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Cultural Conditions for Mycelial Growth and Molecular Phylogenetic Relationship in Different Wild Strains of *Schizophyllum commune*

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The common split-gilled mushroom, *Schizophyllum commune* is found throughout the world on woody plants. This study was initiated to evaluate conditions for favorable vegetative growth and to determine molecular phylogenetic relationship in twelve different strains of *S. commune*. A suitable temperature for mycelial growth was obtained at 30°C. This mushroom grew well in acidic conditions and pH 5 was the most favorable. Hamada, glucose peptone, Hennerberg, potato dextrose agar and yeast malt extract were favorable media for growing mycelia, while Lilly and glucose tryptone were unfavorable. Dextrin was the best and lactose was the less effective carbon source. The most suitable nitrogen sources were calcium nitrate, glycine, and potassium nitrate, whereas ammonium phosphate and histidine were the least effective for the mycelial growth of *S. commune*. The genetic diversity of each strain was investigated in order to identify them. The internal transcribed spacer (ITS) regions of rDNA were amplified using PCR. The size of the ITS1 and ITS2 regions of rDNA from the different strains varied from 129 to 143 bp and 241 to 243 bp, respectively. The sequence of ITS1 was more variable than that of ITS2, while the 5.8S sequences were identical. A phylogenetic tree of the ITS region sequences indicated that the selected strains were classified into three clusters. The reciprocal homologies of the ITS region sequences ranged from 99 to 100%. The strains were also analyzed by random amplification of polymorphic DNA (RAPD) with 20 arbitrary primers. Twelve primers efficiently amplified the genomic DNA. 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 4.5. The size of polymorphic fragments was obtained in the range of 0.2 to 2.3 kb. These results indicate that the RAPD technique is well suited for detecting the genetic diversity in the *S. commune* strains tested.

KEYWORDS : ITS, Mycelial growth, Physicochemical, RAPD, rDNA, *Schizophyllum commune*

Schizophyllum commune one of the most common mushrooms, is widely distributed worldwide and usually grows abundantly during the rainy season. This species frequently appears on dead wood and is a known wood decomposer of over 150 genera of flowering plants [1]. In addition it is a well documented wound parasite of trees and human pathogen of minor but increasing importance [2].

In *S. commune*, single meiospores germinate to produce haploid, monokaryotic mycelia. Two complex mating-type factors control sexual compatibility in the monokaryons and regulate the maintenance of the dikaryotic state [3]. Fusion of sexually compatible haploid monokaryotic mycelia results in the formation of the dikaryotic mycelium. The hyphae of *S. commune* dikaryons develop clamp connections at each septum, while the hyphae of monokaryons do not. The dikaryon is the predominant vegetative structure in *S. commune*. Under appropriate conditions, the dikaryon produces the fruiting bodies within which meiosis occurs. The monokaryotic and dikaryotic mycelia are capable of indefinite growth, allowing for the maintenance and duplication of the genotype of each ploidy state [4]. It is generally recognized that growing mycelia is a defined medium is a rapid and alternative method to obtain fungal biomass for further use [5].

S. commune is a model organism for transmission genetics, developmental biology, and population genetics and has also been used in several different quantitative genetic studies on growth rate [6]. To identify *S. commune* strains and other traits of interest, assessment of genetic and phenotypic diversity is necessary. Recent molecular phylogenetic studies have demonstrated that the internal transcribed spacer (ITS) region of genomic DNA is very useful for assessing phylogenetic relationships at lower taxonomic levels. The ITS of rDNA is considered as a variable region among the species and even among the strains [7]. Random amplification of polymorphic DNA (RAPD) markers has been widely used for assessing genetic diversity, genome mapping, and molecular diagnostics of many fungal species [8]. RAPD was particularly successful when used to verify mushroom strains from various hosts with a wide range of geographical origins [9]. The present study was aimed to assess the genetic diversity and physicochemical requirements for the vegetative growth in different strains of *S. commune*.

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Materials and Methods

Mushroom strains. Twelve wild strains of *S. commune* were collected from the different ecological regions of China (IUM-1763 and IUM-1768) and Korea (IUM-0137, IUM-0157, IUM-0202, IUM-0395, IUM-0548, IUM-2324, IUM-2650, IUM-2659, IUM-3353, and IUM-3566). Pure cultures were deposited in the Culture Collection and DNA Bank of Mushrooms (CCDBM) and acquired the accession number of Incheon University Mushroom (IUM). Seven strains of *S. commune*, AF-249389, AF-249390, AY-573544, AY-636062, EF-488416, FJ-372682, and FJ-372683 were also used as control strains for the phylogenetic comparison with our selected IUM strains. Sequencing data of the control strains were collected from the National Center for Biotechnology Information (NCBI) gene bank data base.

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 *S. commune*. 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 adding 1 N NaOH or HCl before autoclaving. Samples were incubated for 10 days at 25°C. The measurement of mycelial growth was performed according to the described method [10].

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 *S. commune*. The different types of culture media were prepared according to the described method [11]. The media were adjusted to pH 6 before being autoclaved.

Carbon and nitrogen sources. The 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 carbon sources (dextrin, fructose, galactose, glucose, lactose, maltose, mannose, sorbitol, sucrose, and xylose) and ten 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 sources for mycelial growth, each carbon source 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 the concentration of 0.02 M [12]. In both cases, the basal medium was adjusted to pH 6 before autoclaving.

DNA extraction. Genomic DNA was extracted according to the described procedure [13] with some modifications as follows. Fresh mycelia were collected from the 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. 500 μL 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 the samples were vortexed and then centrifuged at 4°C, 10 minutes, 12,000 rpm. Afterwards, the upper phase was transferred to a 1.5 mL micro-tube, 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 [14].

Amplification of the ITS region and sequence analysis. The ITS region of the rDNA in selected strains of *S. commune* was amplified by polymerase chain reaction (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 stage of 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 electrophoreses 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 [15]. The phylogenetic tree was constructed by neighbor-joining method using 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 obtained [16, 17].

RAPD analysis. Genomic DNA was amplified by the RAPD technique [8] in which 20 arbitrary 10-base oligonucleotide primers (Operon Technologies Inc., Alameda, CA, USA) were used to produce amplified fragments.

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

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 72°C. 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 generate 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 [18].

Results and Discussion

Effect of temperature and pH. To determine the most suitable temperature for mycelial growth of *S. commune*, a range from 15~35°C was considered. The highest average mycelial growth was found in 30°C, followed by 35, 25, 20, and 15°C. Among the selected strains, maximum mycelial growth (87.0 mm) was recorded in IUM-0137, 0157, 0395, 1763, and 3353 at 30°C (Table 2). These findings are comparable to the previous studies on *Macrolepiota procera* [19], which was shown to have optimum mycelial growth at 30°C.

pH values ranging from 5~9 were considered to determine the most favorable culture conditions for the mycelial growth of *S. commune*. The highest and lowest level of radial growth of mycelium was found at pH 5 and 9, respectively (Table 3). Mycelial growth decreased with an increase in pH values, thus acidic pH was better for the mycelial growth of *S. commune*. This result agrees with the data from the study of *Pholiota adiposa*, as it grew well in acidic conditions and pH 5 was the most favorable for vegetative growth [20].

Effect of culture media. Ten different types of culture media were used to determine the optimum medium for mycelial growth of the selected *S. commune* strains. Based on mycelial growth, Hamada, glucose peptone, Hennerberg, potato dextrose agar and yeast malt extract were the most favorable, while Lilly and glucose tryptone were the least favorable for the vegetative growth of *S. commune*. The highest and lowest mycelial growth was recorded in

Table 2. Effect of temperature on the mycelial growth in different strains of *Schizophyllum commune*

Strain	Mycelial growth[a] (mm)				
	15°C	20°C	25°C	30°C	35°C
IUM-0137	20.3 ± 2.1	73.3 ± 2.1	79.7 ± 6.4	87.0 ± 0.0	87.0 ± 0.0
IUM-0157	30.7 ± 2.5	70.7 ± 2.1	85.3 ± 2.9	87.0 ± 0.0	87.0 ± 0.0
IUM-0202	19.3 ± 3.8	78.0 ± 3.6	82.7 ± 7.5	82.3 ± 8.1	71.7 ± 2.9
IUM-0395	17.7 ± 2.5	66.3 ± 3.5	79.3 ± 1.5	87.0 ± 0.0	82.7 ± 2.1
IUM-0548	11.7 ± 1.5	43.0 ± 3.0	63.0 ± 1.0	66.3 ± 1.5	72.7 ± 2.1
IUM-1763	19.0 ± 2.6	74.0 ± 3.5	87.0 ± 0.0	87.0 ± 0.0	87.0 ± 0.0
IUM-1768	12.0 ± 1.0	61.0 ± 6.6	66.0 ± 5.3	68.3 ± 3.2	55.9 ± 1.6
IUM-2324	25.0 ± 1.0	76.3 ± 1.5	81.7 ± 1.5	84.7 ± 3.2	70.7 ± 0.7
IUM-2650	18.7 ± 4.5	46.3 ± 0.6	67.7 ± 3.8	86.0 ± 0.0	77.0 ± 2.6
IUM-2659	14.7 ± 3.7	45.3 ± 3.1	64.7 ± 1.5	72.0 ± 4.6	73.0 ± 2.6
IUM-3353	18.7 ± 2.5	67.3 ± 3.5	79.3 ± 1.5	87.0 ± 0.0	81.7 ± 2.1
IUM-3566	23.3 ± 1.2	76.3 ± 2.5	84.0 ± 1.7	86.0 ± 0.6	85.0 ± 0.0
Mean	19.3 ± 2.4	64.8 ± 3.0	76.7 ± 2.9	81.7 ± 1.8	77.6 ± 1.4

[a]Mean of 3 replications.

Table 3. Effect of pH on the mycelial growth in different strains of *Schizophyllum commune*

Strain	Mycelial growth[a] (mm)				
	pH 5	pH 6	pH 7	pH 8	pH 9
IUM-0137	63.7 ± 2.6	61.0 ± 1.7	74.5 ± 2.5	22.0 ± 1.2	18.7 ± 3.7
IUM-0157	85.3 ± 1.5	73.7 ± 0.6	75.0 ± 0.0	68.3 ± 1.4	65.3 ± 1.2
IUM-0202	84.7 ± 1.2	80.3 ± 1.5	74.7 ± 0.6	71.3 ± 1.2	68.7 ± 0.6
IUM-0395	84.3 ± 0.6	80.7 ± 0.6	75.0 ± 1.0	71.0 ± 1.7	69.0 ± 1.0
IUM-0548	70.3 ± 2.5	72.3 ± 1.2	57.7 ± 1.5	63.7 ± 0.6	54.7 ± 3.8
IUM-1763	86.3 ± 1.2	82.7 ± 3.2	69.7 ± 1.5	63.7 ± 1.5	60.0 ± 1.7
IUM-1768	63.3 ± 3.2	53.0 ± 1.0	51.7 ± 4.0	43.3 ± 3.8	36.7 ± 2.5
IUM-2324	64.7 ± 0.6	54.3 ± 1.2	52.3 ± 0.6	50.7 ± 1.7	42.3 ± 1.5
IUM-2650	87.0 ± 0.0	85.3 ± 1.5	81.5 ± 2.4	71.0 ± 1.0	58.3 ± 4.5
IUM-2659	85.0 ± 2.6	72.7 ± 7.1	62.0 ± 7.9	49.3 ± 1.5	46.7 ± 7.4
IUM-3353	87.0 ± 0.0	71.5 ± 5.8	64.0 ± 1.7	27.7 ± 2.3	25.5 ± 0.6
IUM-3566	84.7 ± 4.0	75.3 ± 7.1	72.7 ± 0.6	22.3 ± 2.5	19.5 ± 1.5
Mean	78.9 ± 1.7	71.9 ± 2.7	67.6 ± 2.0	52.0 ± 1.7	47.1 ± 2.5

[a]Mean of 3 replications.

Table 4. Effect of culture media on the mycelial growth in different strains of *Schizophyllum commune*

Strain	Mycelial growth[a] (mm)									
	CZA	GLP	GLT	HAM	HEN	HOP	LIL	MUC	PDA	YEM
IUM-0137	47.0 ± 4.4	81.3 ± 1.5	48.3 ± 2.1	87.0 ± 0.0	75.7 ± 2.5	50.0 ± 2.6	45.0 ± 1.0	75.7 ± 1.5	79.7 ± 6.4	77.7 ± 8.1
IUM-0157	77.0 ± 2.6	81.0 ± 1.0	62.3 ± 2.5	87.0 ± 0.0	87.0 ± 0.0	62.7 ± 2.5	53.0 ± 1.7	82.3 ± 1.5	85.3 ± 2.9	83.0 ± 1.7
IUM-0202	66.0 ± 1.0	84.7 ± 1.5	70.3 ± 4.2	69.0 ± 2.0	87.0 ± 0.0	74.7 ± 3.1	58.7 ± 2.3	78.0 ± 6.1	71.0 ± 1.5	87.0 ± 0.0
IUM-0395	76.7 ± 3.8	87.0 ± 0.0	48.7 ± 3.5	87.0 ± 0.0	81.0 ± 1.7	63.3 ± 1.5	39.0 ± 1.0	78.0 ± 0.0	79.3 ± 1.5	79.7 ± 0.6
IUM-0548	58.0 ± 6.6	72.7 ± 1.5	45.7 ± 5.5	83.3 ± 6.4	75.0 ± 2.0	44.0 ± 1.7	40.0 ± 0.0	69.0 ± 9.4	63.0 ± 1.0	79.7 ± 3.2
IUM-1763	76.3 ± 5.7	87.0 ± 0.0	76.3 ± 1.5	87.0 ± 0.0	87.0 ± 0.0	81.3 ± 2.1	74.3 ± 1.2	87.0 ± 0.0	87.0 ± 0.0	87.0 ± 0.0
IUM-1768	53.3 ± 2.5	68.0 ± 2.6	42.7 ± 2.1	71.7 ± 2.5	73.3 ± 2.1	48.0 ± 5.3	40.3 ± 1.5	58.7 ± 1.5	64.7 ± 1.7	58.0 ± 2.7
IUM-2324	75.7 ± 3.8	87.0 ± 0.0	45.7 ± 4.5	86.0 ± 0.0	80.0 ± 1.7	62.3 ± 1.5	39.0 ± 1.0	70.0 ± 0.0	78.3 ± 1.0	79.7 ± 1.0
IUM-2650	75.7 ± 5.5	87.0 ± 0.0	50.7 ± 5.1	87.0 ± 0.0	87.0 ± 0.0	48.0 ± 2.6	70.0 ± 6.6	87.0 ± 0.0	82.7 ± 2.3	83.7 ± 2.3
IUM-2659	46.0 ± 2.0	71.3 ± 6.5	53.7 ± 6.0	83.0 ± 2.6	56.3 ± 2.5	43.3 ± 2.3	43.7 ± 1.2	59.7 ± 2.5	67.3 ± 2.2	66.0 ± 2.5
IUM-3353	59.7 ± 1.2	63.7 ± 1.2	51.7 ± 3.5	70.0 ± 0.0	67.0 ± 2.0	52.3 ± 1.5	44.0 ± 5.6	61.7 ± 2.5	65.7 ± 1.4	52.0 ± 1.7
IUM-3566	66.7 ± 4.6	74.0 ± 1.7	60.3 ± 0.6	79.7 ± 2.1	77.7 ± 1.7	54.3 ± 2.1	47.0 ± 3.5	73.3 ± 1.5	84.0 ± 1.7	67.3 ± 1.5
Mean	64.8 ± 3.6	78.7 ± 1.5	54.7 ± 3.4	81.5 ± 1.3	77.8 ± 1.4	57.0 ± 2.4	49.5 ± 2.2	73.4 ± 2.2	75.7 ± 2.0	75.1 ± 2.1

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.
[a]Mean of 3 replications.

Table 5. Effect of carbon sources on the mycelial growth in different strains of *Schizophyllum commune*

Strain	Mycelial growth[a] (mm)									
	Dex	Fru	Gal	Glu	Lac	Mal	Man	Sor	Suc	Xyl
IUM-0137	51.0 ± 3.6	46.7 ± 1.5	39.3 ± 1.5	39.7 ± 1.5	29.3 ± 1.2	48.3 ± 2.9	41.0 ± 1.0	41.7 ± 3.2	36.7 ± 2.5	28.3 ± 1.5
IUM-0157	81.7 ± 1.5	79.0 ± 3.6	73.7 ± 1.5	79.7 ± 4.0	34.7 ± 2.3	73.0 ± 1.7	75.0 ± 1.7	68.0 ± 2.0	75.3 ± 6.4	76.3 ± 1.5
IUM-0202	53.3 ± 7.8	53.7 ± 4.0	50.0 ± 6.2	49.7 ± 9.6	29.0 ± 0.0	41.0 ± 3.0	43.0 ± 7.5	52.3 ± 4.9	63.3 ± 6.7	42.0 ± 9.8
IUM-0395	72.0 ± 2.6	79.7 ± 1.5	70.7 ± 9.8	73.7 ± 3.2	42.3 ± 0.6	72.3 ± 4.7	78.3 ± 1.5	66.0 ± 1.7	81.0 ± 1.7	75.7 ± 1.2
IUM-0548	62.3 ± 2.5	66.7 ± 2.1	57.3 ± 1.2	63.0 ± 1.7	44.0 ± 5.3	55.7 ± 4.5	55.0 ± 1.7	50.0 ± 2.0	60.3 ± 2.5	37.7 ± 2.5
IUM-1763	82.7 ± 4.0	84.3 ± 3.8	80.7 ± 9.8	83.0 ± 4.6	58.3 ± 7.6	79.0 ± 8.5	81.7 ± 9.2	80.7 ± 9.8	79.7 ± 9.7	77.7 ± 8.8
IUM-1768	56.0 ± 2.5	50.7 ± 5.6	52.3 ± 1.2	44.0 ± 1.0	41.0 ± 0.0	52.7 ± 3.1	49.7 ± 2.0	45.7 ± 2.5	50.3 ± 1.5	57.0 ± 5.0
IUM-2324	77.3 ± 1.2	34.3 ± 1.2	50.7 ± 1.2	67.3 ± 1.5	29.7 ± 6.4	67.5 ± 1.5	44.0 ± 0.6	37.7 ± 1.5	39.7 ± 1.5	29.7 ± 3.5
IUM-2650	48.3 ± 2.9	41.0 ± 1.0	41.7 ± 3.2	36.7 ± 2.5	28.3 ± 1.5	48.3 ± 4.2	42.7 ± 3.1	41.3 ± 2.3	48.3 ± 1.5	47.7 ± 1.5
IUM-2659	72.0 ± 2.6	79.7 ± 1.5	70.7 ± 8.1	73.7 ± 3.2	42.3 ± 0.6	72.3 ± 4.7	71.2 ± 1.5	66.0 ± 1.7	81.0 ± 1.7	75.7 ± 1.2
IUM-3353	58.7 ± 1.5	54.0 ± 4.0	48.0 ± 4.0	47.7 ± 0.6	36.3 ± 0.6	43.7 ± 1.2	47.2 ± 4.2	48.7 ± 7.1	53.7 ± 3.5	37.7 ± 2.1
IUM-3566	73.0 ± 1.0	75.3 ± 3.1	69.3 ± 3.5	77.7 ± 2.5	31.7 ± 2.1	65.3 ± 2.5	66.3 ± 0.6	65.0 ± 2.0	69.7 ± 2.9	60.0 ± 0.0
Mean	65.7 ± 2.8	62.1 ± 2.7	58.7 ± 4.3	61.3 ± 3.0	37.2 ± 2.4	59.9 ± 3.5	57.9 ± 2.9	55.3 ± 3.4	61.6 ± 3.5	53.8 ± 3.2

Dex, dextrin; Fru, fructose; Gal, galactose; Glu, glucose; Lac, lactose; Mal, maltose; Man, mannose; Sor, sorbitol; Suc, sucrose; Xyl, xylose.
[a]Mean of 3 replications.

Hamada and Lilly, respectively (Table 4). This result is analogous to that of *P. sinclairii* and *P. fumosoroseus* [21], in which mycelial growth was more favorable on Hamada medium.

Effect of carbon and nitrogen sources. Ten different carbon sources were used to monitor mycelial growth. Dextrin was found to be the best for mycelial growth of *S. commune*, followed by fructose, sucrose, and glucose. The lowest growth of mycelium was obtained in lactose (Table 5). This result indicates that mannitol, sorbitol, glucose, and fructose were the best utilized carbon sources having a significant effect on the vegetative growth of *S. commune* [22].

Ten different nitrogen sources were assayed to determine their effects on the vegetative growth of *S. commune*. Among the nitrogen sources, calcium nitrate was found to be the best, followed by glycine and potassium nitrate. The lowest growth was recorded in ammonium phosphate and histidine (Table 6). The most favorable and unfavorable nitrogen sources for the vegetative growth of *M. procera* were glycine and histidine, which is partially similar to our findings [19]. In general, organic nitrogen sources are more effective than inorganic nitrogen sources.

ITS sequence analysis. To investigate the genetic diversity of *S. commune*, twelve wild strains were collected from various ecological regions of China and Korea at different times. The ITS region was amplified using ITS1 and ITS4 primers and then sequenced. Our results revealed that the length of the sequences among the selected strains ranged from 539 to 561 bp. The size of the ITS1 and ITS2 regions varied among the strains from 129 to 143 bp and 241 to 243 bp, respectively. The total G + C and A + T

Table 6. Effect of nitrogen sources on the mycelial growth in different strains of *Schizophyllum commune*

Strain	Mycelial growth[a] (mm)									
	Ala	Ama	Amp	Arg	Can	Gly	His	Met	Pon	Ure
IUM-0137	39.3 ± 3.1	34.7 ± 2.5	31.7 ± 7.2	39.3 ± 1.2	48.0 ± 3.6	45.7 ± 1.2	27.3 ± 2.5	40.0 ± 2.0	27.3 ± 2.9	34.0 ± 6.6
IUM-0157	62.3 ± 7.5	75.3 ± 0.6	56.0 ± 9.4	79.7 ± 4.0	74.0 ± 3.6	76.0 ± 9.5	50.0 ± 4.0	57.0 ± 7.7	61.7 ± 0.6	68.7 ± 5.5
IUM-0202	40.7 ± 1.2	35.3 ± 0.6	35.7 ± 1.5	37.3 ± 0.6	54.0 ± 1.0	41.7 ± 3.5	32.0 ± 1.7	33.0 ± 2.6	40.3 ± 2.5	33.7 ± 4.0
IUM-0395	37.0 ± 3.0	30.7 ± 0.6	28.7 ± 3.1	36.0 ± 2.6	66.7 ± 4.2	37.3 ± 2.3	38.3 ± 2.9	33.3 ± 1.2	53.0 ± 5.0	56.3 ± 7.8
IUM-0548	45.7 ± 5.1	33.0 ± 2.6	25.7 ± 1.5	31.0 ± 2.6	59.7 ± 2.5	36.0 ± 2.6	42.6 ± 6.8	36.7 ± 4.2	41.7 ± 5.7	35.3 ± 8.9
IUM-1763	87.0 ± 0.0	87.0 ± 0.0	55.7 ± 4.5	81.0 ± 3.5	87.0 ± 0.0	87.0 ± 0.0	75.7 ± 2.1	85.3 ± 1.5	87.0 ± 0.0	87.0 ± 0.0
IUM-1768	63.3 ± 1.5	63.0 ± 4.0	45.7 ± 2.5	61.3 ± 1.2	78.0 ± 2.0	68.3 ± 7.4	40.3 ± 0.6	56.7 ± 0.6	59.0 ± 3.0	48.3 ± 3.2
IUM-2324	67.0 ± 9.5	49.0 ± 8.5	32.3 ± 2.1	45.7 ± 3.2	59.0 ± 9.6	60.3 ± 2.5	30.0 ± 0.0	50.3 ± 4.7	62.0 ± 6.9	48.7 ± 3.1
IUM-2650	37.0 ± 3.6	36.0 ± 3.5	29.0 ± 1.0	32.3 ± 1.2	55.7 ± 7.2	46.0 ± 3.4	44.3 ± 6.7	38.0 ± 2.0	46.7 ± 4.2	41.0 ± 1.0
IUM-2659	49.3 ± 7.1	45.0 ± 6.2	36.3 ± 3.2	37.7 ± 2.5	60.7 ± 3.1	42.3 ± 2.9	49.3 ± 5.0	44.0 ± 2.6	59.0 ± 1.7	41.0 ± 1.0
IUM-3353	12.0 ± 2.0	35.3 ± 0.6	13.0 ± 1.0	42.7 ± 2.9	63.7 ± 1.5	65.0 ± 2.0	11.0 ± 1.0	47.7 ± 4.5	42.3 ± 1.2	10.7 ± 1.2
IUM-3566	55.0 ± 4.4	46.0 ± 4.0	38.7 ± 4.7	47.7 ± 2.5	64.3 ± 8.1	53.3 ± 5.7	52.7 ± 8.7	53.7 ± 7.1	54.0 ± 3.3	36.3 ± 1.5
Mean	49.6 ± 4.0	47.5 ± 2.8	35.7 ± 3.5	47.6 ± 2.3	64.2 ± 3.9	54.9 ± 3.6	41.1 ± 3.5	48.0 ± 3.4	52.8 ± 3.1	45.1 ± 3.7

Ala, alanine; Ama, ammonium acetate; Amp, ammonium phosphate; Arg, arginine; Can, calcium nitrate; Gly, glycine; His, histidine; Met, methionine; Pon, potassium nitrate; Ure, Urea.
[a]Mean of 3 replications.

Table 7. Nucleotide distribution, internal transcribed spacer (ITS)1, 5.8S, and ITS2 of rDNA sequences in twelve different strains of *Schizophyllum commune*

Strain	Nucleotide distribution						Sequence information			
	A	C	G	T	G + C (%)	A + T (%)	ITS-1	5.8S	ITS-2	Length (bp)
IUM-0137	132	131	128	160	47.01	52.99	135	151	243	551
IUM-0157	132	133	128	162	47.03	52.97	142	151	243	555
IUM-0202	130	133	128	158	47.54	52.46	137	151	243	549
IUM-0395	130	132	129	160	47.37	52.63	135	151	243	551
IUM-0548	130	130	128	159	47.17	52.83	136	151	241	547
IUM-1763	135	135	130	161	47.24	52.76	143	151	242	561
IUM-1768	131	133	127	162	47.02	52.98	140	151	243	553
IUM-2324	131	133	127	160	47.19	52.81	138	151	243	551
IUM-2650	132	135	128	160	47.39	25.61	143	151	242	555
IUM-2659	129	132	125	153	47.68	52.32	129	151	242	539
IUM-3353	132	133	128	162	47.03	52.97	142	151	243	555
IUM-3566	130	131	127	161	46.99	53.01	136	151	242	549

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

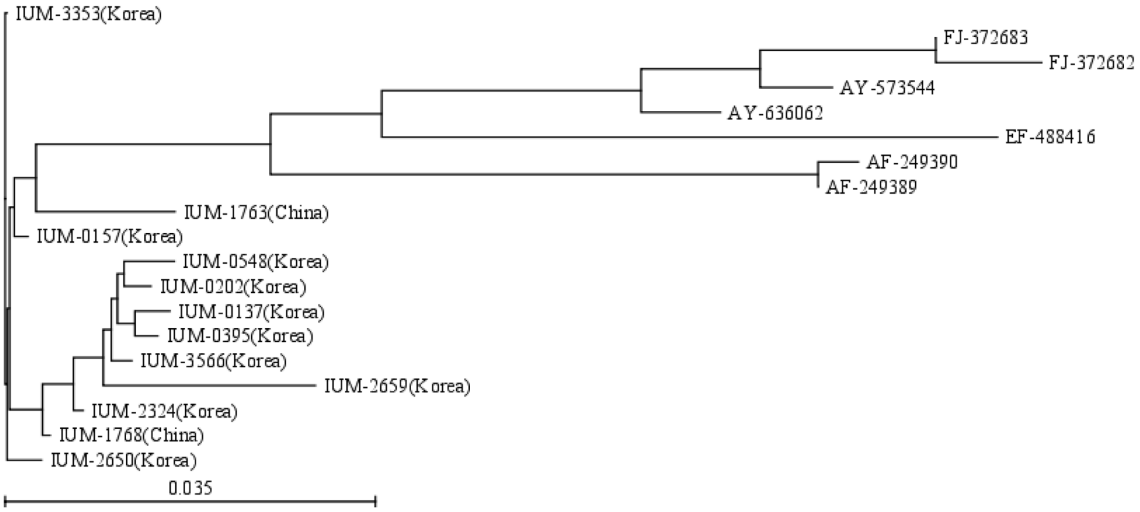

Fig. 1. Phylogenetic tree in nineteen strains of *Schizophyllum commune* based on the nucleotide sequence of the internal transcribed spacer region using neighbor-joining method with 1,000 bootstrapping trails.

content of the ITS region varied from 46.99 to 47.68% and 52.32 to 53.01%, respectively (Table 7). Sequence analysis indicated that the 5.8S of rDNA sequence was identical (151 bp) for all of the strains tested. The size variation was caused by differences in nucleotide sequence, 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 [23].

The phylogenetic tree based on the nucleotide sequence of the ITS region in twelve different strains of *S. commune* was obtained by the neighbor-joining method. Reciprocal homologies of the ITS region sequences ranged from 99 to 100%. The phylogenetic tree out separated into three groups (Fig. 1). The maximum difference was observed between IUM-2659 (Korea) and FJ-372682 (NCBI gene bank strain). IUM-0157 (Korea) and IUM-1763 (China) were similar to all NCBI gene bank strains tested and belongs to the same cluster. The ITS sequences are genetically constant or show little variation within species [24]. The genetic distance exhibited a high level of similarity with identical ITS sequences. The base sequences of the ITS region of rDNA were variable among the strains tested. The genetic variation within groups was greater than that observation 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 [25].

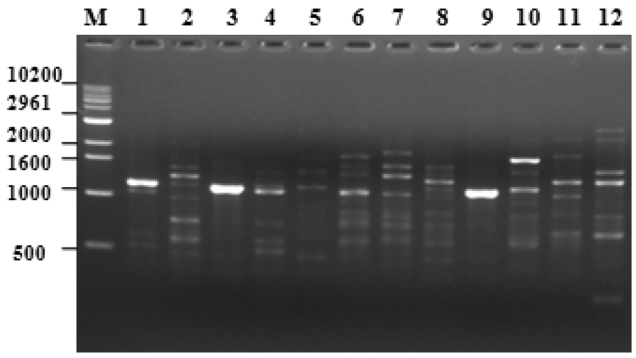

Fig. 2. Random amplification of polymorphic DNA profiles in different strains of *Schizophyllum commune* with primer OPA-1. M, molecular size marker (1 kb DNA ladder); lane 1, IUM-0137; lane 2, IUM-0157; lane 3, IUM-0202; lane 4, IUM-0395; lane 5, IUM-0548; lane 6, IUM-1763; lane 7, IUM-1768; lane 8, IUM-2324; lane 9, IUM-2650; lane 10, IUM-2659; lane 11, IUM-3353; lane 12, IUM-3566.

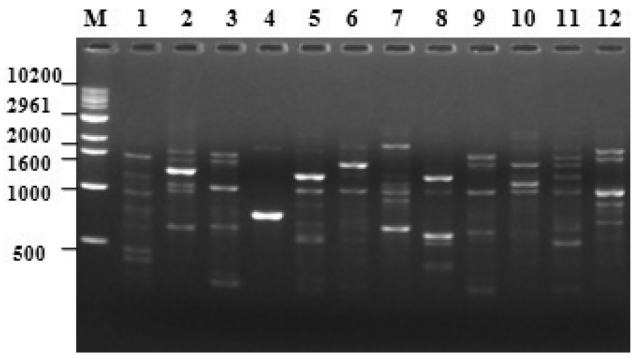

Fig. 3. Random amplification of polymorphic DNA profiles in different strains of *Schizophyllum commune* with primer OPA-3. M, molecular size marker (1 kb DNA ladder); lane 1, IUM-0137; lane 2, IUM-0157; lane 3, IUM-0202; lane 4, IUM-0395; lane 5, IUM-0548; lane 6, IUM-1763; lane 7, IUM-1768; lane 8, IUM-2324; lane 9, IUM-2650; lane 10, IUM-2659; lane 11, IUM-3353; lane 12, IUM-3566.

Table 8. DNA bands in different strains of *Schizophyllum commune* by random amplification of polymorphic DNA assay on 10 base OPA primers

Primers	DNA band (kb)	IUM strains											
		1	2	3	4	5	6	7	8	9	10	11	12
OPA-01	2.5	–	–	–	–	–	–	–	–	–	–	–	+
	1.6	–	+	–	–	–	+	+	+	–	+	+	+
	1.4	+	+	–	+	+	+	+	+	+	+	+	+
	1.0	+	+	+	+	+	+	+	+	+	+	+	+
	0.7	+	+	–	+	–	+	+	+	–	+	+	+
	0.5	+	+	+	+	+	+	+	+	–	+	+	+
OPA-02	1.6	–	+	–	+	–	–	+	–	–	+	+	+
	1.0	+	+	+	–	+	+	+	+	+	+	+	+
	0.8	+	+	+	+	+	+	–	+	+	+	+	+
	0.5	+	+	+	+	+	+	+	+	+	+	+	+
OPA-03	1.7	–	+	+	+	–	+	+	–	+	+	+	+
	1.5	+	+	+	–	+	+	+	+	+	+	+	+
	1.3	+	+	–	–	+	+	+	+	+	+	+	–
	1.0	+	+	+	–	+	+	+	+	+	+	+	+
	0.7	+	+	+	+	+	+	+	+	+	+	+	+
	0.3	+	–	+	–	+	–	–	+	+	–	+	–
OPA-05	1.5	+	+	+	–	–	–	–	–	–	+	+	+
	1.0	+	+	+	+	+	+	+	+	+	+	+	+
	0.5	+	+	+	+	+	+	+	+	+	+	+	+
	0.2	+	+	+	+	+	+	+	+	+	–	+	+
OPA-08	2.7	+	+	+	–	+	+	+	+	+	+	+	+
	1.6	+	+	+	–	+	+	+	+	–	+	+	+
	1.2	+	+	+	+	+	–	+	+	+	+	+	+
	0.7	–	+	+	+	–	–	+	+	+	+	+	+
OPA-09	1.2	+	+	+	–	–	–	+	–	–	+	–	–
	1.0	+	–	+	–	–	+	+	+	–	+	+	+
	0.7	+	+	+	+	+	+	–	+	+	+	+	+
	0.3	+	+	+	+	+	–	–	+	+	–	+	+
OPA-10	1.9	–	+	+	+	–	+	+	+	+	+	+	+
	1.2	+	+	+	–	–	+	–	+	+	+	+	+
	1.0	+	+	+	+	+	+	+	+	+	+	+	+
	0.6	+	+	+	+	+	+	+	+	+	+	–	+
OPA-13	2.4	–	–	–	–	–	+	+	–	–	+	+	+
	2.0	+	+	–	–	+	+	+	–	+	+	+	+
	1.6	+	+	+	+	+	+	+	+	+	+	+	+
	1.0	+	+	+	–	+	+	+	+	+	+	+	+
	0.8	+	+	+	+	+	+	+	+	+	+	+	+
	0.3	–	–	+	+	–	–	–	+	+	–	–	+
OPA-15	1.5	+	+	+	–	+	+	+	+	+	+	+	+
	1.0	+	+	+	–	–	+	+	+	–	+	+	+
	0.8	–	+	+	+	+	+	+	+	–	+	+	+
	0.5	+	+	+	–	–	+	+	+	–	+	+	+
OPA-18	1.5	–	–	–	–	+	+	+	+	–	+	+	+
	1.0	–	+	+	–	+	+	+	+	–	+	+	+
	0.8	+	+	+	+	+	+	+	+	+	+	+	+
	0.5	+	+	+	+	+	+	+	+	+	+	+	+
OPA-19	2.2	+	–	+	+	+	+	+	–	+	+	+	+
	1.3	–	+	+	+	+	+	+	–	–	–	+	+
	1.0	–	+	+	–	+	+	+	–	+	+	+	+
	0.5	+	+	+	+	+	+	+	+	+	+	+	+
OPA-20	1.5	+	+	+	+	+	–	+	–	+	+	+	+
	1.0	+	+	+	+	+	+	+	+	+	+	+	+
	0.7	+	+	+	+	+	–	+	+	+	+	+	+
	0.5	+	+	+	–	+	+	+	+	+	+	+	+

lane 1, IUM-0137; lane 2, IUM-0157; lane 3, IUM-0202; lane 4, IUM-0395; lane 5, IUM-0548; lane 6, IUM-1763; lane 7, IUM-1768; lane 8, IUM-2324; lane 9, IUM-2650; lane 10, IUM-2659; lane 11, IUM-3353; lane 12, IUM-3566.

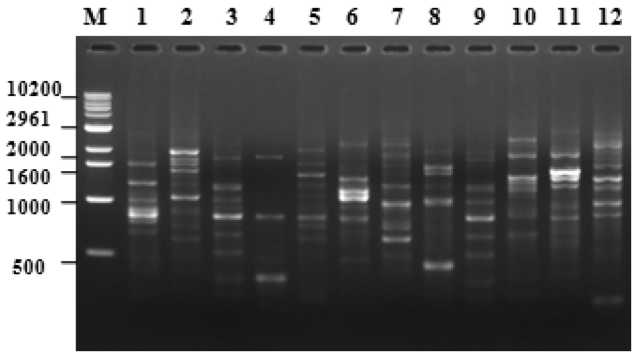

Fig. 4. Random amplification of polymorphic DNA profiles in different strains of *Schizophyllum commune* with primer OPA-13. M, molecular size marker (1 kb DNA ladder); lane 1, IUM-0137; lane 2, IUM-0157; lane 3, IUM-0202; lane 4, IUM-0395; lane 5, IUM-0548; lane 6, IUM-1763; lane 7, IUM-1768; lane 8, IUM-2324; lane 9, IUM-2650; lane 10, IUM-2659; lane 11, IUM-3353; lane 12, IUM-3566.

RAPD analysis. Twenty arbitrary 10 base oligonucleotide primers were used to amplify segments of DNA for selected IUM strains of *S. commune*. Twelve primers, OPA-01, 02, 03, 05, 08, 09, 10, 13, 15, 18, 19, and 20 were found to be efficient for amplifying the genomic DNA (Table 8). These primers showed significant band profiles on the strains tested and high possibilities for screening of each strain (Figs 2~4). RAPD-PCR generated distinct multiple products showing 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 4.5. The size of these polymorphic fragments was obtained in the range from 0.2 to 2.7 kb. The DNA polymorphisms showed the same characteristics in the replication tests. Therefore, if same primers are used for screening DNA polymorphisms, it will be possible to distinguished genetically different strains of *S. commune* (Table 8). To maximize the specificity of the polymorphic patterns, a combined dendrogram was constructed using the RAPD-PCR amplified bands obtained from the twelve RAPD primers. Four putative groups among the 12 strains of *S. commune* were obtained by cluster analysis based on banding patterns and size of amplified products (Fig. 5). Among the twelve strains, 90~100% similarities were found between IUM-3353 (Korea) and IUM-3566 (Korea), IUM-0137 (Korea) and IUM-0157 (Korea), and IUM-0548 (Korea) and IUM-0763 (China), which belong to three different groups. In most of the cases, IUM-0202, IUM-2324, and IUM-0395 showed different band patterns compared to all of the other strains, which was collected from three different ecological regions of Korea. Genetic variability was observed among the tested strains of *S. commune* [11], which could be due to the adaptation of the strains to different environments [23]. Therefore, RAPD is a useful tool for clarifying the genetic relationships among strains.

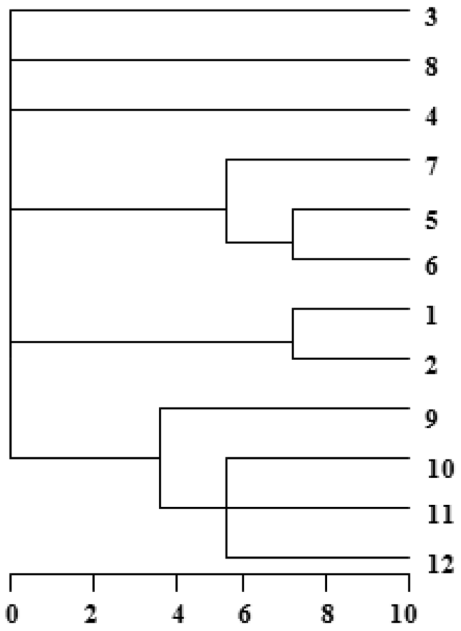

Fig. 5. Dendrogram constructed based on the random amplification of polymorphic DNA markers of *Schizophyllum commune* strains determined by the average linkage cluster. 1, IUM-0137; 2, IUM-0157; 3, IUM-0202; 4, IUM-0395; 5, IUM-0548; 6, IUM-1763; 7, IUM-1768; 8, IUM-2324; 9, IUM-2650; 10, IUM-2659; 11, IUM-3353; 12, IUM-3566.

Acknowledgements

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References

1. Cooke WB. The genus *Schizophyllum*. Mycologia 1961;53: 575-99.
2. Rihs JD, Padhye AA, Good CB. Brain abscess caused by *Schizophyllum commune*: an emerging basidiomycete pathogen. J Clin Microbiol 1996;34:1628-32.
3. Casselton LA, Olesnicky NS. Molecular genetics of mating recognition in basidiomycete fungi. Microbiol Mol Biol Rev 1998;62:55-70.
4. Clark TA, Anderson JB. Dikaryons of the basidiomycete fungus *Schizophyllum commune*: evolution in long-term culture. Genetics 2004;167:1663-75.
5. Yang FC, Liau CB. Effects of cultivating conditions on the mycelial growth of *Ganoderma lucidum* in submerged flask cultures. Bioprocess Eng 1998;19:233-6.
6. Raper CA. *Schizophyllum commune*, a model for genetic studies of the Basidiomycotina. In: Sidhu GS, editor. Genetics of plant pathogenic fungi. London: Academic Press; 1988. p. 511-22.

7. Paul B. ITS region of the rDNA of *Pythium longandrum*, a new species: its taxonomy and its comparison with related species. FEMS Microbiol Lett 2001;202:239-42.
8. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 1990;18:6531-5.
9. Lopandic K, Molnar O, Prillinger H. Application of ITS sequence analysis, RAPD and AFLP fingerprinting in characterising the yeast genus *Fellomyces*. Microbiol Res 2005;160: 13-26.
10. Shim JO, Son SG, Kim YH, Lee YS, Lee JY, Lee TS, et al. The cultural conditions affecting the mycelial growth of *Grifola umbellata*. Kor J Mycol 1997;25:209-18.
11. Alam N, Shim MJ, Lee MW, Shin PG, Yoo YB, Lee TS. Vegetative growth and Phylogenetic relationship of commercially cultivated strains of *Pleurotus eryngii* based on ITS sequence and RAPD. Mycobiology 2009;37:258-66.
12. Sung JM, Kim CH, Yang KJ, Lee HK, Kim YS. Studies on distribution and utilization of *Cordyceps militaris* and *C. nutans*. Kor J Mycol 1993;21:94-105.
13. Lee SB, Taylor JW. Isolation of DNA from fungal mycelia and single spores. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ. editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 282-7.
14. Cubero OF, Crespo A, Fatehi J, Bridge PD. DNA extraction and PCR amplification method suitable for fresh, herbarium stored, lichenized and other fungi. Plant Syst Evol 1999;216: 243-9.
15. Thompson JD, Higgins DG, Gibson TJ. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994;22:4673-80.
16. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 1985;39:783-91.
17. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic tree. Mol Biol Evol 1987;4: 406-25.
18. Nei M, Li WH. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc Natl Acad Sci USA 1979;76:5269-73.
19. Shim SM, Oh YH, Lee KR, Kim SH, Im KH, Kim JW, et al. The characteristics of cultural conditions for the mycelial growth of *Macrolepiota procera*. Mycobiology 2005;33:15-8.
20. Alam N, Jaysinghe C, Cha YJ, Kim JH, Lee TS. Screening of suitable conditions for mycelial growth of wild strains of *Pholiota adiposa*. Bull Life Environ Sci 2008; 2:105-12.
21. Shim SM, Lee KR, Kim SH, Im KH, Kim JW, Lee UY, et al. The optimal culture conditions affecting the mycelial growth and fruiting body formation of *Paecilomyces fumosoroseus*, Mycobiology 2003;31:214-20.
22. Adejoye OD, Adebayo-Tayo BC, Ogunjobi AA, Afolabi OO. Physicochemical studies on *Schizophyllum commune* (Fries) a Nigerian edible fungus. World Appl Sci J 2007;2:73-6.
23. James TY, Moncalvo JM, Li S, Vilgalys R. Polymorphism at the ribosomal DNA spacers and its relation to breeding structure of the widespread mushroom *Schizophyllum commune*. Genetics 2001;157:149-61.
24. White TJ, Bruns T, Lee S, Taylor JW. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, editors. PCR protocols: a guide to methods and applications. San Diego: Academic Press; 1990. p. 315-22.
25. Zervakis GI, Venturella G, Papadopoulou K. Genetic polymorphism and taxonomic infrastructure of the *Pleurotus eryngii* species-complex as determined by RAPD analysis, isozyme profiles and ecomorphological characters. Microbiology 2001;147:3183-94.