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RESEARCH NOTE

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Evaluation of *S*-Adenosyl-L-Methionine Production by *Bifidobacterium bifidum* BGN4

Ji-Youn Kim[1], Joo-Won Suh[2], and Geun-Eog Ji[1,3]*

[1]*Department of Food and Nutrition, Research Institute of Human Ecology, Seoul National University, Seoul 151-742, Korea*
[2]*Myongji University, Yongin, Gyeonggi 449-728, Korea*
[3]*Research Center, Bifido Inc., Hongcheon, Gangwon 250-804, Korea*

Abstract *S*-Adenosyl-L-methionine (SAM) is an important metabolic intermediate in living organisms and participates in many reactions as a methyl group donor. SAM has been used as a dietary supplement and is proposed to have beneficial effects on the liver and brain. The aim of this study was to find lactic acid bacteria with high SAM-producing ability to be used as SAM enhancing probiotics. We used high performance liquid chromatography (HPLC) to quantify the amount of SAM produced, and found that *Bifidobacterium bifidum* BGN4 produced a significantly higher amount of SAM than other *Bifidobacterium* or *Lactobacillus* strains. The effect of various carbon and nitrogen sources on SAM production was examined. This study confirmed that *Bifidobacterium* may be utilized as a source of SAM in the functional food industry.

Keywords: *S*-adenosyl-L-methionine, *Bifidobacterium bifidum* BGN4, probiotics

Introduction

In recent years, *Bifidobacterium* has been added to many probiotic products, since it is beneficial to the human intestinal tract (1-5). The beneficial function and safety of *Bifidobacterium* have been extensively studied (6). S-Adenosyl-methionine (SAM), also known as SAMe or Adomet, is an amino acid derivative normally synthesized in the body and is a metabolite (7) which is involved in over 40 biochemical reactions. SAM plays a key role in metabolism (8-10), including acting as a methyl group donor in various reactions. SAM participates in detoxification reactions and in the production of brain chemicals, antioxidants, joint tissue structures, and many other significant components (11). Furthermore, SAM is an important pharmaceutical component which has a potential role in the prevention of chronic diseases such as osteoarthritis (12), liver disease (13-15), and migraine headaches (16). Additionally, SAM has positive effects as an antidepressant (17-19).

In young healthy people, SAM is distributed throughout the body as a result of its synthesis from methionine. However, in patients with depression (20) and in the elderly, system levels may become depleted, while the activity of methionine adenosyltransferase (MAT), an enzyme involved in the synthesis of SAM from methionine (21), decreases. In Europe, SAM has been used extensively for decades and is an approved prescription medication in Italy, Spain, Germany, and Russia (22). Therapeutic use of SAM has increased as dietary supplements have gained popularity. In this study, we screened the generally recognized as safe (GRAS) lactic acid bacteria and found that *Bifidobacterium bifidum* BGN4 produced the highest amounts of SAM among the lactic acid bacteria tested.

*Corresponding author: Tel: +82-2-880-8749; Fax: +82-2-884-0305
E-mail: geji@snu.ac.kr
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Materials and Methods

Microorganisms and growth conditions All of the bacteria except *B. bifidum* BGN4 and *Lactobacillus rhamnosus* GG used in this study were purchased from the ATCC (Manassas, VA, USA). *B. bifidum* BGN4 (23) and *L. rhamnosus* GG (24) were previously described. The *Bifidobacterium* strains and other lactic acid bacteria used in this study are listed in Table 1. Bacteria were cultivated anaerobically in MRS broth medium (Difco, Lawrence, KS, USA) containing 0.05% cysteine·HCl at 37°C for 18 hr, with the exception of *L. rhamnosus* GG which was incubated at 30°C for 15 hr.

Analysis of SAM by high performance liquid chromatography (HPLC) Lactic acid bacteria which were incubated anaerobically in MRS broth medium were filtered and followed by incubation in a petri dish containing 0.5 M formic acid (Hayachi Pure Industries Ltd., Osaka, Japan) for 1 hr. The formic acid extract was collected, centrifuged to remove cells and debris, and the supernatant was lyophilized. The residue after lyophilization (whole cell extract) was dissolved in 200 μL of HPLC grade water (J.T Baker, Phillipsburg, NJ, USA) and filtered. The samples were then assayed by HPLC (Dionex TCC-100; Germering, Germany) on a C_{18} column (Zorbax Eclipse XDB-C18, Analytical 4×6×250 mm, 5-micron; Agilent, Santa Clara, CA, USA) with a gradient elution system (25). Aliquots of each sample (20 μL) were assessed by a UV detector (Dionex UVD 170U/340U UV/VIS Detector, Germering, Germany) at a wavelength 254 nm.

Production of SAM from *B. bifidum* BGN4 in whey, modified MRS, and milk media with various combinations of carbohydrate and nitrogen sources Carbon sources were: fructo oligosaccharide, sorbose, cellobiose, rhamnose, glucose, lactose, galactose, maltose, sucrose, and arabinose (Sigma Aldrich, St. Louis, MO, USA).

Table 1. Experimental bacterial strains and amount of SAM produced in MRS medium

Species and strains		Amount of SAM (nmol/mL)
Bifidobacterium adolescentis	ATCC 15703	0.58
B. angulatum	ATCC 27535	0.04
B. animalis	ATCC 25527	0.24
B. bifidum	ATCC 15521	0.31
B. bifidum	BGN4	1.22
B. breve	ATCC 15700	0.48
B. catenulatum	ATCC 27539	0.56
B. dentium	ATCC 27534	0.52
B. longum biovar *infantis*	ATCC 15697	0.46
B. longum	ATCC 15707	0.25
B. pseudocatenulatum	ATCC 27919	0.59
B. subtile	ATCC 27537	0.18
B. thermophilum	ATCC 25525	0.59
Enterococcus faecium	ATCC 33314	0.28
Ent. faecalis	ATCC 10881	0.38
Lactobacillus brevis	ATCC 14869	0.61
L. salivarius subsp. *salicinius*	ATCC 11742	0.32
L. acidophilus	ATCC 04356	0.57
L. casei	ATCC 00393	0.34
L. rhamnosus	GG	0.43
Lactococcus. lactis subsp. *cremoris*	ATCC 19257	0.21
Lc. lactis subsp. *lactis*	ATCC 27258	0.54
Pediococcus acidilactici	ATCC 11454	0.56
Streptococcus thermophilus	ATCC 12020	0.32
Weissella confusa	ATCC 27270	0.54

Nitrogen sources were: yeast extract, proteose peptone No. 3, soybean flour, soytone peptone, peptone, soytone, beef extract, yeast nitrogen w/o amino acids (Difco) and special peptone (Oxoid, Lenexa, KS, USA). Vitamin and mineral sources (vit. C, Tween 80, $MgSO_4 \cdot 7H_2O$, $CaCl_2$, $MnSO_4 \cdot H_2O$ (Sigma), KH_2PO_4, and K_2HPO_4 (D.S.P. GR, Korea) were added to the various media. *B. bifidum* BGN4 was pre-cultured anaerobically in MRS broth at 37°C for 20 hr. These cultures were inoculated at 1% into sterilized medium and incubated at 37°C for 20 hr. The growth of bacteria was determined by counting viable cells plated on MRS agar plates (Difco) and by measuring the pH. Plate counts were repeated 3 times. The compositions of each basal and experimental medium are shown in Table 2 and 3, respectively. The pH of each medium was adjusted to 6.7 with NaOH or HCl. Production of SAM was measured as described above.

Results and Discussion

All of the experimental lactic acid bacteria produced SAM. *B. bifidum* BGN4 had much higher levels than any of the other lactic acid bacteria. Our data indicate that the amount of SAM produced by *B. bifidum* BGN4 was at least 2 fold greater than other lactic acid bacteria (Table 1). In the previous studies, SAM was produced at 24 nmol/mg in yeast (26), 10-400 pmol/mL in *Salmonella typhimurium* and *Escherichia coli* (25). However, to our knowledge, there are no studies about SAM production in probiotics including lactic acid bacteria. From this study, in MRS broth, *B. bifidum* BGN4 grew to a maximal concentration of 5×10^9 CFU/mL, weighing 1.02 mg in dry weight, and produced approximately 1.22 nmol/mL SAM. To find the best carbon and nitrogen sources for growth, various combinations were tested. Three media, whey, modified MRS, and milk, were supplemented with various carbohydrate and nitrogen sources (Table 3). The unsupplemented media had minor discrepancies in their components. However, sucrose and galactose were common in all media in which *B. bifidum* BGN4 grew well and produced slightly higher amounts of SAM than any of the other carbohydrates. To find the best nitrogen source, 1% sucrose was added to the basal medium together with various nitrogen sources. Proteose peptone No. 3 proved to be the best nitrogen source for the growth of *B. bifidum* BGN4.

In this study, increases in the cell density of *B. bifidum* BGN4 in culture were correlated with the amount of SAM produced. *B. bifidum* BGN4 in the milk medium showed higher growth than the other media. However, although the bacteria grew well in this medium, assessing the amount of SAM in the fermented milk was not successful. Assessing the amount of SAM in the cells was ineffective because the cells tended to aggregate with components of the milk. *B. bifidum* BGN4 is a strain of *B. bifidum* isolated from human infant fecal material. *B. bifidum* BGN4 produces chiroinositol containing polysaccharides with anti-tumorigenic activity (23) and also has positive effects in alleviating the symptoms of allergy and atopy according to *in vivo* testing in animals (27). In this study, *B. bifidum* BGN4 was found to produce the highest amount of SAM among many

Table 2. Composition of the different basal media

Basal modified MRS medium		Basal whey medium		Basal milk medium	
Tween 80	0.10%	Tween 80	0.10%	Milk	85%
Ammonium citrate	0.20%	Ammonium acetate	0.20%	Vitamin C	0.10%
Sodium acetate	0.50%	$MgSO_4 \cdot 7H_2O$	0.01%		
$MgSO_4 \cdot 7H_2O$	0.01%	$MnSO_4 \cdot H_2O$	0.01%		
$MnSO_4 \cdot H_2O$	0.01%	KH_2PO_4	0.19%		
Dipotassium phosphate	0.20%	Na_2CO_3	0.20%		
L-Cysteine · HCl	0.05%	$CaCl_2$	0.01%		
Glucose	1%	L-Cysteine · HCl	0.05%		
Beef extract	0.25%	Whey powder	3%		
Yeast extract	0.25%				

Table 3. Growth of *Bifidobacterium bifidum* BGN4 and amount of SAM produced in experimental whey, modified MRS, and milk media

Carbohydrate source[1)]	Whey medium		Modified MRS medium		Milk medium
	Growth (CFU/mL)	SAM (nmol/mL)	Growth (CFU/mL)	SAM (nmol/mL)	Growth (CFU/mL)
Sucrose	7.9×10^8	0.17	$<10^8$	0.13	8.1×10^8
Fructooligosaccharide	6.4×10^8	0.11	$<10^8$	0.08	5.1×10^8
Sorbose	2.1×10^8	0.03	$<10^8$	0.06	4.9×10^8
Glucose	3.6×10^8	0.08	$<10^8$	0.07	6.4×10^8
Lactose	4.1×10^8	0.09	$<10^8$	0.08	5.1×10^8
Rhamnose	7.5×10^8	0.15	$<10^8$	0.11	2.2×10^8
Galactose	7.6×10^8	0.15	$<10^8$	0.13	7.4×10^8
Maltose	5.6×10^8	0.11	$<10^8$	0.09	1.5×10^8
Cellobiose	4.6×10^8	0.08	$<10^8$	0.11	2.4×10^8
Arabinose	6.2×10^8	0.11	$<10^8$	0.12	9.2×10^8
Nitrogen source[2)]					
Proteose peptone No.3	5.1×10^9	1.14	9.8×10^8	0.98	7.9×10^9
Yeast nitrogen w/o amino acids	3.1×10^9	0.73	2.0×10^8	0.03	5.9×10^9
Tryptone	2.9×10^9	0.64	3.7×10^8	0.05	7.9×10^8
Beef extract	1.9×10^9	0.57	1.2×10^8	0.03	4.2×10^8
Special peptone	4.9×10^9	1.09	8.5×10^8	0.92	6.2×10^9
Yeast extract	4.1×10^9	0.81	3.5×10^8	0.07	7.5×10^8
Soytone	4.5×10^9	0.99	5.2×10^8	0.12	1.2×10^9
Peptone	4.3×10^9	0.92	6.1×10^8	0.13	4.9×10^8

[1)]Different carbohydrate sources (1%) were added to each basal medium for carbon source comparisons.
[2)]Different nitrogen sources (1%) with 1% sucrose were added to each basal medium for nitrogen source comparisons.

lactic acid bacteria. Even though *B. bifidum* BGN4 produced the highest amount of SAM compared to other lactic acid bacteria, amounts produced in food production (i.e., yogurt fermentation) may not be sufficient to achieve the desired supplemental levels. Therefore, methods for improved large-scale production need to be developed in order to obtain high levels of SAM from *B. bifidum* BGN4 or other GRAS microorganisms. To our knowledge, this has been the first study to test lactic acid bacteria production of SAM. Thus, this study has provided very useful data quantifying the amounts of SAM produced by lactic acid bacteria used in the food industry.

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References

1. Mutai M. Characteristics of 'Mil-Mil', a *Bifidobacterium*-fermented milk. New Food Ind. 20: 17-20 (1978)
2. Hughes DB, Hoover DG. Bifidobacteria: Their potential for use in American dairy products. Food Technol.-Chicago 45: 64-83 (1991)
3. Kim HS. Characterization of lactobacilli and bifidobacteria as applied to dietary adjuncts. Cult. Dairy Prod. J. 23: 6-9 (1988)
4. Jayaprakasha HM, Yoon YC, Paik HD. Probiotic functional dairy foods and health claims: An overview. Food Sci. Biotechnol. 14: 523-528 (2005)
5. Lee CH. Lactic acid fermented foods and their benefits in Asia. Food Sci. Biotechnol. 8: 259-269 (1997)
6. Parvez S, Malik KA, Kang SA, Kim HY. Probiotics and their fermented food products are beneficial for health. J. Appl. Micorbiol. 100: 1171-1185 (2006)
7. Cantoni GL. *S*-Adenosylmethionine: A new intermediate formed enzymatically from L-methionine and adenosine triphosphate. J. Biol. Chem. 204: 403-416 (1953)
8. Lu SC. *S*-Adenosyl-methionine. Int. J. Biochem. Cell. B. 32: 391-395 (2000)
9. Baldessarini RJ. Neuropharmacology of *S*-adenosyl-L-methionine. Am. J. Med. 83: 95-105 (1987)
10. Chiang PK, Gordon RK, Tal J, Zeng GC, Doctor BP, Pardhasaradhi K, McCann PP. *S*-Adenosylmethionine and methylation. FASEB J. 10: 471-480 (1996)
11. Friedel HA, Goa KL, Benfield P. *S*-Adenosyl-L-methionine. A review of its pharmacological properties and therapeutic potential in liver dysfunction and affective disorders in relation to its physiological role in cell membranes. Drugs 38: 389-416 (1989)
12. Bradley JD, Flusser D, Katz BP, Schumacher HR, Brandt KD, Chambers MA, Zonay LJ. A randomized double blind, placebo controlled trial of intravenous loading with s-adenosylmethionine (SAM) followed by oral SAMe therapy in patients with knee osteoarthritis. J. Rheumatol. 21: 905-911 (1994)
13. Purohit V, Russo D. Role of s-adenosyl-L-methionine in the treatment of alcoholic liver disease: Introduction and summary of the symposium. Alcohol 27: 151-154 (2002)
14. Kaye GL, Blake JC, Burroughs AK. Metabolism of exogenous *S*-adenosyl-L-methionine in patients with liver disease. Drugs 40: 124-128 (1990)
15. Lieber CS. *S*-Adenosyl-L-methionine: Its role in the treatment of liver disorders. Am. J. Clin. Nutr. 76: 1183-1187 (2002)
16. Gatto G, Caleri D, Michelacci S, Sicuteri F. Analgesizing effect of a methyl donor (*S*-adenosylmethionine) in migraine: An open clinical trial. Int. J. Clin. Pharm. 6: 15-17 (1986)
17. Agnoli A, Andreoli V, Casacchia M, Cerbo R. Effect of *S*-adenosyl-L-methionine (SAMe) upon depressive symptoms. J. Psychiat. Res. 13: 43-54 (1976)
18. Janicak PG, Lipinski J, Davis JM, Comaty JE, Waternaux C, Cohen B, Altman E, Sharma RP. *S*-Adenosylmethionine in depression. A

literature review and preliminary report. Ala. J. Med. Sci. 25: 306-313 (1988)
19. Jacobsen S, Danneskiold-Samsoe B, Andersen RB. Oral *S*-adenosylmethionine in primary fibromyalgia: Double-blind clinical evaluation. Scand. J. Rheumatol. 20: 294-302 (1991)
20. Bottiglieri T, Godfrey P, Flynn T, Carney MW, Toone BK, Reynolds EH. Cerebrospinal fluid *S*-adenosylmethionine in depression and dementia: Effects of treatment with parenteral and oral *S*-adenosylmethionine. J. Neurol. Neurosurg. Ps. 53: 1096-1098 (1990)
21. Alarcon RD, Tolbert LC, Monti JA, Morere DA, Walter-Ryan WG, Kemp B, Smythie JR. One-carbon metabolism disturbances in affective disorders. A preliminary report. J. Affect. Disorders 9: 297-301 (1985)
22. Bottigliere T, Hyland K, Reynolds EH. The clinical potential of ademetionine (*S*-adenosylmethionine) in neurological disorders. Drugs 48: 137-152 (1994)
23. You HJ, Oh DK, Ji GE. Anticancerogenic effect of a novel chiroinositol-containing polysaccharide from *Bifidobacterium bifidum* BGN4. FEMS Microbiol. Lett. 240: 131-136 (2004)
24. Alvarez-Olmos MI, Oberhelman RA. Probiotic agents and infectious diseases: A modern perspective on a traditional therapy. Clin. Infect. Dis. 32: 1567-1576 (2001)
25. Payne SH, Ames BN. A procedure for rapid extraction and high-pressure liquid chromatographic separation of the nucleotides and other small molecules from bacterial cells. Anal. Biochem. 123: 151-161 (1982)
26. Lee SY, Oh SK, Park SW, Jeon GR, Kim JY, Youn SY, Ji GE. Evaluation of anti-allergic effect of bifidobacteria in murine model of peanut allergy. Pediatr. Allergy Respir. Dis. 16: 131-141 (2006)
27. Rhee JN, Lyoo YW, Choi MU. Laboratory scale preparation of *S*-adenosyl-L-methionine from yeast. Korean J. Appl. Microbiol. Biotechnol. 19: 588-591 (1991)
28. Kim H, Kwack K, Kim DY, Ji GE. Oral probiotic bacterial administration suppressed allergic responses in an ovalbumin-induced allergy mouse model. FEMS Immunol. Med. Mic. 45: 259-267 (2005)