Mycobiology

Research Article

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

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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

Mycobiology 2017 September, **45**(3): 199-203
https://doi.org/10.5941/MYCO.2017.45.3.199
pISSN 1229-8093 • eISSN 2092-9323

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Received August 24, 2017
Revised September 13, 2017
Accepted September 13, 2017

optimal conditions for GABA production in this potent strain were determined, and the anti-hyperglycemic action of GABA was also investigated.

MATERIALS AND METHODS

Yeast 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-picrylhydrazy were also purchased from Sigma-Aldrich. Unless otherwise specified, all chemicals were analytical grade.

Determination of GABA contents. Quantitative determination of GABA with GABase was done as follows. The reaction mixture (cell-free extract from yeast 10 μL, GABase 0.02 units 10 μL, 10 mM β-NADP+ 70 μL, 0.1 M potassium pyrophosphate [pH 8.6] 240 μL, and 0.1 M α-ketoglutarate 10 μL) 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].

***In vivo* test for anti-hyperglycemic effects.** The anti-hyperglycemic effects of the cell-free extracts containing GABA from the selected yeast strains were tested with SD rats following the Guidelines on Animal Breeding for Animal Experiments - Ethics Committee of Paichai University (registration No. 2015. pcu-001).

Male SD rats (age, 6 weeks; weight, 180–200 g) were maintained on a 12-hr light/dark cycle in a temperature and humidity-controlled room for 1 wk. All rats were randomly distributed into experimental groups (n = 5/group). A diabetes inducer streptozotocin was used to induce hyperglycemia in rats. The rats were injected intraperitoneally with streptozotocin (60 mg/kg). Then, various concentrations of the cell-free extract containing GABA from *Pichia silvicola* UL6-1 (1,000 mg/kg and 500 mg/kg) and the commercial anti-diabetic agent acarbose (15 mg/kg) were administered orally.

Each experiment was performed at least three times, and all quantitative data are expressed as the mean ± standard deviation values.

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. sakei* 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

Wild yeasts	GABA content[a] (μg/mL)	Wild yeasts	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 holstii 63-J-1	86.2	*Sporobolomyces carnicolor* 402-JB-1	179.2
Pichia guilliermondii 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

[a]Determined with γ-aminobutyric acid (GABA)-transaminase.

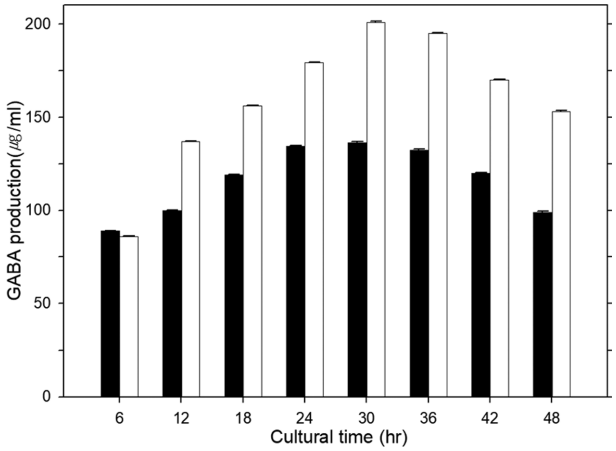

Fig. 1. Effect of the culture time on γ-aminobutyric acid (GABA) production in *Pichia silvicola* UL6-1 (black bar) and *Sporobolomyces carnicolor* 402-JB-1 (white bar).

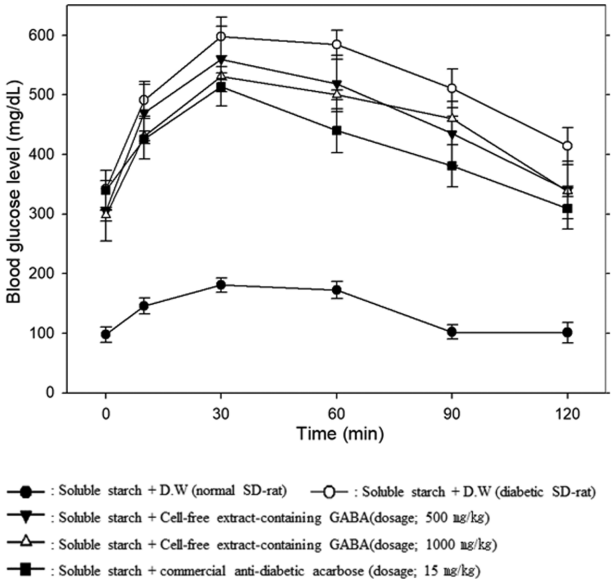

Fig. 2. Changes in blood glucose levels up to 120 min after administration of 3 g/kg soluble starch and various concentrations of the cell-free extract containing the α-glucosidase inhibitor from *Pichia silvicola* UL6-1 in streptozotocin-induced diabetic Sprague-Dawley (SD) rats and normal SD rats. GABA, γ-aminobutyric acid.

ascosporogenous *P. silvicola* UL6-1, even though its production condition was very similar.

Physiological functionality of GABA-producing yeasts. To investigate the application of GABA from yeasts in medicinal foods, several physiological funtionalities of the cell-free extracts from the 1st 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 and *S. carnicolor* 402-JB-1 were investigated in normal rats and streptozotocin-induced diabetic rats.

As shown in Figs. 2 and 3, the blood glucose level at 30 min after the administration of soluble starch (3 g/kg) was significantly increased to 495–600 mg/dL from 300–330 mg/dL in the streptozotocin-induced diabetic rats. However,

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
Nakazawaea 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	66.2 ± 0.2	1.8 ± 0.8	n.d	5.2 ± 0.9	12.2 ± 0.1
Sporobolomyces carnicolor 402-JB-1	54.9 ± 0.5	69.9 ± 0.5	0.2 ± 0.5	n.d	8.1 ± 0.9	11.9 ± 0.2
Sporobolomyces ruberrimus 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 ruberrimus 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 I-converting enzyme; SOD, superoxide dismutase; XOD, xanthine oxidase; n.d, not detected.

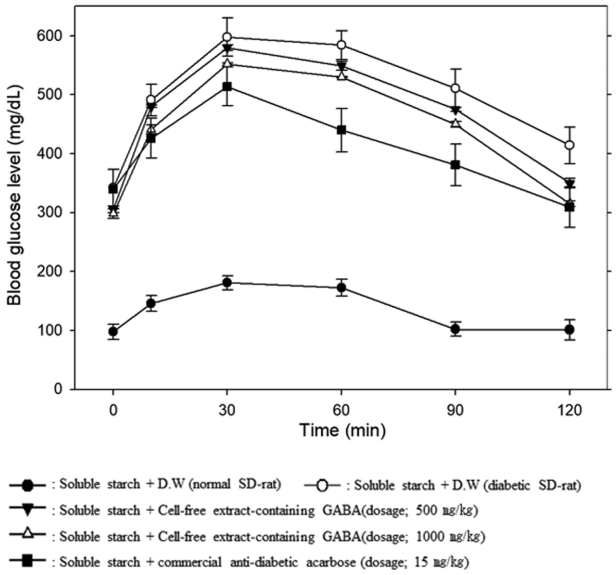

Fig. 3. Changes in blood glucose levels up to 120 min after administration of 3 g/kg soluble starch and various concentrations of the cell-free extract containing the α-glucosidase inhibitor from *Sporobolomyces carnicolor* 402-JB-1 in streptozotocin-induced diabetic Sprague-Dawley (SD) rats and normal SD rats. GABA, γ-aminobutyric acid.

the blood glucose level decreased to 335–350 mg/dL dose-dependently at 120 min after administered the cell-free extract from *P. silvicola* UL6-1. Anti-hyperglycemic effect of the cell-free extract from *S. carnicolor* 402-JB-1 was also very similar tendency that of *P. silvicola* UL6-1.

From these results, we concluded that the cell-free extract-containing GABA from *P. silvicola* UL6-1 and *S. carnicolor* 402-JB-1 have anti-hyperglycemic effects although higher dose were required than that of the commercial anti-diabetic acarbose. Therefore, these two wild yeasts would be very useful in the healthy food industry for development of new anti-diabetic foods.

ACKNOWLEDGEMENTS

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

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