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# Differentiation of *Lentinus edodes* Isolates in Korea by Isozyme Polymorphisms and Random Amplified Polymorphic DNA (RAPD) Analysis

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# Isozyme Polymorphism 및 Random Amplified Polymorphic DNA(RAPD) Pattern에 의한 표고 버섯 품종간 비교

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**ABSTRACT: Sixty-three isolates of *Lentinus edodes* obtained from Korea were used to assess the genetic similarity by isozyme polymorphisms and random amplified polymorphic DNA patterns. The activities of esterase, peroxidase and acid phosphatase displayed 10, 7 and 3 distinct isozyme patterns, respectively. By combining the isozyme patterns obtained with the 3 enzymes, every isolate showed its own distinct electrophoretic phenotypes. A distance matrix calculated between all pairs of 63 electrophoretic phenotypes based on the presence or abscence of isozyme bands were analyzed by the group-average method. Results of the cluster analysis assinged the 63 phenotypes into six major groups. In the analysis of random amplified polymorphic DNA patterns, all isolates of *Lentinus edodes* were devided into five RAPD groups.**

**KEYWORDS:** ***Lentinus edodes*** **isolates, isozyme polymorphism, random amplified polymorphic DNA pattern, cluster analysis.**

*Lentinus edodes* (Berk.) Singer (Pegler, 1975) commonly called "Shitake" is widely cultivated in Asia. Commercial cultivation of this mushroom is now rapidly expanding in other parts of the world because of its superial flavour (Ito, 1978), texture, nutronal value (Crisan and Sands, 1978; Miles and Chang, 1985) and medical properties such as antitumor activity, antiviral activity (Yamamura and Cochrane, 1976) and the ability to reduce serum cholesterol level in humans (Suzuki and Oshima, 1976).

Methods for genotypic identification are important for breeding of commercial mushrooms. Fungal taxonomy relies on differences of morphological characters. Especially, the identification of strains and species for various edible mushrooms has been generally based on morphological characteristics such as the colour, size, and shape of the fruit body and cultivation characteristics including optimal temperature of fruiting body production

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and mycelial growth (Mutoh, 1992). In addition, pairings in culture (Fukuda and Tokimoto, 1991) have been used to identify edible mushrooms. But this resolutions may be difficult because of ambiguous characters or effects from environmental differences. To overcome those disadvantages, various methods have been developed (Fukuda and Tokimoto, 1991).

The electrophoretic separation of proteins has become an important tool in identification and differentiation of species and subspecies in various organisms, including fungi (Chin *et al.*, 1993; Glynn and Reid, 1969; Hanson and Wells, 1991; Lee *et al.*, 1983; Mario *et al.*, 1994; Park *et al.*, 1987; Park *et al.*, 1979, 1986a, 1986b, 1987; Stasz, Weeden and Harman, 1988). Electrophoretic separation of enzymes, which exploits the polymorphism of detectable isozyme forms, is another molecular technique that has been widely used to generate a large number of markers for the assessment of genetic diversity in fungi (Damaj *et al.*, 1993; Kaosiri and Zentmyer, 1980). Damaj *et al.* (1993) reported that fifty isolates of binucleate *Rhizoctonia* representing 12 Japanese and 5 North American anastomosis groups (AGs) were divided into four distinct groups by cluster analysis of isozyme bands. Zervakis and Labarere (1992) reported that isozyme analysis could provide valuable information about the species taxonomy of the genus *Pleurotus*. May and Royse (1988) also discussed genotypic classification of *Pleurotus* using allozyme analysis. Park *et al.* revealed that esterase isozyme resulted in good comparision of *Pleurotus* spp., *Ganoderma lucidum, Colletotrichum* spp. and *Pyricularia oryzae*. Recently, Hyun and Park (1996a) also have reported that thirty-two isolates of *Fusarium oxysporium* f. sp. *fragariae* representing four vegetative compatibility groups (VCGs) were divided into four distinct groups by cluster analysis of isozyme bands.

Williams *et al.* (1990) recently reported a new DNA polymorphism assay based on the random amplification of DNA segments with single primers of arbitrary nucleotide sequences. They suggest that these polymorphisms be called RAPD markers, after Random Amplified Polymorphic DNA. The modification of polymerase chain reaction (PCR) with a single primer of arbitrary nucleotide sequence has been proved useful in detecting intraspecific polymorphisms in various organisms (Folkertsma *et al.*, 1994; William *et al.*, 1990) and useful for genome mapping and identification of isolates (Huff, Bunting and Plumley, 1994). For plant pathogenic fungi, RAPD analysis can provide markers to differentiate races of *Fusarium solani* f. sp. *dianthi* (Manulis *et al.*, 1994; Wright *et al.*, 1990), *F. oxysporum* f. sp. *pisi* (Grajai-Martin, Simson and Muehlbauer, 1993), aggressive and nonaggressive isolates of *Phoma lingam* (Schafer and Wostemeyer, 1992) and *F. oxysporm* f. sp. dianthi (Manulis *et al.*, 1994), and isolates with different geographic origins of *Colletotrichum graminicola* (Guthrie *et al.*, 1992).

Additionally, RAPD markers are used for tracking strains of *F. graminearum* in field experiment (Ouellet and Seifert, 1993) and for investigating evolutionary processes and genetic linkage in the barley powdery mildew pathogene (McDermott *et al.*, 1994). Hyun and Park (1996b) have reported that RAPD assay is very useful method for differentiation of *Fusarium oxysporium* f. sp. *fragariae*.

Recently considerable efforts have been expended to clarify the taxonomy and the varities of this mushroom. Royse *et al.* (1983a, 1983b), Roys and May (1987) and Bowden *et al.* (1991) revealded that examined commercial cultivars of *L. edodes*, through the using of allozyme analysis, have thirty-one loci. And out of thirty-one loci, twenty-three loci

have been polymorphic. By the reports, such a high propotion of polymorphic loci would indicate that there is considerable genetic diversity in this species.

It is considerate that there are many varieties of *L. edodes* in Korea, but there are no data of identification and differentiation of such varieties.

In this study, the genetic diversity in isolates of *L. edodes* was investigated by analyses of isozyme polymorphisms and RAPD markers. In addition, data generated from isozyme patterns were compared with these from RAPD markers.

## Material and Methods

### Isolates of *L. edodes*

Sixty-three isolates (fourteen wild types and forty-nine cultivated types of *L. edodes*) were examined. isolates of *L. edodes* were obtained from the collections of Forest Product Microbial Products Center of National Forestry Cooperatives Federation in Seoul, Korea (Table 1). Mycelia were maintained on PDA (potato dextrose agar) medium. They were grown on MYG (malt extract 5g, yeast extract 10g, glucose 5g, water 1 liter) medium for two weeks at 25°C, harvested by filtration and washed with distilled water for preparation of sample.

### Extraction of protein

Mycelial mats of 10 days old culture in MYG medium were harvested by suction-filtering through filter paper. The mycelial mats were macerated in 0.1 M Tris-HCl buffer (0.1 M Tris, 2 mM EDTA), pH 7.2, by mortar and pestle with sea sand. The homogenates were centrifuged at 15,000g for 30 min at 4°C. The

**Table 1.** Isolates of *L. edodes* arranged by variety, isolate type, geographic source, isozyme polymorphism type and random amplified polymorphic DNA pattern

| Isolate No. | Variety | Isolate type | Geographic source | I. P. T[a] | RAPD pattern |
|---|---|---|---|---|---|
| 1 | LH1 | Registered[b] | | 1a-a | 1[h]a[i]-a[j]-a[k] |
| 2 | LH2 | Registered | | 4a | 1a-a-a |
| 3 | LH3 | Registered | | 2a-a | 3a-a-a |
| 4 | LH4 | Registered | | 2b-a | 4a-a |
| 5 | LH5 | Registered | | 1a-b | 3a-a-a |
| 6 | LH6 | Registered | | 1a-b | 3a-a-a |
| 7 | LH7 | Registered | | 1b-a-a | 1b-a-a |
| 8 | SL1 | Registered | | 1c-a-a | 3a-a-a |
| 9 | SL2 | Registered | | 1a-a | 4b-a-a |
| 10 | SL3 | Registered | | 1a-b | 3a-a-a |
| 11 | SL4 | Registered | | 2a-a | 1a-a-b |
| 12 | SL5 | Registered | | 1a-a | 4a-a |
| 13 | SL6 | Registered | | 1a-a | 4a-a |
| 14 | NG1 | Registered | | 1a-a | 3a-a-a |
| 15 | W1001 | Wild[c] | Kangwon[g] | 2c-a | 4a-a-a |
| 16 | W1002 | Wild | Kangwon | 1d-a | 3a-a-a |
| 17 | W1003 | Wild | Kangwon | 1d-b-a | 3a-a-a |
| 18 | W1004 | Wild | Kangwon | 2b-a | 1a-b |
| 19 | W1005 | Wild | Kangwon | 2c-a | 1a-a-b |
| 20 | W1006 | Wild | Kangwon | 5a | 1a-a-b |

**Table 1.** Continued

| Isolate No. | Variety | Isolate type | Geographic source | I.P.T[a] | RAPD pattern |
|---|---|---|---|---|---|
| 21 | W2001 | Wild | Kyunggi | 1b-a | 1a-b |
| 22 | W2002 | Wild | Kyunggi | 1b-c | 1a-b |
| 23 | W8001 | Wild | Kyungnam | 1b-b | 1a-b-a |
| 24 | W8003 | Wild | Kyungnam | 1c-c | 1a-a-b |
| 25 | W8004 | Wild | Kyungnam | 1c-b-a | 1a-a |
| 26 | W8005 | Wild | Kyungnam | 1d-b-a | 3a-a-a |
| 27 | W8006 | Wild | Kyungnam | 1c-a | 1a-a-c |
| 28 | W8007 | Wild | Kyungnam | 5a | 2a-a |
| 29 | C2002 | Nonregistered[d] | Kyunggi | 1a-a | 3b-a |
| 30 | C4002 | Nonregistered | Chungnam | 1d-b-a | 1a-a-b |
| 31 | C6003 | Nonregistered | Chungnam | 1c-a | 1a-a-c |
| 32 | C7002 | Nonregistered | Kyungbuk | 1d-b | 3a-a |
| 33 | C7003 | Nonregistered | Kyungbuk | 1e-a | 1a-a-c |
| 34 | C7005 | Nonregistered | Kyungbuk | 1c-a-a | 1a-a-c |
| 35 | C7006 | Nonregistered | Kyungbuk | 1b-c | 1a-a-c |
| 36 | C7007 | Nonregistered | Kyungbuk | 1d-a | 1a-a-c |
| 37 | C8001 | Nonregistered | Kyungnam | 1b-b | 2a-a-a |
| 38 | H1001 | Nonregistered | | 1b-b | 1a-b-a |
| 39 | H1002 | Nonregistered | | 2b-a | 1a-b-a |
| 40 | H1003 | Nonregistered | | 1b-c-a | 2b-a |
| 41 | H1004 | Nonregistered | | 4b-a | 2a-b |
| 42 | H1006 | Nonregistered | | 1c-a | 1b-a-a |
| 43 | H1007 | Nonregistered | | 1b-a-a | 1a-a |
| 44 | H1009 | Nonregistered | | 2b-a | 4a-a-a |
| 45 | H1010 | Nonregistered | | 2c-a | 4a-a |
| 46 | H1011 | Nonregistered | | 1b-a | 4a-a-a |
| 47 | H1012 | Nonregistered | | 1c-a | 1b-a-a |
| 48 | H1013 | Nonregistered | | 1c-a | 2a-b |
| 49 | H1014 | Nonregistered | | 4a-a | 2a-a |
| 50 | H1015 | Nonregistered | | 1b-c-a | 2b-a |
| 51 | H1016 | Nonregistered | | 1e-a | 2a-a |
| 52 | H1018 | Nonregistered | | 3a-a | 5a |
| 53 | H1020 | Nonregistered | | 1c-a | 1b-a-a |
| 54 | H1021 | Nonregistered | | 1d-a | 2a-a-a |
| 55 | CH1001 | Hybrid[e] | | 1d-a | 2a-a-a |
| 56 | CH1002 | Hybrid | | 1a-b | 2b-a |
| 57 | CH1003 | Hybrid | | 3a-a | 1b-a |
| 58 | CH1004 | Hybrid | | 3a-a-a | 4a-a-a |
| 59 | CH1005 | Hybrid | | 3a-a-a | 4b-a-a |
| 60 | CH1006 | Hybrid | | 1b-c-b | 2a-a-a |
| 61 | CH1007 | Hybrid | | 6a | 2b-a |
| 62 | No001 | Foreign[f] | | 3a-a | 2a-a-a |
| 63 | As001 | Foreign | | 1b-c-b | 2a-a |

[a]I.P.T.: isozyme polymorphism type, [b]Registered: registered and cultivated isolate in Korea, [c]Wild: wild isolated isolate, [d]Nonregistered: nonregistered but cultivated isolate, [e]Hybrid: hybrided isolate, [f]Foreign: foreign introduced isolate, [g]Kangwon: isolated area, province, 1[h]a-a-a: distinct type at genetic similarity of 0.6, 1a[i]-a-a: distinct type at genetic similarity of 0.7, 1a-a[j]-a: distinct type at genetic similarity of 0.8, 1a-a-a[k]: distinct type at genetic similarity of 1.0.

supernatants were used for electrophoresis.

**Electrophoresis of protein**

Electrophoresis was conducted with 6~26% polyacrylamide porosity gradient slab gels (200×150×2.0 mm) utilizing Poomaphor system according to Stegemann's method (Park and Stegemann, 1979). The 0.125 M tris-borate buffer, pH 8.9 was used as gel buffer and tray buffer. Samples of 100 μg protein per slot were layered onto the gel. The amount of protein was determined by Bradford's method (1976). Electrophoresis was conducted at 100 Volt. for 1 h., followed by 250 Volt. for 18h.

**Isozyme staining**

After electrophoresis, each gel was stained as follows;

**Esterase**

The gel was soaken in 0.1 M tris-HCI buffer, pH 7.2, at room temperature for 30 min, and stained with staining solution (50 mg α-naphthyl acetate, 85 mg Fast blue RR salt, 0.1 M Tris-HCl buffer; pH 7.2) untill the bands appeared and stored in 5% acetic acid.

**Acid phosphatase**

The gel was soaken in 0.1 M acetate buffer, pH 5.2, at room temperature for 30 min. and stained in staining solution (70 mg Fast Garnet GBC salt, 80 mg α-naphthyl acid phosphate, 6 *ml* of 10% $MgCl_2$, 10 *ml* of Acetate buffer; pH 4.5) under dark.

**Peroxidase**

The gel was washed three times with distilled water and stained in benzidin solution (1g benzidin, 9 *ml* acetic acid and 400 *ml* $H_2O$).

**DNA extraction**

DNA was extracted from mycelium by modified method of Yoon *et al.* (1991). Approximately 100 mg of fresh mycelium was ground using a precooled mortar and pestle with 1 *ml* of lysis buffer (50 mM Tris-HCl; pH 7.2, 50 mM EDTA; pH 7.2, 3% sodium dodecyl sulfate and 1% 2-mercaptoethanol). The homogenate was transferred into a 2 *ml* microfuge tube and incubated at 65°C for 1 h., occasionally inverting the tube to ensure complete lysis. The tube was added one volume of chloroform, gently inverted several times, and centrifuged at 14,000g for 15 min. at room temperature. To precipitate DNA, the upper phase was transferred to a new 1.5 *ml* tube, added 0.1 volume of 3 M sodium acetate and I volume of isopropanol, mixed, and centrifuged at 14,000g for 5 min. The supernatant was decanted and the pellet was rinsed with ice-cold 70% ethanol. The pellet was resuspended in 200 μ*l* TE (10 mM Tris-HCl, pH 8.0 and 0.1 mM EDTA, pH 8.0), added 100 mg of CsCl, and centrifuged at 14,000g for 15 min. at room temperature. The upperphase was transferred to a new tube and added 2 volumes of TE. The tube was added one volume of isopropanol and centrifuged at 14,000g for 5 min. at room temperature. The pellet was rinsed with ice-cold 80% ethanol, dried, resuspended in 100 μ*l* TE, and stored at −20°C.

**PCR conditions**

PCR was carried out in 25 μ*l* of a solution containing 1~5 ng of *L. edodes* genomic DNA; 1 mM $MgCl_2$; 0.3 M of primer; 0.5 unit of *Taq* DNA polymerase (Finnzymes Inc., Filand); 100 μM each of dCTP, dGTP, dATP and dTTP (Promega Co., U.S.A.); 100 mM KCl; 0.1 mM EDTA; 1 mM DTT; 160 μg/*ml* BSA; 50% glycerol and 0.1% Triton X-100 under 20 μ*l* of mineral oil. Amplification was performed in a thermal cycler (Perkin-Elmer Cetus, U.S.A.) programmed for one cycle of 2 min at 95°C, followed by 45 cycles of 30 sec at 94°C, 1 min

at 40°C, and 2 min at 72°C and a final incubation at 72°C for 5 min. After PCR, 12.5 μl of the product was electrophoresed in 1.4% agarose gel and visualized by ethidium bromide staining.

**RAPD assay**

Tweenty primers were obtained from Operon Technologies, Inc. (Alameda, CA 94501, USA) (Table 2). Amplification reactions were conducted with each primer on the DNA of the 63 isolates.

**Cluster analysis**

A computer program, NTSYS-pc was used for analysis of the data. Comparison between isolates for each isozyme profile and RAPD markers were made on the basis of the presence versus absence of isozyme band at the same Rf value and RAPD marker of the same distance. Bands of the same distance were scored, and a distance value defined as the Dice coefficient was calculated by the following formulars: $1\text{-}2(N_{XY})/(N_X+N_Y)$, where $N_{XY}$ is the number of isozyme band (RAPD marker) shared between a given pair of phenotypes, and $N_X$ and $N_Y$ are the total numbers of isozyme bands in all enzymes or those of RAPD markers in phenotypes $x$ and $y$. Dendrograms based on the distance values were constructed by group-average cluster analysis (Rohlf, 1990).

**Table 2.** Code and sequence of the 20 primers used, the total number of amplified DNA fragments and size of polymorphic DNA fragments obtained with each primer in random amplified polymorphic DNA (RAPD) experiments

| Code | Sequence 5' to 3' | Numbers of amplified fragments | Size of fragments (bp) |
|---|---|---|---|
| OPA-01 | CAGGCCCTTC | 11 | 1600-400 |
| OPA-02 | TGCCGAGCTG | 21 | 2000-350 |
| OPA-03 | AGTCAGCCAC | 12 | 1500-500 |
| OPA-04 | AATCGGGCTG | 9 | 1700-850 |
| OPA-05 | AGGGGTCTTG | 17 | 2100-500 |
| OPA-06 | GGTCCCTGAC | 20 | 2900-300 |
| OPA-07 | GAAACGGGTG | 14 | 1500-300 |
| OPA-08 | GTGACGTAGG | 13 | 2800-350 |
| OPA-09 | GGGTAACGCC | 9 | 1900-650 |
| OPA-10 | GTGATCGCAG | 15 | 1800-500 |
| OPA-11 | CAATCGCCGT | 16 | 2000-400 |
| OPA-12 | TCGGCGATAG | 11 | 1600-400 |
| OPA-13 | CAGCACCCAC | 17 | 2800-800 |
| OPA-14 | TCTGTGCTGG | 19 | 2600-400 |
| OPA-15 | TTCCGAACCC | 13 | 3200-700 |
| OPA-16 | AGCCAGCGAA | 14 | 1700-400 |
| OPA-17 | GACCGCTTGT | 12 | 1600-300 |
| OPA-18 | AGGTGACCGT | 17 | 2100-300 |
| OPA-19 | CAAACGTCGG | 9 | 1600-300 |
| OPA-20 | GTTGCGATCC | 16 | 1900-300 |

## Result

**Esterase**

A total of 30 isozyme bands was detected. Based on the presence or absence of these bands, the 63 isolates of *L. edodes* were divided into 10 zymogram groups (Fig. 1 and Table 3).

**Acid phosphatase**

In this enzyme, a total of 5 bands were detected and the 63 isolates were divided into 3 zymogram groups (Fig. 2 and Table 3).

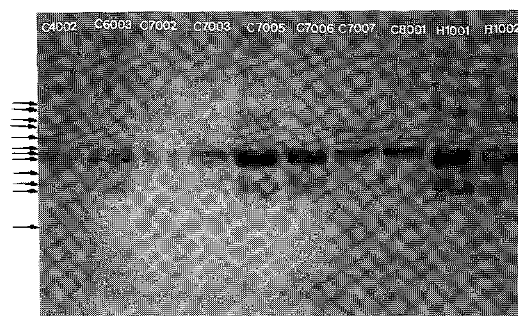


**Fig. 1.** Esterase band patterns of *L. edodes* isolates on 6~26% porosity gradient polyacrylamide gel electrophoresis.

**Table 3.** Electrophoretic phenotypes of zymograms for *L. edodes* isolates in Korea

| Isolate No. | Variety | Types of isozyme patterns | | |
|---|---|---|---|---|
| | | EST[a] | APH[b] | PER[c] |
| 1 | LH1 | a | a | a |
| 2 | LH2 | I | a | a |
| 3 | LH3 | e | a | d |
| 4 | LH4 | f | a | a |
| 5 | LH5 | a | a | a |
| 6 | LH6 | a | a | a |
| 7 | LH7 | a | a | a |
| 8 | SL1 | b | a | a |
| 9 | SL2 | a | a | a |
| 10 | SL3 | a | a | b |
| 11 | SL4 | e | a | a |
| 12 | SL5 | a | a | a |
| 13 | SL6 | a | a | a |
| 14 | NG1 | a | a | d |
| 15 | W1001 | g | a | a |
| 16 | W1002 | c | a | d |
| 17 | W1003 | c | a | d |
| 18 | W1004 | f | a | b |
| 19 | W1005 | g | a | d |
| 20 | W1006 | k | b | a |
| 21 | W2001 | b | a | a |
| 22 | W2002 | b | a | d |
| 23 | W8001 | b | a | a |
| 24 | W8003 | c | a | a |
| 25 | W8004 | b | a | a |
| 26 | W8005 | b | a | c |
| 27 | W8006 | b | a | a |
| 28 | W8007 | l | a | a |
| 29 | C2002 | a | c | a |
| 30 | C4002 | c | a | a |
| 31 | C6003 | b | a | a |
| 32 | C7002 | c | a | a |
| 33 | C7003 | d | a | a |
| 34 | C7005 | b | a | a |
| 35 | C7006 | b | a | a |
| 36 | C7007 | c | a | a |
| 37 | C8001 | b | a | a |
| 38 | H1001 | b | a | a |
| 39 | H1002 | f | a | a |
| 40 | H1003 | b | a | a |
| 41 | H1004 | j | c | e |
| 42 | H1006 | b | a | a |
| 43 | H1007 | b | a | a |
| 44 | H1009 | f | a | a |
| 45 | H1010 | g | a | a |
| 46 | H1011 | b | a | a |
| 47 | H1012 | b | a | c |
| 48 | H1013 | b | a | a |
| 49 | H1014 | I | c | g |
| 50 | H1015 | b | a | d |
| 51 | H1016 | d | a | d |
| 52 | H1018 | h | a | f |
| 53 | H1020 | b | a | d |
| 54 | H1021 | c | a | d |
| 55 | CH1001 | c | a | a |
| 56 | CH1002 | h | a | a |
| 57 | CH1003 | h | a | a |
| 58 | CH1004 | h | a | a |
| 59 | CH1005 | h | a | a |
| 60 | CH1006 | b | a | a |
| 61 | CH1007 | m | a | a |
| 62 | No001 | h | a | d |
| 63 | As001 | b | a | a |

[a]EST: esterase, [b]APH: acid phosphatase, [c]PER: peroxidase. Same alphabet means the same group of each enzyme.

**Peroxidase**

A total of 12 band appeared and 2 bands out of these bands were found to be common to all isolates examined. Based on the presence and absence of remaining isozyme bands, 7 different zymogram groups were produced from the 63 isolates (Fig. 3 and Table 3).

**Cluster analysis of zymograms**

When the isozyme patterns of the three enzymes described above were combined. It was shown that 63 isolates had their own distinct electrophoretic phenotypes (Table 1). Distance values were calculated between all

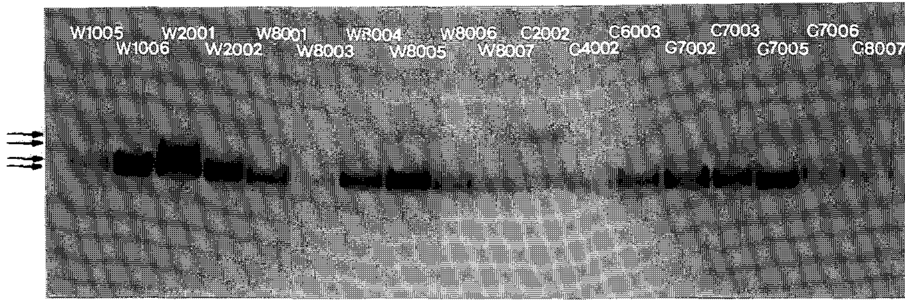


**Fig. 2.** Acid phosphatase band patterns of *L. edodes* isolates on 6~26% porosity gradient polyacrylamide gel electrophoresis.

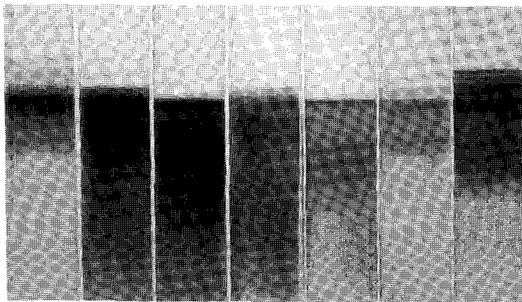

**Fig. 3.** Peroxidase band patterns of *L. edodes* isolates on 6~26% porosity gradient polyacrylamide gel electrophoresis.

pairs of the 63 electrophoretic phenotypes. Distance values ranged from 0.423 to 1.000. Based on the distance matrix, a dendrogram was constructed by group-average analysis (Fig. 4).

According to the dendrogram, the 63 electrophoretic phenotypes fall into six major clusters. The first group, isozyme polymorphism type (IPT) 1, included 43 isolates out of total 63 isolates. The second group, IPT 2, included 9 isolates. The third group, IPT 3, included 5 isolates. The fourth group, IPT 4 included 3 isolates. IPT 5 included 2 isolates. Finally, IPT 6 included 1 hybrid isolate, CH 1007. All of isolates were not corresponding with geographic origin. Of the isozyme polymorphism types, IPT 1 could be divided into five subgroups at the similarity level of 0.7. Ten isolates (LH1, SL1, NG1, SL5, SL2, C2002, LH5, CH1002, LH6 and SL3) were in-

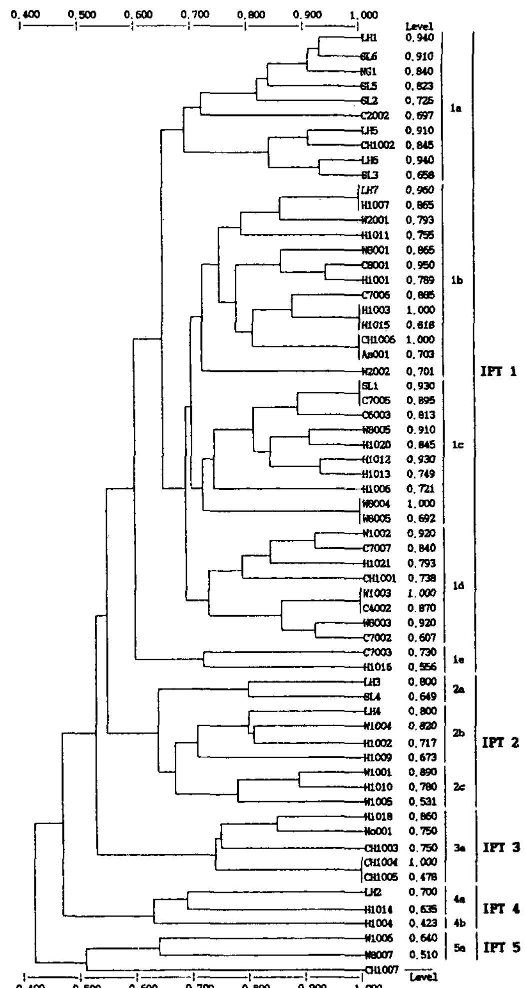


**Fig. 4.** Phenogram showing relationships among the 63 isolates of *L.edodes*. Genetic similarities were obtained by combination of the isozyme patterns of esterase, peroxidase and acid phosphatase.

cluded in subgroup 1. Most isolates in subgroup 1 were registered isolates except one unregistered isolate (C2002) and one hybrid isolate (CH1002). Subgroup 2 of IPT 1 included 13 isolates (LH7, H1013, W2001, H 1011, W8001, C8001, H1001, C7006, H1003, H1015, CH1006, As001 and W2002). Isolates LH7, H1003 showed identical band pattern with H1013, H1015, respectively. Distance values within most isolates of subgroup 2 ranged from 1.000 to 0.701. Subgroup 3 included 10 isolates (LH8, C7005, C6003, W 8005, CH1004, H1012, H1013, H1006, W8004 and W8005). Two isolates (W8004 and W8005) showed the same band patterns. Most isolates of subgroup 3 have the distance values ranging from 0.930 to 0.692. Subgroup 4 included 8 isolates (W1002, C7007, H1021, CH 1001, W1003, C4002, W8003 and C7002). Most isolates of subgroup 4 were unregisterd isolate and the distance values ranged from 0.920 to 0.607. Two isolates (W1003 and C 4002) showed the same band pattern. Subgroup 5 included two isolates (C7003 and H 1016). The genetic similarity between IPT 1 and 2 was 0.556 that among IPT 1, 2 and 3 was 0.531, that among IPT 1, 2, 3 and 4 was 0.478 and that among IPT 1, 2, 3, 4 and 5 was 0.423.

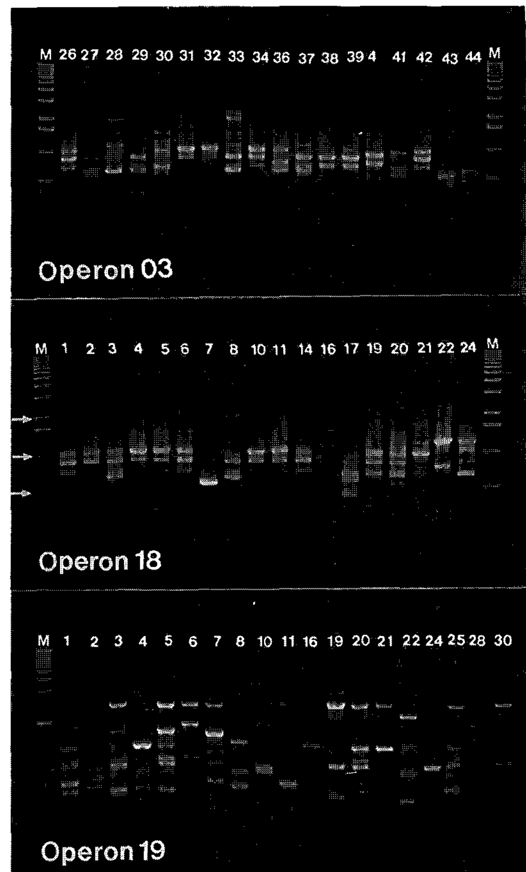


**Fig. 5.** Electrophoresis patterns showing amplification products generated from the *L. edodes* isolates with primer Operon 03, Operon 18 and Operon 19 on 1.2% agarose gel. Lanes show amplification products from isolates of *L. edodes*. Lane M shows 1 kb DNA ladder (GIBCO BRL) for size marker.

**RAPD assay**

Concentrations of DNA template, primer, $MgCl_2$ and dNTP were determined in preliminary trials to get unambiguous amplification patterns. Out of 20 primers, three primers (OPA-03, OPA-18, OPA-19) produced DNA fragment bands for all 63 isolates (Fig. 5), whereas other 17 primers could produce bands for parts of the isolates. The three primers revealed polymorphisms useful for differentiating isolates. Distance values were calculated among all pairs of the 63 electrophoretic phenotypes. Distance values ranged from 0.620 to 1.000. Based on the distance matrix, a dendrogram was constructed by group-average analysis (Fig. 6). The sixty three isolates of electrophoretic phenotype fall into five major cluster. The first group, RAPD I, included twenty two isolates and the genetic similarities between isolates were 1.000 to 0.741. Of the RAPD 1 isolates, two isolates (LH1 and LH2), five isolates (SL4, W 1005, W1006, W8003 and C4002), two isolates (W2001 and W2002), two isolates (H 1001 and H1002), and three isolates (LH7,

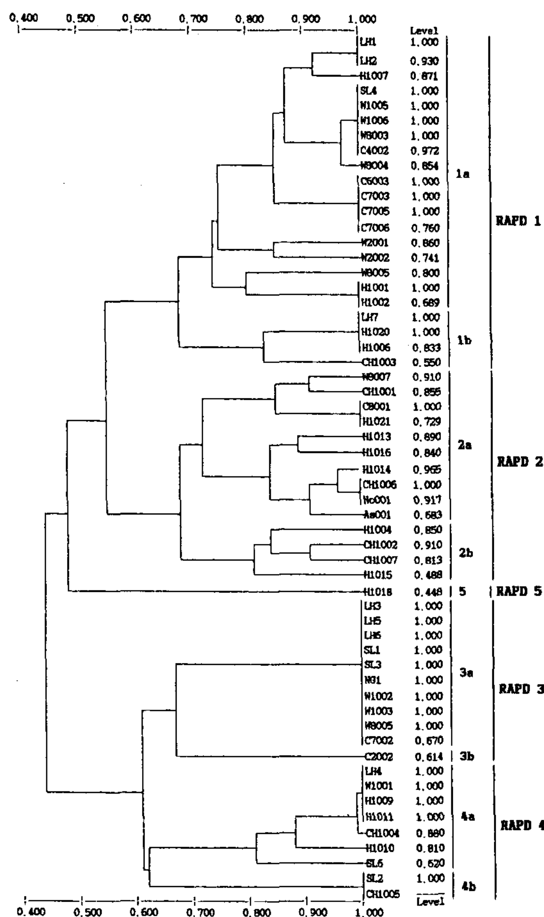


**Fig. 6.** Phenogram showing relationships among the 63 isolates of *L. edodes*. Genetic similarities were obtained by random amplified polymorphic DNA analysis with combination of 3 primers.

CH1004 and H1006) shared the same band patterns. The second group, RAPD 2, included fourteen isolates and the genetic similarities between isolates were 1.000 to 0.683. Among the isolates of RAPD 2, two isolates (C8001 and H1021), and two isolates (CH1006 and No001) shared same band patterns. The third group, RAPD 3, included eleven isolates and the genetic similarities among isolates were 1.000 to 0.670. Among the isolates of RAPD 3, ten isolates (LH3, LH5, LH6, SL1, SL3, NG1, W1002, W1003, W8005 and C7002) shared same band patterns. The fourth group, RAPD 4, included seven isolates and the genetic similarities among isolates were 1.000 to 0.620. Among the isolates of RAPD 4, four isolates (LH4, W1001, H1009 and H1011), and two isolates (SL2 and CH1005) shared same band patterns. Finally, the fifth group, RAPD 5, included only one isolate, H1018. The genetic similarity between RAPD groups was as follows. Between RAPD group 1 and 2 was 0.550, RAPD group 1, 2 and 5 was 0.448, RAPD 3 and 4 was 0.614 and RAPD group 1, 2, 5 and 3, 4 was 0.448.

**Relationship between isozyme polymorphism types and RAPD patterns**

Data generated from each of isozyme polymorphisms and RAPD markers were analyzed to produce phenograms according to a simple matching coefficient (Fig. 4, 5).

At genetic similarity of 0.600, five distinct groups were differentiated from the 63 isolates of *L. edodes* by RAPD markers, whereas six group were revealed by isozyme polymorphism type. The RAPD I included 17 isolates (LH1, LH7, C4002, C6003, C7003, C7005, C 7006, H1001, H1006, H1007, H1020, W2001, W2002, W8003, W8004, W8005, and CH1003) assigned to IPT 1, isolates of SL4, W1005 and H1002 assigned to IPT 2, isolate LH2 assigned to IPT 4 and the isolate W1006 assinged to IPT 5. RAPD 2 included isolates of H1013, H1015, H1016, H1021, C8001, CH 1001, CH1002, CH1006 and As001 assigned to IPT 1, isolate of No001 assinged to IPT 3, isolates of H1004 and H1014 assinged to IPT 4, isolate of W8007 assinged to IPT 5 and isolate of CH1007 also assinged to IPT 6. Most of isolates of RAPD 3 were assinged to IPT 1 that were isolates of LH5, LH6, SL1, SL3, NG1, W1002, W1003, W8005, C7002 and C 2002. One of the isolates of RAPD 3, LH3 was only included into IPT 2. RAPD 4 included isolates of SL6, SL2 and H1011 assinged to IPT 1, isolates of LH4, H1009, W

1001 and H1010 assinged to IPT 2 and isolates of CH1004 and CH1005 assinged to IPT 3. RAPD 5 included only one isolaes, H1018 assinged to IPT 1.

## Discussion

Successful cultivation and breeding of commercial mushrooms require the methods for efficient identification of useful varieties. In general, the identification of strains and species for various edible mushrooms largely based upon morphological and cultural criteria. However, this method has been experienced not to be satisfactory identification of different varieties due to similar morphological and cultural characters. In this study, we could differentiate the varieties of *L. edodes* by analyzing isozyme polymorphisms and random amplified polymorphic DNA patterns. We used the electrophoretic patterns of esterase, acid phosphatase and peroxidase, that provided reproducible isozyme patterns to acess the genetic diversity among 63 isolates of *L. edodes*. The high variation was observed in esterase loci as previously reported in *Lentinus edodes* (Fukuda and tokimoto, 1991). Fukuda and Tokimoto reported that the esterase isozyme pattern by isoelectric focusing can be used as biochemical markers to discriminate cultivars of *L. edodes*. Such a high polymorphisms in esterase were also shown in various studies on *Pleurotus* spp., *Ganoderma lucidum, Colletotrichum* spp. and *Pyricularia oryzae* (Chin *et al.*, 1993; Lee *et al.*, 1983; Park *et al.*, 1987; Park *et al.*, 1979, 1986a, 1986b, 1987).

Total 30 isozyme bands were detected by three enzyme systems. Phenetic analysis of these bands separated the 63 isolates into twelve zymogram groups.

In the assay of acid phosphatase band patterns, 5 bands were revealed and 3 zymogram groups were produced from the 63 isolates. Peroxidase system produced 12 bands, 3 of which were found to be common to all isolates. Esterase system seperated the 63 isolates into 7 different zymogram groups. Combined data generated from three enzyme systems resulted in six groups of electrophoretic phenotype. The first group, isozyme polymorphism type (IPT) 1, included 43 isolates which were differentiated into 5 subgroups; ten registered isolates, twenty unregistered (but cultivated) isolates, nine wild isolates, one introduced foreign isolate and four hybrid isolates. Most of Korean isolates including registered and unregistered isolates included in IPT 1, showing close relationship. The second group, IPT 2, including nine isolates were seperated into three subgroups; three registered isolate, three unregistered isolates and three wild isolates. The third group, IPT 3, included 5 isolates and were differentiated into three subgroup; one registered isolate, one unregistered isolate and three hybrid isolates. The fourth group, IPT 4 included 3 isolates that were two unregistered isolates and one registered isolate. IPT 5 included 2 wild isolates. IPT 6 included hybrid isolate.

Of the isozyme polymorphism types, IPT 1 divided into five subgroups. Most of isolates of subgroup 1 were registered isolates except one unregistered isolate and one hybrid isolate. Subgroup 2 of IPT 1 also included thirteen isolates. Of the isolates, LH7 and H1013, H1003 and H1015 shared the same band patterns. Most of isolates of subgroup 2, distance value that were ranged from 1.000 to 0.701. By the result of this distance value, it is assumed that all of isolates of subgroup 2 were closely related. Subgroup 3 included ten isolates. Two isolates, W8004 and W8005 also shared the same band patterns. Most of isolates of subgroup 3 have the distance value that were ranged from 0.930 to 0.692. Subgroup 4 included eight isolates. Most of iso-

lates of subgroup 4 were unregisterd isolates and was ranged from 0.920 to 0.607. Two isolates, W1003 and C4002 shared the same band pattern. Subgroup 5 included two isolates. Most of isolates were independent. According to the phenogram, groups of electrophoretic phenotypes do not correspond with geographic origins of isolates. Especially, isolates from Kangwon province had not genenetic relationship. These results seem to be consistent with the previous study (William *et al.*, 1990). Their results did not show any association between geographic origin and vegetative compatibility, isozyme phenotype or pathotype of *F. oxysporum*. The random amplified polymorphic DNA patterns were analyzed for the 63 isolates with 20 primers. Differentiation of isolates was not successful with all primer due to inefficiency of certain primers. However, three primers (OPA-03, OPA-18 and OPA-20) resulted in clear differentiation of isolates. It is suggested that RAPD markers were useful tools for the identification of varieties of *L. edodes*. According to the dendrogram, 63 isolates fall into five major cluster. The first group, RAPD I, included twenty two isolates and the genetic similarities between isolates as 1.000 to 0.741. A second group, RAPD 2, included fourteen isolates. and the genetic similarities between isolates were 1.000 to 0.683. A third group, RAPD 3, included eleven isolates and the genetic similarities between isolates were 1.000 to 0.670. A fourth group, RAPD 4, included seven isolates and the genetic similarities between isolates were 1.000 to 0.620. Finally, a fifth group, RAPD 5, included only one isolate, H1018.

The combined data from all isolates were analysed by a simple marching coefficient to produce a phenograms of isozyme polymorphisms and RAPD patterns. At genetic similarity level of 0.600, five groups resulted from 63 isolates. This result is different from that from isozyme data, which generated 6 groups. However, there was similar aspect between the dendrograms of isozyme and RAPD analysis at the main group 1.

Relationship between isozyme polymorphism types and RAPD patterns were as follows; the first group, RAPD I, included 17 isolates which were assigned to IPT 1, 3 isolates were assigned to IPT 2, 1 isolate was assigned to IPT 4 and the isolate W1006 was assinged to IPT 5. A second group, RAPD 2, included 9 isolates which were assigned to IPT 1, 1 isolate assinged to IPT 3, 2 isolates assinged to IPT 4, 1 isolate assinged to IPT 5 and 1 isolate also assinged to IPT 6. Most of isolates of third group, RAPD 3 were assinge to IPT 1. Isolate LH3 was only included into IPT 2. A fourth group, RAPD 4 included 3 isolates assinged to IPT 1, 4 isolates which were assinged to IPT 2 and 2 isolates assinged to IPT 3. A fifth group, RAPD 5 included only one isolaes, H1018 assinged to IPT 1.

In conclusion, the isolates of *L. edodes* in Korea could be differentiated by the analysis of isozyme polymorphism and random amplified polymorphic DNA (RAPD) patterns. All isozyme polymorphisma and RAPD patterns were useful means for differentiation and identification of *L. edode* isolates but little relationship was dectected between data generated from isozyme and RAPD patterns. According to the report of Yli-Mattila *et al.* (1996) some of the dissimilarities at subgroup level between isozyme and RAPD analyses may be due to the greater number of strain-specific eletrophoretic bands obtained by the RAPD method. High sensitivity of RAPD to polymorphisms would be useful to differentiate isolates with genetically close affinity.

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## 적 요

Isozyme PAGE에 의한 isozyme polymorphism 및 random amplified polymorphic DNA(RAPD) pattern의 비교를 통하여 한국에서 수집된 63개의 표고 버섯(*Lentinus edodes*) 품종의 유연관계를 조사하여 보았다. NTSYS PC program을 통하여 비교에 사용되어진 3가지 isozyme 즉 esterase, peroxidase, acid phophatase 등은 각각 10, 7, 3개의 isozyme polymorphism type으로 나뉘어질 수 있었고, 이러한 3개의 isozyme type들은 group-average method를 이용하여 최종적으로 6개의 집단으로 구분되어질 수 있었다. 또한 RAPD를 통한 표고 품종간 유연관계 비교는 사용되어진 총 20개의 random primer 들중 3개의 primer(OPA 03, OPA 18, OPA 19)가 품종간 polymorphism을 보였고 사용되어진 전체 random primer의 PCR product들의 집단 분석을 통해 최종적으로 5개의 집단으로 구분할 수 있었다. 사용되어진 두 가지 방법 즉, isozyme polymorphism 및 RAPD pattern에 의해 구분되어진 63개 표고 버섯 품종의 subgroup level에서의 절대적인 유사성은 관찰할 수 없었다. Yii-Mattila (1996) 등에 의하면 품종간 비교시 isozyme analyses에서와는 달리 RAPD analyses에서는 많은 수의 strain-specific band들이 얻어질 수 있고 이것이 두 방법간의 유사성을 관찰하지 못하게 하는 원인이라고 보고하였다.

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