

# Extraction and Characterization of an Anti-hyperglycemic $\alpha$ -Glucosidase Inhibitor from Edible Mushroom, *Pleurotus cornucopiae*

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The extraction and purification of the anti-hyperglycemic  $\alpha$ -glucosidase inhibitor from an edible mushroom, *Pleurotus cornucopiae*, were investigated. The inhibitor was maximally extracted when the *P. cornucopiae* fruiting body was treated with distilled water at 30°C for 12 h. Purification was achieved using Sephadex G-100 and G-50 filtration chromatography, pepsin hydrolysis, and reverse-phase HPLC. The compound's solid yield and inhibitory activity were 12.2% and 9.10 mg/ml of IC<sub>50</sub>, respectively. The purified inhibitor contained two hexapeptides with Thr-Ile-Ala-Phe-Ile-Asp (A) and Tyr-Tyr-Ala-Ile-Gly-Asp (B) sequences and molecular weights of 678.79 Da (A) and 643.7 Da (B). The purified inhibitor showed a mixed inhibition pattern to  $\alpha$ -glucosidase and a dose-dependent anti-hyperglycemic effect in a streptozotocin-induced diabetic Sprague-Dawley rat model, exhibited by decreased blood glucose levels at doses of 50 and 300 mg/kg.

**Keywords:** Anti-hyperglycemic,  $\alpha$ -glucosidase inhibitor, diabetic rat, edible mushroom, *Pleurotus cornucopiae*

## Introduction

Recently, the health-enhancing effects of some mushrooms have been reported in addition to the taxonomy, reproduction, and cultivation of mushrooms [4, 6, 7, 12, 22]. *Pleurotus cornucopiae* is classified as a part of the *Pleurotus* genus of the *Pleurotus* family in the Basidiomycetes. This mushroom is found exclusively in Korea, China, Japan, Turkey, Europe, and North America. It also generally grows well in the leaned or stumps of latifoliate trees from summer to autumn. Some medicinal and healthy properties of *Pleurotus cornucopiae* such as anti-AIDSs, antibacterial, antitumor, anti-obesity [3, 21],

and antihypertensive angiotensin I - converting enzyme inhibitors have been studied [6].

$\alpha$ -Glucosidase (EC3.2.1.20) plays a role in controlling the glucose contents in the blood and it is the key enzyme that catalyzes the degradation of disaccharides and oligosaccharides to glucose [2]. Some commercial  $\alpha$ -glucosidase inhibitors such as acarbose [23], nojirimycin [18], voglibose [14], and 1-deoxynojirimycin [1] have been developed and used as diabetic drugs. However, these chemicals have side effects such as insomnia, headaches, flatulence, vomiting, and diarrhea [11, 19]. Even though some studies have been performed to find effective  $\alpha$ -glucosidase inhibitors from microorganisms [8, 9, 11, 19] and plants [20], a potent industrial  $\alpha$ -glucosidase inhibitor without side effects has not been developed yet.

In the previous paper [19], we selected potent *Neolentinus lepideus* and *Pleurotus cornucopiae*, which

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showed high  $\alpha$ -glucosidase inhibitory activity, to develop a new anti-hyperglycemic agent from edible mushrooms. In this study, we investigated the extraction conditions of the  $\alpha$ -glucosidase inhibitor from *Pleurotus cornucopiae*. Furthermore, the  $\alpha$ -glucosidase inhibitor was purified and characterized.

## Materials and Methods

### Mushroom, chemicals and rats

*Pleurotus cornucopiae* was obtained from the Mushroom Research Station, Gyeonggi-do Agricultural Research and Extension Service in Gwangju, South Korea.

$\alpha$ -Glucosidase from baker's yeast was purchased from Sigma Chemical Co. (USA). Sephadex G-50 and G-100 were purchased from Pharmacia Fine Chemicals (Uppsala, Sweden), and acarbose, streptozotocin as a diabetic-induced chemical, and ammonium formate were purchased from Sigma Chemical Co. (USA). Acetonitrile was purchased from J.T. Baker (NJ, USA). Unless otherwise specified, all chemicals and solvents were of analytical grade.

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

### Extraction of $\alpha$ -glucosidase inhibitor

Dried fruiting body (1 g) of *Pleurotus cornucopiae* was pulverized and then added to 30 ml distilled water. After being extracted at 30°C for 12 h by stirring, the extracts were centrifuged at 5000 g for 30 min and filtrated with a Whatman No. 41 filter paper. The supernatant was lyophilized.

### Assay of $\alpha$ -glucosidase inhibitory activity

$\alpha$ -Glucosidase inhibitory activity was assayed by using the method described in a previous paper [7]. A substrate, pNPG and  $\alpha$ -glucosidase were dissolved in a 0.1 M potassium phosphate buffer (pH 6.8), respectively.  $\alpha$ -Glucosidase 50  $\mu$ l solution (0.2 units/ml) was preincubated at 37°C for 5 min with 50  $\mu$ l of the sample solution (dissolved in a potassium phosphate buffer, pH 6.8 at 20 mg/ml). A potassium phosphate buffer was used as the blank solution. The reaction was initiated by the addition of 30  $\mu$ l of pNPG (0.2 mM), and the mixture was

incubated for 20 min at 37°C. The reaction was stopped by the addition of 100  $\mu$ l of sodium carbonate solution (0.1 M, pH 9.8).

The  $\alpha$ -glucosidase inhibitory activity was determined by measuring the optical density (OD) of the p-nitrophenol released from pNPG at 405 nm using an ELISA reader and calculated as following formula.

$$\text{Inhibitory activity (\%)} \\ = 100 \times (\text{Sample OD} - \text{Blank OD}) / (\text{Control OD} - \text{Blank OD})$$

### Purification of the $\alpha$ -glucosidase inhibitor

The water extracts of *Pleurotus cornucopiae* fruiting body applied to a Sephadex G-100 column (3.0  $\times$  35 cm) were equilibrated with distilled water and eluted with distilled water at a flow rate of 1.5 ml/min. The  $\alpha$ -Glucosidase inhibitory activity of all elutes were determined and the active fractions were obtained.

In order to investigate the effects of various proteases and carbohydrases on the increase of the  $\alpha$ -glucosidase inhibitory activity, the active fraction was digested with 1% pepsin, pancreatin, trypsin,  $\alpha$ -amylase and maltase under their optimal reaction temperature and pH. Then, the reactions were stopped by heating in boiling water at 80°C for 10 min. After the precipitate was removed by centrifugation at 5,000 g for 10 min at 4°C, the filtrates was lyophilized, and further  $\alpha$ -glucosidase inhibitory activity of the resolubilized solution was determined.

The active fraction was then applied to a Sephadex G-50 column (3.0  $\times$  35 cm) equilibrated with distilled water and eluted at a flow rate of 1.5 ml/min distilled water. The active fraction was applied to a reverse phase-high performance liquid chromatography column (FORTIS, C18 column, 5  $\mu$ m, 4.6  $\times$  250 mm; FORTIS Technology Co., IL, USA) equilibrated with acetonitrile. The column's absorbance was monitored at 210. The active fraction was performed acetonitrile/0.1% acetic acid in water (20:80) under isocratic conditions at a flow rate of 1.0 ml/min. Finally, the purified  $\alpha$ -glucosidase inhibitor was obtained [9].

### Determination of molecular weight and amino acid sequence

The purified  $\alpha$ -glucosidase inhibitor which was solubilized in distilled water eluted on an Acclaim RSLC 120 C18 (2.1  $\times$  100 mm, 2.2  $\mu$ m, DIONEX) at a flow rate of

200  $\mu$ l/min by applying a gradient 0–95% acetonitrile for 45 min. All mass spectrometry (MS) and tandem mass spectrometry (MS/MS) spectra in the Micro Q-TOF III Mass Spectrometer (Bruker Daltonics, 255748 Germany) were obtained in ESI + MS/MS. For peptide identification, the MS/MS spectra were performed using a De-novo sequencing program [9].

#### Determination of inhibition pattern on $\alpha$ -glucosidase

The purified  $\alpha$ -glucosidase inhibitor was added to each reaction mixture of various concentrations of substrate, pNPG, and  $\alpha$ -glucosidase. All of the  $\alpha$ -glucosidase activities were measured. The kinetics of  $\alpha$ -glucosidase in the presence of the inhibitor was determined by Lineweaver-Burk plots [9].

#### In vivo anti-hyperglycemic action

The anti-glycemic effect of the purified  $\alpha$ -glucosidase inhibitor from the *Pleurotus cornucopiae* fruiting body was performed using Sprague-Dawley (SD) rats under the Guidelines on the Animal Breeding of Animal Experiment-Ethics Committee of Pai Chai University (Registration No 2014.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 week. All of the rats were randomly distributed into experimental groups ( $n = 5$ / group). The diabetes inducer, streptozotocin, was used to induce hyperglycemic rats [5, 10, 13, 15–17]. The rats were injected intraperitoneally with streptozotocin (65 mg/kg) for 3 days. Then, 50 mg/kg and 300 mg/kg of the purified  $\alpha$ -glucosidase inhibitor from *Pleurotus*

*cornucopiae* and 15 mg/kg of the commercial anti-diabetic agent, acarbose, were administered orally. After 0, 10, 30, 60, 90 and 120 min of administration, the glucose contents of each of the rats' bloods were determined.

#### Statistical analysis

Each experiment was performed three times or more and their quantitative data were expressed as mean  $\pm$  SD values.

## Results and Discussion

#### Extraction condition of $\alpha$ -glucosidase inhibitor from *Pleurotus cornucopiae*

In the previous paper [19], we selected edible *Pleurotus cornucopiae* as a potent mushroom- containing anti-diabetic  $\alpha$ -glucosidase inhibitor. In this study, the effects of temperature on the extraction of the  $\alpha$ -glucosidase inhibitor from *Pleurotus cornucopiae* were investigated from 20°C to 70°C. The water extracts from the 30°C extraction showed a higher  $\alpha$ -glucosidase inhibitory activity (49.1%) than those of 20°C extracts (38.3%) and 50°C extracts (19.4%) and 70°C extracts (5.1%) (data not shown). The effect of time on the extraction of the  $\alpha$ -glucosidase inhibitor at 30°C were investigated in the range of 30 min to 48 h. The water extracts from the 12 h extraction showed the highest  $\alpha$ -glucosidase inhibitory activity (77.9%, IC<sub>50</sub> 23.2 mg/ml) and its solid yield was 42.7% (Table 1). The  $\alpha$ -glucosidase inhibitory activity was lower than that of 95% ethanol extracts (86.3%) from *Neolentinus lepideus* [19], whereas its extraction time was shorter than that of *Neolentinus lepideus* [19]. It was also higher than those of *Pichia burtonii* Y257-7 (55.6%) [11] and *Aspergillus oryzae* N159-1 (65.9%) [9].

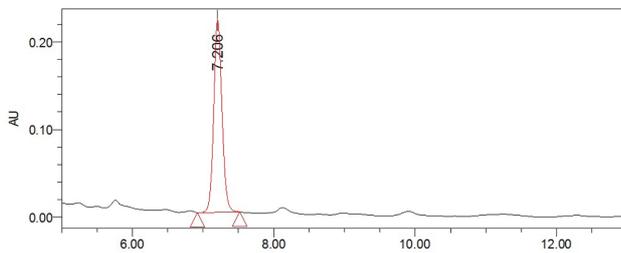
#### Purification of the $\alpha$ -glucosidase inhibitor

Sephadex G-100 filtration chromatography was performed on the water extracts of *Pleurotus cornucopiae* and an active fraction with an  $\alpha$ -glucosidase inhibitory activity of 17.30 mg/ml of IC<sub>50</sub> was obtained. After the active fraction was hydrolyzed by proteases and amylase, we obtained an active pepsin hydrolysate (IC<sub>50</sub>: 11.88 mg/ml). The active hydrolysate was then subjected to a Sephadex G-50 filtration chromatography. The active elutes had IC<sub>50</sub>, 10.72 mg/ml of  $\alpha$ -glucosidase inhibitory activity. The active elutes were applied to RP-

**Table 1. Effect of extraction time on the  $\alpha$ -glucosidase inhibitory activities of water extract from *Pleurotus cornucopiae* fruiting body.**

Extraction time (h)*	$\alpha$ -Glucosidase inhibitory activity of water extracts (%)
0.5	27.8 $\pm$ 0.1
1.0	38.8 $\pm$ 0.8
3.0	55.7 $\pm$ 0.6
6.0	64.9 $\pm$ 0.1
12.0	77.9 $\pm$ 0.8 (Yield; 42.7%)
24.0	48.5 $\pm$ 0.4
48.0	42.3 $\pm$ 0.7

\*Extraction temp.; 30°C.



**Fig. 1.** RP-HPLC chromatography on FORTIS C18 column of active fraction P-2-1 from Sephadex G-50 chromatography. Separation was performed isocratically by 20% acetonitrile in 0.1% acetic acid.

**Table 2.** Purification summary of  $\alpha$ -glucosidase inhibitor from *Pleurotus cornucopiae*.

Purification steps	$\alpha$ -Glucosidase inhibitory activity (IC <sub>50</sub> : mg/ml)	Solid yield (%)	Purification fold (times)
Water extract	23.20	42.7	1
Sephadex G-100 column chromatography	17.30	37.4	1.4
Pepsin hydrolysates	11.88	30.0	2.0
Sephadex G-50 column chromatography	10.72	12.5	2.2
RP-HPLC	9.10	12.2	2.6

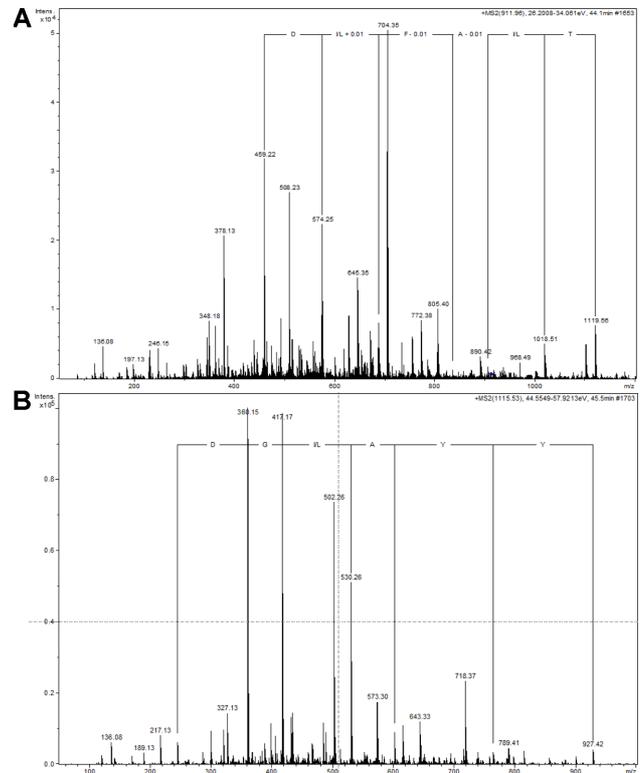
HPLC by using a Vydac protein/peptide reverse-phase 218T P54 column. The purified  $\alpha$ -glucosidase inhibitor also obtained a solid yield of 12.2% and an  $\alpha$ -glucosidase inhibitory activity of IC<sub>50</sub>, 9.10 mg/ml (Fig. 1, Table 2).

The inhibitory activity of the purified  $\alpha$ -glucosidase inhibitor was lower than that of *Aspergillus oryzae* (IC<sub>50</sub> 3.1 mg/ml) [9] and *Pichia burtonii* (IC<sub>50</sub> 0.82 mg/ml) [11].

#### Characterization of the $\alpha$ -glucosidase inhibitor

The purified  $\alpha$ -glucosidase inhibitor was analyzed by LC-MS/MS spectrometry and, finally, two hexapeptides that had sequences of Thr-Ile-Ala-Phe-Ile-Asp (A) and Tyr-Tyr-Ala-Ile-Gly-Asp (B) were obtained (Fig. 2). Their two A, B oligopeptides were chemically synthesized by Cosmogenetech. Co., Korea and their inhibitory activities were investigated. The inhibitory activities of their chemically synthesized oligopeptides showed IC<sub>50</sub>, 10.98 mg/ml (A) and 11.01 mg/ml (B), respectively. The molecular weight of the A and B oligopeptides were also estimated to be 678.79 Da and 643.7 Da, respectively.

Kang *et al.* [9] reported that the purified  $\alpha$ -glucosidase



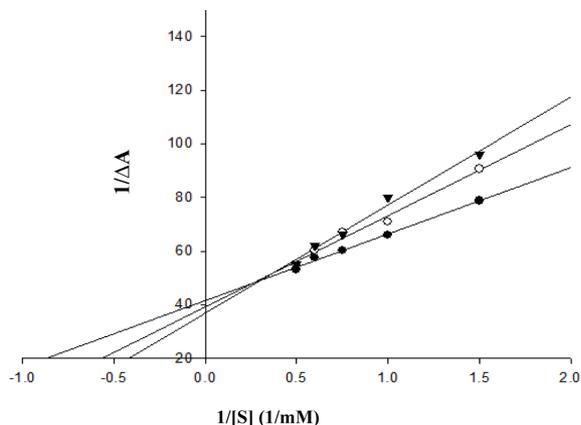
**Fig. 2.** Amino acid sequence of the two purified  $\alpha$ -glucosidase inhibitory peptides using liquid chromatography-tandem mass spectrometry. \*Reverse: (A) Thr-Ile-Ala-Phe-Ile-Asp, \*Reverse: (B) Tyr-Tyr-Ala-Ile-Gly-Asp.

inhibitor from *Aspergillus oryzae* was a tripeptide with a sequence of Pro-Phe-Pro and its molecular weight was 350.1 Da. Jeon [8] also reported that the  $\alpha$ -glucosidase inhibitor from *Streptomyces* sp. CK-4416 was purified by ion exchange chromatography and two kinds of purified  $\alpha$ -glucosidase inhibitors with C<sub>25</sub>H<sub>43</sub>NO<sub>20</sub> (M.W, 677.61 Da) and C<sub>37</sub>H<sub>63</sub>NO<sub>30</sub> (M.W, 1001.30 Da) were obtained. The inhibitor also showed inhibitory effects against blood glucose increment.

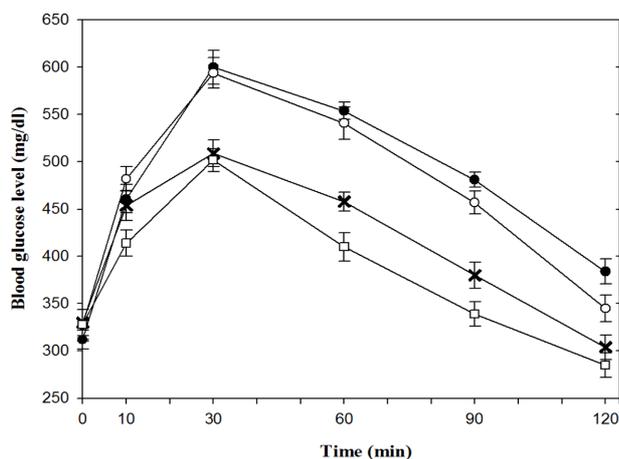
Furthermore, the purified  $\alpha$ -glucosidase inhibitor from *Pleurotus cornucopiae* showed a typical mixed-inhibition type to  $\alpha$ -glucosidase from the Lineweaver-Burk plot (Fig. 3).

#### Antihyperglycemic effect of the purified $\alpha$ -glucosidase inhibitor

The antihyperglycemic action of the purified  $\alpha$ -glucosidase inhibitor from RP-HPLC chromatography was evaluated in streptozotocin-induced diabetic rats.



**Fig. 3.** Lineweaver-Burk plot of the purified  $\alpha$ -glucosidase inhibitor on  $\alpha$ -glucosidase at different concentrations of chemical substrate, pNPG.  $\blacktriangledown$ : 1.0 mg of the purified inhibitor;  $\circ$ : 0.5 mg of the purified inhibitor;  $\bullet$ : control.



**Fig. 4.** Changes of blood glucose levels up to 120 minute after administration of 3 g/kg soluble starch and various concentration of the purified  $\alpha$ -glucosidase inhibitor from *Pleurotus cornucopiae* in streptozotocin-induced diabetic SD-rat.  $\bullet$ : Soluble starch + D.W.,  $\circ$ : Soluble starch + purified  $\alpha$ -glucosidase inhibitor (dosage; 50 mg/kg),  $\times$ : Soluble starch + purified  $\alpha$ -glucosidase inhibitor (dosage; 300 mg/kg),  $\square$ : Soluble starch + commercial anti-diabetic acarbose (dosage; 15 mg/kg).

As shown in Fig. 4, the blood glucose level at 30 min after administering the soluble starch (3 g/kg) significantly increased to 465–600 mg/dl from 315 mg/dl in diabetic rats and  $\alpha$ -glucosidase inhibitor-treated diabetic rats. After 120 min, the blood glucose level decreased to 325–360 mg/dl dose-dependently.

This suggests that the water extracts from the *Pleurotus cornucopiae* fruiting body containing the  $\alpha$ -glucosi-

dase inhibitor has an antiglycemic effect in diabetic rats at a dosage of 50 mg/kg. This tendency of decreased blood glucose contents in  $\alpha$ -glucosidase inhibitor-treated diabetic rats 120 min after oral administration was similar to those of the commercial anti-diabetic drug, acarbose, and water extracts (50–300 mg/kg) from *Neolentinus lepideus* fruiting body- containing  $\alpha$ -glucosidase inhibitors [19].

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## References

- Asano N, Oseki K, Tomioka E, Kizu H, Matsui, K. 1994. N-containing sugars from *Morus alba* and their glycosidase inhibitory activities. *Carbohydr. Res.* **259**: 243–255.
- Fatmawati S, Shimizu K, Kondo R. 2011. Ganoderol B: a potent  $\alpha$ -glucosidase inhibitor isolated from the fruiting body of *Ganoderma lucidum*. *Phytomed.* **18**: 1053–1055.
- Gutiérrez A, Caramelo L, Prieto A, Martínez MJ, Martínez AT. 1994. Anisaldehyde production and aryl-alcohol oxidase and dehydrogenase activities in ligninolytic fungi of the genus *Pleurotus*. *Appl. Environ. Microbiol.* **60**: 1783–1788.
- Gern RM, Wisbeck E, Rampinelli JR, Ninow JL, Furlan SA. 2008. Alternative medium for production of *Pleurotus ostreatus* biomass and potential antitumor polysaccharides. *Bioresour. Technol.* **99**: 76–82.
- Hue JJ, Kim JS, Kim JH, Nam SY, Yun YW, Jeong JH, et al. 2010. Anti-glycemic effect of L-carnosine in streptozotocin-induced diabetic mice. *Korean J. Vet. Res.* **50**: 105–111.
- Jang JH, Jeong SC, Kim JH, Lee YH, Ju YC, Lee JS. 2011. Characterization of a new antihypertensive angiotensin I-converting enzyme inhibitory peptide from *Pleurotus cornucopiae*. *Food. Chem.* **127**: 412–418.
- Jang IT, Hyun SH, Shin JW, Lee YH, Ji JH, Lee JS. 2014. Characterization of an anti-gout xanthine oxidase inhibitor from *Pleurotus ostreatus*. *Mycobiol.* **42**: 296–300.
- Jeon HS. 2001. Studies on the structural property of an  $\alpha$ -glucosidase inhibitor from *Streptomyces* sp. and its inhibitory characteristics of blood glucose increment [dissertation]. Seoul: Korea University.
- Kang MG, Yi SH, Lee JS. 2013. Production and characterization of a new  $\alpha$ -glucosidase inhibitory peptide from *Aspergillus oryzae* N159-1. *Mycobiol.* **41**: 149–154.
- Kim GY, Yoon YJ, Kim EJ. 2013. Improvement of lipid metabolism and antihyperglycemic by *Lentinus edodes* in high fat-fed and streptozotocin-treated rats. *Korean J. Orient. Physiol. Pathol.* **27**: 196–201.

11. Kim YH, Shin JW, Lee JS. 2014. Production and anti-hyperglycemic effects of  $\alpha$ -glucosidase inhibitor from yeast, *Pichia burtonii* Y257-7. *Korean J. Microbiol. Biotechnol.* **42**: 219–224.
12. Lee DH, Kim JH, Park JS, Choi YJ, Lee JS. 2004. Isolation and characterization of a novel angiotensin I-converting enzyme inhibitory peptide derived from the edible mushroom *Tricholoma giganteum*. *Peptides.* **25**: 621–627.
13. Marcelo BM, Cristiane MF, Kamilla FE, Marx ANL, Nilsa SYW, Erna EB. 2014. Effect of dietary supplementation with *Agaricus sylvaticus* schaeffer on glycemia and cholesterol after streptozotocin-induced diabetes in rats. *Evid. Complement. Altern. Med.* **2014**: 10–20.
14. Murai A, Iwamura K, Takada M, Ogawa K, Usui T, Okumura J. 2002. Control of postprandial hyperglycaemia by galactosyl maltobionolactone and its novel anti-amylase effect in mice. *Life. Sci.* **71**: 1405–1415.
15. Park HS, Lee YS, Choi SJ, Kim JK, Lee YL, Kim HG, et al. 2009. Effects of herbal complex on blood glucose in streptozotocin-induced diabetic rats and in mice model of metabolic syndrome. *Korean J. Pharmacogn.* **40**: 196–204.
16. Park JH, Chu WM, Lee JM, Park HR, Park EJ. 2011. Antihyperglycemic of *Gleditschia Spina* extracts in streptozotocin-nicotinamide induced type 2 diabetic rats. *J. Korean Soc. Food. Sci. Nutr.* **40**: 321–326.
17. Park YK, Kim JS, Jeon EJ, Kang MH. 2009. The improvement of Chaga mushroom (*Inonotus obliquus*) extract supplementation on the blood glucose and cellular DNA damage in streptozotocin-induced diabetic rats. *Korean J. Nutr.* **42**: 5–13.
18. Reese ET, Parrish FW, Ettlinger M. 1971. Nojirimycin and D-glucono-1,5-lactone as inhibitors of carbohydrases. *Carbohydr. Res.* **18**: 381–388.
19. Shin JW, Bae SM, Han SM, Lee YH, Jeong YK, Lee JS. 2015. Extraction and antihyperglycemic action of  $\alpha$ -glucosidase inhibitor from basidiomycetes, *Neolentinus lepideus*. *Korean J. Mycol.* **43**: 174–179.
20. Shipp GM. 2012. Purification of alpha-glucosidase inhibitors from grape extract [dissertation]. Wayne State University.
21. Sumisa F, Iijima N, Ando A, Shiga M, Kondo K, Amano K, et al. 2004. Properties of mycelial aggregate-specific lectin of *Pleurotus cornucopiae* produced in *Pichia pastoris*. *Biosci. Biotechnol. Biochem.* **4**: 959–960.
22. Wang H, Gao J, Ng TB. 2000. A new lectin with highly potent anti-hepatoma and antisarcoma activities from the oyster mushroom *Pleurotus ostreatus*. *Biochem. Biophys. Res. Commun.* **275**: 810–816.
23. Wehmeier UF, Piepersberg W. 2004. Biotechnology and molecular biology of the alpha-glucosidase inhibitor acarbose. *Appl. Microbiol. Biotechnol.* **63**: 613–625.

## 국문초록

### 식용버섯인 노랑느타리버섯으로부터 혈당상승억제성 $\alpha$ -glucosidase 저해제의 추출 및 특성

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식용버섯인 노랑느타리버섯으로부터  $\alpha$ -glucosidase 저해제의 추출과 정제에 대하여 연구하였다.  $\alpha$ -glucosidase 저해제는 노랑느타리버섯 자실체를 증류수로 30°C에서 12시간 처리하였을 때 가장 많이 추출되었다.  $\alpha$ -glucosidase 저해제를 sephadex G-100 여과 크로마토그래피와 펩신 가수분해, sephadex G-50 여과 크로마토그래피, 역상 HPLC로 정제한 결과 수율과  $\alpha$ -glucosidase 저해활성은 각각 12.2%와 IC<sub>50</sub> 9.10 mg/ml이었다. 정제한  $\alpha$ -glucosidase 저해제는 Thr-Ile-Ala-Phe-Ile-Asp (A)과 Tyr-Tyr-Ala-Ile-Gly-Asp (B)의 서열을 갖고 있는 두개의 헥사펩타이드를 함유하였고 이들의 분자량은 각각 (A)가 678.79 Da, (B)가 643.7 Da 이었다. 정제한  $\alpha$ -glucosidase 저해제는  $\alpha$ -glucosidase에 대하여 혼합형 저해양상을 보였고 streptozotocin으로 유도된 당뇨 쥐 모델에서 50 mg/kg과 300 mg/kg 투여시 혈당함량을 낮추어 주는, 농도 의존적 혈당상승억제 효과를 보였다.