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Production of Antihypertensive Angiotensin I-Converting Enzyme Inhibitor-Enriched Edible Yeast Using Gugija (*Lycium chinesis Mill*)

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To produce bioactive compound enriched yeast using medicinal Gugiga (*Lycium chinensis Mill*), several edible *Saccharomyces* species were cultured in Gugija extracts added yeast extract, peptone and dextrose medium (GE - YEPD medium) at 30°C for 24 hr, and their growth were determined. Growth of *Saccharomyces cerevisiae* K-7 and *Sacchromyces cerevisiae* ACTC 7904 were better than those of the other yeasts. Two yeasts were selected and then determined their some physiological functionalities after cultivated the yeasts in the GE - YEPD medium and compared those grown on YEPD medium. Antihypertensive angiotensin I-converting enzyme (ACE) inhibitory activity of *S. cerevisiae* K-7 grown on GE - YEPD medium was about 20% higher than that grown on YEPD medium. Superoxide dismutase-like activity of *S. cerevisiae* ACTC 7904 was also about 12% more high. However, the other physiological functionalities were almost same or lower. Optimal addition concentration of Gugija extract was 10%, and maximally growth and ACE inhibitory activity of *S. cerevisiae* K-7 were shown when the strain was cultured in 10% Gugija extracts containing YEPD medium at 30°C for 12 hr.

KEYWORDS : Antihypertensive ACE inhibitory activity, Gugija (*Lycium chinesis Mill*) extract, *Saccharomyces cerevisiae*

Yeasts have been used in the production of various alcoholic beverages, breads as well as research materials, and recently they also have been used in the production of bioactive compounds such as antihypertensive angiotensin I-converting enzyme (ACE) inhibitors [1] and antidementia β-secretase inhibitor [2], etc [3, 4]. Furthermore, selenium [5], germanium [6] and ginsenoside-Rg3 [7] enriched yeasts were produced by means of bioconversion [8, 9] or by incorporation [10-12] of selenium and germanium into the yeast cells during fermentation.

The *Lycii fructus*, fruit of *Lycii chinensis*, is one of medicinal plants which is cultivated in far east Asia. It is known that it contains carotenoid, cholin, meliscic acid, zeaxanthin, physalier, betaine (choline derivatives), β-sitosterol, vitamin B_1 and unsaturated fatty acid [4, 13]. *Lycii folium* and *Lycii cortex* also contain nicotianamaine, glutamic acid, proline, rutin, vitamin C and cinnamic acid, diterpene sugiol, steroid, β-sitosterol, betaine, vitamin B_2, kukoamine A, respectively [4]. Therefore, the *Lycii fructus* including *Lycii cortex* has been used to the prevention of many diseases including hypertension, liver toxicity and aging [13, 14]. However, there are few reports on the development of new ACE inhibitor from yeast grown on *Lycii chinensis*. In the previous study, we described cardiovascular functional activity and antioxidant activity of Gugija (*Lycium chinensis Mill*) species and its hybrids [15]. Especially, water extracts from *Lycii cortex* of Cheonnyang No 7 (84.1%) and methanol extracts from *Lycii fructus* of Do148-72 (A11), hybrid of *Lycii chinensis,* showed high antihypertensive ACE inhibitory activity of 84.1% and 96.9%, respectively [15]. Physiological functionality of Gugija products and an *in vivo* test on antihypertension were also reported [16].

In this study, effects of Gugija extracts on the preparation of antihypertensive ACE inhibitor enriched yeast were investigated for development of high value Gugija - yeast products.

Materials and Methods

Yeasts and chemicals. *Saccharomyces cerevisiae* K-7 and the other *Saccharomyces cerevisiae* were obtained from Laboratory of Biotechnology at Paichai University and Korea Research Institute of Bioscience and Biotechnology, respectively.

The ACE was extracted from rabbit lung acetone powder (Sigma Chemical Co., St. Louise, Mo., USA), and hippuric acid - histidine - leucine, and 1,1-diphenyl-2-picryl hydrazyl (DPPH) were purchased from Sigma Chemical Co. Unless otherwise specified, all the chemicals were of analytical grade.

Assay of physiological functionality. Cell-free extract of yeasts was prepared by cell disruption and centrifugation (9,000 ×g, 20 min). The antihypertensive ACE inhibitory activity was assayed according to the Cushman and

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Cheung's method [17]. A mixture containing 100 mM sodium borate buffer (pH 8.3), 300 mM NaCl, 3 units of ACE, and an appropriate amount of the cell-free extract was preincubated for 10 min at 37°C. The reaction was initiated by adding 50 μL of Hip-His-Leu at a final concentration of 5 mM and terminated after 30 min of incubation by the addition of 250 μL of 1.0 N HCl. The liberated hippuric acid was extracted with 1 mL of ethyl acetate, and 0.8 mL of the extract was dried with a Speed Vac Concentrator (EYELA Co., Tokyo, Japan). The residue was then dissolved in 1 mL of the sodium borate buffer. The absorbance at 228 nm was measured in order to estimate the ACE inhibitory activity.

The antioxidant activity was assayed by the method of Blois [18] with DPPH. A 0.8 mL DPPH solution (12.5 mg of DPPH dissolved in 100 mL of ethanol) was added to 0.2 mL of the sample, shaken for 10 sec, and left for 10 min. The absorbance at 525 nm was then determined and calculated as follows:

$$[(A_{525} \text{ of reaction mixture} - A_{525} \text{ of sample alone})/A_{525} \text{ of blank}] \times 100.$$

Superoxide dismutase (SOD)-like activity was assayed by the method of Marklund and Marklund [19]. A 20 mL sample was added to 20 mL of 55 mM tris-cacodylic acid buffer (TCB, pH 8.2), and then the mixture was homogenized for 2 min and centrifuged at 4°C for 30 min at 12,000 rpm. The supernatant was adjusted to pH 8.2 and then increased in volume up to 50 mL (sample extracts). 5 μL of 24 mM pyrogallol containing 10 mM HCl (substrate) was added to 0.95 mL of the sample extracts, and then absorbance at 420 nm was measured for the first 2 min. SOD-like activity was calculated by the following equation:

$$\text{SOD-like activity (\%)} = [(A - B)/A] \times 100$$

where A is the increase in absorbance of TCB (blank) and B is the increase in absorbance of sample.

Sensory evaluation. Sensory evaluation of the edible yeasts was estimated by 10 trained sensory panels, which they evaluated the taste and odor of the yeasts after dilution of freeze dried yeast by D.W. (10%) [7]. Total acceptability was scored 1 to 5 using the mean value of a hedonic scale.

Results and Discussion

Growth of yeasts in the Gugija extract-added yeast extract, peptone and dextrose (GE - YEPD) medium. Table 1 shows growth of yeasts in the Gugija extract-added medium (GE - YEPD medium). Almost all of yeasts were grew well in the GE - YEPD medium and especially, *S. cerevisiae* K-7 and *Saccharomyces cerevisiae* ACTC 7904 show more higher growth than the other yeasts. Therefore, we first selected these two yeasts for further studies.

Physiological functionality of the selected yeasts. Some physiological functionalities of the 1st selected yeasts, *S. cerevisiae* K-7 and *S. cerevisiae* ACTC 7904 were investigated (Table 2). Antihypertensive angiotensin I-converting enzyme (ACE) inhibitory activity of *S. cerevisiae* K-7 show the highest of 77.0%, showing higher than that of grown on YEPD medium (56.0%). ACE inhibitory activity of Gugija extract itself was 23.9% [17] and that of *S. cerevisiae* K-7 grown on YEPD medium was 56.0%, suggesting that ACE inhibitor of Gugija extract was absorbed and stored into the yeast cell as selenium [12] and germanium [10] or produced newly during fermentation by bioconversion [8].

SOD-like activity of *S. cerevisiae* K-7 was high (65.0%), however it was lower than that of grown on YEPD medium. ACE inhibitory activity of *S. cerevisiae* ACTC

Table 1. Growth of various edible yeasts in the GE - YEPD medium

Yeasts		Growth (A_{660})[a]
Saccharomyces cerevisiae	K-2	0.96 (1.11)[b]
	K-7	1.20 (1.16)
S. cerevisiae	C-1	0.95 (0.90)
	C-2	0.87 (0.93)
S. cerevisiae	ACTC 7904	1.00 (0.98)
	ACTC 7919	0.95 (1.00)

GE - YEPD medium, yeast extract, peptone and dextrose medium containing 10% Gugija extracts.
[a]Growth after 24 hr cultivation at 30°C.
[b]Growth in the YEPD medium.

Table 2. Physiological functionalities of *Saccharomyces cerevisiae* K-7 and *Saccharomyces cerevisiae* ACTC 7904 grown on GE - YEPD medium

		ACE inhibitory activity (%)	Antioxidant activity (%)	SOD-like activity (%)
S. cerevisiae	K-7	77.0 (56.0)[a]	2.6 (9.4)	65.0 (76.3)
	ACTC 7904	63.6 (64.0)	9.4 (13.2)	35.4 (23.2)

GE - YEPD medium, 10% Gugija extract containing yeast extract, peptone and dextrose medium; ACE, angiotensin I-converting enzyme; SOD, superoxide dismutase.
[a]YEPD medium-yeast extract (1%), peptone (2%) and dextrose (2%).

Table 3. Effect of addition concentration of Gugija extracts on the growth and physiological functionality of *Saccharomyces cerevisiae* K-7

Addition concentration (%)	Growth (A_{660})	ACE inhibitory activity (%)	Antioxidant activity (%)	SOD-like activity (%)
5.0	0.82	62.5	1.5	57.0
10	1.19	77.0	2.4	65.0
30	1.25	73.1	5.2	41.5
50	1.30	64.0	7.3	27.0

ACE, angiotensin I-converting enzyme; SOD, superoxide dismutase.

7904 was 63.6%, similar with that of grown on YEPD medium (64.0%). Antioxidant activities of two yeasts were very low. From these results, we selected finally *S. cerevisiae* K-7, showing the highest ACE inhibitory activity.

Effects of increasing the Gugija extract concentration. The effects of increasing the Gugija extract concentration on the growth and physiological functionality of *S. cerevisiae* K-7 were investigated using GE - YEPD medium (Table 3). As the amount of Gugija extract addition into YEPD medium was increased from 5.0% to 50%, growth was increased at 30% addition and then not charged. However, ACE inhibitory activity was increased about 15% at 10% addition and then decreased about 13% at 50% addition, respectively. Therefore, optimal amount of Gugija extract addition was 10% for production of ACE inhibitor enriched *S. cerevisiae* K-7. Meanwhile, SOD-like activity has also shown 65% at 10% addition, increased about 8%. Antioxidant activity was very weak and slightly increased.

Effect of culture time on the ACE inhibitory activity. Effect of cultivation period of *S. cerevisiae* K-7 on the ACE inhibitory activity was investigated (Fig. 1). As the culture time was increased, the ACE inhibitory activity was also increased and maximal ACE inhibitory activity of *S. cerevisiae* K-7 was shown in the cell-free extract from 12 hr cultivation (stationary phase). After that, as increased to 36 hr, its ACE inhibitory activity was not significantly changed. These results were different with that ACE inhibitory activity of *S. cerevisiae* was the highest (42.1%) when it was cultured in YEPD medium at 30°C for 24 hr [1].

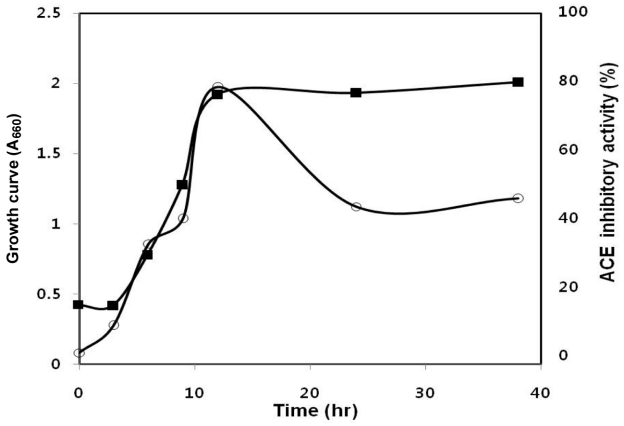

Fig. 1. Effect of cultural time on the growth and ACE inhibitory activity of *Saccharomyces cerevisiae* K-7 in Gugija extract medium (■, ACE inhibitory activity; ○, Growth).

Sensory evaluation and physicochemical properties of *S. cerevisiae* K-7. Sensory evaluation of ACE inhibitor enriched *S. cerevisiae* K-7 which was grown on GE - YEPD medium was investigated and compared those of *S. cerevisiae* K-7 grown on YEPD medium and *S. cerevisiae* ACTC 7904 grown on GE - YEPD medium (Table 4). ACE inhibitor enriched *S. cerevisiae* K-7 showed unique fermentative and slight herbal flavor. The yeast has also moderate bitter, sweet and sour taste rather than *S. cerevisiae* K-7 grown on YEPD medium. Total acceptability was the best in the ACE inhibitor enriched *S. cerevisiae* K-7.

Table 4. Sensory characteristics of *Saccharomyces cerevisiae* K-7 grown on Gugija exract (GE) medium

Yeasts	Sensory characteristics[a]	Total acceptability (order)[b]
S. cerevisiae K-7 grown on GE medium	Fermentative, slight herbal flavor, moderate bitter, sweet and sour taste	1
S. cerevisiae ACTC 7904 grown on GE medium	Fermentative, slight herbal flavor, slight bitter taste	3
S. cerevisiae K-7 grown on YEPD medium	Strong fermentative flavor, strong bitter and astringent	2

YEPD medium, yeast extract, peptone and dextrose medium.
[a]Sensory characteristics were described on 10% yeast solution by 10 panels.
[b]Total acceptability was scored 1~5 point on 10% yeast solution, and the order was presented with mean score.

We prepared cell-free extracts of ACE inhibitor enriched *S. cerevisiae* k-7 by cell digestion and centrifugation, and then its some physicochemical properties were determined. The cell-free extracts was yellow reddish and also soluble by water. The cell-free extracts had also maximal absorption spectrum at 225 nm (data not shown).

From our previous results on *in vivo* antihypertensive effects of Gugija extracts [16] and *S. cerevisiae* K-7 [1] using spontaneous hypertensive rat (SHR), we suggest Gugija ACE inhibitor enriched *S. cerevisiae* K-7 may also show good anti-hypertensive effects in SHR. Therefore, further study is necessary on *in vivo* test and purification of the ACE inhibitor.

From this study, we obtained antihypertensive ACE inhibitor-enriched *S. cerevisiae* K-7 from 12 hr cultivation in 10% Gugija extracts containing YEPD medium. *S. cerevisiae* K-7 also shows good acceptability. Therefore, we think that this *S. cerevisiae* K-7 might be used very usefully in the production of antihypertensive traditional alcoholic beverages or new antihypertensive agents for medical or functional food industry.

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