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Mycelial Growth Conditions and Molecular Phylogenetic Relationships of *Pleurotus ostreatus*

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Abstract: Commercially cultivated strains of *Pleurotus ostreatus* were collected from different geographical regions of Korea, China and Taiwan. This study was initiated to screening the suitable conditions for mycelial growth and molecular phylogenetic relationship of the selected strains. Suitable temperature and pH for mycelial growth were obtained at 25°C and 7. Glucose peptone, Hamada and yeast malt extract media were the favorable. Dextrin and ammonium acetate were the best carbon and nitrogen sources for the vegetative growth of *P. ostreatus*. Investigation of genetic diversity is necessary to identify the strains. The internal transcribed spacer (ITS) regions of rDNA were amplified using polymerase chain reaction (PCR). The size of ITS1 and ITS2 region varied among the strains and ITS2 was more variable than that of ITS1, whereas the 5.8S sequences were identical. Phylogenetic tree of the ITS region sequences indicate that selected strains were classified into five clusters. The strains were also analyzed by random amplification of polymorphic DNA (RAPD) using 20 arbitrary primers. Ten primers were efficiently amplified the genomic DNA. The numbers of amplified bands were varied based on the primers and strains, with polymorphic fragments ranging from 0.1 to 2.0 kb. The results revealed that tested strains were genetically similar with some variations, RAPD and ITS techniques were well competent for detecting the genetic diversity of all tested strains of *P. ostreatus*.

Key words: ITS sequences · Mycelial growth · *Pleurotus ostreaus* · Phylogenetic relationship · RAPD

INTRODUCTION

Pleurotus ostreatus is a commercially important edible fungus, commonly known as oyster mushroom. This mushroom is widespread in various geographical regions of Korea, China and Taiwan. It has very good abilities to grow at a wide range of temperatures utilizing various lignocelluloses, so that is becoming more popular throughout the world [1-3]. *P. ostreatus* is a good source of dietary fiber and other valuable nutrients [4]. This mushroom contain a number of biologically active compounds with therapeutic activities such as modulation of the immune system, hypoglycemic and antithrombotic activities, decreasing blood lipid concentrations, prevention of high blood pressure and atherosclerosis [5-7]. It has a tetrapolar system of sexual compatibility and a well defined haplo dikaryotic life cycle [8]. Two compatible monokaryotic hyphae fuse and produce a dikaryotic mycelium in which the two parental nuclei remain independent throughout the vegetative growth. The mycelial growth rate exhibits continuous variation and is presumably under the control of a polygenic genetic system [9]. Mycelium cultivation is enhanced by different environmental and nutritional factors as well as propagation of mycelium is an earlier and essential step to cultivate fruiting bodies of mushrooms [10].

P. ostreatus has complicated morphological variations of basidiospores, resulting in taxonomic confusion and difficulties in delimiting species boundaries [11]. Recent molecular phylogenetic studies have demonstrated that ITS region of genomic DNA is very useful for assessing phylogenetic relationships at lower taxonomic levels. ITS of rDNA is considered as a variable region among the species and even among the strains [12]. Among the molecular approaches, RAPD is a convenient method for detecting genetic diversity [13, 14]. Recent genetic analysis on the fungal species has shown that RAPD was superior to rDNA sequence based methods, when distinguishing strains within species. RAPD was particularly successful when applied for verifying mushroom strains from various hosts with a wide range of geographical origins [15]. The intention of this study was to elucidate the favorable environmental and nutritional conditions for the mycelial growth and to

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assess the genetic relationships of the Korean, Chinese and Taiwanese cultivated strains of *P. ostreatus* using both ITS1-5.8S rDNA-ITS2 regions and RAPD analysis.

MATERIALS AND METHODS

Mushroom Strains: Eleven different strains of *P. ostreatus* were acquired from Culture Collection and DNA Bank of Mushrooms (CCDBM), University of Incheon. The accession number (IUM) of strains was University of Incheon mushroom, which was collected from different ecological regions of Korea (IUM-1316, IUM-1319, IUM-1395 and IUM-1721), China (IUM-1932, IUM-2013 and IUM-2036) and Taiwan (IUM-2679, IUM-4143, IUM-4162 and IUM-4171). AB-115051, AF-423120, AY-854077, DQ-077884, EF-514247 and EU-162048 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: Temperatures, 15, 20, 25, 30 and 35°C, were used to find the suitable mycelial growth of *P. ostreatus*. A 5 mm diameter agar plug was removed from 10 days 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 [16].

Culture Media: 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 *P. ostreatus*. The different types of culture media were prepared according to the described method [17].

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 each of ten 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 the 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 [18]. In both cases, the basal medium was adjusted to pH 6 before autoclaving.

DNA Extraction: Genomic DNA was extracted according to the described procedure [19] with some modifications. Fresh mycelia were collected from 10 days old culture 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 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 [20].

Amplification of the ITS Region and Sequence Analysis: The ITS region of the rDNA in selected strains of *P. ostreatus* 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 [21]. 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 [22, 23].

RAPD Analysis: Genomic DNA was amplified by the RAPD technique [24], in which 20 arbitrary 10-base oligonucleotide primers, (OPA-1-CAGGCCCTTC, OPA-2-TGCCGAGCTG, OPA-3-AGTCAGCCAC, OPA-4-AATCGGGCTG, OPA-5-AGGGGTCTTG, OPA-6-GGTCCCTGAC, OPA-7-GAAACGGGTG, OPA-8-GTGACGTAGG, OPA-9-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, Operon Technologies Inc., Alameda, CA, USA) were used to produce amplified fragments. RAPD-PCR reaction was performed using a thermal cycler (Veriti thermal cycler; Applied Biosystems, Foster City, CA, USA) 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 h 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 [25].

RESULTS AND DISCUSSION

Effect of Temperature and pH: To investigate the most favorable temperature for the vegetative growth of *P. ostreatus*, a range from 15-35°C was considered. The optimal mycelial growth (77.03 mm) was recorded at 25°C and the lowest (24.18 mm) at 15°C (Fig. 1). In case of maximum mycelial growth, there are no significant difference between the temperature of 25 and 30°C. Therefore, experimental results indicate that temperature range, 25-30°C was the best for the mycelial growth of *P. ostreatus*. The highest radial growth of mycelium was found at pH 7 (Fig. 2). There was no significance variation between the ranges of pH 6-8 on the mycelial growth of *P. ostreatus*. This result is agrees with the data from studies on *P. eryngii* and *P. adiposa* [17, 26]. Present results indicated that *P. ostreatus* grew very well at wide range of pH and temperature.

Effect of Culture Media: Ten different culture media were used for evaluating favorable mycelial propagation of the selected strains of *P. ostreatus*. Based on the mycelial growth the results indicate that, glucose peptone, Hamada and yeast malt extract were the most favorable, which was statistically similar, whereas Hoppkins and Lilly were the least favorable for the mycelial propagation of *P. ostreatus* (Fig. 3). This result is analogous to that of *P. sinclairii* and *P. fumosoroseus* [27], where mycelial growth was favorable on Hamada and glucose peptone media.

Effect of Carbon and Nitrogen Sources: Ten different carbon sources were assayed for screening effective mycelial growth of *P. ostreatus*. Dextrin was found to be the best and followed by fructose, maltose, sucrose and mannose. On the other hand lactose and xylose were the most unfavorable carbon sources (Fig. 4). Among the nitrogen sources, ammonium acetate was found to be the best and followed by glycine and arginine. The lowest level of mycelial growth was recorded in histidine and alanine (Fig. 5). These findings are comparable to the previous studies on *P. eryngii* [17], in which dextrin and arginine were the most effective carbon and nitrogen sources for the mycelial growth.

ITS Sequence Analysis: To investigate the genetic characteristics of selected strains of *P. ostreatus*, the ITS region were amplified using ITS1 and ITS4 primers and then sequenced. Results indicate that the length of the sequences among the selected strains ranged from 580 to 604 bp. The size of the ITS1 and ITS2 regions varied among the strains from 206 to 221 bp and 166 to 200 bp, respectively. The total G+C and A+T content of the ITS region varied from 43.8 to 46.5% and 53.5 to 56.2% (Table 1). Sequence analysis indicated that the 5.8S of rDNA sequences were identical (158 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

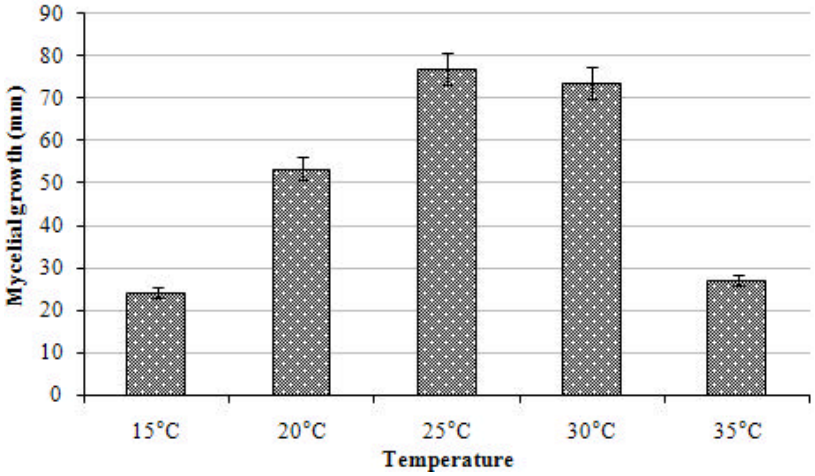

Fig. 1: Effect of temperature on the mycelial growth of *Pleurotus ostreatus* on PDA after 10 days of incubation. Vertical bars show standard error.

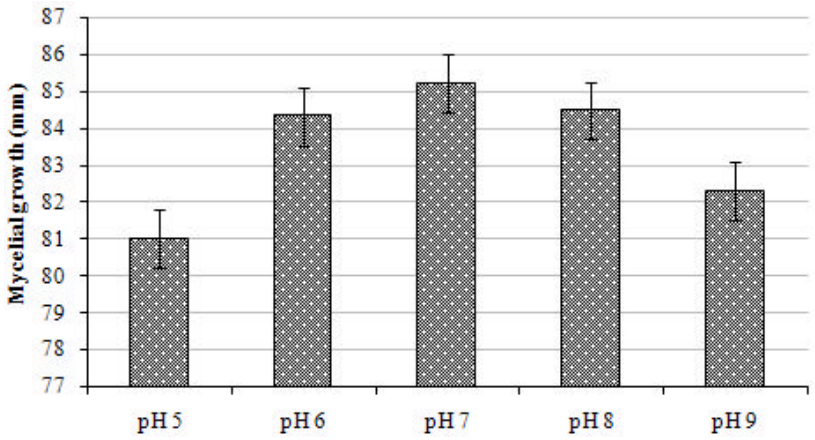

Fig. 2: Effect of pH on the mycelial growth of *Pleurotus ostreatus* on PDA after 10 days of incubation at 25 ºC. Vertical bars show standard error.

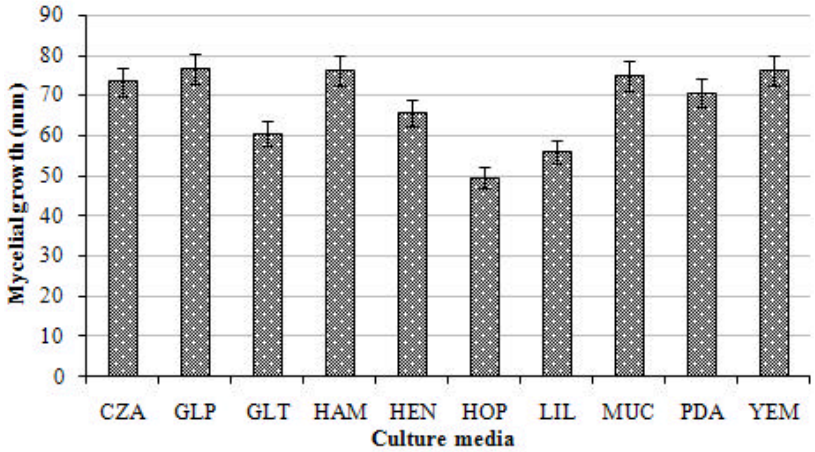

Fig. 3: Effect of different media on the mycelial growth of *Pleurotus ostreatus* after 10 days of incubation at 25 ºC. Vertical bars show standard error. CZA- Czapek Dox, HAM - Hamada, HEN - Hennerberg, HOP- Hoppkins, GLP- glucose peptone, GLT- glucose tryptone, LIL- Lilly, MUC- mushroom complete, PDA-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

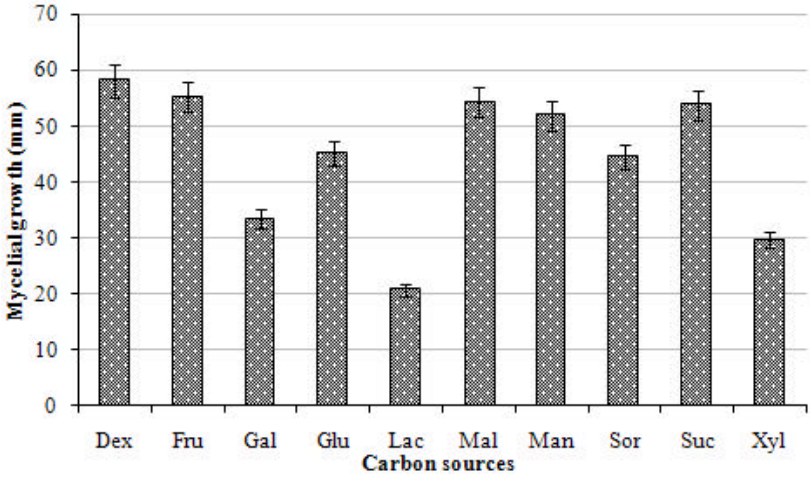

Fig. 4: Effect of carbon sources on the mycelial growth of *Pleurotus ostreatus* on basal medium after 10 days of incubation at 25 ºC. Vertical bars show standard error. Dex- dextrin, Fru- fructose, Gal- galactose, Glu- glucose, Lac- lactose, Mal - maltose, Man- mannose, Sor- sorbitol, Suc- sucrose,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

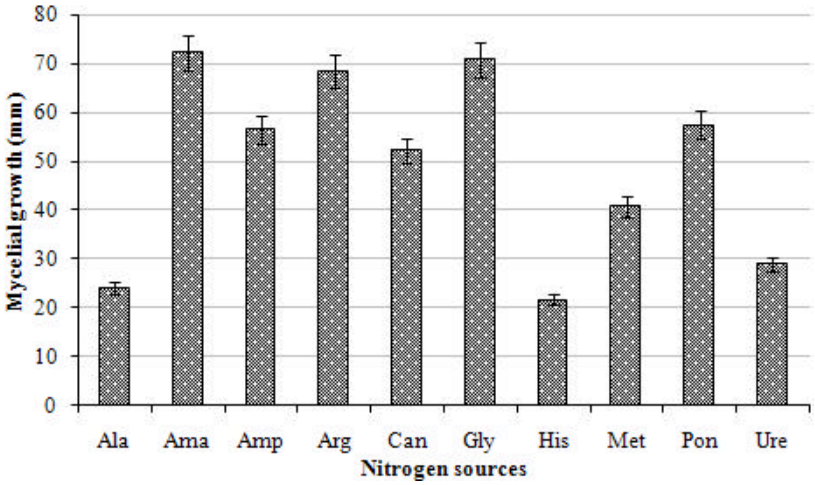

Fig. 5: Effect of nitrogen sources on the mycelial growth of *Pleurotus ostreatus* on basal medium after 10 days of incubation at 25ºC. Vertical bars show standard error. Ala- alanine, Ama- ammonium acetate, Amp- ammonium phosphate, Arg- arginine, Can- calcium nitrate, Gly - glycine, His - histidine,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

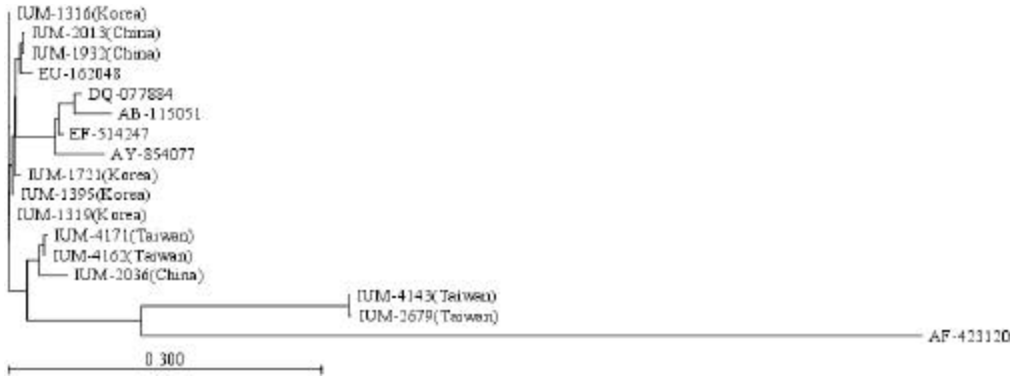

Fig. 6: Phylogenetic tree of seventeen strains of *Pleurotus ostreatus* based on the nucleotide sequences of the ITS regions using neighbor-joining method with 1,000 boot-strapping trails.

Table 1: Nucleotide distribution, ITS1, 5.8S and ITS2 of rDNA sequences in different strains of *Pleurotus ostreatus*

	Nucleotide distribution					Sequence information				
Strain	A	C	G	T	G+C (%)	A+T (%)	ITS1	5.8S	ITS2	Length (bp)
IUM-1316	150	130	132	185	44.2	55.8	220	158	200	600
IUM-1319	150	133	132	185	44.2	55.8	220	158	200	600
IUM-1395	149	133	131	185	44.1	55.9	220	158	200	598
IUM-1721	148	133	129	184	44.1	55.9	221	158	200	594
IUM-1932	146	135	132	185	44.1	55.4	216	158	200	598
IUM-2013	147	135	132	185	44.6	55.4	216	158	200	599
IUM-2036	150	130	124	176	43.8	56.2	206	158	166	580
IUM-2679	137	142	139	186	46.5	53.5	216	158	200	604
IUM-4143	137	142	138	185	46.5	53.5	216	158	200	602
IUM-4162	150	130	127	180	43.8	56.2	219	158	166	587
IUM-4171	150	130	127	180	43.8	56.2	220	158	166	587

A- adenine, C- cytosine, G- guanine, T- thymine

Table 2: DNA bands in different strains of *Pleurotus ostreatus* by random amplification of polymorphic DNA assay using 10-base OPA primers

		IUM Strains										
Primers	DNA band(kb)	1	2	3	4	5	6	7	8	9	10	11
OPA-1	2.0	-	-	-	-	+	+	-	+	+	+	+
	1.5	+	+	+	+	+	+	+	-	+	-	-
	1.2	+	+	+	+	+	+	-	-	-	+	+
	0.9	+	+	+	+	+	+	+	+	+	+	+
	0.7	-	-	-	-	+	+	+	+	+	+	+
	0.5	-	-	-	-	-	-	+	+	-	-	-
	0.1	+	+	+	+	-	-	-	-	+	-	-
OPA-2	1.5	+	+	+	+	+	+	-	-	+	-	-
	1.0	+	+	+	+	+	+	-	-	+	+	+
	0.8	+	+	+	+	+	+	-	+	+	+	+
	0.5	-	-	-	-	+	+	-	+	+	+	+
	0.2	-	-	-	-	+	+	+	+	+	+	+
OPA-3	1.5	+	+	+	+	+	+	+	-	+	+	+
	1.0	+	+	+	+	+	+	+	-	+	+	+
	0.8	+	+	+	+	+	+	+	+	+	+	+
	0.5	+	+	+	+	+	+	+	+	+	+	+
	0.3	+	+	+	+	+	+	+	+	+	-	-
OPA-4	1.2	-	-	-	-	+	+	-	+	-	-	-
	1.0	+	+	+	+	+	+	-	+	+	+	+
	0.8	+	+	+	+	+	+	-	+	+	+	+
	0.3	+	+	+	+	+	+	+	-	+	+	+
OPA-5	1.4	+	+	+	+	+	+	+	+	+	-	-
	0.7	-	-	-	-	+	+	+	+	+	+	+
	0.5	-	-	-	-	+	+	+	+	+	+	+
OPA-7	1.6	+	+	+	+	-	-	-	+	+	+	+
	1.3	+	+	+	+	-	-	-	+	+	+	+
	0.6	+	+	+	+	+	+	+	-	-	+	+
OPA-11	1.3	+	+	+	+	-	-	-	-	+	+	+
	1.0	-	-	-	-	+	+	+	+	-	-	-
	0.3	-	-	-	-	+	+	+	-	+	+	-
OPA-13	1.6	+	+	+	+	+	+	+	+	+	+	+
	1.1	+	+	+	+	+	+	+	-	-	-	-
	0.7	-	-	-	-	+	+	+	-	-	-	+
	0.5	-	-	-	-	-	-	-	+	+	+	-
OPA-17	1.0	+	+	+	+	+	+	+	+	+	+	+
	0.5	+	+	+	+	+	+	+	-	-	-	-
	0.3	+	+	+	+	-	-	-	-	-	-	-
OPA-18	1.5	-	-	-	-	+	+	+	+	+	+	+
	1.0	+	+	+	+	+	+	-	-	-	-	-
	0.3	+	+	+	+	+	+	-	+	+	+	+

lane 1- IUM-1316, lane 2- IUM-1319, lane 3- IUM-1395, lane 4- IUM-1721, lane 5- IUM-1932, lane 6- IUM-2013, lane 7- IUM-2036, lane 8- IUM-2679, lane 9- IUM-4143, lane 10- IUM-4162, lane 11- IUM-4171, - indicate absence of DNA band, + indicate presence of DNA band

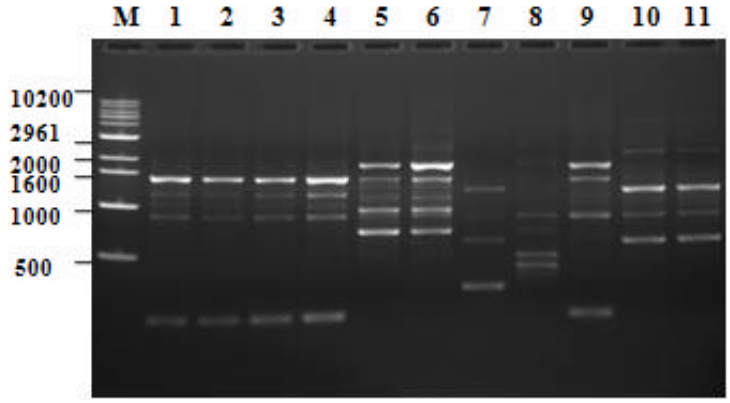

Fig. 7: Random amplification of polymorphic DNA profiles in different strains of *Pleurotus ostreatus* using OPA-1 primer. M- molecular size marker (1 kb DNA ladder), lane 1- IUM-1316, lane 2- IUM-1319, lane 3- IUM-1395, lane 4- IUM-1721, lane 5- IUM-1932, lane 6- IUM-2013, lane 7- IUM-2036, lane 8- IUM-2679, lane 9- IUM-4143, lane 10- IUM-4162, lane 11- IUM-4171.

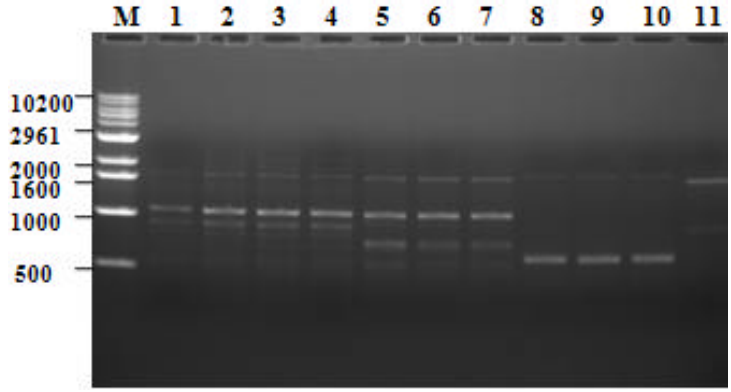

Fig. 8: Random amplification of polymorphic DNA profiles in different strains of *Pleurotus ostreatus* with OPA-11 primer. M- molecular size marker (1 kb DNA ladder), lane 1- IUM-1316, lane 2- IUM-1319, lane 3- IUM-1395, lane 4- IUM-1721, lane 5- IUM-1932, lane 6- IUM-2013, lane 7- IUM-2036, lane 8- IUM-2679, lane 9- IUM-4143, lane 10- IUM-4162, lane 11- IUM-4171.

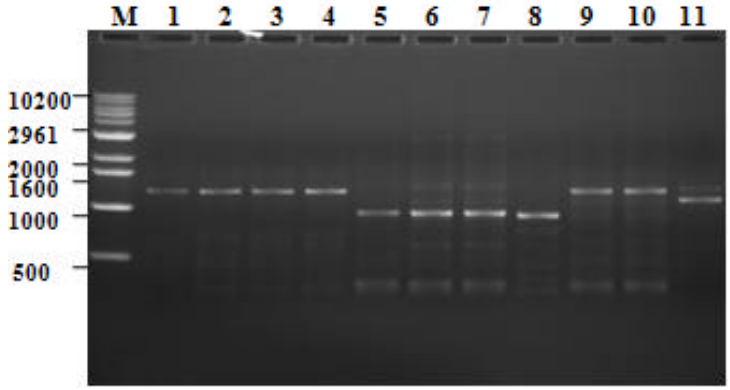

Fig. 9: Random amplification of polymorphic DNA profiles in different strains of *Pleurotus ostreatus* with OPA-13 primer. M- molecular size marker (1 kb DNA ladder), lane 1- IUM-1316, lane 2- IUM-1319, lane 3- IUM-1395, lane 4- IUM-1721, lane 5- IUM-1932, lane 6- IUM-2013, lane 7- IUM-2036, lane 8- IUM-2679, lane 9- IUM-4143, lane 10- IUM-4162, lane 11- IUM-4171.

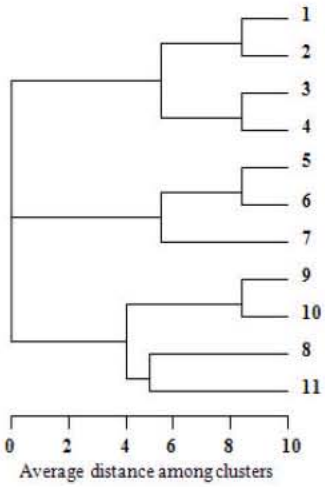

Fig. 10: Dendrogram constructed based on the random amplification of polymorphic DNA markers of *Pleurotus ostreatus* strains determined by the average linkage cluster. 1- IUM-1316, 2- IUM-1319, 3- IUM-1395, 4- IUM-1721, 5- IUM-1932, 6- IUM-2013, 7- IUM-2036, 8- IUM-2679, 9- IUM-4143, 10- IUM-4162, 11- IUM-4171

insertion or deletion polymorphisms of the base position [28]. Phylogenetic tree based on the nucleotide sequences of the ITS regions in seventeen different strains of *P. ostreatus* was constructed by neighbor-joining method. The phylogenetic tree was separated into five groups (Fig. 6). Maximum difference was observed between AB-115051 and AF-423120 of NCBI GenBank strain, while maximum similarity (99-100%) was recorded in between IUM-4143 (Taiwan) and IUM-2679 (Taiwan), IUM-4171 (Taiwan) and IUM-4162 (Taiwan) and IUM-2013 (China) and IUM-1932 (China). The results also indicate that most of the NCBI GenBank strains were very similar to IUM tested 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 genetic diversity was detected within groups is probably due to an efficient gene flow and to a high genetic compatibility within the strains tested [30].

RAPD Analysis: Twenty arbitrary 10-base oligonucleotide primers were used to amplify segments of genomic DNA for selected IUM strains of *P. ostreatus*. Ten primers (OPA-1, 2, 3, 4, 5, 7, 11, 13, 17 and 18) were found to be efficient for amplification of genomic DNA (Table 2). These primers show significant band profiles on the tested strain, which made them good candidates for screening of each strain (Fig. 7, 8 and 9). RAPD-PCR generated distinct multiple products showing considerable variability among the tested strains. The number of amplified bands varied depending on the primers used or the strains tested. The size of these polymorphic fragments was obtained in the range from 0.1 to 2.0 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 could be possible to distinguish genetically different strains of *P. ostreatus*. To maximize the specificity of polymorphic patterns, a combined dendrogram was constructed by using RAPD-PCR amplified bands obtained from the ten RAPD primers. Three putative groups among the 11 strains of *P. ostreatus* were obtained by cluster analysis based on banding patterns and the size of amplified products (Fig. 10). Among the eleven strains, 99-100% similarities were observed between IUM-1316 (Korea) and IUM-1319 (Korea), IUM-1395 (Korea) and IUM-1721 (Korea), IUM-1932 (China) and IUM-2013 (China), IUM-4143 (Taiwan) and IUM-4162 (Taiwan) and IUM-2679 (Taiwan) and IUM-4171 (Taiwan), all of which belong to three different groups. Therefore, our results are comparable to the study made by Alam *et al*. [17] and support RAPD as a useful tool for clarifying the genetic relationships among strains.

Therefore, it could be concluded that *P. ostreatus* has a wide range of temperature, pH, culture media, carbon and nitrogen sources for the suitable mycelial growth. This mushroom grew very well at acetic or neutral or alkaline conditions. Dextrin and ammonium acetate were the best carbon and nitrogen sources for the mycelial growth of *P. ostreatus*. Molecular results suggested that tested strains were genetically similar with some variations, RAPD and ITS techniques were well suited for detecting the genetic diversity of all tested strains of *P. ostreatus*.

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