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Growth Characteristics and Physiological Functionality of Yeasts in Pear Marc Extracts

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***Kluyveromyces fragilis* KCTC 7260 and *Saccharomyces cerevisiae* KCTC 7904, which both grew well in pear marc extract, were selected and their growth profiles and physiological functionalities were determined. Both of the selected yeasts established maximal growth by 20 hr of cultivation at 30°C in pear marc extract. The cell-free extracts showed high antihypertensive angiotensin I-converting enzyme inhibitory activity of 68.9% and 52.1%, respectively. The extracts also displayed 9.2 U/mL and 12.0 U/mL of protease activity, respectively.**

KEYWORDS : ***Kluyveromyces fragilis*, Pear marc extract, Physiological functionality, *Saccharomyces cerevisiae***

Pear contains 10~13% sugar, various organic acids and vitamin C [1]. Especially, pear contains flavonoid compounds such as quercetin and leuteolin, and polyphenol compounds including chlorogenic acid, catechin and arbutin. These constituents bestow various physiological functionalites such as antioxidant activity, immunostimulating activity, cholesterol-lowering activity, antihypertension activity and anti-inflammatory activity [1, 2].

A large amount of pear marc is produced during the preparation of pear juice, pear paste and pear *makgeolli*. Pear marc contains useful substances, such as phenolic compounds, organic acid and edible fiber. However, only a small amount of pear marc is used as animal feed. The bulk of pear marc is discharged in the field, causing environmental pollution. It is imperative, therefore, to improve the efficiency of pear marc utilization and to develop high value bioactive agents from pear marc.

Yeast has some industrial advantages, including rapid growth, ease of cultivation and the capacity to be grown in a cheap medium containing agricultural by-products [3]. Recently, bioactive compounds such as an antihypertensive angiotensin I-converting enzyme (ACE) inhibitor [3], ribonucleotides [4, 5] chitosan [6], an anti-angiogenic compound [7] and an antidementia β-secretase inhibitor [8] have been produced and characterized from yeasts including *Saccharomyces cerevisiae.*

In this paper, we describe the growth characteristics, physiological functionality and enzyme activity of yeasts grown on pear marc extracts for the efficient utilization and prevention of environmental pollution of pear marc.

Materials and Methods

Strains, pear marc and chemicals. Eighty six strains of yeast including *S. cerevisiae* KCTC 7904, *Candida krusei* KCTC 7213, *Torulopsis sphaerica* KCTC 7138, *Kluyveromyces fragilis* KCTC 7260 and *Zygosaccharomyces rouxii* KCCM 12066 were used in this study. Pear marc containing 80% moisture was obtained from the Wooil pear processing plant, Gwangju, Chonnam Province, South Korea. Two liter of distilled water were added to 100 g of pear marc and shaken for 24 hr at 30°C. After centrifugation at 10,000 ×g for 15 min, the extracts were harvested. ACE was extracted from rabbit lung acetone powder (Sigma-Aldrich, St. Louis, MO, USA). Hippuric acid-histidine-leucine, fibrin, pyrogallol, and 2,2-diphenyl-1-picrylhydrazy (DPPH) were also purchased from Sigma-Aldrich. Unless otherwise specified, all the chemicals were of analytical grade.

Assay of growth and physiological functionality. The selected yeasts were cultured in the pear marc extract at 30°C for 20 hr and growth was determined by absorbance at 660 nm. After centrifugation at 10,000 ×g for 15 min,

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the cells were harvested and disrupted by vortexing with glass beads. After centrifugation at 12,000 ×g for 20 min, the cell-free extract was obtained and physiological functionalities were determined as follows.

ACE inhibitory activity was assayed by the method of Cushman and Cheung [9]. 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 was 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, Tokyo, Japan). The residue was dissolved in 1 mL of sodium borate buffer. The absorbance at 228 nm was measured.

Fibrinolytic activity was assayed by the method of Fayek and El-Sayed [10]. Each sample of 0.5 mL was added to 3 mL of a substrate solution (0.6% fibrin in 0.1 M Mcllvaine buffer, pH 7.0) and incubated at 40°C for 10 min. The reaction was stopped by adding 3 mL of 0.4 M trichloroacetic acid for 30 min. The mixture was then filtered through Whatman No. 2 filter paper (Whatman, Newton, MA, USA). A reaction mixture of 1 mL of the filtrate, 5 mL of 0.4 M Na_2CO_3 and 1 mL of 1 N Folin reagent was placed at room temperature for 30 min. The amount of tyrosin released from the fibrin as a substrate was determined from the tyrosine standard curve by measuring the absorbance at 660 nm.

Antioxidant activity was also assayed by the method of Lee *et al.* [11, 12] using DPPH as substrate. A 0.8 mL volume of DPPH solution (12.5 mg DPPH dissolved in 100 mL 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 antioxidant activity was calculated as [(A_{525} of reaction mixture – A_{525} of sample alone)/A_{525} of blank] × 100.

Superoxide dismutase (SOD)-like activity was assayed as described previously [11]. A 20 mL sample was added to 20 mL of 55 mM tris-cacodylic acid buffer (TCB, pH 8.2), after which 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). Five μL of 24 mM pyrogallol containing 10 mM HCl (substrate) was added to 0.95 mL of the sample extracts, after which absorbance at 420 nm was measured for the first 2 min. SOD-like activity (%) was calculated as [(A – B)/A] × 100, where A is the increase in absorbance of TCB (blank) and B the increase in absorbance of the sample.

Tyrosinase inhibitory activity was assayed by the method of Kim *et al.* [13]. The conversion of L-DOPA to a red-colored oxidation product, dopachrome, was measured spectrophotometrically. A 0.1 mL sample was incubated for 5 min at 30°C. At time zero, 1 mL of L-DOPA solution (4 mg/mL) was rapidly added while stirring, and the absorbance was measured at 475 nm. After incubation for an additional 5 min, the mixture was shaken again, and a second reading was determined. Tyrosinase inhibitory activity (%) was determined as [1 – (A_{475} of sample reaction mixture)/A_{475} blank] × 100.

Xanthine oxidase inhibitory activity was determined by a modification of a previously-described method [14]. Xanthine oxidase (0.2 U/mL) 100 μL was added to a mixture containing 0.6 mL of 0.1 M potassium phosphate buffer (pH 7.5) and 100 μL of sample (1 mg/mL) and 200 μL of 1 mM xanthine. After reacting the mixture at 37°C for 5 min, the reaction was stopped by the addition of 200 μL of 1 N HCl. Protein was removed by centrifugation at 12,000 rpm for 10 min and then amount of the released uric acid was determined by absorbance at 292 nm.

α-Glucosidase inhibitory activity was assayed as follows. α-Glucosidase and p-nitrophenyl-β-D-glucophylanoside (PNPG) were dissolved in 0.1 M potassium phosphate buffer (pH 6.8). An enzymatic reaction mixture composed of 100 μL α-glucosidase (0.2 U/mL), 100 μL of 1.5 mM PNPG and sample was incubated at 37°C for 25 min. The reaction was terminated by adding 100 μL of sodium carbonate solution (0.1 M, pH 9.8). The inhibitory activity of sample was determined by measuring the remaining activity of α-glucosidase at a concentration of 50 μM. Enzymatic activity was measured by the amount of the released p-nitrophenol detected by absorbance at 405 nm.

Results and Discussion

Growth characteristics of yeasts. Eighty six strains of yeast were cultivated in pear marc extract at 30°C for 48 hr and five strains grew in the pear marc extract (Table 1). Among these, *K. flagilis* KCTC 7260 and *S. cerevisiae* KCTC 7904 grew especially well in the pear marc extract. Their growth was better than those of *C. krusei* KCTC 7213 (A_{660}; 0.14), *K. fragilis* KCTC 7260 (A_{660}; 0.13) and *S. cerevisiae* T-71 (A_{660}; 0.32) grown on ginseng-steaming effluent [5].

Effect of cultural time on the growth of *K. flagilis*

Table 1. Growth of the selected yeasts in pear marc extract

Yeasts	Growth[a] (A_{660})
Saccharomyces cerevisiae KCTC 7904	0.51
Candida krusei KCTC 7213	0.25
Torulopsis sphaerica KCTC 7138	0.20
Kluyveromyces fragilis KCTC 7260	0.67
Zygosaccharomyces rouxii KCCM 12066	0.26

[a]Growth was determined after cultivation in pear marc extract for 20 hr at 30°C.

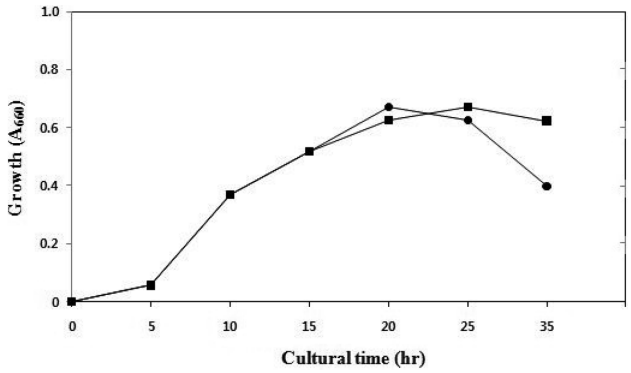

Fig. 1. Time course of growth of *Kluyveromyces fragilis* KCTC 7260 in the pear marc extract (-●-, pear macr extract; -■-, YEPD medium). YEPD, yeast extract, peptone, dextrose.

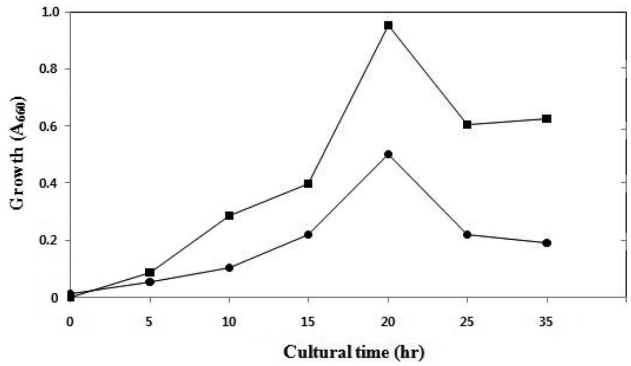

Fig. 2. Time course of growth of *Saccharomyces cerevisiae* KCTC 7904 in the pear marc extract (-●-, pear macr extract; -■-, YEPD medium). YEPD, yeast extract, peptone, dextrose.

KCTC 7260 and *S. cerevisiae* KCTC 7904 in pear marc extract was investigated. Maximum growth of *K. flagilis* KCTC 7260 was attained at 20 hr of cultivation, very similar to growth in yeast extract, peptone, dextrose (YEPD) medium (Fig. 1). *S. cerevisiae* KCTC 7904 also displayed maximal growth after 20 hr of cultivation in pear marc extract (Fig. 2). However, its growth was lower than attained in YEPD medium and compared to *K. flagilis* KCTC 7260. In another study, *Hansenula anomala* KCCM 11473 displayed maximal growth after 6 hr at 30°C in ginseng-steaming effluent [5].

Physiological functionalities of yeasts. The physiological functionalities of cell-free extract from the selected yeasts were investigated (Table 2). The antihypertensive ACE inhibitory activity of *K. flagilis* KCTC 7260 grown on pear marc extract was 68.9%, which was about 16% higher than that of the cell-free extract from *S. cerevisiae* KCTC 7904. These results were higher than those of the biomass from *Pichia anomala* (31.0%) [5] and its mutant HA-2 (16.0%) [4] grown on ginseng-steaming effluent and the biomass from *S. cerevisiae* (42.1%) grown on YEPD medium [3]. However, it was lower than that of *P. anomala* KCCM 11473 (72.0%) grown on ginseng-steaming effluent [15]. Recently, various ACE inhibitors with antihypertensive effects have been isolated from various protein hydrolysates, sake and its by-products, Korean traditional rice wines, cereals and legumes, and microbes such as yeast and mushrooms including *Pleurotus cornucopiae* [3, 16]. From our reports on ACE inhibitory activity of pear *yakju* (57.2%) [1], pear wine (32.2%) and pear-strawberry wine (70.8%) [2], and from pear marc extract itself (49.5%), we surmise that the high ACE inhibitory activity of the cell-free extract from the selected yeasts grown on pear marc extract is caused by transfer of ACE inhibitor from pear marc extract or production of ACE inhibitor during cultivation of yeasts. Several ACE inhibitors are peptides [3]. Therefore, cell-free extract of *K. fragilis* KCTC 7260 was treated by pepsin under optimal reaction conditions and the ACE inhibitory activity was determined. The ACE inhibitory activity of *K. fragilis* KCTC 7260 increased about 9% after treatment of cell-free extract with pepsin (77.5%) (data not shown), indicating the peptide nature of the ACE inhibitor of cell-free extract from *K. fragilis* KCTC 7260. This result was similar to that of alcohol fermentative *S. cerevisiae* KCTC 7904 [3]. Further purification and characterization of the ACE inhibitor from *K. fragilis* KCTC 7260 is reguired.

Almost 30% of antigout xanthine oxidase inhibitory activity was detected only in *K. fragilis* KCTC 7260, whereas 39.0% of SOD-like activity was showed in *S. cerevisiae* KCTC 7904. However, the other functionalities

Table 2. Physiological functionalities of yeasts grown on pear marc extract

	ACE inhibitory activity (%)	XOD inhibitory activity (%)	SOD like activity (%)	Antioxidant activity (%)	Fibirinolytic activity (%)	α-Glucosidase inhibitory activity (%)	Tyrosinase inhibitory activity (%)
Saccharomyces cerevisiae KCTC 7904	52.1 ± 0.5	1.9 ± 1.0	39.0 ± 2.0	4.7 ± 2.0	n.d.	n.d.	n.d.
Kluyveromyces fragilis KCTC 7260	68.9 ± 0.8	21.7 ± 0.8	n.d.	n.d.	n.d.	n.d.	8.0 ± 0.5

ACE, angioteansin I-converting enzyme; XOD, xanthine oxidase; SOD, superoxide dismutase; n.d., not detected.

Table 3. Enzyme activities (uint/mL) of the cell-free extract from the selected yeasts

	α-Amylase activity	Lipase activity	Protease activity
Saccharomyces cerevisiae KCTC 7904	1.2 ± 0.5	n.d.	12.0 ± 0.1
Kluyveromyces fragilis KCTC 7260	1.5 ± 0.1	n.d.	9.2 ± 0.4

n.d., not detected.

were either not detected or were weakly evident (< 10% of control).

The activities of α-amylase, lipase and neutral protease were spectrophotometrically determined by using 1% soluble starch, triolein and 0.6% skim milk, respectively [11, 17]. Neutral protease activity of the cell-free extract from *K. fragilis* KCTC 7260 and *S. cerevisiae* KCTC 7904 were 9.2 U/mL and 12.0 U/mL, respectively. However, α-amylase activity and lipase activity were very low or not detected (Table 3).

We have previously reported on the production of bioactive ribonucleotide [5] and chitosan [6] from yeasts grown on ginseng-steaming effluent. Presently, *K. fragilis* KCTC 7260 and *S. cerevisiae* KCTC 7904 grew exuberantly when supplied with pear marc extract. The present findings may be beneficial in the production of high value antihypertensive agents and prevention of environmental pollution arising from pear marc disposal. Further studies are necessary on the application the yeasts biomass and some bioactive compounds from this study into functional foods or medicinal compounds.

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References

1. Song JH, Jang JH, Na KC, Kim HK, Lee JS. Screening of yeast for brewing of Korean traditional pear *yakju* and optimal fermentation condition. Kor J Mycol 2010;38:184-8.
2. Song JH, Chun JP, Na KC, Moon JH, Kim WS, Lee JS. Optimal fermentation condition for development of high quality pear wine and characteristics of pear wines. Korean J Microbiol Biotechnol 2009;37:213-8.
3. Kim JH, Lee DH, Jeong SC, Chung KS, Lee JS. Characterization of antihypertensive angiotensin 1-converting enzyme inhibitor from *Saccharomyces cerevisiae*. J Microbiol Biotechnol 2004;14:1318-23.
4. Lee JS, Hyun KW, Jeong SC, Kim JH, Choi YJ, Miguez CB. Production of ribonucleotides by autolysis of *Pichia anomala* mutant and some physiological activities. Can J Microbiol 2004;50:489-92.
5. Kim JH, Lee BH, Lee JS. Production of ribonucleotides by autolysis of *Hansenula anomala* grown on Korean ginseng-steaming effluent. J Biosci Bioeng 2002;93:318-21.
6. Kim JH, Lee KS, Kim NM, Lee JS. Production and characterization of chitosan from ginseng-steaming effluents by *Mucor miehei*. J Microbiol Biotechnol 2002;12:760-5.
7. 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.
8. Lee DH, Lee DH, Lee JS. 2006. Production and characterization of antidementia β-secretase inhibitor from *Saccharomyces cerevisiae*. In: 2006 Annual Meeting and International Symposium; 2006 Jun 18-20; Busan, Korea. Seoul: The Korean Society for Microbiology and Biotechnology; 2006.
9. Cushman DW, Cheung HS. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. Biochem Pharmacol 1971;20:1637-48.
10. Fayek KI, El-Sayed ST. Purification and properties of fibrinolytic enzyme from *Bacillus subtilis*. Z Allg Mikrobiol 1980;20:375-82.
11. 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.
12. 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.
13. 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.
14. 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.
15. Kim NM, So SH, Lee SG, Song JE, Seo DS, Lee JS. Physiological functionality and enzyme activity of biomass from *Pichia anomala* grown on ginseng-steaming effluent. Mycobiology 2008;36:148-51.
16. Jang JH, Jeong SC, Kim JH, Lee YH, Ju YC, Lee JS. Characterization of a new antihypertensive angiotensin I-converting enzyme inhibitory peptide from *Pleurotus cornucopiae*. Food Chem 2011;127:412-8.
17. Lee JK, Kang MG, Lee JS. Nutritional characteristics and physiological functionality of lipase inhibitor-containing *Desmodium oxyphyllum* DC. extracts. Korean J Food Nutr 2011;24:153-8.