Mycobiology 38(2) : 89-96 (2010)
DOI:10.4489/MYCO.2010.38.2.089

Mycelial Propagation and Molecular Phylogenetic Relationships of Commercially Cultivated *Agrocybe cylindracea* based on ITS Sequences and RAPD

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(Received April 12, 2010. Accepted April 16, 2010)

This study evaluated the optimal vegetative growth conditions and molecular phylogenetic relationships of eleven strains of *Agrocybe cylindracea* collected from different ecological regions of Korea, China and Taiwan. The optimal temperature and pH for mycelial growth were observed at 25°C and 6. Potato dextrose agar and Hennerberg were the favorable media for vegetative growth, whereas glucose tryptone was unfavorable. Dextrin, maltose, and fructose were the most effective carbon sources. The most suitable nitrogen sources were arginine and glycine, whereas methionine, alanine, histidine, and urea were least effective for the mycelial propagation of *A. cylindracea*. The internal transcribed spacer (ITS) regions of rDNA were amplified using PCR. The sequence of ITS2 was more variable than that of ITS1, while the 5.8S sequences were identical. The reciprocal homologies of the ITS sequences ranged from 98 to 100%. The strains were also analyzed by random amplification of polymorphic DNA (RAPD) using 20 arbitrary primers. Fifteen primers efficiently amplified the genomic DNA. The average number of polymorphic bands observed per primer was 3.8. The numbers of amplified bands varied based on the primers and strains, with polymorphic fragments ranging from 0.1 to 2.9 kb. The results of RAPD analysis were similar to the ITS region sequences. The results revealed that RAPD and ITS techniques were well suited for detecting the genetic diversity of all *A. cylindracea* strains tested.

KEYWORDS : ***Agrocybe cylindracea*, ITS, Mycelial growth, RAPD, rDNA**

Agrocybe cylindracea, known as black poplar mushroom, belongs to the family Bolbitiaceae of the order Agaricales [1]. *A. cylindracea* is a newly cultivated mushroom in East Asia that has become increasingly popular, due to its delicious taste and unique texture. It is cultivated mainly on low cost substrates derived from agricultural and forest wastes, barley, wheat straw, orange peel, grape stalks, rice husks and sawdust of broad leaf trees, as well as on the stumps of cottonwoods [2]. Sirand-Pugnet and Labarère [3] reported that pileus coloration, the number of spores produced per basidium and the size of basidiospores are all variable. Moreover, fruiting body size is also usually variable within a species. *A. cylindracea* is currently being widely studied for its medicinal properties, especially for its antioxidant, antimutagenic, antitumor, antifungal, hypercholesterolemic, and hyperlipidemic properties, its ability to decrease blood sugar [4], and its immuno-stimulating and lipid peroxidation inhibitory activities [5, 6]. Within *A. cylindracea*, the antifungal protein agrocybin inhibits the activity of HIV-1 reverse transcriptase [7, 8]. Therefore, this mushroom could be used to combat human diseases. Mycelium cultivation is enhanced by different environmental and nutritional factors as well as propagation of mycelia is an earlier and essential step to artificial cultivation of mushrooms [9].

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Molecular phylogenetic studies have demonstrated that the internal transcribed spacer (ITS) region has generally been considered for the molecular identification of fungi at species level [10]. Among the molecular approaches, random amplification of polymorphic DNA (RAPD) is a convenient method for detecting genetic diversity [11, 12], and it was particularly successful when applied to the verification of mushroom strains from a wide range of geographical origins [13, 14]. The purpose of this study was to investigate the optimal culture conditions for the vegetative growth and molecular phylogenetic relationships of the selected strains of *A. cylindracea*. The various environmental and nutritional parameters were studied in these experiments.

Materials and Methods

Mushroom strains. Eleven cultivated strains of *A. cylindracea* were collected from different ecological regions of China (IUM-1437, IUM-1571, IUM-1901, and IUM-2028), Korea (IUM-0737, IUM-1665, IUM-1803, and IUM-1811), and Taiwan (IUM-1389, IUM1590, and IUM-2147). Pure cultures were deposited in the Culture Collection and DNA Bank of Mushrooms (CCDBM), and an accession number was acquired for Incheon University Mushroom (IUM). AY-168826, FJ-869183, FJ-869184, and EU-487011 were used as control strains for phylogenetic comparison with

selected IUM strains. Sequencing data of the control strains were collected from the National Center for Biotechnology Information (NCBI) GenBank database.

Temperature and pH. Five different temperatures, 15, 20, 25, 30, and 35°C, were used to find the optimum temperature for the mycelial growth of *A. cylindracea*. A 5 mm diameter agar plug was removed from 10 day old cultures and placed in the center of a potato dextrose agar (PDA) plate. The medium was adjusted to pH 6 and incubated for 10 days at 15, 20, 25, 30, and 35°C. To determine the optimum pH, the medium was adjusted to pH 5, 6, 7, 8, and 9 by the addition of 1 N NaOH or HCl before autoclaving. Samples were incubated for 10 days at 25°C. Mycelial growth was measured according to a previously described method [15].

Culture media. Ten different types of culture media, (Czapek dox, glucose peptone, glucose tryptone, Hamada, Hennerberg, Hoppkins, Lilly, mushroom complete, potato dextrose agar, and yeast malt extract) were used to investigate the mycelial growth of *A. cylindracea*. The different types of culture media were prepared according to a previously described method [16]. The media were adjusted to pH 6 before being autoclaved.

Carbon and nitrogen sources. Experiments were performed on basal medium (0.05 g $MgSO_4$, 0.46 g KH_2PO_4, 1.0 g K_2HPO_4, 120 μg thiamine-HCl, 20 g agar, and 1 litter of distilled water) supplemented with one of 10 available carbon sources (dextrin, fructose, galactose, glucose, lactose, maltose, mannose, sorbitol, sucrose, and xylose) and 10 nitrogen sources (alanine, ammonium acetate, ammonium phosphate, arginine, calcium nitrate, glycine, histidine, methionine, potassium nitrate, and urea). To evaluate the most favorable carbon and nitrogen source for mycelial growth, each carbon source along with 5 g of peptone was added to the basal medium separately at the concentration of 0.1 M and mixed thoroughly. Each nitrogen source along with 20 g of glucose was supplemented to the basal medium at a concentration of 0.02 M [17]. In both cases, the basal medium was adjusted to pH 6 before autoclaving.

DNA extraction. Genomic DNA was extracted according to a previously described procedure [18] with some modifications. Fresh mycelia were collected from 10 day old cultures grown on PDA medium and frozen with liquid nitrogen. Frozen mycelia were ground with a sterilized mortar-pestle and kept in 1.5 mL micro-tubes. Five hundred microliters of extraction buffer (equal volumes of 50 mM Tris-HCl [pH 7.5], 50 mM EDTA [pH 8], and 1% sarkosyl) was added to each of the micro-tubes and incubated at 65°C for 30 min. After incubation, the same volume of PCI (25 mL phenol; 24 mL chloroform; 1 mL isoamyl-alcohol) was added, and samples were vortexed and centrifuged at 4°C and 12,000 rpm for 10 min. Afterwards, the upper phase was transferred to a 1.5 mL microtube, 1,000 μL of 99.9% alcohol was added and then it was centrifuged at 4°C, 5 min 12,000 rpm. Subsequently, the supernatant was removed, 500 μL of 70% alcohol was added to the precipitated DNA, and then it was vortexed and centrifuged at 4°C, 5 min 12,000 rpm. Again the supernatant was the removed and the residual alcohol evaporated. The DNA pellet was resuspended in 500 μL of sterilized distilled water. The DNA concentration was measured using spectrophotometer [19].

Amplification of the ITS region and sequence analysis. The ITS region of the rDNA in selected strains of *A. cylindracea* was amplified by PCR using universal primers ITS1 (5'-TCCGTAGGTGAACCTGCG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Amplification reactions were performed in a total volume of 20 μL containing 2 μL 10 × PCR buffer, 1.6 μL dNTP, 0.5 μL of each primer, 0.2 μL Taq polymerase, 1 μL of genomic DNA, and 14.2 μL of sterilized distilled water. The PCR was performed using a thermal cycler (Veriti thermal cycler; Applied Biosystems, Foster City, CA, USA) with an initial denaturation for 5 min at 95°C, followed by 35 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 52°C, extension for 1 min at 72°C, and a final 10 min extension at 72°C. Amplification products were analyzed by gel electrophoresis on a 1.5% agarose gel with a 1 kb DNA ladder as a marker. ITS sequences were aligned for phylogenetic analysis using the program Cluster W [20]. The phylogenetic tree was constructed by neighbor-joining method using the CLC free Workbench program. Bootstrap analysis was repeated 1,000 times to examine the reliability of the interior branches and the validity of the trees [21, 22].

RAPD analysis. Genomic DNA was amplified by the RAPD technique [23], in which 20 arbitrary 10-base oligonucleotide primers (Operon Technologies Inc., Alameda, CA, USA) were used to produce amplified fragments. The primer sequences are listed in Table 1. RAPD-PCR reaction was performed using a thermal cycler with an initial denaturation stage of 5 min at 94°C, followed by 35 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 36°C, extension for 2 min at 72°C, and a final 7 min extension at 72ºC. RAPD products were run on a 1.4% agarose gel in 1 × Tris-acetate-EDTA buffer for 1.15 hr at 100 V along with a 1 kb DNA ladder as a size marker. The gel was then stained with an ethidium bromide (EtBr) solution (0.5% μg/mL). The stained gels were visualized and photographed using a UV transilluminator. RAPD bands were recorded as present (1) or absent (0) to gener-

Table 1. Random amplification of polymorphic DNA primers used in this study

Primers	Sequence (5' to 3')
OPA-01	CAGGCCCTTC
OPA-02	TGCCGAGCTG
OPA-03	AGTCAGCCAC
OPA-04	AATCGGGCTG
OPA-05	AGGGGTCTTG
OPA-06	GGTCCCTGAC
OPA-07	GAAACGGGTG
OPA-08	GTGACGTAGG
OPA-09	GGGTAACGCC
OPA-10	GTGATCGCAG
OPA-11	CAATCGCCGT
OPA-12	TGCGCGATAG
OPA-13	CAGCACCCAC
OPA-14	TCTGTGCTGG
OPA-15	TTCCGAACCC
OPA-16	AGCCAGCGAA
OPA-17	GACCGCTTGT
OPA-18	AGGTGACCGT
OPA-19	CAAACGTCGG
OPA-20	GTTGCGATCC

ate the data matrix. The similarity coefficients (S) were calculated between isolates across bands for all primers using the formula S = 2Nxy/(Nx + Ny), where Nx and Ny are the number of bands shared by the two strains [24].

Results and Discussion

Effect of temperature and pH. A range of 15~35°C was considered to find the favorable temperature for mycelial propagation. The optimal mycelial growth (83.8 mm) was observed at 25°C and the lowest (16.6 mm) at 15°C (Fig. 1). Mycelial growth was not observed at 35°C. To determine the suitable pH for the mycelial growth of *A. cylindracea*, a pH range of 5~9 was tested. The optimal

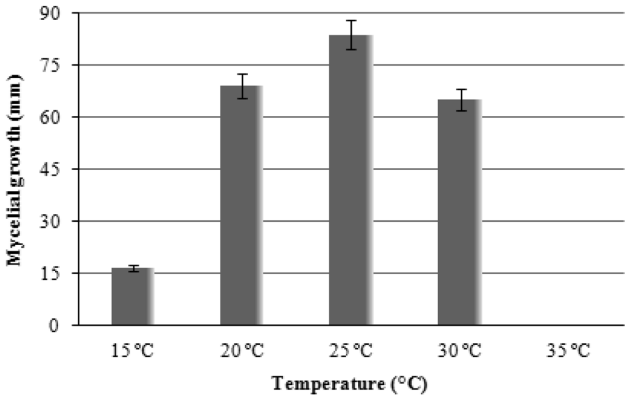

Fig. 1. Effect of temperature on the mycelial growth of *Agrocybe cylindracea* on potato dextrose agar after 10 days of incubation. Vertical bars show standard error.

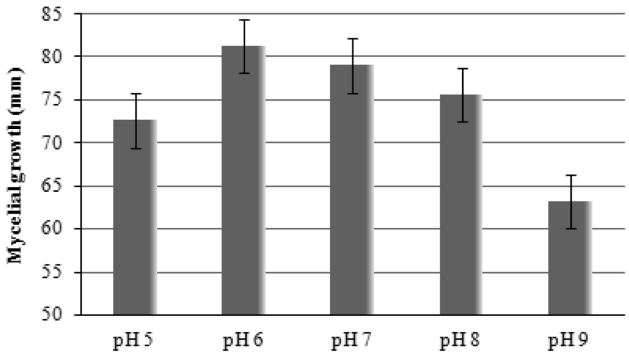

Fig. 2. Effect of pH on the mycelial growth of *Agrocybe cylindracea* on potato dextrose agar after 10 days of incubation at 25°C. Vertical bars show standard error.

radial growth of mycelium was found at pH 6 (Fig. 2). There was no significant variation in the range of pH 6~7 on the mycelial growth of *A. cylindracea*. This result agrees with the data from studies on *Paecilomyces fumosoroseus* [25, 26].

Effect of culture media. Ten different types of culture media were used to evaluate the optimal vegetative growth of *A. cylindracea*. Based strictly on mycelial growth, PDA was found to be the best followed by Hennerberg, yeast malt extract, mushroom complete, and Czapek dox. Glucose tryptone and Hamada were the most unfavorable for the mycelial propagation of this mushroom (Fig. 3). These findings are comparable to the previous studies on *Macrolepiota procera* [27] in which PDA and yeast malt extract were found to be the most suitable, whereas glucose peptone and glucose tryptone were unfavorable.

Effect of carbon and nitrogen sources. Ten different carbon sources were assayed to determine their effects on

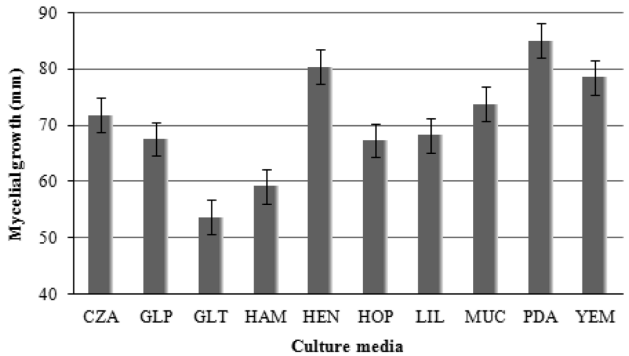

Fig. 3. Effect of different media on the mycelial growth of *Agrocybe cylindracea* after 10 days of incubation at 25°C. Vertical bars show standard error. CZA, Czapek Dox; GLP, glucose peptone; GLT, glucose tryptone; HAM, Hamada; HEN, Hennerberg; HOP, Hoppkins; LIL, Lilly; MUC, mushroom complete; PDA, potato dextrose agar; YEM, yeast malt extract.

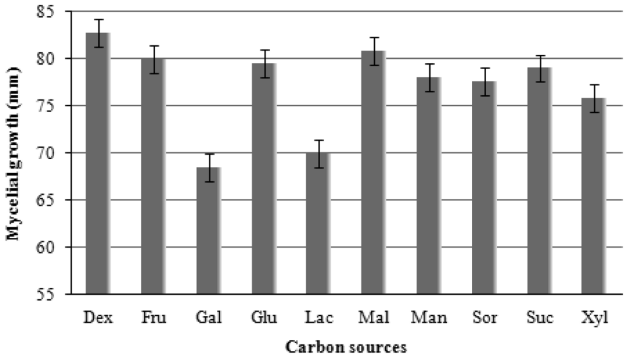

Fig. 4. Effect of carbon sources on the mycelial growth of *Agrocybe cylindracea* on basal medium after 10 days of incubation at 25°C. Vertical bars show standard error. Dex, dextrin; Fru, fructose; Gal, galactose; Glu, glucose; Lac; lactose; Mal, maltose; Man, mannose; Sor, sorbitol; Suc, sucrose; Xyl, xylose.

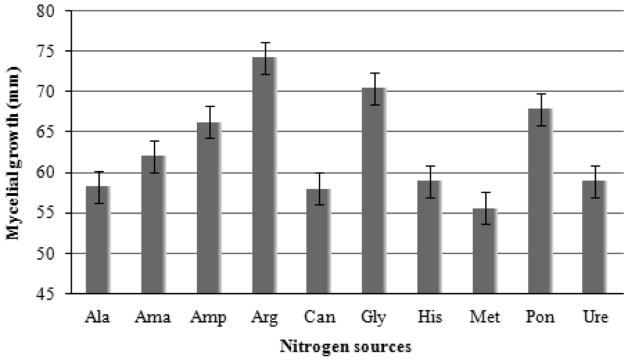

Fig. 5. Effect of nitrogen sources on the mycelial growth of *Agrocybe cylindracea* on basal medium after 10 days of incubation at 25°C. Vertical bars show standard error. Ala, alanine; Ama, ammonium acetate; Amp, ammonium phosphate; Arg, arginine; Can, calcium nitrate; Gly, glycine; His, histidine; Met, methionine; Pon, potassium nitrate; Ure, urea.

the vegetative growth of *A. cylindracea*. Dextrin was found to be the best for mycelial propagation, followed by maltose, fructose, glucose, sucrose, mannose, and sorbitol. The lowest growth level was obtained in galactose and lactose (Fig. 4). Among the nitrogen sources, arginine was found to be the best, followed by glycine and potassium nitrate. The lowest level of vegetative growth was recorded in methionine and calcium nitrate (Fig. 5). These findings are comparable to the previous studies on *Pleurotus eryngii* [16] in which dextrin and arginine were the most effective carbon and nitrogen sources for the mycelial growth. In general, organic nitrogen sources are more effective then inorganic nitrogen sources.

ITS sequence analysis. To study the genetic variation of selected strains of *A. cylindracea*, the ITS regions were

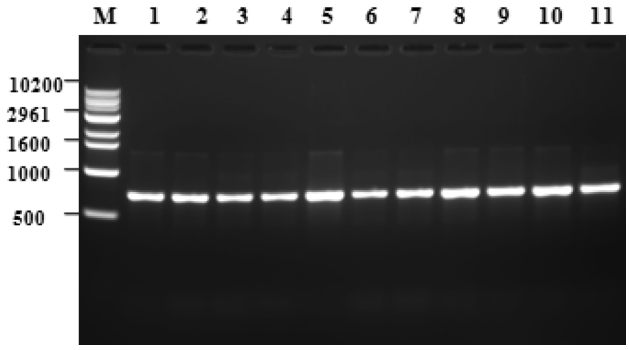

Fig. 6. PCR products of the internal transcribed spacer regions in 11 different strains of *Agrocybe cylindracea*. M, molecular size marker (1 kb DNA ladder); lane 1, IUM-0737; lane 2, IUM-1389; lane 3, IUM-1437; lane 4, IUM-1571; lane 5, IUM-1590; lane 6, IUM-1665; lane 7, IUM-1803; lane 8, IUM-1811; lane 9, IUM-1901; lane 10, IUM-2028; lane 11, IUM-2147.

amplified using ITS1 and ITS4 primers and then sequenced. Results indicate that the length of the sequences among the selected strains ranged from 628 to 652 bp (Fig. 6). The size of the ITS1 and ITS2 regions varied among the strains from 214 to 244 bp and 174 to 238 bp, respectively. The total G + C and A + T content of the ITS regions varied from 46.7 to 48.1% and 51.9 to 53.3%, respectively (Table 2). Sequence analysis indicated that the 5.8S of rDNA sequences were identical (153 bp) for all of the strains tested. The size variation was caused by differences in the number of nucleotides, revealing that these strains are clearly distinguishable from each other based on the ecological distribution, substitution, and insertion or deletion polymorphisms of the base position [28].

The phylogenetic tree based on the nucleotide sequences of the ITS regions in fifteen different strains of *A. cylindracea* was constructed by the neighbor-joining method. The phylogenetic tree was separated into five groups (Fig. 7). Maximum differences were observed between IUM-1389 (Taiwan) and FJ-869183 (NCBI GenBank strain), while maximum similarity (98~100%) was recorded in between IUM-2028 (China) and IUM-1901 (China), IUM-1437 (China) and IUM-1571 (China), IUM-0737 (Korea) and IUM-1803 (Korea), and IUM-1389 (Taiwan) and IUM-1590 (Taiwan). The results indicate that all Chinese strains belonged to same group and are very similar to NCBI gene bank strains. The ITS sequences are genetically constant or show little variation within species [29]. The genetic distance exhibited a high level of similarity with identical ITS sequences. The sequences of the ITS regions of rDNA were variable among the strains tested. The genetic variation between clusters was greater than that observed between groups. The high genetic diversity detected within groups is probably due to an efficient gene flow and a high genetic compatibility within the strains tested [30].

Table 2. Nucleotide distribution, internal transcribed spacer (ITS)1, 5.8S, and ITS2 of rDNA sequences in eleven different strains of *Agrocybe cylindracea*

Strain	Nucleotide distribution						Sequence information			
	A	C	G	T	G + C (%)	A + T (%)	ITS-1	5.8S	ITS-2	Length (bp)
IUM-0737	130	154	151	200	48.0	52.0	216	153	174	635
IUM-1389	128	153	149	198	48.1	51.9	214	153	174	628
IUM-1437	123	153	154	222	47.1	52.9	242	153	238	652
IUM-1571	122	150	147	217	46.7	53.3	226	153	238	636
IUM-1590	128	154	149	199	48.1	51.9	216	153	174	630
IUM-1665	129	154	151	200	48.1	51.9	216	153	174	634
IUM-1803	130	155	151	201	48.0	52.0	216	153	174	637
IUM-1811	131	155	151	200	48.0	52.0	216	153	174	637
IUM-1901	124	153	152	221	46.9	53.1	244	153	238	650
IUM-2028	122	152	152	221	47.0	53.0	241	153	238	647
IUM-2147	129	154	151	200	48.1	51.9	216	153	174	634

A, adenine; C, cytosine; G, guanine; T, thymine.

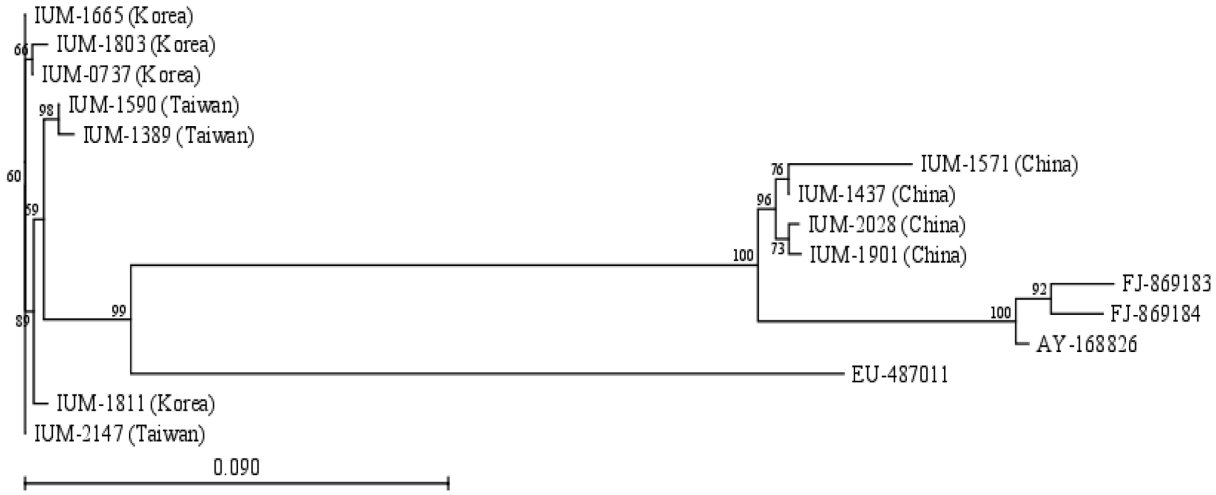

Fig. 7. Phylogenetic tree in 15 strains of *Agrocybe cylindracea* based on the nucleotide sequences of the internal transcribed spacer regions using neighbor-joining method with 1,000 boot-strapping trails.

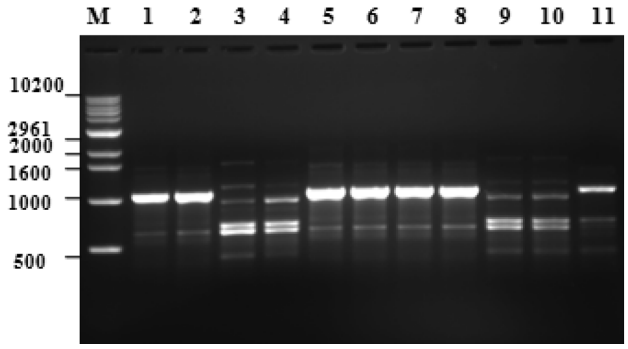

Fig. 8. Random amplification of polymorphic DNA profiles in different strains of *Agrocybe cylindracea* using OPA-1 primer. M, molecular size marker (1 kb DNA ladder); lane 1, IUM-0737; lane 2, IUM-1389; lane 3, IUM-1437; lane 4, IUM-1571; lane 5, IUM-1590; lane 6, IUM-1665; lane 7, IUM-1803; lane 8, IUM-1811; lane 9, IUM-1901; lane 10, IUM-2028; lane 11, IUM-2147.

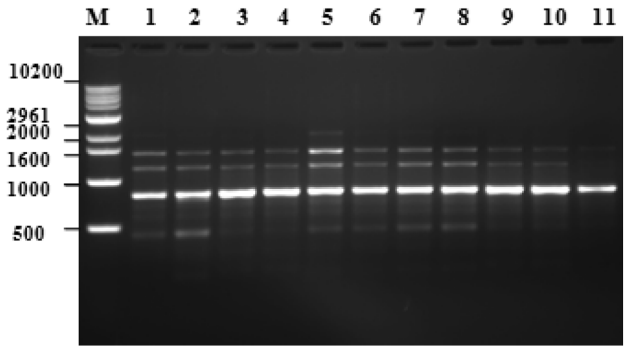

Fig. 9. Random amplification of polymorphic DNA profiles in different strains of *Agrocybe cylindracea* using OPA-5 primer. M, molecular size marker (1 kb DNA ladder); lane 1, IUM-0737; lane 2, IUM-1389; lane 3, IUM-1437; lane 4, IUM-1571; lane 5, IUM-1590; lane 6, IUM-1665; lane 7, IUM-1803; lane 8, IUM-1811; lane 9, IUM-1901; lane 10, IUM-2028; lane 11, IUM-2147.

Table 3. DNA bands in different strains of *Agrocybe cylindracea* by random amplification of polymorphic DNA assay using 10-base OPA primers

Primers	DNA band (kb)	IUM strains										
		1	2	3	4	5	6	7	8	9	10	11
OPA-1	1.7	–	–	+	+	–	–	–	–	+	+	–
	1.0	+	+	+	+	+	+	+	+	+	+	+
	0.8	–	–	+	+	–	–	–	–	+	+	+
	0.7	+	+	+	+	+	+	+	+	+	+	+
	0.5	–	–	+	+	–	–	–	–	+	+	+
OPA-2	1.7	+	–	+	+	+	+	+	+	+	+	+
	1.6	+	–	+	+	+	+	+	+	+	+	+
	1.4	+	+	+	+	+	+	+	+	+	+	+
	0.8	+	+	+	+	+	+	+	+	+	+	–
	0.4	+	+	–	–	+	+	+	+	–	–	–
OPA-3	1.5	+	+	–	–	+	+	+	+	–	–	–
	1.0	+	+	+	+	+	+	+	+	+	+	+
	0.7	+	+	+	–	+	+	+	+	+	+	+
	0.5	–	–	–	+	–	–	–	–	+	+	+
OPA-4	2.0	+	+	+	+	+	+	+	+	+	+	–
	1.4	+	+	+	+	+	+	+	+	+	+	–
	1.0	+	+	–	–	+	+	+	+	–	–	+
	0.6	+	+	+	+	+	+	+	+	+	+	+
OPA-5	2.3	–	–	–	–	+	–	–	–	–	–	–
	1.6	+	+	+	+	+	+	+	+	+	+	+
	1.3	+	+	+	+	+	+	+	+	+	+	–
	0.9	+	+	+	+	+	+	+	+	+	+	+
	0.4	+	+	–	–	+	+	+	+	–	–	–
OPA-7	1.8	–	–	+	+	–	–	–	–	+	+	–
	1.4	+	+	+	+	+	+	+	+	+	+	–
	0.8	+	+	–	–	+	+	+	+	–	–	+
	0.1	–	–	+	+	–	–	–	–	+	+	–
OPA-8	1.7	+	+	+	+	+	+	+	+	+	+	+
	1.4	+	+	+	–	+	+	+	+	–	–	+
	0.3	–	+	+	+	–	–	–	–	+	+	+
OPA-10	1.2	+	+	+	–	+	+	+	+	+	+	+
	0.8	–	+	+	+	+	+	+	+	+	+	+
	0.6	–	+	+	+	+	+	+	+	+	+	–
OPA-11	1.2	+	+	+	+	+	+	+	+	+	+	+
	0.7	+	+	+	+	+	+	+	+	+	+	+
	0.4	–	–	+	+	–	–	–	–	+	+	–
OPA-13	1.6	–	–	+	+	+	+	+	–	+	+	–
	1.3	+	+	+	+	+	+	+	+	+	+	+
	0.7	+	+	+	+	+	+	+	+	+	+	+
OPA-15	2.9	–	–	+	+	–	–	–	–	+	+	–
	1.4	–	–	+	+	–	–	–	–	+	+	+
	1.0	+	+	–	–	+	+	+	+	–	–	–
	0.8	–	–	+	+	–	–	–	–	+	+	+
	0.5	–	–	+	+	–	–	–	–	+	+	–
OPA-16	2.0	+	+	+	+	+	+	+	+	+	+	–
	1.2	+	+	–	–	+	+	+	+	–	–	+
	0.9	+	+	+	+	+	+	+	+	+	+	–

RAPD analysis. Twenty arbitrary 10-base oligonucleotide primers were used to amplify segments of genomic DNA for selected IUM strains of *A. cylindracea*. Fifteen primers (OPA-1, 2, 3, 4, 5, 7, 8, 10, 11, 13, 15, 16, 17, 18, and 19) were found to be efficient for amplification of genomic DNA (Table 3). These primers show significant band profiles on the tested strain, which made them good candidates for screening each strain (Figs. 8~10). RAPD-PCR generated distinct multiple products with considerable variability among the strains tested. The number of amplified bands varied depending on the primers used or the strains tested. The average number of polymorphic bands observed per primer was 3.8. The size of these polymorphic fragments was obtained in the range from 0.1 to 2.9 kb. The DNA polymorphisms showed the same characteristics in the replication tests. Therefore, if same primers are used for the screening of DNA polymorphisms, it would be possible to distinguish genetically different strains of *A. cylindracea*. To maximize the speci-

Table 3. Continued

Primers	DNA band (kb)	IUM strains										
		1	2	3	4	5	6	7	8	9	10	11
OPA-17	1.6	–	–	–	–	–	–	–	–	–	–	+
	1.3	–	–	+	+	+	–	–	–	+	–	+
	1.0	+	+	+	+	+	+	+	+	+	+	–
OPA-18	0.7	+	+	+	+	+	+	+	+	+	+	+
	0.5	+	+	–	–	+	+	+	+	–	–	–
	0.3	+	+	+	+	+	+	+	+	+	+	–
OPA-19	2.0	+	+	+	+	+	+	+	+	+	+	+
	1.4	+	+	+	+	+	+	+	+	+	+	+
	1.2	+	+	+	+	+	+	+	+	+	+	+
	0.8	–	–	–	–	–	–	–	–	–	–	+

Lane 1, IUM-0737; lane 2, IUM-1389; lane 3, IUM-1437; lane 4, IUM-1571; lane 5, IUM-1590; lane 6, IUM-1665; lane 7, IUM-1803; lane 8, IUM-1811; lane 9, IUM-1901; lane 10, IUM-2028; lane 11, IUM-2147.

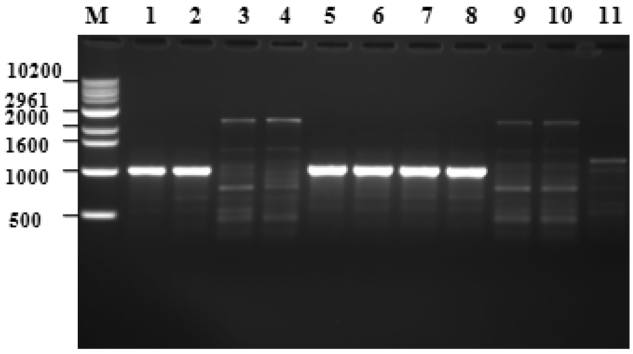

Fig. 10. Random amplification of polymorphic DNA profiles in different strains of *Agrocybe cylindracea* using OPA-15 primer. M, molecular size marker (1 kb DNA ladder); lane 1, IUM-0737; lane 2, IUM-1389; lane 3, IUM-1437; lane 4, IUM-1571; lane 5, IUM-1590; lane 6, IUM-1665; lane 7, IUM-1803; lane 8, IUM-1811; lane 9, IUM-1901; lane 10, IUM-2028; lane 11, IUM-2147.

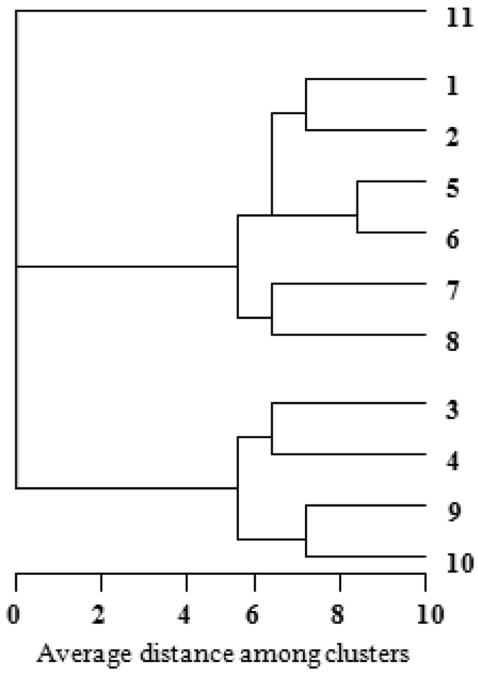

Fig. 11. Dendrogram constructed based on the random amplification of polymorphic DNA markers of *Agrocybe cylindracea* strains determined by the average linkage cluster. 1, IUM-0737; 2, IUM-1389; 3, IUM-1437; 4, IUM-1571; 5, IUM-1590; 6, IUM-1665; 7, IUM-1803; 8, IUM-1811; 9, IUM-1901; 10, IUM-2028; 11, IUM-2147.

ficity of the polymorphic patterns, a combined dendrogram was constructed using the RAPD-PCR amplified bands obtained from the 15 different RAPD primers. Three putative groups among the 11 strains of *A. cylindracea* were obtained by cluster analysis based on banding patterns and the sizes of amplified products (Fig. 11). Among the 11 strains, 98~100% similarities were found between IUM-1901 (China) and IUM-2028 (China), IUM-1437 (China) and IUM-1571 (China), IUM-1803 (Korea) and IUM-1811 (Korea), IUM-1590 (Taiwan) and IUM-1665 (Korea), and IUM-0737 (Korea) and IUM-1389 (Taiwan), all of which belong to two different groups. In most of the cases, IUM-2147 (Taiwan) showed different band patterns compared to other strains. The results indicate that all Chinese strains belonged to the same group and that the results of the RAPD analysis were similar to those of the ITS region analysis. Therefore, our results are comparable to the study made by Alam *et al.* [14] and support RAPD as a useful tool for clarifying the genetic relationships among strains.

Acknowledgements

This research was supported by research grant from University of Incheon in 2009.

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