J. Microbiol. Biotechnol. (2006), **16**(5), 757–763

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Production and Characterization of Antihypertensive Angiotensin I-Converting Enzyme Inhibitor from *Pholiota adiposa*

KOO, KYO-CHUL[1], DAE-HYOUNG LEE[1], JAE-HO KIM[2], HYUNG-EUN YU[1], JEONG-SIK PARK[3], AND JONG-SOO LEE[1]*

[1]*Department of Life Science and Genetic Engineering, Paichai University, Daejeon 302-735 Korea*
[2]*Bae Sang Myun Brewery Co., Ltd. Pocheon, Kyungi-do, 487-840, Korea*
[3]*Institute of Agricultural Science and Technology, Suwon 441-707, Korea*

Received: November 4, 2005
Accepted: December 29, 2005

Abstract Angiotensin I-converting enzyme (ACE) inhibitors have generally been very useful to remedy or prevent hypertension. This study describes the extraction and characterization of an ACE inhibitor from the fruiting body of *Pholiota adiposa* ASI 24012, which can be used as an antihypertensive drug. The maximal ACE inhibitory activity (IC_{50}; 0.25 mg) was obtained when the fruiting body of *Pholiota adiposa* ASI 24012 was extracted with distilled water at 30°C for 12 h. After the purification of ACE inhibitor with ultrafiltration, Sephadex G-25 column chromatography, and reverse-phase HPLC, an active fraction with an IC_{50} of 0.044 mg was obtained. The purified ACE inhibitory peptide was a novel pentapeptide, showing very little similarity to other ACE inhibitory peptide sequences. The molecular mass of the purified ACE inhibitor was estimated to be 414 daltons with a sequence of Gly-Glu-Gly-Gly-Pro, and showed a clear antihypertensive effect on spontaneously hypertensive rats (SHR) at a dosage of 1 mg/kg.

Key words: Antihypertensive angiotensin I-converting enzyme inhibitor, *Pholiota adiposa*

Angiotensin I-converting enzyme (ACE, dipeptidyl carboxy peptidase I, kinase II, E.C 3.4.15.1) is the key enzyme in the renin-angiotensin system, which catalyzes production of the active hypertensive hormone angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) from the inactive pro-hormone angiotensin I (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu). Bradykininogen from the liver is converted to active hypotensive bradykinin, but it turns into inactive fragments by kinase II. Thus, a constant blood pressure is normally maintained by a hypertensive (high pressure) peptide, angiotensin II, and a hypotensive (low pressure) peptide, bradykinin. This conversion action endows ACE with a very important role in regulating blood pressure through the direct action of angiotensin II on blood vessels, sympathetic nerves, and adrenal glands [10].

There are some known ways to prevent or remedy hypertension, such as the utilization of the ACE inhibitor or receptor blocker of angiotensin II. However, ACE inhibitors have long been very useful because they inhibit both the ACE in the renin-angiotensin system and the kallikrein-kinin system.

Since the ACE inhibitor was first discovered in snake venom [9], many antihypertensive ACE inhibitors have been isolated and characterized from various natural sources and microorganisms [8] including *Doratomyces putredinis*, *Nocardia orientalis*, *Streptomycetes*, *Actinomycetes*, *Actinomadura spiculosopora*, and *Actinomadura* sp. ACE inhibitory peptides have also been isolated from food or the enzymatic digestion of food proteins [2] including gelatin [24], casein [23], fish [3, 29, 30], fig tree latex [21], α-zein [22], and seven brown algae species such as *Ecklonia cava*, *Ishige akamurae*, *Sargassum fulvellum*, *Sargassum horneri*, etc. [4]. Other ACE inhibitors were found in sake and its by-products [28], Korean traditional rice wines and liquors [12], cereals and legumes [26], and microbes such as yeasts [14] and mushrooms [18].

In addition to these ACE inhibitors, Kohama *et al.* [17] isolated three kinds of ACE inhibitory peptides from Baker's yeast glyceraldehyde-3-phosphate dehydrogenase. Among them, YG-1 (Gly-His-Lys-Ile-Ala-Thr-Phe-Gln-Glu-Arg, IC_{50}: 0.4 μM) is the most potent yeast ACE inhibitor, and its sequences are quite different from those of the venom peptide family. Some research papers have reported on the antihypertensive effects from some ACE inhibitors: *Grifola frondosa* [5], *Ganoderma lucidum* [23], and *Tricholoma giganteum* [19]. Morigiwa *et al.* [23] isolated strong antihypertensive triterpene compounds, such as ganoderal

*Corresponding author
Phone: 82-42-520-5388; Fax: 82-42-520-5388;
E-mail: biotech8@pcu.ac.kr

A, ganoderols A and B, and ganoderic acids K and S from a 70% methanol extract of *Ganoderma lucidum*. Recently, Lee *et al.* [19] isolated an ACE inhibitory peptide from *Tricholoma gigantum*, which is a novel tripeptide with the sequence of (Gly-Glu-Pro) and shows very little similarity to the sequences of other ACE inhibitory peptides.

Even though many natural and synthetic ACE inhibitors, such as captopril, enalapril, and lisinopril, are remarkably effective as antihypertensive drugs, they show certain disadvantages, such as easy digestion by protease in the body, and side effects such as coughing, allergies, taste disturbances, and skin rashes. Therefore, the development of novel ACE inhibitors that have a strong activity and strong resistance to various protease attacks without side effects is essentially required.

Recently, mushrooms have received attention, because they are nutritious foods with health-stimulating properties and medicinal effects [12, 15, 16, 18, 25, 32, 34]. *Pholiota adiposa kumm,* commonly called "yellow-cap fungus," is classified as part of the *Pholiota* genus of the *Strophariaceae* family. This mushroom is found in Korea, Japan, China, Europe, and North America. Few studies have been done on the pharmaceutical effects of *Pholiota adiposa*, except for its antibiotic and antitumor activities.

In this paper, we describe the extraction and characterization of a novel ACE inhibitor from the fruiting body of *Pholiota adiposa*, which can be used as an antihypertensive drug.

Material and Methods

Materials and Chemicals

The Korean mushrooms used were obtained from the National Institute of Agriculture Science and Technology in Suwon, South Korea. The angiotensin I-converting enzyme (ACE) used in this study was extracted overnight from rabbit lung acetone powder using 100 mM sodium borate buffer (pH 8.3) containing 300 mM NaCl at 4°C. Hippuric acid-Histidine-Leucine (Hip-His-Leu) was used as a substrate in the determination of ACE activity. Unless otherwise specified, all chemicals and solvents were of analytical grade. The rabbit lung acetone powder for ACE and Hip-His-Leu were obtained from Sigma Chemical Co. (St. Louis, U.S.A.), and Sephadex G-25 was purchased from Pharmacia Fine Chemicals (Uppsala, Sweden). Trypsin, trifluoroacetic acid, and acetonitrile were purchased from Sigma Chemical Co. (St. Louis, U.S.A.). Spontaneously hypertensive male rats (SHR), Sam:TacN(SHR)fBR, weighing 280 to 300 g and 11 weeks old, were purchased from Samtaco Bio-Korea Co. (Osan, Korea).

Extraction of Mushroom

The dried fruiting bodies of mushrooms (5 g) were pulverized, and they were extracted with 200 ml each of water, ethanol, and methanol. The extractions were carried out at 30°C, while stirring for 12 h. The mixtures were centrifuged at 10,000 $\times g$ for 20 min and filtered with Whatman No. 41 filter paper. Each supernatant was lyophilized for analysis.

Assay of the ACE Inhibitory Activity

The ACE inhibitory activity was assayed according to the modified method of Cushman and Cheung [7]. A mixture containing 100 mM sodium borate buffer (pH 8.3), 300 mM NaCl, 3 units of ACE from rabbit lungs, and an appropriate amount of the inhibitor solution 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 it was terminated after 30 min of incubation by adding 250 µl of 1.0 M HCl. The hippuric acid liberated was extracted with 1 ml of ethyl acetate, and 0.8 ml of the extract was evaporated using a Speed Vac Concentrator (EYELA Co., Japan). The residue was then dissolved in 1 ml of sodium borate buffer. Absorbance at 228 nm was measured to estimate the ACE inhibitory activity. The concentration of the ACE inhibitor required to inhibit 50% of the ACE activity under the above assay conditions was defined as IC_{50}.

Purification of ACE Inhibitor from *Pholiota adiposa*

The water extract solution was ultrafiltrated with a 5,000 M.W. cut-off filter (Labscale TFF System, Millipore Co., U.S.A.), and the ACE inhibitory activities in the filtrates and the solution of the filter cake were determined. The active fraction was lyophilized, and 10 g in distilled water was applied to a Sephadex G-25 column (3.0×80 cm; Pharmacia Fine Chemicals, Sweden) equilibrated and eluted with the same buffer at a flow rate of 24 ml/h. The active fractions obtained were then applied to a preparative reverse-phase high-performance liquid chromatography (µBondapack C_{18} column) equilibrated with acetonitrile. A linear gradient elution was carried out with 0.1% trifluoroacetic acid (TFA) in water from 0 to 100%. The active fractions were collected and lyophilized immediately. The active fractions obtained were then subjected to an analytical reverse-phase high-performance liquid chromatography (µ Vydac protein/peptide reversed-phase 218Tp column) equilibrated with 0.1% TFA in water. A linear gradient elution was carried out with 0.1% TFA in the water from acetonitrile 0 to 100%.

Molecular Weight and Amino acid Sequence of the ACE Inhibitor

The molecular mass of the purified ACE inhibitor was determined using MALDI-MS (Voyager-DES TR) [22], and the amino acid sequence was determined by the Edman method using an Applied Biosystems 491A automatic protein sequencer [31].

Table 1. ACE inhibitory activities of the various extracts from mushrooms.[d]

Species	Strain (ASI)[b]	ACE inhibitory activity (IC_{50}, mg)[a]		
		Water extract	Ethanol extract	Methanol extract
Pholiota adiposa	24002	0.57±0.07[c]	>1	>1
	24004	0.49±0.04	>1	>1
	24005	0.81±0.05	>1	>1
	24007	0.76±0.03	>1	>1
	24008	0.42±0.04	>1	>1
	24010	0.95±0.05	>1	>1
	24012	0.21±0.03	>1	>1
	24017	0.28±0.06	>1	>1
	24018	0.29±0.07	>1	>1
	24022	0.38±0.04	>1	>1
	24024	0.45±0.05	>1	>1
	24027	0.31±0.04	>1	>1
Inonotus obliquus	MA	>1	0.36±0.04	0.42±0.06
Grifola frondosa	9012	0.31±0.04	>1	>1
	9021	0.28±0.05	0.39±0.06	>1

[a]Samples showing IC_{50} of 1 mg below after secondary screening.
[b]Strain No of National Institute of Agricultural Science and Technology.
[c]Mushroom (1 g) were extracted from water, ethanol, and methanol at 30°C for 12 h.
[d]Values are means±S.D. of three determinations.

Determination of Inhibition Pattern on ACE

To investigate the inhibition pattern of the ACE, the ACE inhibitory activity was measured with different concentrations of the substrate. The kinetics of ACE in the presence of the inhibitor were determined by the Lineweaver-Burk plot [5].

Antihypertensive Action of the Purified ACE Inhibitor

The purified ACE inhibitor from *Pholiota adiposa* was orally administered to the SHRs at a dosage of 1 mg/kg, and then the systolic blood pressure was measured before as well as after 0.15 h, 2 h, 4 h, and 6 h of administration from rat tail using a specially devised Blood Pressure Monitoring System (IWORX, U.S.A.). Each experimental group consisted of 4 SHRs, and a negative and a positive control group were also provided: The positive control group was administered a commercial antihypertensive drug, captopril (ACE inhibitor), at a dose of 1 mg/kg, whereas saline was administered to the negative control group. Prior to the administration of the purified ACE inhibitor, the blood pressures of the SHRs were measured four times during a one-week period, and the test groups were selected according to their average blood pressure. While the ACE inhibitor was being administered, the blood pressure of each group was measured three times during every test.

Results and Discussion

ACE Inhibitory Activities of Various Extracts from Mushrooms

To select a potent ACE inhibitor-producing mushroom, extracts (water, ethanol, and methanol extracts) from the fruiting bodies of 63 edible mushrooms were prepared to determine their ACE inhibitory activities. As shown in Table 1, the water extracts from all mushrooms tested had higher ACE inhibitory activities than those from other extracts. The water extracts from *Pholiota adiposa* ASI 24012 showed the greatest inhibitory activity, and its IC_{50} value was determined to be 0.21 mg. Therefore, *Pholiota adiposa* ASI 24012 was selected as a producer of ACE inhibitors.

The ACE inhibitory activity of *Pholiota adiposa* was higher than those of water extracts from *T. giganteum* (61.3%±0.5), *G. frondosa* (58.7%±0.2, IC_{50}: 0.28 mg), *I. obliquus* and *F. fraxinea* (IC_{50}>1 mg), *C. versicolor* (37.7%±0.3), and *P. coccinea* (37.5%±0.5) [18], whereas it was lower than those of hydrolysates of some cereals and legumes [26] and Flaourzyme digests of brown algae, *Ecklonia cava* protein (IC_{50}: 0.23 μg/ml) [4]. Since the ACE inhibitor was first discovered from snake venom, many antihypertensive ACE inhibitors have been isolated from natural sources, including food or enzymatic digestion of food proteins and mushrooms. However, ACE inhibitors from only a few kinds of mushrooms, such as *Pholiota adiposa*, *T. giganteum*, *G. frondosa*, and *Ganderma lucidum*, have been characterized [18, 19, 23].

Optimal extraction conditions of the ACE inhibitor from *Pholiota adiposa* ASI 24012 were investigated in the range of 10°C–70°C and from 1 to 18 h. As the extraction time increased to 12 h, the ACE inhibitory activity of the water extracts increased, and then remained constant without any change. The highest ACE inhibitory activity of water extracts from the *Pholiota adiposa* fruiting body (67.6%, IC_{50}; 0.21 mg) was obtained when the extraction was

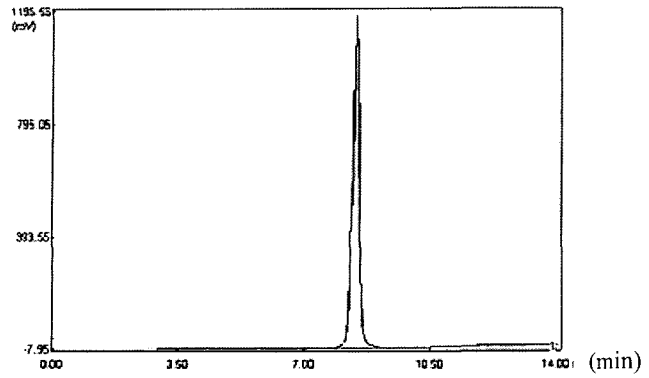

Fig. 1. Reverse-phase HPLC profile of the active fraction from μ Vydac protein/peptide reversed-phase 218Tp column.

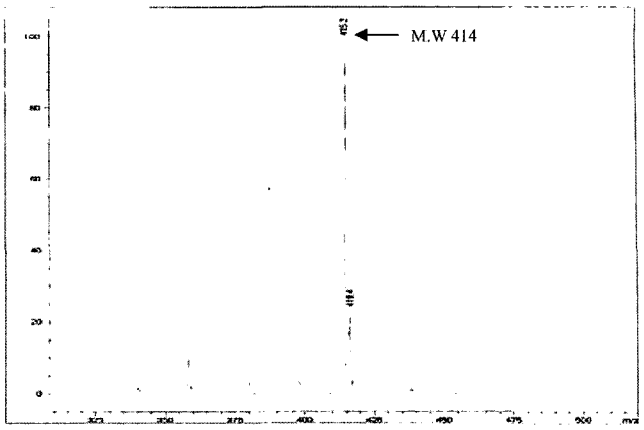

Fig. 2. Mass spectrum of the ACE inhibitor purified from *Pholiota adiposa.*

performed at 30°C for 12 h, and the hot water extraction showed lower activity, suggesting that higher temperatures may degrade the ACE inhibitor (data not shown).

Purification of the ACE Inhibitor

The extracts of *Pholiota adiposa* ASI 24012 were ultrafiltrated with a 5,000 M.W. cut-off filter (Labscale TFF System, Millipore Co., U.S.A.), and the ACE inhibitory activity of the filtrates was then determined. The active fraction was concentrated by lyophilization. The ACE inhibitory activity of the filtrates from 5,000 M.W. cut-off ultrafiltration of the water extracts was 0.21 mg of IC_{50}. After Sephadex G-25 column chromatography, the active fraction showed 0.19 mg of IC_{50}. The active fractions from the above column chromatography were collected and subjected to a preparative reverse-phase HPLC using a μBondapack C_{18} column. One peak containing ACE inhibitory activity was obtained (IC_{50}: 0.12 mg). After that, two reverse-phase HPLCs were performed for the active fraction. When subjected to the reverse-phase HPLC using a μVydac protein/peptide reverse-phase 218TP column, a single peak was obtained (Fig. 1), and the ACE inhibitor with an IC_{50} of 0.044 mg was obtained (Table 2).

Molecular Weight of the ACE Inhibitor

The molecular weight of the purified ACE inhibitor from *Pholiota adiposa* ASI 24012 was 414 daltons, estimated by MALDI-MS analysis (Fig. 2). Although its molecular weight was very small compared with the others, which mostly are oligopeptides, the ACE inhibitor purified from *Pholiota adiposa* ASI 24012 is considered to be able to prevent degradation in the stomach by some proteases and further, more suitable for absorption in the intestine.

Table 2. Summary of the purification of an ACE inhibitor from *Pholiota adiposa* ASI 24012.

Purification step	ACE inhibitory activity (IC_{50}: mg)
Water extract	0.21
Ultrafiltraction	0.28
Sephadex G-25	0.15
Prep-RP-HPLC	0.12
First-RP-HPLC	0.098
Second-RP-HPLC	0.044

Amino Acid Sequence of the ACE Inhibitor

The amino acid composition of the ACE inhibitor was identified as Pro (20%), Glu (20%), Gly (60%), and its sequence was found to be Gly-Glu-Gly-Gly-Pro. This peptide was composed of a hydrophobic amino acid at the amino-terminal, a neutral Q amino acid residue at the center, and a proline at the carboxy-terminal. This peptide is a novel ACE inhibitor, which has not previously been found in any proteins. It is known that almost all ACE inhibitors are peptides, except for triterpene from *G. lucidum* [23] and a complex of glycoprotein and polyphenol from *Ecklonia cava* [4]. There are many various sequences of ACE inhibitors reported, ranging from tripeptide to oligopeptides [14, 18]. Some peptapeptides of ACE inhibitors are listed in Table 3 and compared with ACE inhibition. Although the ACE inhibitor in this study was weaker than captopril, a commercial antihypertension drug, and some peptides, it is the first reported unique pentapeptide with a sequence of GQGGP and with no other homologies. Furthermore, based on their interaction with ACE, substrates, and inhibitory peptides [11, 20], our purified peptide among the three groups of ACE inhibitory peptides can be considered to be a true ACE inhibitor, because the inhibitory activities (IC_{50} value) for the purified peptide before and after preincubation with ACE were found to be the same.

Determination of ACE Inhibitory Pattern and Resistance to Protease

The inhibitory pattern of the purified ACE inhibitor was investigated using a Lineweave-Burk plot (Fig. 3). It was

Table 3. Comparison of sequence and ACE inhibitory activity between ACE inhibitory pentapeptides from *Pholiota adiposa* ASI 24012 and hydrolysate of several proteins.

Peptides	Source	Preparation	IC_{50} (μM)[a]	References
VHLPP	γ-Zein	Synthesis	18	[18]
IWHHT	Bonito muscle	Thermolysin	5.1	[30]
ALPHA	Bonito muscle	Thermolysin	10	[30]
LKPNM	Bonito muscle	Thermolysin	17	[30]
MNPPK	Porcine myosin	Thermolysin	945.5	[1]
ITTNP	Porcine myosin	Thermolysin	549.0	[1]
IYPRY	Sake lees	Protease	4.1	[25]
GQGGP	*Pholiota adiposa* ASI 24012	-	254.0	This study
Captopril			1.8	Commercial drug

[a]The concentration of an ACE inhibitor required to inhibit 50% of ACE activity.

found that this ACE inhibitor had a competitive inhibitory pattern, similar to that of *T. giganteum*, *G. frondosa*, and others such as fibrinogen pentapeptides, casein fragments, porcine plasma tripeptides, tuna muscle octapeptides, etc. [3]. Cheung *et al.* [6] reported that tryptophan, tyrosine, proline, or phenylalanine at the carboxy-terminal and branched-chain aliphatic amino acids at the amino-terminal are necessary for a peptide to bind to an ACE as a competitive inhibitor.

When the ACE inhibitor was treated with pepsin and trypsin, the ACE inhibitory activities decreased only slightly to 97.8% and 96.5%, respectively, compared with control (without treatment of protease) (data not shown), because there is no digestive site for these proteases in the ACE inhibitory peptide from *Pholiota adiposa* ASI 24012. Furthermore, the results were similar to that of ACE inhibitory peptides from *T. giganteum* [19], indicating that it would be stable in the stomach when the ACE inhibitory peptide was orally administered.

Antihypertensive Action of the Purified ACE Inhibitor

As shown in Fig. 4, the average blood pressure of the rats in the ACE inhibitor group was about 190 mmHg just before administration of the ACE inhibitor. Two hours after administering the ACE inhibitor at a dose of 1 mg/kg/rat, the blood pressure measured decreased to 168 mmHg; and the average blood pressure then increased slightly. It was similar to that of the commercial antihypertensive drug, captopril. This suggests that the purified ACE inhibitor produces a clear antihypertensive effect in SHRs at a dose of 1 mg/kg. Although the ACE inhibitor from *Pholiota adiposa* ASI 24012 was weaker *in vivo* than the commercial antihypertension drug, captopril, the present ACE inhibitor is a novel peptide derived from a mushroom that is eaten

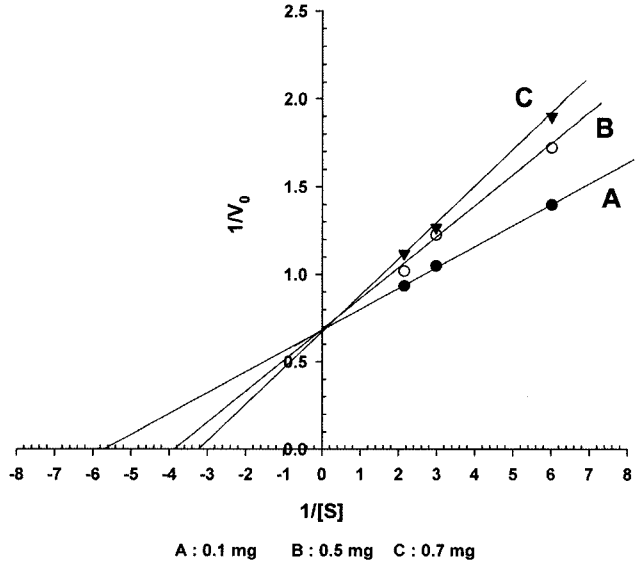

Fig. 3. Lineweaver-Burk plot of ACE activity in the presence of inhibitor, P1.

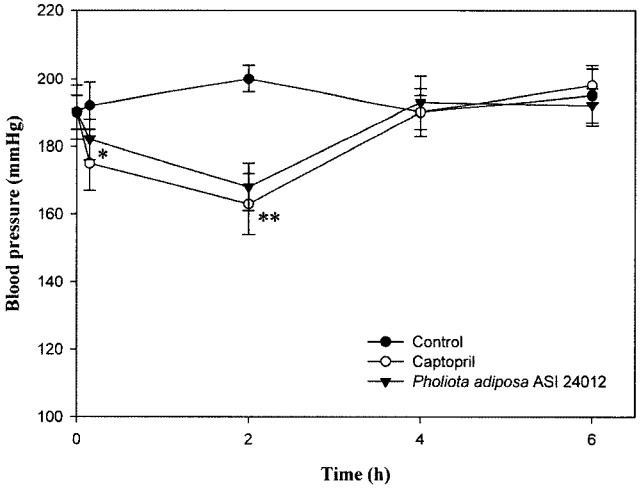

Fig. 4. Effect of orally administered ACE inhibitor from *Pholiota adiposa* on blood pressure in SHRs.
(▼) ACE inhibitor 1 mg/kg, (○) positive control (captopril) 1 mg/kg, (●) negative control. (*, **) Significantly different from test group at $p<0.05$ by Tukey's test.

daily, and it is also stable toward gastrointestinal proteases. Therefore, the ACE inhibitory peptide from *Pholiota adiposa* ASI 24012 would be very useful in the preparation of antihypertensive drugs and functional foods.

Acknowledgment

This study was supported by ARPC (Agriculture R&D Promotion Center) Technology Development Program for Agriculture and Forestry, Ministry of Agriculture and Forestry, Republic of Korea.

References

1. Arihara, K., Y. Nakashima, T. Mukai, S. Ishikawa, and M. Itoh. 2001. Peptide inhibitors for angiotensin I-converting enzyme from enzymatic hydrolysates of porcine skeletal muscle proteins. *Meat Sci.* **57:** 319–324.
2. Ariyosh, Y. 1993. Angiotensin converting enzyme inhibitors derived from food protein. *Trends Food Sci. Technol.* **4:** 139–144.
3. Astawan, M., M. Wahyuni, T. Yasuhara, K. Yamada, T. Tadokoro, and A. Maekawa. 1995. Effects of angiotensin-I converting enzyme inhibitory substance derived from Indonesian dried-salted fish on blood pressure of rats. *Biosci. Biotechnol. Biochem.* **59:** 425–429.
4. Athukorala, Y. and Y. J. Jeon. 2005. Screening for angiotensin I-converting enzyme inhibitory activity of *Ecklonia cava*. *Korean J. Food Sci. Nutr.* **10:** 134–139.
5. Choi, H. S., H. Y. Cho, H. C. Yang, K. S. Ra, and H. J. Suh. 2001. Angiotensin I-converting enzyme inhibitor from *Grifola frondosa*. *Food Res. Int.* **34:** 177–182.
6. Cheung, H. S., F. L. Wang, M. A. Ondetti, E. F. Sabo, and D. W. Cushman. 1980. Binding of peptide substrates and inhibitors of angiotensin-converting enzyme. Importance of the COOH-terminal dipeptide sequence. *J. Biol. Chem.* **255:** 401–407.
7. Cushman, D. W. and H. S. Cheung. 1971. Spectrophotometric assay and properties of the angiotensin converting enzyme of rabbit lung. *Biochem. Pharmacol.* **20:** 1637–1648.
8. Demain, A. L., G. A. Somkuti, J. C. Hunter-Cevera, and H. W. Rossmoore. 1989. *Novel Microbial Products for Medicine and Agriculture*. Elsevier Science Publisher Co.
9. Ferreira, S. H., D. C. Bartelt, and L. J. Greene. 1970. Isolation of bradykinin-potentiating peptides from *Bothrops jararaca* venom. *Biochemistry* **9:** 2583–2592.
10. Folkow, B., B. Johansson, and S. Mellander. 1961. The comparative effects of angiotensin and noradrenaline on consecutive vascular sections. *Acta Physiol. Scand.* **53:** 99–104.
11. Fujita, H. and M. Yoshikawa. 1999. A prodrug-type ACE-inhibitory peptide derived from fish protein. *Immunopharmacology* **44:** 123–127.
12. Jeong, S. C., S. P. Cho, B. K. Yang, Y. T. Jeong, K. S. Ra, and C. H. Song. 2004. Immunomodulating activity of the exopolymer from submerged mycelial culture of *Phellinus pini*. *J. Microbiol. Biotechnol.* **14:** 15–21.
13. Kim, J. H., D. H. Lee, S. Y. Choi, and J. S. Lee. 2002. Characterization of physiological functionalities in Korean traditional liquors. *Korean Food Sci. Technol.* **34:** 118–122.
14. Kim, J. H., D. H. Lee, S. C. Jeong, K. S. Chung, and J. S. Lee. 2004. Characterization of antihypertensive angiotensin I-converting enzyme inhibitor from *S. cerevisiae*. *J. Microbiol. Biotechnol.* **14:** 1318–1323.
15. Kim, K. B., S. I. Kim, and K. S. Song. 2003. Neuraminidase inhibitors from mushroom *Microphorus affinis*. *J. Microbiol. Biotechnol.* **13:** 778–782.
16. Kim, H. H., J. G. Na, Y. K. Chang, G. T. Chun, S. J. Lee, and Y. H. Jeong. 2004. Optimization of submerged culture conditions for mycelial growth and exopolysaccharides production by *Agaricus blazei*. *J. Microbiol. Biotechnol.* **14:** 944–951.
17. Kohama, Y., Y. Nagase, H. Oka, T. Nakagama, T. Teramoto, N. Murayama, H. Tsujibo, Y. Inamori, and T. Mimura. 1990. Production of angiotensin-converting enzyme inhibitors from Baker's yeast glyceraldehyde-3-phosphate dehydrogenase. *J. Pharmacobio-Dyn.* **13:** 766–771.
18. Lee, D. H., J. H. Kim, J. C. Cheang, W. S. Gong, Y. B. Yoo, J. J. Park, C. H. Yoo, and J. S. Lee. 2003. Screening of mushrooms having antihypertensive angiotensin I-converting enzyme inhibitor. *Korea J. Mycol.* **3:** 148–154.
19. Lee, D. H., J. H. Kim, J. S. Park, C. H. Yoo, and J. S. Lee. 2004. Isolation and characterization of a novel angiotensin I-converting enzyme inhibitory peptide derived from the edible mushroom *Tricholoma giganteum*. *Peptides* **25:** 621–627.
20. Marczak, E. D., H. Usui, H. Fujita, Y. Yang, M. Yokoo, A. W. Lipkowski, and M. Yoshikawa. 2003. New antihypertensive peptides isolated from rapeseed. *Peptides*. **24:** 791–798.
21. Maruyama, S., S. Miyoshi, and H. Tanaka. 1989. Angiotensin converting enzyme inhibitor derived from *Ficus carica*. *Agric. Biol. Chem.* **53:** 2763–2769.
22. Miyoshi, S., H. Ishikawa, T. Kaneko, F. Fukui, H. Tanaka, and S. Maruyama. 1991. Structures and activity of angiotensin-converting enzyme inhibitors in an α-zein hydrolysate. *Agric. Biol. Chem.* **55:** 1313–1318.
23. Morigiwa, A., A. Kitabatake, Y. Fujimoto, and N. Ikekawa. 1986. Angiotensin converting enzyme inhibitory triterpenes from *G. lucidum*. *Chem. Pharmaceut. Bull.* **34:** 3025–3028.
24. Ondetti, M. A., B. Rubin, and D. W. Cushman. 1982. Enzyme of the renin-angiotensin system and their inhibitors. *Annu. Rev. Biochem.* **51:** 283–308.
25. Park, J. S., K. W. Hyun, S. B. Seo, S. M. Cho, C. H. Yoo, and J. S. Lee. 2003. Detection of platelet aggregation inhibitors and fibrinolytic substances from mushrooms. *Korea J. Mycol.* **31:** 114–116.
26. Rhyu, M. R., Y. J. Nam, and H. Y. Lee. 1996. Screening of angiotensin converting enzyme inhibitors in cereals and legumes. *Foods Biotechnol.* **5:** 334–337.
27. Saito, Y., K. Wanezaki, A. Kawato, and S. Imayasu. 1992. Angiotensin-I converting enzyme inhibitor in sake and its by-product. *Nippon Nogeikagaku Kaishi* **66:** 1081–1087.

28. Saito, Y., K. Wanezaki, A. Kawato, and S. Imayasu. 1994. Structure and activity of angiotensin I converting enzyme inhibitory peptides from sake and sake lees. *Biosci. Biotechnol. Biochem.* **58:** 767–1771.
29. Sugiyama, K., K. Takada, M. Egawa, I. Yamamoto, H. Onzuka, and K. Oba. 1991. Hypertensive effect of fish protein hydrolysate. *Nippon Nogeikagaku Kaishi* **65:** 35–41.
30. Sun, H. J., S. J. Cho, J. H. Whang, H. Lee, and H. C. Yang. 1997. Characterization of angiotensin converting enzyme inhibitor from squid hydrolysate. *Foods Biotechnol.* **6:** 122–128.
31. Totowa, N. J. 1994. *Basic Protein and Peptide Protocols.* Humana Press, New Jersey, U.S.A.
32. Yang, B. K., M. A. Wilson, K. Y. Cho, and C. H. Song. 2004. Hypoglycemic effect of exo- and endo-biopolymers produced by submerged mycelial culture of *Ganoderma lucidum* in streptozotocin-induced diabetic rats. *J. Microbiol. Biotechnol.* **14:** 972–977.
33. Yokoyama, K., H. Chiba, and M. Yoshikawa. 1992. Peptide inhibitors for angiotensin I-converting enzyme from thermolysin digest of dried bonito. *Biosci. Biotech. Biochem.* **56:** 1541–1545.
34. Zhao, M. W., J. Y. Zhong, W. Q. Liang, N. Wang, M. J. Chen, D. B. Zhang, Y. J. Pan, and S. C. Jong. 2004. Analysis of squalene synthase expression during the development of *Ganoderma lucidum*. *J. Microbiol. Biotechnol.* **14:** 116–120.