties of human lactobacilli strains to be used in the gastrointestinal tract. *J Appl Microbiol* 2003;94:449–455.

20. Erkkila S, Petaja E: Screening of commercial meat starter cultures at low pH and in the presence of bile salts for potential probiotic use. *Meat Sci* 2000;55:279–300.
21. Ouwehand AC, Kirjavainen PV, Shortt C: Salminen S: Probiotics; mechanisms and established effects. *Int Dairy J* 1999;9: 43–52.
22. Lim SD, Kim KS, Do JR: Physiological Characteristics and GABA production of Lactobacillus acidophilus RMK567 Isolated from raw milk. *Korean J Food Sci Anim Resour* 2009;29: 15–23.
23. Yu JJ, Kim SG, Seo KW, Oh SH: Isolation, identification, and characterization of ornithine-producing *Enterococcus faecalis* OA18 from Kefir grain. *Korean J Microbiol* 2011;47:218–224.
24. Park SY, Yoo JC, Seong CN, Cho SS: Isolation and characterization of *Bacillus subtilis* MP56 with antimicrobial activity against MDR (multi drug resistant) strains. *J Korean Microbiol* 2013;49:90–94.
25. Kim JW, Doo HS, Kwon TH, Kim YS, Shin DH: Quality characteristics of *Doenjang* meju fermented with Aspergillus species and Bacillus subtilis during fermentation. *Korean J Food Preserv* 2011;18:397–406.
26. Rye HS, Shon MY, Cho SJ, Park SK, Lee SW: Characterization of antibacterial substance-producing bacillus subtilis isolated from traditional Doenjang. *J Korean Soc Appl Biol Chem* 2007;50:87–94.
27. Park IK, Yang SH, Choi YS: Quality characteristics of soy ice cream prepared with fermented soybean powder base and oligosaccharide and its blood glucose lowering effect. *Korean J Food Sci Technol* 2008;40:88–95.