THE KOREAN JOURNAL OF MYCOLOGY
Vol. 27, No 5, p328-336 October 1999

Printed in S. KOREA

Genetic Variation of the *Pleurotus ostreatus* Complex Based on Isozyme Analysis

Hee Kyung Lee[1], Young Bok Yoo[2] and Kyung Hee Min*

[1]Chungbuk Agricultural Research and Extension Services, Ochang 363-880, Korea
[2]Division of Applied Microbiology, National Institute of Agricultural Science and Technology RDA, Suweon 441-707, Korea
**Department of Biology, Sookmyung Women's University, Seoul 140-742, Korea*

동위효소 분석에 의한 *Pleurotus ostreatus* Complex의 유전적 변이

이희경[1] · 유영복[2] · 민경희*

*[1]충북농업기술원, [2]농업과학기술원 응용미생물과, *숙명여자대학교 생명과학과*

ABSTRACT: Isozyme comparisons of mycelial extracts from *Pleurotus ostreatus* were undertaken using isoelectric focusing. Enzyme isozyme patterns were used to describe the extent of geographical diversity and degree of intraspecific variation in these extracts. A total of 77 bands were resolved from six different enzymes. Cluster analyses were performed using the zymograms for esterase(EST), glucose phosphate isomerase (GPI), leucine aminopeptidase (LAP), malate dehydrogenase(MDH), peroxidase (POX), and phosphoglucomutase (PGM). EST gave multiple banding patterns, while less variability was observed for GPI, MDH, and PGM. Cluster analyses demonstrated that strains of *P. ostreatus* from geographically different origins are genetically divergent, supporting the idea that there is little or no gene flow between these geographically distant population groups.

KEYWORDS: *Pleurotus ostreatus* complex, Isoelectronic focusing, Isozyme pattern

Pleurotus ostreatus, the oyster mushroom, is a common decay fungus of hardwood logs that is highly cultivated in several countries throughout the world because of its taste and flavor. Unfortunately, certain aspects of the nomenclature and taxonomy of the *Pleurotus* species have been ambiguous, including an especially high level of taxonomic confusion surrounding the *P. ostreatus-florida-pulmonarius* species complex. These taxonomic problems are revealed a high degree of morphological variability in the species that can be attributed to many factors, including environmental conditions, phenotypic plasticity, and genetic variation. As pleurotus production now accounts for nearly a quarter of the worldwide mushroom production (Chang and Miles, 1991), a better understanding of their taxonomy should be contributed increasingly important for breeding studies and preservation of the native germ plasm.

Electrophoretic profiles of soluble proteins have been valuable in taxonomy because they reflect the genetic constitution of the cell (Shechter, 1973), and variations in isozyme patterns within different strains of the same fungal species have been reported (Franke, 1973; May *et al.*, 1979; Kulkarni *et al.*, 1986). Taxonomic research on Basidiomycetes isozymes has concentrated on the commercially important edible species (Royse and May, 1982; Toyomasu and Zennyozi 1981), such as *Lentinus edodes* (Itavaara, 1988) and *Agaricus bisporus* strains (Royse and May, 1982). Using restriction fragment length polymorphism analysis of mtDNA, Teruyuki and Yukitaka (1995) revealed that mtDNA divergence in *P. ostreatus* correlates significantly with its geographical distribution.

In the present study we examined the total protein profile of strains of the *P. ostreatus* complex obtained from different populations found in the world. Isozyme electrophoresis was used to examine genetic variation between six different enzymes and their cluster analysis of *P. ostreatus* complex strains from geographical different origin.

Materials and Methods

Strains and culture conditions

The *Pleurotus ostreatus* strains used in this study were obtained from the Microbial Genetics Laboratory, National Institute of Agricultural Science and Technology (MGLIAS, Korea). Table 1 shows identification of the strains on the basis of morphological examination.

The mycelia were incubated at 25°C in Petri dishes containing Mushroom Complete Medium (MCM, 0.2% yeast extract; 0.2% peptone; 0.05% $MgSO_4 \cdot 7H_2O$; 0.046% KH_2PO_4;

*Corresponding author

Table 1. *Pleurotus ostreatus* complexused in this study

Isolate No.	Species	MGLIAS No.[2]	Geographic origin	Source
1	*P. pulmonarius*	1149	Kyonggi(KG), Korea	Tissue culture
2	*P. ostreatus*	1201	Kyonggi(KG), Korea	Tissue culture
3	*P. ostreatus*	1042	Kangwon(KW), Korea	Tissue culture
4	*P. ostreatus*	1045	Kangwon(KW), Korea	Tissue culture
5	*P. ostreatus*	1041	Chungbuk(CB), Korea	Tissue culture
6	*P. ostreatus*	1067	Chungbuk(CB), Korea	Tissue culture
7	*P. ostreatus*	1036	Chungnam(CN), Korea	Tissue culture
8	*P. ostreatus*	1046	Chungnam(CN), Korea	Tissue culture
9	*P. ostreatus*	1069	Jeonbuk(JB), Korea	Tissue culture
10	*P. ostreatus*	1081	Jeonbuk(JB), Korea	Tissue culture
11	*P. pulmonarius*	1136	Jeonbuk(JB), Korea	Tissue culture
12	*P. pulmonarius*	1035	Kyongbuk(KB), Korea	Tissue culture
13	*P. ostreatus*	1037	Kyongbuk(KB), Korea	Tissue culture
14	*P. ostreatus*	1040	Kyongnam(KN), Korea	Tissue culture
15	*P. florida*	1230	Kyonggi(KG), Korea	Tissue culture
16	*P. ostreatus*	1031	Kyonggi(KG), Korea	Tissue culture
17	*P. ostreatus*	1014	Unknown	Commercial strain, Japan
18	*P. ostreatus*	1058	Unknown	Commercial strain, Japan
19	*P. ostreatus*	1061	Unknown	Commercial strain, Japan
20	*P. ostreatus*	1100	Unknown	Commercial strain, Japan
21	*P. pulmonarius*	1019	Japan	Tissue culture
22	*P. ostreatus*	1056	Unknown	Commercial strain, Japan
23	*P. ostreatus*	1176	Unknown	Commercial strain, Germany
24	*P. ostreatus*	1179	Unknown	Commercial strain, Germany
25	*P. ostreatus*	1187	Unknown	Commercial strain, Netherland
26	*P. ostreatus*	1030	Unknown	Commercial strain, Denmark
27	*P. ostreatus*	1194	Unknown	Commercial strain, Canada
28	*P. ostreatus*	1162	Unknown	Commercial strain, U. S. A.
29	*P. ostreatus*	1158	Unknown	Commercial strain, U. S. A.
30	*P. florida*	1196	Unknown	Commercial strain, Taiwan
31	*P. ostreatus*	1197	Unknown	Commercial strain, Taiwan
32[1]	Hybrid PO+PF+PO, Wonhyeong	1208	Korea	Commercial strain, Korea
33	*P. florida*	1189	Unknown	Commercial strain, Netherland
34	*P. florida*	1183	Unknown	Commercial strain, Germany
35	*P. florida*	1029	Unknown	#2A, F. Zadrazil, Germany
36	*P. florida*	1209	Thailand	Tissue culture

[1]No. 32: Somatic hybrid between P5-M43-*arg rib* (Hybrid of *P. ostreatus* and *P. florida*) and *P. ostreatus* 2-13-*pro orn* by protoplast fusion (Yoo *et al.*, 1993).

[2]MGLIAS: Microbial Genetics Laboratory, National Institute of Agricultural Science and Technology, RDA, Suweon, Korea.

0.1% K_2HPO_4; 2% glucose; 1.5% agar). After a period of vigorous growth, mycelial plugs were transferred to 50 mL of MCM broth in 100 mL Erlenmyer flasks and incubated at 25°C under static conditions for 1 week.

Protein extraction and isoelectric focusing electrophoresis

The mycelial mat in each flask was harvested by filtration through Whatman No. 2 filter paper and rinsed several times with chilled distilled water. The quantity of mycelia obtained varied from 3 to 4 g per flask, depending on the strain. The mycelium was homogenized with a mortar and pestle. Liquid nitrogen was added to facilitate efficient cell disruption, and then polyvinyl polypyrrolidone (PVP) 40,000 was added to remove phenolic substances. After centrifugation (30 min, 4°C, 12,000 rpm), the supernatants containing the soluble proteins were used for isozyme analysis.

Isoelectric focusing was performed horizontally in ultrathin (0.5 mm) 6% polyacrylamide gels containing ampholytes (Pharmacia, pH 3.0-10.0 and pH 4.0-6.5). The electrode buffers used were: 0.8M ethylenediamine for the cathode and 0.7M phosphoric acid for the anode. Isoelectric focusing was carried out at 4°C on a flat bed apparatus. Prefocusing lasted 15 min. at 100V. Then, 50 u*l* of proten extract per isolate were deposited on the surface of the gel with 5×10 mm filter-paper wicks (3MM, Whatman), along a line 1 cm away from the anode. Focusing was carried out at 300V for 1h and then gradually increased to 1200V, and the gel was run for a further 2h. The focusing plate was

Table 2. Composition of staining mixture

Enzyme (EC number)[a]	Abbreviation	Buffer, substrate and dye
Esterase (3.1.1.1)	EST	0.2M phosphate buffer (pH 7.0) 100 m*l*, α-naphtyl acetate(15 mg) in aceton 3 m*l*, fast blue RR salt 10 mg in
Glucosephosphate isomerase (5.3.1.9)	GPI	0.2M Tris-HCl (pH 8.0) 80 m*l*, 0.5M $MgCl_2$ 1 m*l*, glucose-6-phosphate dehydrogenase 20 unit, 1% NADP 1 m*l*, 1% PMS 0.5 m*l*, 1% MTT 0.5 m*l*, 1.5% agarose 10 m*l*
Leucine aminopeptidase (3.4.11.1)	LAP	0.2M Tris-malate buffer (pH 6.0) 100 m*l*, 1% L-leucyl-β-naphthylamide 3 m*l*, fast garnet GBC salt 10 mg
Malate dehydrogenase (1.1.1.37)	MDH	0.2M Tris-base (pH 8.0) 90 m*l*, L-malic acid 0.45 g, 1% NAD 2 m*l*, 0.5M $MgCl_2$ 1 m*l*, 1% PMS 0.5 m*l*, 1% MTT 1 m*l*
Peroxidase (1.11.1.7)	POX	0.1M acetic buffer(pH 4.5) 100 m*l*, 1M $CaCl_2$ 2 m*l*, 1% benzidine 4 m*l*, H_2O_2 100 u*l*
Phosphoglucomutase (2.7.5.1)	PGM	0.1M Tris-HCl (pH 8.0) 90 m*l*, 0.5M $MgCl_2$ 1 m*l*, 1% NADP 1 m*l*, glucose-1-phosphate 0.15 g, glucose-6-phosphate dehydrogenase 20 unit, 1% PMS 0.5 m*l*, 1% MTT 1 m*l*

[a]Enzyme Commission Numbers: Nomenclature Committee of the International Union of Biochemistry (1984).

cooled with running tap water.

After isoelectric focusing, the gels were stained by direct immersion in the substrate solutions. After the bands had developed they were washed with running tap water to stop the reaction, immersed in preservation solution (Winter and Andersson, 1977), and dried under vacuum. Table 2 shows the enzyme staining solutions for each of the isozymes.

Phenetic analysis

Isoenzyme banding patterns were recorded according to their migration along the pH gradient polyacrylamide gel. The presence of a band at a particular position (pI) was designated '1'; if absent at a particular position, the absent band was designated '0' (Smith and Anderson, 1989). Variations in staining intensity were not taken into account in the construction of this matrix. Genetic relationships were performed using F values generated from the following formula (Nei, 1987): $F = 2m_{xy}/(m_x + m_y)$, in which m_{xy} equals the number of bands in common to samples x and y, and m_x and m_y represent the number of unique bands. Genetic relationships were subjected to phenetic analyses using the software package NTSYS-pc (Rohlf, 1993).

Results and Discussion

In the present study, strains were obtained from various geographical areas throughout the world. Most strains showed enzyme activity and clear resolution of bands for the six enzyme systems selected. Some strains exhibited variant zymograms for EST, but invariant zymograms for GPI, LAP, MDH, POX and PGM.

EST: Over a pH range of 3.0-10.0, a total of 34 distinct bands from *P. ostreatus* mycelia were observed for this highly polymorphic enzyme (Fig. 1). Individual strains produced between one to 34 EST bands. Korean *P. ostreatus*

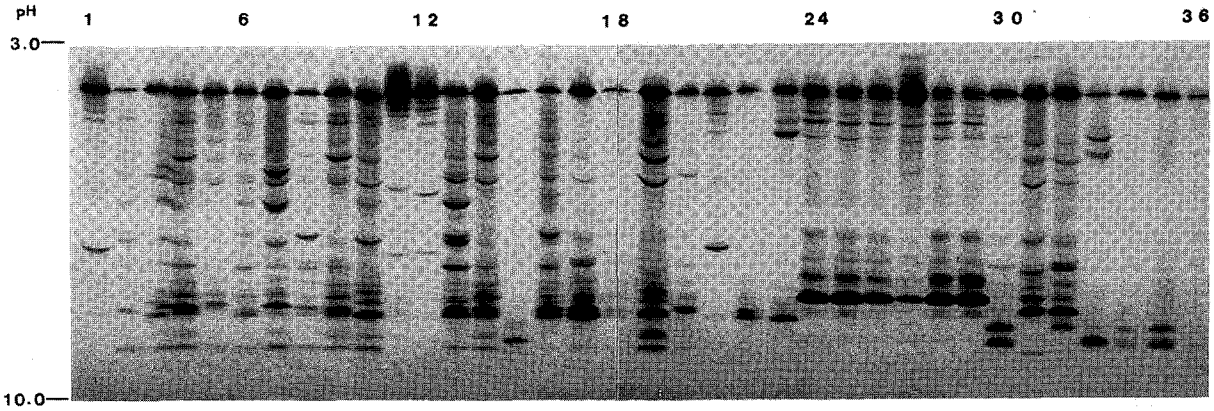

Fig. 1. Esterase patterns of *P. ostreatus* (2-10, 13-20, and 22-31), somatic hybrid Wonhyeong (32), *P. florida* (33-36), and *P. pulmonarius* (1, 11, 12, and 21).

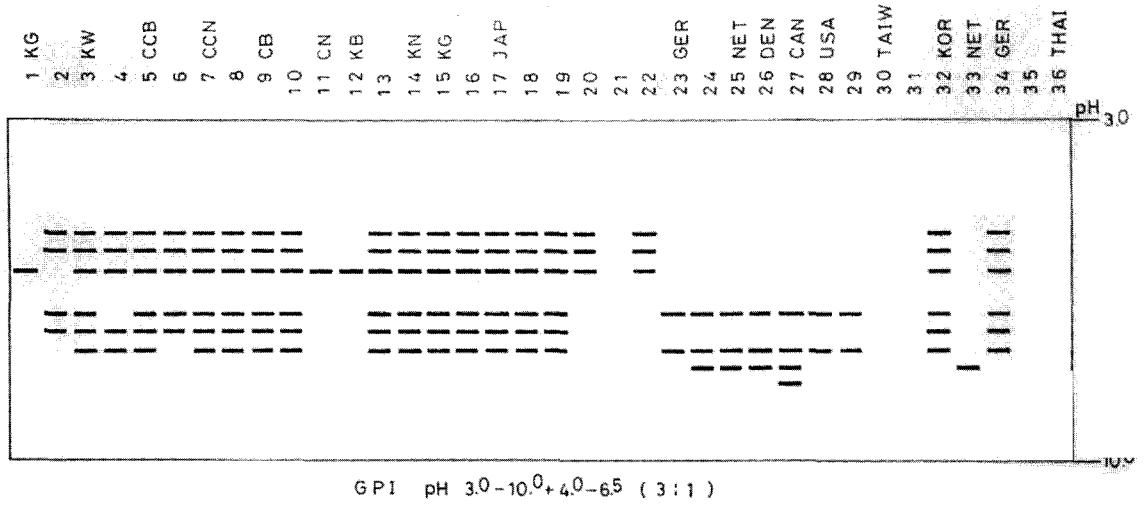

Fig. 2. Diagramatic representation of glucosephosphate isomerase profiles of *P. ostreatus* (2-10, 13-20, and 22-31), somatic hybrid Wonhyeong (32), *P. florida* (33-36), and *P. pulmonarius* (1, 11, 12, and 21).

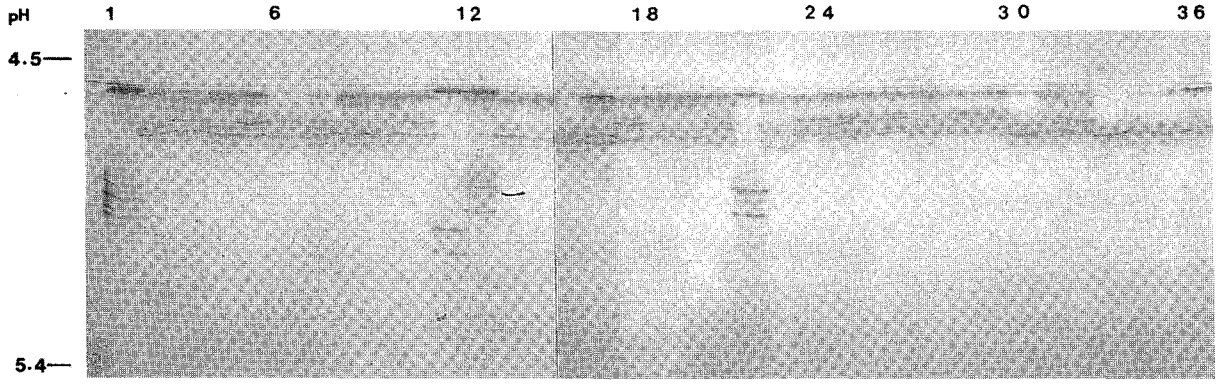

Fig. 3. Isozyme banding patterns of *P. ostreatus* (2-10, 13-20, and 22-31), somatic hybrid Wonhyeong (32), *P. florida* (33-36), and *P. pulmonarius* (1, 11, 12, and 21) for the leucine aminopeptidase.

strains showed the highest degree of variability. *P. ostreatus* strains from Germany (strain 24), Netherlands (strain 25), Denmark (strain 26), and the USA (strains 28 and 29) produced the same banding patterns.

GPI: Eight bands were detected for GPI in the pH 3.0-10.0 gels (Fig. 2). The strains tested produced one to six bands each. No band was apparent for Taiwanese *P. ostreatus* (strains 30 and 31) and Japanese *P. ostreatus* (strain 21). In those strains that did produce GPI bands, the bands were very slight and unstable, and diffused after 3-5 min. This result suggests that GPI band analysis has be performed immediately after staining.

LAP: A total of 14 bands were recorded for LAP, with pI values between 4.5 and 5.4 (Fig. 3). Each strain produced from three to six bands. *Pleurotus pulmonarius* strains (strains 1, 11, 12, and 21) gave a specific banding pattern compared to the other strains analyzed in this study, and can consequently be included in the same cluster. The similar banding patterns were found between strain 12 from Kyongsangbuk-do (Korea) and strain 21 from Japan.

MDH: Three MDH bands from the mycelia of *P. ostreatus* were detected in the pH 4.0-6.5 gel (Fig. 4). No bands were apparent in strains 17, 20, 22, 23, 25, 28 and 29.

POX: A total of 13 bands with POX activity were found in a pH range 3.0-10.0 (Fig. 5). POX bands were noted on the isoelectric focusing gels, but their activity was unstable and the bands disappeared after 5-10 min. Thus, analysis of POX variation was performed immediately after staining. Banding patterns were similar to each strain assessed. No bands were apparent for strain 12.

PGM: Five PGM bands were detected in the pH 4.0-6.5 gel (Fig. 6). Variability in *P. ostreatus* strains was very slight for this enzyme, producing two to five bands each. Identical phenotypes were observed for the following strains: 1, 28, 29, 31; 2, 3, 4, 33; 5, 23, 25, 26, 27; 6, 7, 9, 13, 14, 15, 16, 18, 19; 8, 10; 11, 12; 17, 36; and 20, 22.

Analysis of variation, based on the detection of six isozyme activities, enabled us to estimate the degree of genetic relatedness between the strains of the *P. ostreatus* complex. Following isoelectric focusing and protein blotting, valid

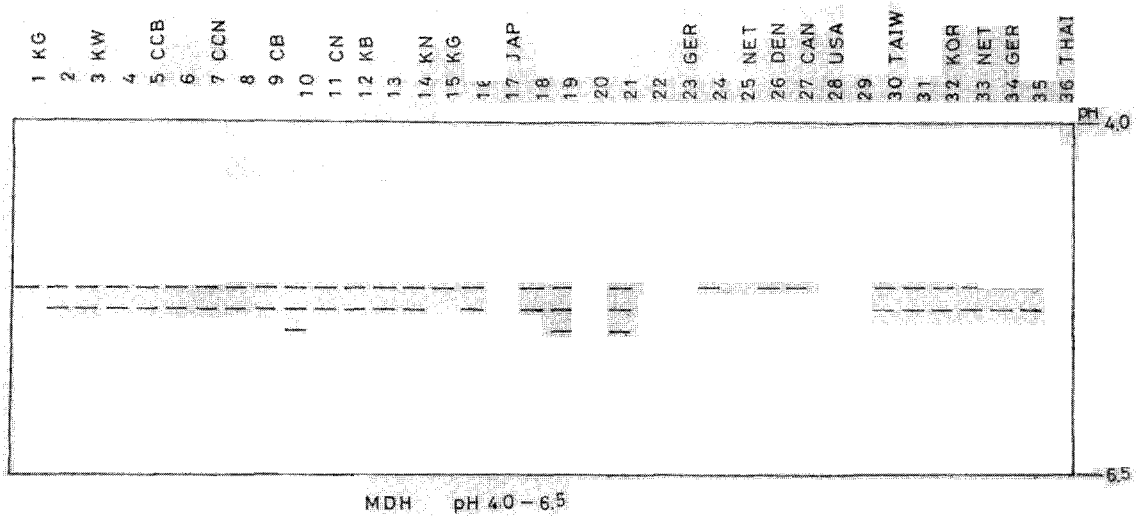

Fig. 4. Electromorphs of malate dehydrogenase found in isolates of *P. ostreatus* (2-10, 13-20, and 22-31), somatic hybrid Wonhyeong (32), *P. florida* (33-36), and *P. pulmonarius* (1, 11, 12, and 21).

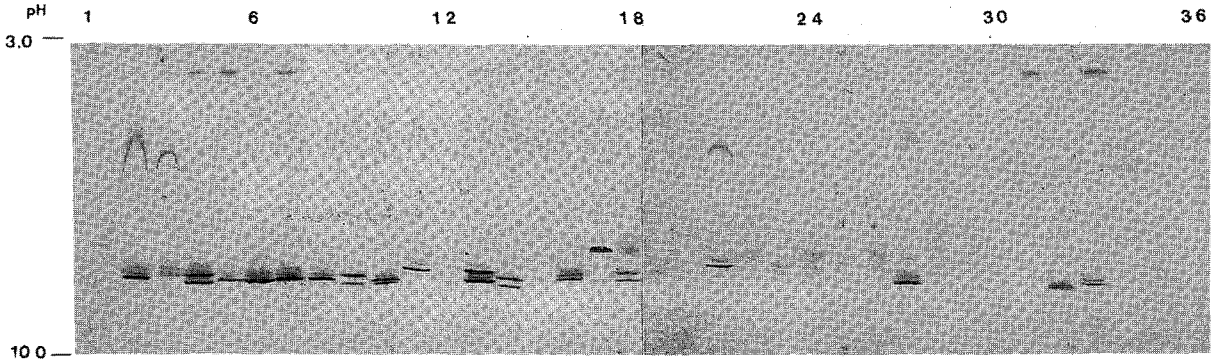

Fig. 5. Isozyme banding patterns of peroxidase of *P. ostreatus* (2-10, 13-20, and 22-31), somatic hybrid Wonhyeong (32), *P. florida* (33-36), and *P. pulmonarius* (1, 11, 12, and 21).

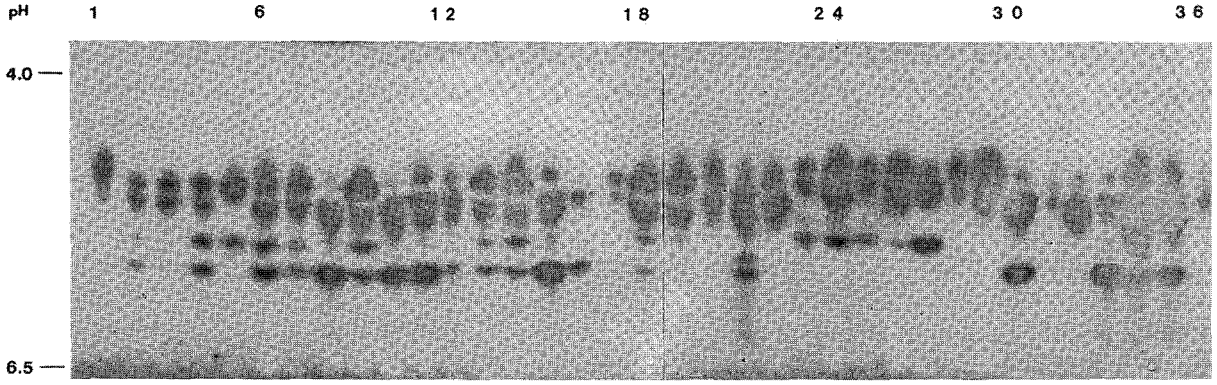

Fig. 6. Phosphoglucomutase patterns of *P. ostreatus* (2-10, 13-20, and 22-31), somatic hybrid Wonhyeong (32), *P. florida* (33-36), and *P. pulmonarius* (1, 11, 12, and 21).

biochemical criteria were used for taxonomic classification of *P. ostreatus*. Estimates of diversity were found to be high in general. The highest estimates were obtained from EST analysis, for which a large number of electromorphs were detected. The high degree of EST polymorphism seems to be common in Basidiomycetes (Roux and Labarere, 1991). Because of their relatively high concentration and their multiple banding patterns, EST electromorphs were often chosen as markers for the identification of microorganisms (Engvild and Nielsen, 1985; Goullet and Picard, 1986). Conversely, the lowest estimates were observed from GPI, MDH, and PGM analysis, because of the small number of

electromorphs detected. Moderate levels of diversity were found for LAP and POX.

Apparently, such activities might potentially serve as biochemical markers for the registration and protection of *P. ostreatus* strains, as previously demonstrated for *Lentinus edodes* (Royse *et al*, 1983) and *Agaricus bitorquis* (Roux and Labarere, 1990).

Cluster analysis was performed on the results from the six enzyme systems that were resolved successfully by isoelectric focusing. A total of 77 different electromorph types were observed among the 27 strains of *P. ostreatus*, four strains of *P. florida*, four strains of *P. pulmonarius* and one strain of somatic hybrid Wonhyeong (the fusant of *P. florida*×*P. ostreatus*×*P. ostreatus*).

Taxonomic relationships, based on the electrophoretic phenotypes of *P. ostreatus P. florida, P. pulmonarius*, and the somatic hybrid fusant of *P. florida* and *P. ostreatus*, were generated using Nei's coefficients (Table 3). The level of relatedness between the strains is shown in Fig. 7. The dendrogram groups the 77 different electromorphs into five major clusters: (I) four European strains and four North American strains; (II) twelve strains from Korea and five from Japan; (III) six *P. florida* strains; (IV) one Taiwanese strain of *P. ostreatus* and the somatic hybrid strain; and (V) four strains of *P. pulmonarius*. The present work demonstrates that *P. pulmonarius* and *P. ostreatus* may be a different species, and *P. pulmonarius is* more likely to correspond to *P. florida*. Throughout this study, *P. florida* was linked with *P. pulmonarius* to a greater extent than *P. ostreatus*. This outcome is in accordance with the findings of previous taxonomic studies which have applied morphological and physiological criteria (Zervakis and Balis,

Table 3. Coefficients of similarity between 27 *Pleurotus ostreatus*, 1(hybrid Wonhyung), 4 *P. florida*, and 4 *P. pulmonarius* strains based on isozymes for six enzymes. Matrix calculated from 77 isozyme bands

Strains	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.000																	
2	0.545	1.000																
3	0.506	0.753	1.000															
4	0.493	0.792	0.779	1.000														
5	0.571	0.714	0.779	0.740	1.000													
6	0.480	0.779	0.714	0.727	0.779	1.000												
7	0.454	0.779	0.792	0.753	0.857	0.818	1.000											
8	0.545	0.766	0.753	0.766	0.818	0.779	0.779	1.000										
9	0.519	0.766	0.779	0.870	0.844	0.805	0.857	0.870	1.000									
10	0.467	0.714	0.701	0.740	0.792	0.753	0.779	0.896	0.844	1.000								
11	0.727	0.636	0.597	0.610	0.584	0.571	0.571	0.584	0.584	0.506	1.000							
12	0.792	0.623	0.610	0.571	0.623	0.584	0.584	0.571	0.571	0.519	0.883	1.000						
13	0.532	0.753	0.740	0.805	0.831	0.818	0.818	0.909	0.935	0.857	0.571	0.558	1.000					
14	0.532	0.701	0.714	0.805	0.831	0.766	0.792	0.883	0.935	0.857	0.545	0.558	0.948	1.000				
15	0.649	0.740	0.727	0.662	0.766	0.753	0.727	0.714	0.740	0.662	0.688	0.727	0.727	0.727	1.000			
16	0.493	0.766	0.727	0.740	0.844	0.831	0.831	0.818	0.870	0.844	0.532	0.545	0.883	0.857	0.714	1.000		
17	0.558	0.701	0.662	0.675	0.805	0.714	0.714	0.831	0.779	0.805	0.519	0.532	0.818	0.818	0.727	0.831	1.000	
18	0.532	0.701	0.636	0.727	0.805	0.766	0.766	0.831	0.857	0.805	0.519	0.532	0.870	0.896	0.701	0.883	0.844	1.000
19	0.545	0.766	0.701	0.766	0.844	0.779	0.805	0.844	0.896	0.870	0.584	0.597	0.857	0.883	0.766	0.896	0.805	0.909
20	0.649	0.662	0.701	0.662	0.818	0.701	0.727	0.766	0.740	0.714	0.636	0.675	0.779	0.779	0.766	0.766	0.831	0.753
21	0.727	0.636	0.597	0.636	0.584	0.571	0.571	0.584	0.610	0.558	0.844	0.805	0.597	0.571	0.636	0.558	0.545	0.545
22	0.662	0.701	0.714	0.701	0.831	0.688	0.740	0.753	0.779	0.727	0.675	0.688	0.766	0.766	0.779	0.779	0.818	0.740
23	0.688	0.727	0.662	0.623	0.753	0.610	0.636	0.675	0.675	0.623	0.649	0.662	0.662	0.636	0.753	0.675	0.740	0.636
24	0.584	0.675	0.584	0.649	0.649	0.610	0.584	0.623	0.675	0.623	0.571	0.558	0.636	0.610	0.649	0.649	0.610	0.610
25	0.610	0.675	0.610	0.623	0.649	0.584	0.584	0.623	0.675	0.597	0.571	0.584	0.636	0.610	0.675	0.623	0.610	0.584
26	0.623	0.688	0.623	0.636	0.662	0.597	0.597	0.636	0.688	0.610	0.584	0.597	0.649	0.623	0.688	0.636	0.597	0.597
27	0.571	0.636	0.571	0.584	0.662	0.623	0.571	0.584	0.610	0.610	0.532	0.545	0.597	0.597	0.688	0.662	0.649	0.623
28	0.636	0.675	0.610	0.623	0.649	0.584	0.584	0.649	0.675	0.623	0.597	0.610	0.636	0.610	0.675	0.623	0.636	0.584
29	0.636	0.675	0.610	0.623	0.649	0.584	0.584	0.649	0.675	0.623	0.597	0.610	0.636	0.610	0.675	0.623	0.636	0.584
30	0.662	0.675	0.662	0.649	0.701	0.636	0.636	0.675	0.675	0.649	0.727	0.766	0.636	0.662	0.753	0.623	0.636	0.636
31	0.584	0.597	0.584	0.571	0.727	0.610	0.636	0.623	0.649	0.597	0.597	0.636	0.636	0.636	0.675	0.675	0.610	0.610
32	0.480	0.623	0.662	0.597	0.701	0.636	0.688	0.675	0.701	0.701	0.519	0.584	0.714	0.714	0.727	0.727	0.740	0.688
33	0.610	0.753	0.688	0.701	0.701	0.688	0.662	0.675	0.675	0.623	0.701	0.714	0.662	0.636	0.753	0.649	0.636	0.636
34	0.636	0.753	0.792	0.701	0.831	0.740	0.740	0.753	0.779	0.701	0.701	0.740	0.766	0.766	0.935	0.753	0.740	0.740
35	0.688	0.727	0.688	0.623	0.753	0.688	0.662	0.675	0.675	0.623	0.727	0.792	0.662	0.662	0.831	0.675	0.662	0.662
36	0.740	0.649	0.636	0.597	0.701	0.610	0.610	0.623	0.597	0.597	0.727	0.792	0.584	0.584	0.779	0.597	0.662	0.558

Table 3. Continued

Strains	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36
1																		
2																		
3																		
4																		
5																		
6																		
7																		
8																		
9																		
10																		
11																		
12																		
13																		
14																		
15																		
16																		
17																		
18																		
19	1.000																	
20	0.740	1.000																
21	0.636	0.636	1.000															
22	0.779	0.961	0.675	1.000														
23	0.701	0.727	0.675	0.766	1.000													
24	0.649	0.623	0.597	0.662	0.818	1.000												
25	0.623	0.675	0.571	0.714	0.844	0.948	1.000											
26	0.636	0.662	0.584	0.701	0.831	0.961	0.987	1.000										
27	0.662	0.636	0.558	0.675	0.805	0.857	0.831	0.844	1.000									
28	0.623	0.701	0.597	0.740	0.844	0.922	0.974	0.961	0.805	1.000								
29	0.623	0.701	0.597	0.740	0.844	0.922	0.974	0.961	0.805	1.000	1.000							
30	0.701	0.753	0.727	0.766	0.740	0.662	0.688	0.701	0.675	0.714	0.714	1.000						
31	0.649	0.727	0.571	0.740	0.662	0.988	0.714	0.727	0.701	0.740	0.740	0.766	1.000					
32	0.701	0.675	0.519	0.688	0.636	0.610	0.610	0.623	0.597	0.636	0.636	0.714	0.740	1.000				
33	0.701	0.675	0.753	0.688	0.766	0.688	0.662	0.675	0.701	0.636	0.636	0.792	0.662	0.610	1.000			
34	0.805	0.779	0.675	0.792	0.792	0.636	0.662	0.675	0.675	0.662	0.662	0.818	0.662	0.766	0.792	1.000		
35	0.727	0.753	0.727	0.766	0.844	0.688	0.688	0.701	0.701	0.688	0.688	0.870	0.714	0.688	0.870	0.896	1.000	
36	0.623	0.805	0.701	0.818	0.792	0.688	0.740	0.727	0.675	0.766	0.766	0.818	0.740	0.636	0.792	0.766	0.844	1.000

1991), mating reactions (Hilber, 1982), and isoelectric focusing (Lee *et al*, 1998) to distinguish strains from these two species.

Strains 15 and 30 (cluster III) were identified as *P. ostreatus* by analysis of their morphological characters, but the present results group them with *P. florida*. The fruiting body of strain 15 had been collected on platanus and was tissue cultured. The large cluster, cluster II, contained 17 strains from Korea and Japan. It could be separated into two subgroups: the first subgroup containing three strains from Korea, the second subgroup containing the remaining 14 Korean and Japanese strains. The present work showed the separation of *P. pulmonarius* from *P. ostreatus* strains as their respective clusters were found to be distinct. This outcome is in accordance with the findings of a preceding taxonomic study which applied morphological and physiological criteria to distinguish strains from these two species (Zervakis and Balis, 1991). The taxonomic position of *P. florida* appeared to be a slightly higher similarity with *P. pulmonarius* than with *P. ostreatus*. The present results show that *P. ostreatus* isoenzyme phenotypes can be separated into two distinct groups that are correlated with geographical origin.

A large diversity among *P. ostreatus* strains has been previously described (Kulkarni *et al.*, 1986; May and Royse, 1988). The isozyme banding patterns for 15 enzymes were evaluated to generate a dendrogram to illustrate the relationships between strains. However they used different enzyme system from those in this study. Therefore it can not be discussed with our result. Within the species of other genera, genetic distance has been reported, including *Agaricus campestris* (Royse and May, 1982), *Lentinus edodes* (Royse *et al.*, 1989), *Botrytis cineria* (Backhouse *et al.*, 1984), and *Phytophthora cambivora* (Oudemans and

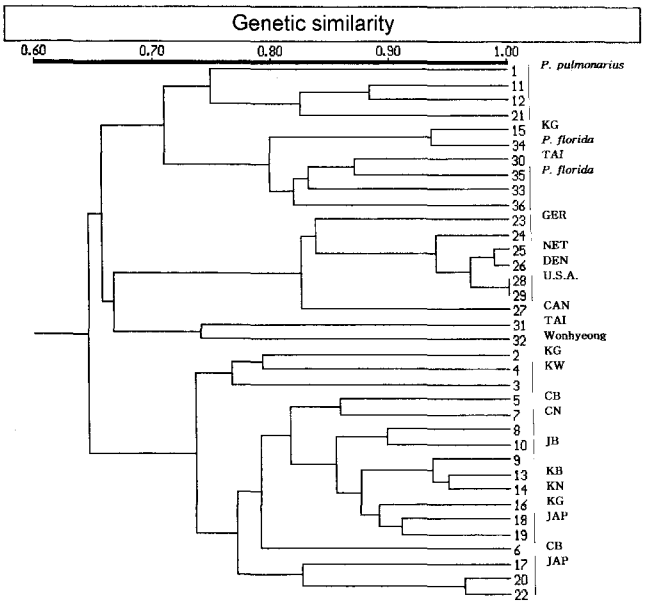

Fig. 7. Dendrogram demonstrating the genetic similarity among 77 electrophoretic types, which represent 36 isolates from *Pleurotus ostreatus, P. florida*, and *P. pulmonarius*. The dendrogram illustrates the five main clusters.

Coffey, 1991). Allozyme markers have been used to routinely identity is homokaryons and heterokaryons among regenerated single protoplasts, commercial and research work maintained lines of *A. bisporus* (Rouse and May, 1989).

In the present study, although the relatively high degree of polymorphism obtained was due to the resolving power of the electrophoretic method applied (Righetti, 1986), and most of all to the wide geographical distribution of the species has been analyzed. In summary, it may be possible to accept that strains of *P. ostreatus* from geographically different origins are genetically divergent, supporting the idea that there is little or no gene flow between these geographically distant population groups. This suggests that geographically separated strains may provide a genetic resource for breeding purposes.

The use of isoelectric focusing analysis of zymograms in fungal taxonomy has been reported, and the present study clearly demonstrates its suitability for this purpose. This study has presented the evidence that isozyme patterns can be used with great confidence for the identification of *P. ostreatus* strains. These results also suggest another potential application of enzyme isoelectric focusing in fungal systematics: distinction of electrophoretic phenotypes by particular enzyme bands. This method might be appropriate for the development of molecular markers for systematics.

References

Backhouse, D., Willets, H. J. and Adam, P. 1984. Electrophoretic studies of *Botrytis* species. *Trans. Brit. Mycol. Soc.* **82**: 625-630.

Chang, S. T. and Miles, P. G. 1991. Recent trends in world production of cultivated edible mushrooms. *Mushroom Journal* **504**: 15-18.

Engvild, K. C. and Nielsen G. 1985. Strain identification in *Rhizobium* by starch gel electrophoresis of isoenzymes. *Plant Soil* **87**: 251-256.

Franke, R. G. 1973. Electrophoresis and taxonomy of saprophytic fungi. In:"Symposium on the use of electrophoresis in the taxonomy of algae and fungi," *Bull. Torrey Botanical Club* **100**: 287-296.

Goullet, P. and Picard B. 1986. Comparative esterase electrophoretic polymorphism of *Escherichia coli* isolates obtained from animal and human sources. *J. Gen. Microbiol.* **132**: 1843-1851.

Hilber, O. 1982. Die Gattung *Pleurotus* (Fr) Kummer unter besonderer Berucksichtigung des *Pleurotuseryngii*-Formenkomplexes. *Bibliotheca Mycologica* **87**: 1-448.

Itavaara, M. 1988. Identification of shiitake strains and some other basidiomycetes: Protein profile, esterase and acid phosphatase zymograms as an aid in taxonomy. *Trans. Brit. Mycol. Soc.* **91**: 295-304.

Kulkarni, R. K., Kamerath, C.D. and Allred, K. L. 1986. Genetic diversity between isolates of *Pleurotus ostreatus* as revealed by isozyme analysis. In Developments in Crop Science 10: Cultivating Edible Fungi (eds. P. J. Wuest, D. J. Royse and R. B. Beelman), pp. 171-181. Elsevier Press: Amsterdam.

Lee, H. K., Yoo, Y. B., Cha, D. Y. and Min, K. H. 1998. Interspecific relationships within the fungal genus *Pleurotus* by isozyme analysis. *Kor. J. Mycol.* **26**: 163-172.

May, B. and Royse, D. J. 1982. Genetic variation and joint segregation of biochemical loci in the common meadow mushroom, *Agaricus campestris. Biochem. Genet.* **20**: 1165-1173.

May, B. and Royse, D. J. 1988. Interspecific allozyme variation within the fungal genus *Pleurotus. Trans. Brit. Mycol. Soc.* **90**: 29-36.

Nei, M. 1987. Molecular evolutionary genetics. pp. 106-107. Columbia University Press: New York.

Oudemans, P. and Coffey, M. D. 1991. Isoenzyme comparision within and among worldwide sources of three morphologically distinct species of *Phytophthora. Mycol. Res.* **95**: 19-30.

Righetti, P. G. 1986. Isoelectric Focusing : Theory, Methodology and Application. (Ed. T.S. Works and Burdon, R. H.) Elsevier Biomedical: Amsterdam.

Rohlf, F. J. 1993. NTSYS-pc. Numerical Taxonomy and Multivariate Analysis System. Version 1.80. Exeter Software: Setauket, New York.

Roux, P. and Labarere, J. 1990. Isozyme characterization of dikaryotic strains of the edible Basidiomycete *Agaricus bitorquis* (Quel.) Sacc. (syn. *Agaricus edulis). Exper. Mycol.* **14**: 101-112.

Roux, P. and Labarere, J. 1991. Determination of genes and

subunit composition of three isozyme activities in *Agaricus bitorquis*. *Mycol. Res.* **95**: 851-860.

Royse, D. J. and May, B. 1982. Use of isozyme variation to identify genotypic classes of *Agaricus brunnescens*. *Mycologia* **74**: 93-102.

Royse, D.J. and May, B. 1989. Identification and use of three new biochemical markers in *Agaricus bisporus*. *Agri. Biol. Chem.* **53**: 2861-2866.

Royse, D. J., Spear, M. C. and May, B. 1983. Cell line authentication and genetic relatedness of lines of the shiitake mushroom, *Lentinus edodes*. *J. Gen. Appl. Microbiol.* **29**: 205-216.

Shechter, Y. 1973. Symposium on the use of electrophoresis in the taxonomy of algae and fungi. *Bull. Torrey Bot. Club* **100**: 253-303.

Smith, M. L. and Anderson, J. B. 1989. Restriction fragment length polymorphisms in mitochondrial DNAs of *Armillaria*: identification of North American biological species. *Mycol. Res.* **93**: 247-256.

Totomasu, T. and Zennyozi, A. 1981. On the application of isoenzyme electrophoresis to identification strains in *Lentinus edodes* (shiitake). *Mushroom Science* **11**: 675-685.

Winter, A. Ek, K. and Anderson, U. B. 1977. Analytical electrofocusing in thin layers of polyacrylamide gels. LKB Application Note 250.

Teruyuki M. and Yukitaka F. N. 1995. Mitochondrial DNA restriction fragment length polymorphisms and phenetic relationships in natural populations of the oyster mushroom, Pleurotus ostreatus. *Mycol. Res.* **99**: 562-566.

Zervakis, G. and Balis, C. 1991. *Pleurotus* species of Greece: an evaluation of their morphological and physiological characteristics. *Mushroom Science* **13**: 537-544.