Production and Its Anti-hyperglycemic Effects of γ-Aminobutyric Acid from the Wild Yeast Strain Pichia silvicola UL6-1 and Sporobolomyces carnicolor 402-JB-1

Sang-Min Han and Jong-Soo Lee*
Department of Biomedicinal Science and Biotechnology, Paichai University, Daejeon 35345, Korea

Abstract This study was done to produce γ-aminobutyric acid (GABA) from wild yeast as well as investigate its anti-hyperglycemic effects. Among ten GABA-producing yeast strains, Pichia silvicola UL6-1 and Sporobolomyces carnicolor 402-JB-1 produced high GABA concentration of 134.4 μg/mL and 179.2 μg/mL, respectively. P. silvicola UL6-1 showed a maximum GABA yield of 136.5 μg/mL and 200.8 μg/mL from S. carnicolor 402-JB-1 when they were cultured for 30 hr at 30°C in yeast extract-peptone-dextrose medium. The cell-free extract from P. silvicola UL6-1 and S. carnicolor 402-JB-1 showed very high anti-hyperglycemic α-glucosidase inhibitory activity of 72.3% and 69.9%, respectively. Additionally, their cell-free extract-containing GABA showed the anti-hyperglycemic effect in streptozotocin-induced diabetic Sprague-Dawley rats.

Keywords Anti-hyperglycemic effects, Gamma-aminobutyric acid, Pichia silvicola UL6-1, Sporobolomyces carnicolor 402-JB-1, Wild yeast

Generally, yeasts are heterotrophic, facultative anaerobes with relatively simple nutritional needs. They are wildly distributed in natural habitats such as in flowers, fruits, and cereals as well as in plant debris found on the surface area of soils. Most yeasts were isolated from various fermentation foods or their raw materials including meju [1] and more recently from flowers and soil samples from the mountains, islands and inlands of Korea [2-5].

Yeasts have long been used to prepare alcoholic beverages [6], soy sauces [7], etc. Recently, they have received much attention because of their various physiological activities such as anti-gout, anti-hypertensive, and anti-diabetic activities as well as other activities [8-13].

Gamma-aminobutyric acid (GABA) is a non-protein amino acid that is widely distributed in plants and animals [14] and also produced by microorganisms [15-17].

GABA is produced by decarboxylation through glutamate decarboxylase with the cofactor pyridoxal-5-phosphate. It acts as a major neurotransmitter in the mammalian central nervous system. Additionally, GABA has hypotensive, tranquilizing and diuretic effects and can prevent diabetes [18-21]. Furthermore, GABA may improve the concentration of plasma growth hormones and the rate of protein synthesis in the brain [22] and inhibit small airway-derived lung adenocarcinoma [23]. Therefore, GABA has potential as a bioactive component in foods and pharmaceuticals.

The GABA are produced from various microorganisms including Saccharomyces cerevisiae [15], Rhodotorula mucilaginosa and Debaryomyces hansenii [16], and Lactobacillus buchneri, L. brevis, and L. sakei from Kimchi [17, 24-27], etc. However, their GABA productivity was low, and the physiological activities of the GABA in those studies were not investigated for the preparation of functional foods and biomedicines.

In a previous paper, ten GABA-producing yeast strains were screened and their microbiological characteristics were investigated [28]. In this study, a potent yeast strain with a high GABA content with anti-hyperglycemic effects was finally selected for further investigation. Moreover, the
optimal conditions for GABA production in this potent strain were determined, and the anti-hyperglycemic action of GABA was also investigated.

**MATERIALS AND METHODS**

**Yeasts strains, rats, and chemicals.** Ten yeast strains that were screened as GABA-producing yeasts in a previous paper [28] were used in this study.

Sprague-Dawley (SD) male rats, weighing 180–200 g and 7 weeks old, were purchased from Orientbio Co., Seongnam, Korea.

Angiotensin 1-converting enzyme from rabbit lung acetone powder, tyrosinase, xanthine oxidase, and γ-aminobutyric acid transaminase (GABase) from *Pseudomonas fluorescens* were purchased from Sigma-Aldrich (St. Louis, MO, USA). β-NADP+, hippuric acid-histidine-leucine, pyrogallol, and 2,2-diphenyl-1-picrylhydrazyl were also purchased from Sigma-Aldrich. Unless otherwise specified, all chemicals were analytical grade.

Determinantiation of GABA contents. Quantitative determination of GABA with GABase was done as follows. The reaction mixture (cell-free extract from yeast determination of GABA with GABase was done as follows.

The reaction mixture (cell-free extract from yeast was kept at 37°C for 60 min after which the absorbance was measured at 340 nm with a enzyme-linked immunosorbent assay reader. The GABA contents were calculated with a GABA standard curve.

**Assay of physiological functionalities.** The physiological activities of the cell-free extracts containing GABA from the selected yeast strains were determined as follows. Antihypertensive angiotensin I-converting enzyme (ACE) inhibitory activity was assayed by the method of Cushman and Cheung [29] using ACE from rabbit lung. Antioxidant activity was assayed with DPPH as the substrate [30], and superoxide dismutase-like activity was assayed by the method of Lee et al. [31] using pyrogallol. Tyrosinase inhibitory activity was measured by conversion of L-DOPA to a red-colored oxidation product dopachrome spectrophotometrically [32]. Xanthine oxidase inhibitory activity was determined by the modification method of Noro et al. [33]. α-Glucosidase inhibitory activity was assayed using α-glucosidase and p- nitrophenyl α-D-glucopyranoside [10].

**RESULTS AND DISCUSSION**

**Selection of potent GABA-producing yeast strains and production of GABA.** The GABA contents of ten yeast strains including *Kazachstania unispora* SY14-1, were determined with GABase (Table 1). The cell-free extracts of asporogenous *Sporobolomyces carnicolor* 402-JB-1 had the highest GABA content of 179.2 μg/mL. Ascosporogenous *P. silvicola* UL6-1 was also produced high content of GABA (134.4 μg/mL) even though lower than that of *S. carnicolor* 402-JB-1. Finally, *P. silvicola* UL6-1 and *S. carnicolor* 402-JB-1 were selected as potent GABA-producing yeasts. These GABA contents also were similar or higher than that of *L. plantarum* K74 from Kimchi (134.52 μg/mL) [34] while they were lower than that of Bokbunja wine (330 μg/mL) [15], and *L. sake* A156 (15.81 ± 0.98 mg/mL) and *Lactobacillus zymae* GU240 (16.94 ± 1.14 mg/mL) [35].

Meanwhile, the effect of the culture time on GABA production in *P. silvicola* UL6-1 and *S. carnicolor* 402-JB-1 was investigated (Fig. 1). The maximum yield of GABA (200.8 μg/mL, 136.5 μg/mL from *S. carnicolor* 402-JB-1 and *P. silvicola* UL6-1 were achieved when their wild yeast strains were cultured for 30 hr at 30°C in yeast extract-peptone-dextrose media, respectively. Asporogenous *S. carnicolor* 402-JB-1 was higher produced GABA than

**Table 1. Quantitative GABA contents of the first 10 screened yeast strains**

| Yeast Strains | GABA Content (μg/mL) | Yeast Strains | GABA Content (μg/mL) |
|---------------|----------------------|---------------|----------------------|
| Kazachstania unispora SY14-1 | 99.1 | Pichia silvicola UL6-1 | 134.4 |
| Metschnikowia reukaufii SY20-7 | 99.9 | Sporobolomyces carnicolor 374-CO-1 | 145.6 |
| Nakazawaea holsti 63-J-1 | 86.2 | Sporobolomyces carnicolor 402-JB-1 | 179.2 |
| Pichia guillermondii 89-J-1 | 98.2 | Sporobolomyces ruberrimus 73-D-3 | 109.4 |
| Pichia scolyti YJ14-2 | 126.7 | Sporobolomyces ruberrimus 121-Z-3 | 136.0 |

* Determined with γ-aminobutyric acid (GABA)-transaminase.
Physiological functionality of GABA-producing yeasts. To investigate the application of GABA from yeasts in medicinal foods, several physiological functionalities of the cell-free extracts from the first screened ten yeasts were investigated (Table 2). The cell-free extract from *P. silvicola* UL6-1 had the highest anti-hyperglycemic α-glucosidase inhibitory activity at 72.3%, and *S. carnicolor* 402-JB-1 had high anti-hyperglycemic α-glucosidase inhibitory activity at 69.9% and anti-hypertensive angiotensin 1-converting enzyme inhibitory activity at 54.9%.

These α-glucosidase inhibitory activities were higher than that of Makgeolli made by *Saccharomyces cerevisiae* Y111-5 (42.0%) [36] while they were lower than those of *Bullera coprosmaensis* JS00600 (94.7%) [37] and *P. burtonii* Y257-7 (90.9%) [10].

Finally, *Pichia silvicola* UL6-1 and *S. carnicolor* 402-JB-1, which had high GABA contents as well as a high anti-hyperglycemic effect, were selected as potent yeast strains for the medicinal foods industry.

**Anti-hyperglycemic effect of GABA from *Pichia silvicola* UL6-1 and *Sporobolomyces carnicolor* 402-JB-1.** The anti-hyperglycemic action of the cell-free extract-containing GABA from *P. silvicola* UL6-1 in streptozotocin-induced diabetic Sprague-Dawley (SD) rats and normal SD rats. GABA, γ-aminobutyric acid.

---

**Table 2.** Physiological activities of the cell-free extracts from the first 10 screened yeast strains

| Yeast strain                  | ACE inhibitory activity (%) | α-Glucosidase inhibitory activity (%) | Antioxidant activity (%) | SOD-like activity (%) | XOD inhibitory activity (%) | Tyrosinase inhibitory activity (%) |
|------------------------------|-----------------------------|---------------------------------------|--------------------------|-----------------------|-----------------------------|-----------------------------------|
| *Kazachstania unispora* SY14-1 | 14.2 ± 0.6                  | 50.3 ± 0.9                            | 1.9 ± 0.1                | n.d                   | 3.6 ± 0.8                    | 11.3 ± 0.1                        |
| *Metschnikowia reukaufii* SY20-7 | 31.0 ± 0.2                  | 42.5 ± 0.5                            | 1.1 ± 0.3                | n.d                   | 7.6 ± 0.3                    | 14.3 ± 0.7                        |
| *Nakazawae holstii* 63-J-1    | 28.0 ± 0.8                  | 59.2 ± 0.1                            | 4.1 ± 0.1                | n.d                   | n.d                         | 12.6 ± 0                          |
| *Pichia guilliermondii* 89-J-1 | 21.8 ± 0.7                  | 59.8 ± 0.2                            | 2.0 ± 0.8                | n.d                   | 7.3 ± 0.1                    | 13.2 ± 0.1                        |
| *Pichia scolyti* YJ14-2       | 22.0 ± 0.9                  | 62.5 ± 0.4                            | 0.8 ± 0.9                | n.d                   | 4.7 ± 0.1                    | 12.0 ± 0.7                        |
| *Pichia silvicola* UL6-1      | 24.4 ± 0.1                  | 72.3 ± 0.7                            | 0.6 ± 0.9                | n.d                   | 8.0 ± 0.3                    | 18.2 ± 0.8                        |
| *Sporobolomyces carnicolor* 374-CO-1 | 39.9 ± 0.0                  | 66.2 ± 0.2                            | 1.8 ± 0.8                | n.d                   | 5.2 ± 0.9                    | 12.2 ± 0.1                        |
| *Sporobolomyces ruberrimus* 402-JB-1 | 54.9 ± 0.5                  | 69.2 ± 0.5                            | 0.2 ± 0.5                | n.d                   | 8.1 ± 0.9                    | 11.9 ± 0.2                        |
| *Sporobolomyces ruberrinus* 73-D-3 | 29.1 ± 0.5                  | 65.2 ± 0.4                            | 1.0 ± 0.9                | n.d                   | 6.8 ± 0.5                    | 16.2 ± 0.8                        |
| *Sporobolomyces ruberrinus* 121-Z-3 | 40.3 ± 0.9                  | 65.6 ± 0.1                            | n.d                     | n.d                   | 13.9 ± 0.4                   | 13.3 ± 0.9                        |

ACE, angiotensin 1-converting enzyme; SOD, superoxide dismutase; XOD, xanthine oxidase; n.d, not detected.
the blood glucose level decreased to 335–350 mg/dL dose-dependently at 120 min after administered the cell-free extract containing the α-glucosidase inhibitor from Sporobolomyces carnicolor 402-JB-1 in streptozotocin-induced diabetic Sprague-Dawley (SD) rats and normal SD rats. GABA, γ-aminobutyric acid.

ACKNOWLEDGEMENTS

This work was supported by the research grant of PaiChai University in 2017.

REFERENCES

1. Kim JH, Kim NM, Lee JS. Physiological characteristics and ethanol fermentation of thermostolerant yeast Saccharomyces cerevisiae OE-16 from traditional Meju. Korean J Food Nutr 1999;12:490-5.

2. Min H, Ryu J, Kim HK, Lee JS. Isolation and identification of yeasts from wild flowers in Gyeoksan, Oseosan and Beaksan of Korea. Kor J Mycol 2013;41:47-51.

3. Hyun SH, Min JH, Kim SA, Lee JS, Kim HK. Yeasts associated with fruits and blossoms collected from Hanbat arboretum, Daejeon, Korea. Kor J Mycol 2014;42:178-82.

4. Hyun SH, Lee JG, Park WJ, Kim HK, Lee JS. Isolation and diversity of yeasts from fruits and flowers of orchid in Sinam-nyeon of Yesan-gun, Chungcheongnam-do, Korea. Kor J Mycol 2014;42:21-7.

5. Hyun SH, Han SM, Lee JS. Isolation and physiological functionality of yeasts from wild flowers in Seonyudo of Gogunsanyeokdo, Jeollabuk-do, Korea. Kor J Mycol 2014;42:201-6.

6. Yi SH, Ann YG, Choi JS, Lee JS. Development of peach fermented wine. Korean J Food Nutr 1996;9:409-12.

7. Lee TS, Lee SK. Studies on the yeasts for the brewing of soy sauce (I). Isolation, identification and classification of the yeasts in the soy sauce koji. J Korean Agric Chem Soc 1970;13:97-103.

8. Han SM, Hyun SH, Kim NM, Lee JS. Antioxidant activity and inhibitory activities of xanthine oxidase and tyrosinase of yeasts from wild flowers in Korea. Kor J Mycol 2015;43:99-103.

9. Kim JH, Lee DH, Jeong SC, Chung KS, Lee JS. Characterization of antihypertensive angiotensin I-converting enzyme inhibitor from Saccharomyces cerevisiae. J Microbiol Biotechnol 2004;14:1318-23.

10. Kim YH, Shin JW , Lee JS. Production and antihyperglycemic effects of α-glucosidase inhibitor from yeast, Pichia burtonii Y257-7. Korean J Microbiol Biotechnol 2014;42:219-24.

11. Lee DH, Lee DH, Lee JS. Characterization of a new antidiementia β-secretase inhibitory peptide from Saccharomyces cerevisiae. Enzyme Microb Technol 2007;42:83-8.

12. Lee DH, Lee DH, Yi SH, Lee HS. Production of the acetylcholinesterase inhibitor from Yarrowia lipolytica S-3. Mycobiol 2008;36:102-5.

13. Jeong SC, Lee DH, Lee JS. Production and characterization of an anti-angiogenic agent from Saccharomyces cerevisiae K-7. J Microbiol Biotechnol 2006;16:1904-11.

14. Ueno H. Enzymatic and structural aspects on glutamate decarboxylase. J Mol Catal B Enzym 2000;10:67-79.

15. Kim JH. Isolation and characterization of high GABA producing yeast isolated from fermented Bokbunja wine [dissertation]. Jeonju: Chonbuk National University; 2014.

16. Lee SH. Study on a gamma-aminobutyric acid (GABA) producing yeast isolated from Korean fermented soybean product, meju and optimal condition for GABA synthesis [dissertation]. Jeonju: Chonbuk National University; 2014.

17. Cho YR, Chang JY, Chang HC. Production of γ-aminobutyric acid (GABA) by Lactobacillus buchneri isolated from kimchi and its neuroprotective effect on neuronal cells. J Microbiol Biotechnol 2007;17:104-9.

18. Hayakawa K, Kimura M, Kasahara K, Matsumoto K, Sansawa H, Yamori Y. Effect of a gamma-aminobutyric acid-enriched dairy product on the blood pressure of spontaneously hypertensive and normotensive Wistar-Kyoto rats. Br J Nutr 2004;92:411-7.

19. Inoue K, Shirai T, Ochiai H, Kasao M, Hayakawa K, Kimura M, Sansawa H. Blood-pressure-lowering effect of a novel fermented milk containing gamma-aminobutyric acid (GABA) in mild hypertensives. Eur J Clin Nutr 2003;57:490-5.

20. Jakobs C, Jæcken J, Gibson KM. Inherited disorders of GABA metabolism. J Inherit Metab Dis 1993;16:704-15.
21. Wong CG, Bottiglieri T, Snead OC 3rd. GABA, gamma-hydroxybutyric acid, and neurological disease. Ann Neurol 2003;54 Suppl:S3-12.

22. Tujio K, Ohsuni M, Horie K, Kim M, Hayase K, Yokogoshi H. Dietary gamma-aminobutyric acid affects the brain protein synthesis rate in ovariectomized female rats. J Nutr Sci Vitaminol (Tokyo) 2009;55:75-80.

23. Schuller HM, Al-Wadei HA, Majidi M. Gamma-aminobutyric acid, a potential tumor suppressor for small airway-derived lung adenocarcinoma. Carcinogenesis 2008;29:1979-85.

24. Park KB, Oh SH. Isolation and characterization of Lactobacillus buchneri strains with high γ-aminobutyric acid producing capacity from naturally aged cheese. Food Sci Biotechnol 2006;15:86-90.

25. Park KB, Oh SH. Cloning, sequencing and expression of a novel glutamate decarboxylase gene from a newly isolated lactic acid bacterium, Lactobacillus brevis OPK-3. Bioresour Technol 2007;98:312-9.

26. Ueno Y, Hayakawa K, Takahashi S, Oda K. Purification and characterization of glutamate decarboxylase from Lactobacillus brevis IFO 12005. Biosci Biotechnol Biochem 1997;61:1168-71.

27. Nomura M, Kimoto H, Someya Y, Suzuki I. Novel characteristic for distinguishing Lactococcus lactis subsp. lactis from subsp. cremoris. Int J Syst Bacteriol 1999;49(Pt 1):163-6.

28. Han SM, Jeon SJ, Lee HB, Lee JS. Screening of γ-aminobutyric acid (GABA)-producing wild yeasts and their microbiological characteristics. Kor J Mycol 2016;44:87-93.

29. Cushman DW, Cheung HS. Spectrophotometric assay and properties of angiotensin-converting enzyme of rabbit lung. Biochem Pharmacol 1971;20:1637-48.

30. Lee SE, Seong NS, Bang JK, Kang SW, Lee SW, Chung TY. Inhibitory effect against angiotensin converting enzyme and antioxidant activity of Panax ginseng C. A. Meyer extracts. Korean J Med Crop Sci 2003;11:236-45.

31. Lee JS, Yi SH, Kown SJ, Ahn C, Yoo JY. Enzyme activities and physiological functionality of yeasts from traditional Meju. Korean J Appl Microbiol Biotechnol 1997;25:448-53.

32. Kim JK, Cha WS, Park JH, Oh SL, Cho YJ, Chun SS, Choi C. Inhibition effect against tyrosinase of condensed tannins from Korean green tea. Korean J Food Sci Technol 1997;29:173-7.

33. Noro T, Oda Y, Miyase T, Ueno A, Fukushima S. Inhibitors of xanthine oxidase from the flowers and buds of Daphne genkwa. Chem Pharm Bull (Tokyo) 1983;31:3984-7.

34. Park SY, Shim HY, Kim KS, Lim SD. Physiological characteristics and GABA production of Lactobacillus plantarum K74 isolated from Kimchi. Korean J Dairy Sci Technol 2013;31:143-52.

35. Sa HD, Park JY, Jeong SJ, Lee KW, Kim JH. Characterization of glutamate decarboxylase (GAD) from Lactobacillus sakei A156 isolated from Jeot-gal. J Microbiol Biotechnol 2015;25:696-703.

36. Jang IT, Kang MG, Yi SH, Lim SI, Kim HR, Ahn BH, Lee JS. Physiological functionality of Nuruk, Makgeolli and Cheonggukjang made with fungi and bacteria isolated from Korean traditional fermented foods. Kor J Mycol 2012;40:164-73.

37. Han SM, Hyun SH, Lee HB, Lee HW, Kim HK, Lee JS. Isolation and identification of yeasts from wild flowers collected around Jangeong lake in Jeollanam-do, republic of Korea, and characterization of the unrecorded yeast Bullera coprosmaensis. Mycobiology 2015;43:266-71.