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Enhanced Production of γ-Aminobutyric Acid Using Rice Bran Extracts by *Lactobacillus sakei* B2-16

Kook, Moo-Chang[1,2†], Myung-Ji Seo[3†], Chan-Ick Cheigh[2], Yu-Ryang Pyun[1,2,4], Seok-Cheol Cho[2], and Hoon Park[5*]

[1]*Department of Marine Biotechnology, Anyang University, Incheon 417-833, Korea*
[2]*R&D Division, Biovan Co. Ltd., YERC B120F, Yonsei University, Seoul 120-749, Korea*
[3]*Department of Chemistry, Box H, Brown University, Providence, RI 02912-9108, U.S.A.*
[4]*Department of Biotechnology, Yonsei University, Seoul 120-749, Korea*
[5]*Department of Food Science, Sunmoon University, Asan 336-708, Korea*

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An efficient and simple fermentation process was developed for the production of γ-aminobutyric acid (GABA) by *Lactobacillus sakei* B2-16. When the *L. sakei* B2-16 was cultivated in the rice bran extracts medium containing 4% sucrose, 1% yeast extract, and 12% monosodium glutamate, the maximum GABA concentration reached 660.0 mM with 100% conversion yield, showing the 2.4-fold higher GABA concentration compared with the modified MRS medium without the rice bran extracts. The GABA production was scaled-up from a laboratory scale (5 l) to a pilot (300 l) and a plant (5,000 l) scale to investigate the application possibility of GABA production to industrial fields. The production yields at the pilot and plant scales were similar to the laboratory scale using rice bran extracts medium, which could be effective for the low-cost production of GABA.

Keywords: γ-Aminobutyric acid, *Lactobacillus sakei*, monosodium glutamate, rice bran extracts, scale-up fermentation

γ-Aminobutyric acid (GABA), a four-carbon non-protein amino acid that is widely found in nature, is produced by the α-decarboxylation of glutamic acid catalyzed by a glutamate decarboxylase (GAD) [10, 12]. GABA is functionally involved in the induction of hypotensive, diuretic, and tranquilizing effects, playing a principal inhibitory neurotransmitter in the central nervous system [4, 5]. In addition, GABA has been known to be effective to regulate several neurological disorders such as Parkinson's disease, Huntington's chorea, and Alzheimer's disease [10]. Owing to the physiological functions of GABA, increased interest has been focused on the development of functional foods containing the highly accumulated GABA and mass production of GABA as a bioactive food component. So far, there have been various studies on the GABA production by fermentation methods using bacteria, fungi, and yeast [3, 8, 13]. Specifically, there have been several studies on the isolation of lactic acid bacteria (LAB) having the potential of rich GABA sources from traditional fermented food and on optimized GABA production using LAB for the industrial purpose [2, 7]. It is commercially useful to produce GABA using LAB, because the LAB can be used as starters of functional fermented food. Recently, we isolated the highest GABA-producing *Lactobacillus sakei* B2-16 from *kimchi*, a Korean traditional fermented food (unpublished). So far, several LAB-producing GABA have been isolated from *kimchi*; *L. buchneri*, *L. brevis*, and *Lactococcus lactis* [1, 6, 9]. Since GABA production is affected by several factors including carbon and nitrogen sources, and fermentation conditions, these factors have to be taken into account in the design of efficient GABA production processes, applicable in industrial fields.

In this study, we obtained the high GABA concentration from *L. sakei* B2-16 by optimizing the culture conditions for carbon and nitrogen sources, and monosodium glutamate (MSG). Additionally, the utilization of by-products of agricultural crops was investigated for the industrial application of GABA production. Finally, the scale-up fermentation was attempted from a laboratory scale (5 l) to a pilot (300 l) and a plant (5,000 l) scales.

*Corresponding author
Phone: +82-41-530-2262; Fax: +82-41-530-2917;
E-mail: hpark@sunmoon.ac.kr

†Kook and Seo contributed equally to this work.

L. sakei B2-16 was cultured in the defined MRS medium containing 3% food-grade MSG (purity >99%; Daesang, Korea) as a basal medium at 30°C for 4 days, and the produced GABA concentration was analyzed by using HPLC after derivatization with *o*-phthaldialdehyde (OPA). After cultivation, the cells were removed by centrifugation and the supernatants were derivatized with OPA reagent, which was prepared in a mixture of methanolic OPA, borate buffer, and 2-mercaptoethanol [100:4,000:1 (v/v/v)]. The derivatized samples were analyzed by HPLC on an Xterra column (RP18, 4.6×150 mm, 5 μm; Waters, U.S.A.). The chromatogram was developed with the solvent A (0.1 M sodium acetate, pH 7.2) to the solvent B [0.1 M sodium acetate–acetonitrile–methanol, 46:44:10 (v/v/v), pH 7.2] at a flow rate of 1.0 ml/min for 60 min and monitored at 338 nm.

To enhance the GABA production by *L. sakei* B2-16, the culture conditions for the carbon and nitrogen sources were optimized in a 500-ml Erlenmeyer flask containing 200 ml of modified MRS medium supplemented with 3% MSG. The effect of a carbon source on GABA production was investigated in the modified MRS medium, where 2% glucose was eliminated to exclude the effect of glucose as carbon source in the defined MRS medium, adding 2% various carbon sources (glucose, lactose, sucrose, xylose, fructose, maltose, galactose, and arabinose). As the results, the highest GABA concentration reached 165.0 mM with 4% sucrose as a carbon source (data not shown). To determine the nitrogen source optimized for the high GABA production, three nitrogen sources (1% proteose peptone No. 3, 1% beef extract, and 0.5% yeast extract) were removed in the modified MRS medium, where 4% sucrose replaced 2% glucose as carbon source. The various nitrogen sources (beef extract, tryptone, soytone, yeast extract, peptone, casitone, casamino acid, and proteose peptone No. 3) were added instead of the above-mentioned three nitrogen sources. The nitrogen sources except yeast extract decreased the GABA concentration. Since the high concentration of yeast extract did not seem to be significantly effective to enhance the GABA concentration, we considered the 1% yeast extract as a nitrogen source, producing 165.0 mM GABA, from the point of view of a low-cost production of GABA (data not shown). The effect of MSG concentration on GABA production was also investigated, showing that the highest GABA concentration was 269.7 mM in the modified MRS medium containing 4% sucrose, 1% yeast extract, and 5% MSG (MRS–MSG; data not shown).

The maximum GABA yields obtained from preceding studies were not sufficient and the optimized MRS medium was not well applied for the commercial GABA production in industrial fields in terms of a competitive price, because the cost of MRS medium was too expensive to be applied to industrial fields. Therefore, it was required to develop the effective mass-production of GABA using a cost-effective medium. The by-products of agricultural crops were the materials of interest because they could be obtained inexpensively and utilized as an extra GAD source. The rice bran extract (RBE) was chosen as the medium for mass-production of GABA, because it is an abundant agricultural by-products from the rice-polishing process in Korea and can be, therefore, obtained inexpensively. Rice bran (Kimpo, Korea) soaked in a 10-fold volume of deionized water was extracted at 55°C for 12 h. The extracts were centrifuged at 8,000 ×*g* for 15 min to eliminate solid fractions in the rice bran extracts and the supernatants were used as the commercial RBE medium. The effect of MSG concentration on GABA production was examined in 5-l jar fermentor (KF-5, Ko-biotech, Korea) with the RBE medium containing 4% sucrose and 1% yeast extract, although the 5% MSG was optimized for the highest GABA production in the MRS–MSG medium. The fermentor was operated with slow agitation (50 rpm) at 30°C without aeration. The pH of the culture broth was adjusted initially to a predetermined value of pH 6.0 and not maintained during fermentation. The GABA concentration was significantly enhanced according to the increase of MSG concentration from 3% to 12%, showing that the highest GABA concentration reached 660.0 mM with 12% MSG, although it was slightly decreased at the highest MSG concentration of 15% (Table 1). This significant GABA production might be mainly due to the GAD from *L. sakei* B2-16, where the GAD activity is dependent on the MSG concentration, even though the rice bran-derived GAD might be able to take a little effect on the GABA conversion from MSG [11]. In addition, it should be noted that the optimal MSG concentration was 12% in the RBE medium, whereas it was 5% in the modified MRS medium, resulting in that the GABA production could be enhanced by adding more MSG in the RBE medium. To examine if the GABA production in the RBE medium containing 4% sucrose, 1% yeast extract,

Table 1. Effects of MSG concentration on GABA production by *L. sakei* B2-16 in the RBE medium containing 4% sucrose and 1% yeast extract.

MSG concentration (%)	Cell growth (CFU/ml)	GABA production	
		Concentration (mM)	Conversion yield (%)
3	2.5×10^9	165.0	100
5	1.9×10^9	275.0	100
7	3.3×10^9	385.0	100
10	2.3×10^9	550.0	100
12	3.0×10^9	660.0	100
15	1.4×10^9	648.3	78.6

GABA conversion yield was calculated by the percentage of the produced GABA concentration over the supplied MSG concentration.

Table 2. Comparison of GABA production between laboratory-, pilot-, and plant-scale fermentations of *L. sakei* B2-16.

Fermentation	Medium	Cell growth (CFU/ml)	GABA production	
			Concentration (mM)	Conversion yield (%)
Laboratory scale (5 l)	MRS−MSG	1.03×10^9	272.1	98.9
Laboratory scale (5 l)	RBE−MSG	3.93×10^9	660.0	100
Pilot scale (300 l)	RBE−MSG	1.93×10^9	660.0	100
Plant scale (5,000 l)	RBE−MSG	1.08×10^9	660.0	100

MRS−MSG medium is the modified MRS medium containing 4% sucrose, 1% yeast extract, and 5% MSG. RBE−MSG medium is composed of 4% sucrose, 1% yeast extract, 12% MSG, and 100% rice bran extracts. GABA conversion yield was calculated by the percentage of the produced GABA concentration over the supplied MSG concentration.

and 12% MSG (RBE−MSG) could have potential for commercial production in industrial fields, the laboratory-scale production of GABA was increased to pilot- and plant-scale productions in a 300-l fermentor (KF-300, Ko-biotech, Korea) and 5,000-l fermentor (KSB6231, Ko-biotech, Korea), respectively. The agitation speed and temperature during the fermentation were 30 rpm and 30°C, respectively. The pH was not controlled during the fermentation after setting the initial pH of 6.0. The dissolved oxygen level was also not controlled without aeration. The production yields at the pilot and plant scales were similar to the laboratory scale. The highest concentration (660.0 mM) of GABA was obtained in RBE−MSG medium with a conversion yield of 100% from the supplied MSG (Table 2 and Fig. 1). This concentration was significantly higher than 272.1 mM GABA produced from the MRS−MSG medium, suggesting that the RBE−MSG medium was very effective for GABA production.

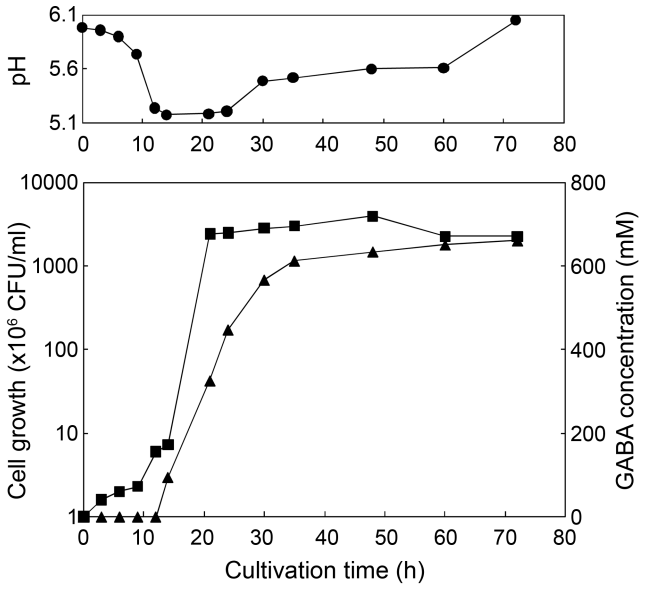

Fig. 1. Time profile of cell growth and GABA concentration by *L. sakei* B2-16 using RBE−MSG medium in plant-scale (5,000 l) fermentation.
Symbols: circle, pH; square, cell growth; triangle, GABA concentration.

In conclusion, we developed a process for GABA production using rice bran, agricultural by-products. In fact, various studies have been conducted to enhance GABA concentration by several microorganisms and processes. *L. paracasei* NFRI 7415 isolated from Japanese traditional fermented fish, *funa-sushi*, produced 302 mM GABA by addition of pyridoxal phosphate as a coenzyme of GAD [7]. To improve GABA production, it was also used to encapsulate *L. brevis* GABA 057 by alginate beads, where the GABA concentration reached 223 mM [2]. As far as we know, this study achieved the highest GABA concentration yet reported. Our LAB strain, *L. sakei* B2-16, produced 660.0 mM GABA with a 100% conversion yield using rice bran, which is easily and inexpensively obtained. The GABA production using rice bran would be cost-effective and valuable as an effective utilization of by-products of agricultural crops [11]. In addition, the successful result of GABA production up to plant scale strongly suggests that the commercial GABA production by *L. sakei* B2-16 in the RBE−MSG medium can be applied to industrial fields.

REFERENCES

1. Cho, Y. R., J. Y. Chang, and H. C. Chang. 2007. Production of γ-aminobutyric acid (GABA) by *Lactobacillus buchneri* isolated from *kimchi* and its neuroprotective effect on neuronal cells. *J. Microbiol. Biotechnol*. **17:** 104−109.
2. Choi, S. I., J. W. Lee, S. M. Park, M. Y. Lee, G. E. Ji, M. S. Park, and T. R. Heo. 2006. Improvement of γ-aminobutyric acid (GABA) production using cell entrapment of *Lactobacillus brevis* GABA 057. *J. Microbiol. Biotechnol*. **16:** 562−568.
3. Hao, R. and J. C. Shimit. 1993. Cloning of the gene for glutamate decarboxylase and its expression during conidiation in *Neurospora crassa. Biochem. J.* **293:** 735−738.
4. Inoue, K., T. Shirai, H. Ochiai, M. Kasao, K. Hayakawa, M. Kimura, and H. Sansawa. 2003. Blood-pressure-lowering effect of a novel fermented milk containing γ-aminobutyric acid (GABA) in mild hypertensives. *Eur. J. Clin. Nutr*. **57:** 490−495.
5. Jakobs, C., J. Jaeken, and K. M. Gibson. 1993. Inherited disorders of GABA metabolism. *J. Inherit. Metab. Dis*. **16:** 704−715.
6. Kim, J. Y., M. Y. Lee, G. E. Ji, Y. S. Lee, and K. T. Hwang. 2009. Production of γ-aminobutyric acid in black raspberry

juice during fermentation by *Lactobacillus brevis* GABA100. *Int. J. Food Microbiol.* **130:** 12–16.
7. Komatsuzaki, N., J. Shima, S. Kawamoto, H. Momose, and T. Kimura. 2005. Production of γ-aminobutyric acid (GABA) by *Lactobacillus paracasei* isolated from traditional fermented foods. *Food Microbiol.* **22:** 497–504.
8. Kono, I. and K. Himeno. 2000. Changes in γ-aminobutyric acid content during *beni-koji* making. *Biosci. Biotechnol. Biochem.* **64:** 617–619.
9. Lu, X., Z. Chen, Z. Gu, and Y. Han. 2008. Isolation of γ-aminobutyric acid-producing bacteria and optimization of fermentative medium. *Biochem. Eng. J.* **41:** 48–52.
10. Manyam, B. V., L. Katz, T. A. Hare, K. Kaniefski, and R. D. Tremblay. 1981. Isoniazid-induced elevation of cerebrospinal fluid (CSF) GABA levels and effects on chorea in Huntington's disease. *Ann. Neurol.* **10:** 35–37.
11. Ohtsubo, S., S. Asano, K. Sato, and I. Matsumoto. 2000. Enzymatic production of γ-aminobutyric acid using rice (*Oryza sativa*) germ. *Food Sci. Technol. Res*. **6:** 208–211.
12. Ueno, H. 2000. Enzymatic and structural aspects on glutamate decarboxylase. *J. Mol. Catal. B Enzym*. **10:** 67–69.
13. Yokoyama, S., J. Hiramatsu, and K. Hayakawa. 2002. Production of γ-aminobutyric acid from alcohol distillery lees by *Lactobacillus brevis* IFO 12005. *J. Biosci. Bioeng*. **93:** 95–97.