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Comparisons of Soluble Protein Bands for *Pleurotus* Species and Interspecific Crosses of *Pleurotus* Species

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느타리버섯속균과 교잡종에 대한 단백질 밴드에 의한 분류

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ABSTRACT: In comparison of three *Pleurotus* species and their selfed and crossed isolates using SDS-polyacrylamide gel electrophoresis of total soluble proteins, *Pleurotus ostreatus* 201 showed low similarity to selfed or *P. ostreatus* 201 crossed ones. *Pleurotus ostreatus* 2042 showed low similarity to selfed or *P. ostreatus* 2042 crossed ones. However, *P. ostreatus* 2042×*P. ostreatus* 202, *P. ostreatus* 2042×*P. sajor-caju*, and *P. ostreatus* 2042×*P. ostreatus* 900 showed high similarity. *Pleurotus ostreatus* 202 showed low similarity to selfed or crossed ones. *Pleurotus sajor-caju* showed low similarity to selfed or crossed ones. *Pleurotus ostreatus* 900 showed low similarity to selfed or crossed ones. However, selfed *P. ostreatus* and *P. ostreatus*×*P. florida* showed high similarity. *Pleurotus florida* and selfed *P. florida* showed high similarity, too.

KEYWORDS: *Pleurotus ostreatus*, Soluble proteins

The production and consumption of the edible fungi have increased rapidly during the last decades in Korea. Among the edible fungi, *Pleurotus* is a popular mushroom for its delicious taste and good flavor. *Pleurotus ostreatus, P. florida*, and *P. sajor-caju* are available for the production of commercially cultivated mushroom.

Pleurotus species belong to basidiomycetes and is called oyster mushroom that gives lilac to grayish-tinged spore print on white paper. *Pleurotus ostreatus* forms four terminal basidiospores on the club-shaped and unicellular basidia. Mycelium germinated from a basidiospore is monokaryon with single nucleus. Mycelial anastomosis between compatible two different hyphae can produce dikaryotic mycelia with two nuclei per one cell compartment (Chang and Miles, 1989).

Recently, electrophoresis methods have been used to identify different fungal species (Chen *et al.*, 1991; Lee, 1993; Park and Hyun, 1992). In this study, electrophoresis method was used to analyse genetic relationships among *Pleurotus* species and crossed isolates of *Pleurotus*. The objective of this study was to compare *Pleurotus* species and their selfed and crossed isolates using SDS-polyacrylamide gel electrophoresis of total soluble proteins.

Materials and Methods

Collection and maintenance of cultures

Pleurotus isolates were obtained from In-

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Table 1. Isolates of *Pleurotus* species and their crossed isolates of *Pleurotus* species used for SDS-Polyacrylamide gel electrophoresis (12%)

Single or crossed *Pleurotus* species	Isolate Number	Origin
Pleurotus ostreatus 201	1	Korea
P. ostreatus 201×*P. ostreatus* 2042	2	-
P. ostreatus 201×*P. ostreatus* 202	3	-
P. ostreatus 201×*P. sajor-caju*	4	-
P. ostreatus 201×*P. ostreatus* 900	5	-
P. ostreatus 201×*P. florida*	6	-
P. ostreatus 201×*P. ostreatus* 201	7	-
P. ostreatus 2042	8	Unknown
P. ostreatus 2042×*P. ostreatus* 202	9	-
P. ostreatus 2042×*P. sajor-caju*	10	-
P. ostreatus 2042×*P. ostreatus* 900	11	-
P. ostreatus 2042×*P. florida*	12	-
P. ostreatus 2042×*P. ostreatus* 2042	13	-
P. ostreatus 202	14	Unknown
P. ostreatus 202×*P. sajor-caju*	15	-
P. ostreatus 202×*P. ostreatus* 900	16	-
P. ostreatus 202×*P. florida*	17	-
P. ostreatus 202×*P. ostreatus* 202	18	-
P. sajor-caju	19	India
P. sajor-caju×*P. ostreatus* 900	20	-
P. sajor-caju×*P. florida*	21	-
P. sajor-caju×*P. sajor-caju*	22	-
P. ostreatus 900	23	Unknwon
P. ostreatus 900×*P. florida*	24	-
P. ostreatus 900×*P. ostreatus* 900	25	-
P. florida	26	Germany
P. florida×*P. florida*	27	-

stitute of Rural Development Agency of Kangwon-Do. Their selfings and crosses through mycelial fusion were made on Potato Dextrose Agar (PDA), and each isolate number was designated as shown in Table 1. Identification of all *Pleurotus* isolates was re-confirmed based on morphological characteristics. Three isolates of *Pleurotus* species, their self-crossed isolates of six, and fifteen crossed isolates were studied. Isolates were maintained on Potato Dextrose Agar at room temperature throughout the experiment.

Cultural conditions and protein extraction

Single isolate cultures were grown on PDA for seven to ten days at room temperature. Agar plugs were taken from actively growing colonies and transferred to a Potato Dextrose Broth (PDB) with mininal agar. This broth medium conatined the following per 1,000 m*l*: 20 g PDA (Sigma, St Louis, MS USA), 20 g dextrose, and 1 g Bacto-agar. Four agar plugs were inoculated into 100 m*l* of broth medium in 250-m*l* flasks (four flasks per isolate) and incubated on a rotary shaker (100-110 rpm) at room temperature for seven to ten days . During the extraction procedure, samples were always placed on ice unless otherwise indicated. Mycelial mats were collected by filtering through filter paper (Whatman no. 1), washed with chilled, sterile, deionized distilled water, and frozen at $-20^{\circ}C$. The frozen mycelial

mats were then lyophilized overnight, ground to a fine powder with a chilled mortar and pestle, and transferred to centrifuge tube. Extraction buffer (0.1 M Sodium Phosphate Buffer, pH 8.3) was then added at a rate of 1 m*l* per 0.5 g mycelium, and the suspensions were vortexed every 10 min for 40 min. The extraction mixture was then centrifuged at 16,000× g for 40 min at 4°C. A portion of the supernatant was used for SDS-polyacrylamide electrophoresis. The other portion was stored in small aliquots in microcentrifuge tubes at −20°C and used for starch gel electrophoresis. Each aliquot was used only once. All samples were used within 3 months after extraction.

Polyacrylamide gel electrophoresis of soluble proteins

Protein concentrations in the supernatant were estimated before protein samples were treated with SDS reducing buffer at 95°C for 4 min at the concentrations of 5 mg/m*l*. Polyacrylamide gels, consisting of a 10% separating gel and a 4% stacking gel, were 1 mm thick and the size was 10×8 cm long. The SDS-treated protein samples were applied to the polyacrylamide gel at 25 μg protein per sample and separated with an electric current of 15 mA per gel for 6-7 hrs. At the end of the electrophoretic run, gels were fixed and stained for 1 hr in 0.1% Coomassie blue, 40% methanol, and 10% acetic acid and destained with 40% methanol and 10% acetic acid (Hames and Rickwood, 1981). The presence and absence of a particular band was recorded as 1 and 0, respectively, and this formed a data matrix for further analysis. The relative intensity of protein bands was ignored in cluster and principle analysis.

Data analysis

A computer program, NTSYS-pc Version

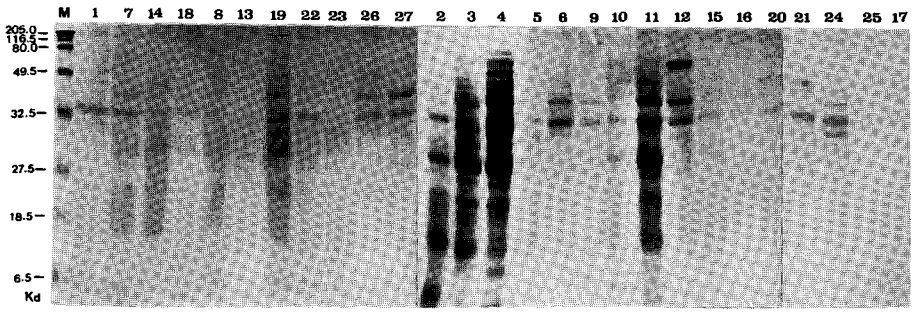

Fig. 1. Protein banding patterns of *Pleurotus* isolates after discontinuous SDS-Polyacrylamide gel (12%) electrophoresis and staining with Coomassie blue. M, marker; lane 1, isolate 201 of *Pleurotus ostreatus*; lane 7, selfed isolate of *P. ostreatus* 201; lane 14, isolate of *P. ostreatus* 202; lane 18, selfed isolate of *P. ostreatus* 202; lane 8, isolate of *P. ostreatus* 2042; lane 13, selfed isolate of *P. ostreatus* 2042; lane 19, isolate *P. sajor-caju*; lane 22, selfed isolate of *P. sajor-caju*; lane 23, isolate 900 of *P. ostreatus*; lane 26, isolate of *P. florida*; lane 27, selfed isolate of *P. florida*; lane 2, crossed isolate of 201 and 2042 of *P. ostreatus*; lane 3, crossed isolate of 201 and 202 of *P. ostreatus*; lane 4, crossed isolate of *P. ostreatus* 201 and *P. sajor-caju*; lane 5, crossed isolate of 201 and 900 of *P. ostreatus*; lane 6, crossed isolate of *P. ostreatus* 201 and *P. florida*; lane 9, crossed isolate of 2042 and 202 of *P. ostreatus*; lane 10, crossed isolate of *P. ostreatus* 2042 and *P. sajor-caju*; lane 11, crossed isolate of 2042 and 900 of *P. ostreatus*; lane 12, crossed isolate of *P. ostreatus* 2042 and *P. florida*; lane 15, crossed isolate of *P. ostreatus* 202 and *P. sajor-caju*; lane 16, crossed isolate of 202 and 900 of *P. ostreatus*; lane 20, crossed isolate of *P. ostreatus* 900 and *P. sajor-caju*; lane 21, crossed isolate of *P. sajor-caju* and *P. florida*; lane 24, crossed isolate of *P. ostreatus* 900 and *P. florida*; lane 25, selfed isolate of *P. ostreatus* 900; lane 17, crossed isolate of *P. ostreatus* 202 and *P. florida*.

Table 2. Similarity matrix based on the number of shared bands by the compared *Pleurotus* species and their crossed isolates of *Pleurotus* species on SDS-Polyacrylamide gel electrophoresis (12%)[a]

	7	14	18	8	13	19	22	23	26	27	2	3	4	5	6	9	10	11	12	15	16	20	21	24	25	17[b]
1[b]	0.21	0.11	0.29	0.00	0.43	0.52	0.11	0.35	0.14	0.00	0.22	0.21	0.14	0.13	0.20	0.30	0.33	0.20	0.10	0.22	0.13	0.35	0.10	0.29	0.16	0.10
7		0.35	0.15	0.17	0.00	0.18	0.11	0.25	0.00	0.00	0.12	0.11	0.00	0.29	0.11	0.00	0.00	0.00	0.11	0.12	0.00	0.25	0.21	0.10	0.33	0.33
14			0.00	0.18	0.17	0.29	0.12	0.27	0.33	0.18	0.12	0.35	0.17	0.15	0.11	0.11	0.25	0.00	0.35	0.12	0.15	0.13	0.11	0.11	0.17	0.24
18				0.00	0.25	0.24	0.00	0.18	0.00	0.00	0.33	0.00	0.00	0.00	0.00	0.14	0.00	0.14	0.00	0.00	0.00	0.00	0.14	0.00	0.15	0.15
8					0.00	0.25	0.17	0.40	0.29	0.33	0.18	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.25	0.00	0.15	0.00	0.00	0.17
13						0.24	0.00	0.18	0.50	0.00	0.33	0.31	0.00	0.22	0.14	0.29	0.33	0.14	0.31	0.17	0.44	0.18	0.14	0.40	0.00	0.00
19							0.18	0.30	0.24	0.37	0.29	0.18	0.12	0.11	0.17	0.26	0.19	0.26	0.09	0.14	0.22	0.10	0.52	0.18	0.21	0.48
22								0.25	0.00	0.17	0.24	0.22	0.31	0.43	0.21	0.00	0.12	0.11	0.22	0.12	0.10	0.25	0.21	0.10	0.17	0.22
23									0.18	0.00	0.13	0.25	0.18	0.00	0.12	0.12	0.13	0.12	0.12	0.13	0.00	0.14	0.35	0.00	0.18	0.25
26										0.29	0.17	0.31	0.00	0.22	0.00	0.15	0.17	0.14	0.29	0.00	0.44	0.00	0.14	0.13	0.00	0.15
27											0.36	0.35	0.00	0.31	0.33	0.33	0.12	0.31	0.17	0.18	0.50	0.00	0.15	0.00	0.00	0.33
2												0.35	0.00	0.31	0.33	0.33	0.12	0.56	0.35	0.37	0.62	0.27	0.11	0.42	0.35	0.47
3													0.31	0.43	0.53	0.32	0.35	0.42	0.67	0.59	0.43	0.37	0.21	0.40	0.25	0.11
4														0.22	0.14	0.14	0.33	0.14	0.15	0.17	0.00	0.36	0.29	0.00	0.00	0.00
5															0.27	0.00	0.00	0.13	0.43	0.31	0.40	0.17	0.13	0.25	0.20	0.14
6																0.70	0.67	0.70	0.63	0.89	0.40	0.24	0.20	0.29	0.24	0.15
9																	0.67	0.70	0.32	0.67	0.27	0.35	0.20	0.29	0.08	0.11
10																		0.56	0.59	0.62	0.31	0.40	0.11	0.21	0.00	0.00
11																			0.42	0.78	0.53	0.24	0.10	0.29	0.24	0.21
12																				0.59	0.57	0.25	0.11	0.30	0.17	0.11
15																					0.46	0.27	0.22	0.32	0.26	0.12
16																						0.17	0.13	0.25	0.10	0.29
20																							0.12	0.33	0.09	0.12
21																								0.10	0.24	0.42

[a] Data matrix was made by scoring the presence or absence of the bands as 1 and 0, respectively. Similarity coefficients between two isolates were then calculated with modified formula of Sneath and Sokal (1973) [Ssm=m/(m+u)], where m=the number of bands found in common between two isolates, and u=the total number of bands unique to each sample.

[b] Isolate numbers

1.61 (Rohlf, 1990), was used for analysis of the electrophoretic data. Simple matching coefficients (Ssm) for each pair of isolates were calculated as described by Sneath and Sokal (1973) by formular: $S_{sm}=m/(m+u)$ where m=the number of bands found in common between two isolates, and u=the total number of bands unique to each sample. The matrix of similarity coefficients was then subjected to a clustering algorithm, the unweighted pair-group method with arithmatic average (UPGMA), that was then used to generate a dendrogram (Romesburg, 1984; Sneath and Sokal, 1973).

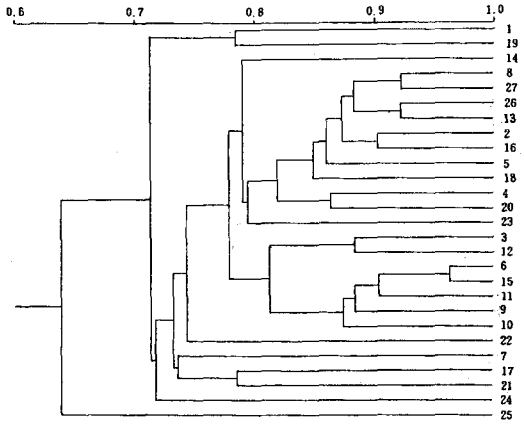

Fig. 2. UPGMA dendrogram showing the relationship among the twenty seven Pleurotus isolates based on the bands formed in SDS-PAGE (12%).

Results and Discussion

The Coomassie blue stain gave very good results with sharp, clear protein bands. The silver staining procedure did not increase the number of bands detected and it stained non-proteinaceous macromolecules (gels not shown). Only gels stained with Coomassie blue were used for further analysis. Protein banding patterns for twenty seven isolates are presented in Fig. 1. Several prominent protein bands were present in all isolates. The number of protein bands were different among isolates *P. ostreatus* 201, 2042, 202, 900, *P. sajor-caju* and *P. florida*. Among these single isolates, *P. sajor-caju* were totally different in their number of bands from others, and *P. florida* showed few common bands with isolates of *P. ostreatus*.

Similarity matrix and UPGMA dendrogram showing the relationships among the 27 isolates based on the number of shared bands by the compared isolate were presented in Table 2 and Figure 2, respectively.

Pleurotus ostreatus 201 showed low similarity with other isolates. *Pleurotus ostreatus* 201 showed the highest level of similarity with *P. ostreatus* 900 among compared single isolates, and the lowest level with *P. ostreatus* 2042. *Pleurotus ostreatus* 202 showed the highest similarity with *P. ostreatus* 201×*P. ostreatus* 202 and *P. ostreatus* 2042×*P. florida*, and the lowest similarity with *P. ostreatus* 2042×*P. ostreatus* 900 and *P. ostreatus* 202×*P. ostreatus* 202. *Pleurotus ostreatus* 2042 showed the highest similarity with *P. florida*×*P. florida* and no similarity with *P. ostreatus* 2042×2042, *P. ostreatus* 201×*P. ostreatus* 202, *P. ostreatus* 201×*P. sajor-caju*, *P. ostreatus* 201×*P. ostreatus* 900, *P. ostreatus* 201×*P. florida*, *P. ostreatus P. sajor-caju* 2042×*P. ostreatus* 202, and *P. ostreatus* 2042×*P. ostreatus* 2042×*P. florida*, *P. ostreatus* 202×*P. sajor-caju*, *P. sajor-caju*×*P. ostreatus* 900, *P. ostreatus* 900×*P. florida*, and *P. ostreatus*×*P. ostreatus* 900. *Pleurotus sajor-caju* showed the highest similarity with *P. sajor-caju*×*P. florida*, and the lowest similarity with *P. ostreatus* 2042×*P. florida*. *Pleurotus ostreatus* 900 showed the highest similarity with *P. sajor-caju*×*P. florida* and no similarity with *P. florida*×*P. florida*, *P. ostreatus* 201×*P. ostreatus* 900, *P. ostreatus* 202×*P. ostreatus* 900, *P. ostreatus* 900×*P. florida*. *Pleurotus florida* showed the highest similarity with *P. ostreatus* 202×*P. ostreatus*

900, and no similarity with *P. ostreatus* 201×*P. sajor-caju, P. ostreatus* 201×*P. florida, P. ostreatus* 2902×*P. sajor-caju, P. ostreatus sajor-caju*×*P. ostreatus* 900, and *P. ostreatus* 900×*P. ostreatus* 900. Based on the results from this study, selfed or crossed isolates of *Pleurotus* showed different genetic characteristics that can be used for the development of new species of *Pleurotus*. Further studies on the genetic relationships among these selfed or crossed *Pleurotus* species based not only on the isozymes but also on the DNA analyses including RFLP, RAPD and base sequence analysis should be performed as described previously for different *Pleurotus* species (Bae *et al.*, 1996; Song *et al.*, 1996).

적 요

느타리버섯 균주(*Pleurotus* isolates)와 이들 균주들을 교잡하여 얻어진 균사에서 추출한 단백질을 전기영동하여 균주간의 유전적 유연관계를 밝힌 결과, *P. ostreatus* 201은 자가교잡 균주 혹은 타가교잡 균주들과 낮은 유연관계를 나타냈다. *P. ostreatus* 2042도 역시 동일한 결과를 나타냈다. 그러나, *P. ostreatus* 2042×*P. ostreatus* 202, *P. ostreatus* 2042×*P. sajor-caju*, 그리고 *P. ostreatus* 2042×*P. ostreatus* 900은 높은 상관관계를 나타냈다. *P. ostreatus* 202와 *P. ostreatus* 900은 자가교잡 균주 혹은 타가 교잡된 균주들과 낮은 상관관계를 나타내었다. 그러나 자가 교잡된 *P. ostreatus*와 *P. ostreatus*×*P. florida,* 그리고 *P. florida*와 자가교잡된 *P. florida*는 각각 서로 높은 상관관계를 나타냈다.

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