Nuclear selection and nuclear substitution in fully compatible di-mon matings in *Pleurotus ostreatus*

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Nuclear behavior in fully compatible dikaryon-monokaryon matings in *Pleurotus ostreatus* was examined with esterase isozymes as markers, which are located at the EST1 locus independent of mating type factors A and B. Monokaryotic strains mMA91 and mOW89 were obtained from the stocks MA91 from Japan and OW89 from Thailand, respectively. The mMA91 strains represented genotype EST1 (100), and the mOW89 strains represented either EST1 (86) or EST1 (93). Most of the stocks derived from mMA91 × OW89 represented the genotype EST1 (93) + EST1 (100). This result suggests that the donor nucleus of OW89 carrying EST1 (93) migrated preferentially into the monokaryon mMA91 to conjugate with the recipient nucleus of the latter. Some of the stocks from mOW89 × MA91 represented the genotype EST1 (100) + EST1 (100). This seems to have resulted from substitution of a nucleus of the recipient mOW89 by a pair of nuclei of the donor MA91. The variations in mycelial products, mycelial growth rate, and wood degrading ability among the D1 populations of stocks from mMA91 × OW89 were significantly smaller than those from mOW89 × MA91, reflecting the selective nuclear migration from OW89 to mMA91.

Key Words——dikaryon-monokaryon matings; esterase isozymes; nuclear selection; nuclear substitution; *Pleurotus ostreatus*.

Introduction

The sexuality of most basidiomycetous fungi shows heterothallism (Whitehouse, 1949), in which the mating is genetically regulated by an extensive series of multiple alleles of mating factor(s) (Kniep, 1920; Raper, 1953, 1966; Raper, C. A., 1978). This system limits the mating potential by the fact that a fertile dikaryon is established only between monokaryotic mycelia carrying different mating type factors from each other, e.g., A1B1 × A2B2→(A1B1+A2B2) in tetrapolar (bifactorial) fungi.

The dikaryotization of a monokaryotic mycelium by a dikaryotic mycelium was first demonstrated by Buller (1930, 1931) in *Coprinus lagopus* (Fr.) Fr. and was commonly referred to as the "Buller Phenomenon" by Quintanilha (1937) or "di-mon mating", an abbreviation of "dikaryon-monokaryon mating" by Papazian (1950). This mode of dikaryotization produces a new pair of conjugate nuclei following nuclear migration from the dikaryon into the monokaryon. In fully compatible di-mon matings, two kinds of dikaryons must occur with equal frequency, i.e., A1B1 × (A2B2+A3B3)→(A1B1+A2B2) and (A1B1+A3B3). Intensive studies on this mating, however, revealed that the nucleus of dikaryon frequently did not conjugate randomly with the nucleus of a monokaryotic mycelium in fully compatible di-mon matings. In incompatible di-mon mating combination, dikaryotization occurred accompanying genetic recombi-

nation between conjugate nuclei of the donor dikaryotic mycelium (Papazian, 1950; Crowe, 1960; Ellingboe, 1963, 1964; Ellingboe and Raper, 1962a; Parag, 1962), or nuclear substitution of a pair of nuclei of the dikaryon (Papazian, 1950). Consequently, a fertile dikaryon can result from all the combinations of mating factors in di-mon matings. These phenomena were revealed using morphological and biochemical mutants of *Schizophyllum commune* Fr. or *Coprinus* species (Raper and Miles, 1958), but few studies were conducted using cultivated edible basidiomycetes. High mating potential in di-mon mating is effective for taxonomic identification of fungal specimens (Rizzo and Harrington, 1993; Kinugawa et al., 1994) and for producing hybrids in mushroom breeding (Eger, 1978; Kinugawa et al., 1994).

In di-mon matings of *Pleurotus ostreatus* (Jacq.: Fr.) Kummer, we used esterase isozymes as markers of the nuclei concerned to trace their migration into the counterpart mycelium in mating. We found selective migration of a particular nucleus of OW89 into mMA91 in mating, resulting in diminution of the variation of some quantitative traits of the derived dikaryotic progenies.

Materials and Methods

Fungi In this study the terms "dikaryotic stock" or "stock" are used for any mycelium having a clamp connection in each septum, and the terms "monokaryotic strain" or "strain" for any mycelium having one kind of

nucleus, as proposed by Raper (1966). Two cultivated stocks of *Pleurotus ostreatus* were collected at geographycally different sites, stock MA91 from Japan and stock OW89 from Thailand. The monokaryotic strains of mMA91 and mOW89 were obtained by dilution culture of basidiospores of MA91 and OW89, respectively. Selfed dikaryotic stocks (S1) of OW89 occurred spontaneously on the culture plates during monospore culture.

All stocks and strains were maintained at 13°C in the dark on MYP agar slants consisting of 7 g of malt extract (Difco), 1 g of soytone (Difco), 0.5 g of yeast extract (Difco), and 15 g of agar in 1000 ml of distilled water (Bandoni and Johri, 1972).

Crosses Mon-mon matings were carried out by reciprocal intercrosses between 13 strains of mMA91 and 14 strains of mOW89 in all possible combinations. Mating was performed on the MYP agar plates containing 25 ml of medium in a 90 mm petri dish. Tiny mycelial masses of each monokaryotic strain to be crossed were inoculated at opposite sides of the plate, 10 mm from the wall. Three or six days later, when mycelia came into contact with each other, tiny masses of colony were transferred from both sides of inocula onto slants. Mycelia that posessed clamp connections were taken as F1 dikaryotic mycelia. The reciprocal F1 stocks were represented as mMA91 (×mOW89) for an isolate from the colony of mMA91, and as mOW89 (×mMA91) for that from mOW89. The strain in parenthesis was the nuclear donor.

Di-mon matings were carried out between 99 strains of mMA91 and OW89 (crosses mMA91 × OW89), and between 84 strains of mOW89 and MA91 (crosses mOW89 × MA91). The derived dikaryons (D1 stocks) were represented as mMA91 (×OW89) and mOW89 (×MA91), respectively.

Electrophoresis Sample preparation and electrophoresis followed Pasteul et al. (1988).

(1) Culture and sample preparation: A tiny agar disk containing mycelia excised with a cork borer (5 mm diam) from a colony on an agar plate sub-cultured for 10 days at 25°C was inoculated on 30 ml of MYP liquid medium in a 100 ml Erlenmeyer flask. Vegetative mycelium was cultured for 7 to 28 days at 25°C in the dark, and collected by filtration through doubled nylon stocking cloth. It was washed twice with distilled water, then frozen and stored at −20°C until use. Frozen mycelium was ground with glass beads and a few drops of grinding buffer (see below). Mycelial extract was absorbed onto rectangles (4 × 8 mm) of filter paper (Advantec; type Ananashi-26) and used directly as a crude enzyme solution for electrophoresis.

(2) Electrophoretic conditions: The rectangles were inserted into starch gel (12%) and subjected to horizontal electrophoresis at 4°C. After a pre-run at 40 mA for 20 min, the rectangles were removed and the gel was run at 190 V for 5-6 h, until the dye marker bromophenol blue had moved 95 mm.

(3) Buffer system and staining schedule: Buffer solutions and staining solution for esterase isozymes were based on those of Soltis et al. (1983).

a) Grinding buffer: 0.05 M Tris-HCl, pH 7.2, containing 0.1% 2-mercapto-ethanol and 10% polyvinyl pyrrolidone (Sigma, PVP-40T).

b) Electrode buffer: 0.038 M LiOH, 0.188 M boric acid, pH 8.3.

c) Gel buffer: 0.0045 M Tris-HCl, 0.007 M citric acid, 0.004 M LiOH, 0.019 M boric acid, pH 8.3.

The staining solution consisted of 40 mg of α-naphthyl acetate and 40 mg of β-naphthyl acetate dissolved in 2 ml of acetone, and 100 mg of fast blue RR salt as azo-dye in 10 ml of 1.0 M phosphate buffer (pH 6.0) and 90 ml of water.

Genetic nomenclature of isozyme The abbreviation EST refers to esterase isozyme. Following May et al. (1979), a numeral following EST indicates the locus and one in parentheses indicates the allele, which is represented by the relative mobility of the isozyme. The EST genotype of a dikaryotic stock is represented by two allelic genes located separately on conjugate nuclei that are linked with a +sign.

Quantitative traits

(1) Mycelial biomass: Vegetative mycelium was cultured in 30 ml of MYP liquid medium in a 100 ml Erlenmeyer flask at 25°C for 20 days. The mycelium was collected, dried (at ca. 40°C for 48 h, then at 105°C for 1 h) and weighed.

(2) Mycelial growth rate: Expansion of colony diameter was measured on the MYP agar medium in a 90 mm petri dish at 25°C. Growth rate of mycelium is represented by a regression coefficient calculated from the diameter and the number of days from inoculation during the period in which the colony expanded linearly.

(3) Wood weight loss: Weight loss (%) of a wood block (*Fagus crenata* Blueme) by fungal degradation was measured after 80 days of culture on an MYP agar plate (Tanesaka et al., 1993).

Statistical analysis Significant differences in the segregation ratio of esterase isozymes in the progenies were examined by the χ^2-test. For quantitative traits, differences in means and variances between two populations, and between means of each pair of reciprocal F1 stocks were calculated by the F-test and the t-test for paired comparisons, respectively (Sokal and Rohlf, 1981).

Results

Crosses After reciprocal mon-mon matings of 162 combinations, 160 F1 stocks from mMA91 × mOW89, and 150 F1 stocks from mOW89 × mMA91 were obtained, indicating that the two parent stocks MA91 and OW89 were fully compatible with each other.

In di-mon matings, 80 D1 stocks from 84 combinations of the crosses mMA91 × OW89, and 97 D1 stocks from 99 combinations of the crosses mOW89 × MA91 were obtained.

Estearase isozymes in comparison Electrophoretic patterns of esterase isozymes were different between MA91 and OW89, and these patterns were segregated in the monokaryotic progenies (Fig. 1). Among the isozyme
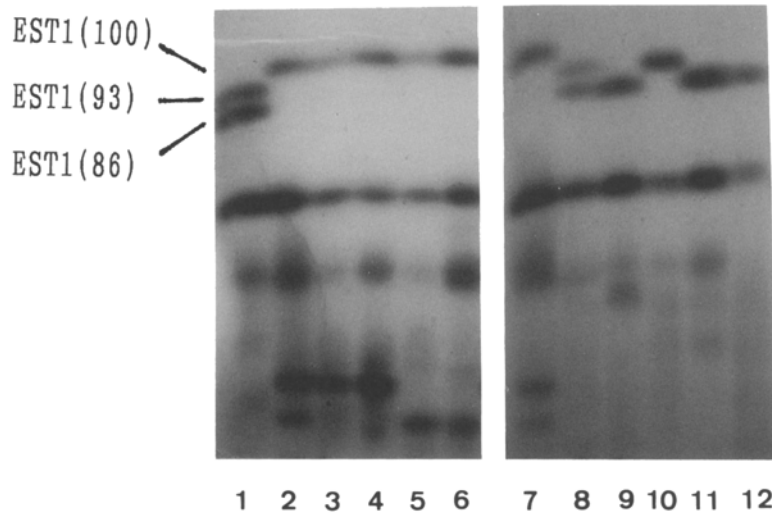
Fig. 1. Esterase isozyme pattern in monokaryotic strains of MA91 and OW89.
Lanes 1 and 8: OW89; lanes 2 and 7: MA91; lanes 3–6: mMA91; lanes 9–12: mOW89.

bands, EST1(100) from MA91, and EST1(86) and EST1(93) from OW89 moved faster than others, and these were used in this study because of their highly stable activity over 7 to 28 days of culture.

Genotypes of parental MA91 and OW89 mMA91 expressed only EST1(100) and mOW89 expressed either EST1(86) or EST1(93). The segregation ratios of EST1(86) and EST1(93) in the mOW89 population agreed with the expected 1 : 1 ratio (Table 1). Further investigation using dikaryotic stock carrying EST1(86) and EST1(100) revealed that the two isozymes were segregated in a 1 : 1 ratio in its monokaryotic population (data not shown). These results indicate that EST1(86), EST1(93), and EST1(100) were under allelic control, and that the genotype of MA91 at the EST1 locus was EST1(100)+EST1(100), while that of OW89 was EST1(86)+EST1(93).

Isozyme patterns in free selfed stocks (S1) of OW89 Esterase isozyme patterns of S1 stocks are shown in Fig. 2. They represent three genotypes: EST1(86) +EST1(86), EST1(86)+EST1(93), and EST1(93) +EST1(93). The segregation ratio of these genotypes agrees with the 1 : 2 : 1 ratio that is expected for loci that are independent of mating-type factors (Table 2).

Behavior of EST1 alleles in di-mon matings Esterase isozyme patterns and their segregation ratio in the D1 progenies are shown in Fig. 3 and Table 3, respectively.

Among the genotypes of D1 stocks mMA91 (×OW89), EST1(93)+EST1(100) was prevalent, while only one example of EST1(86)+EST1(100) was found. The observed ratio was far from the expected 1 : 1 ratio in which each of the conjugate nuclei of OW89 randomly enter mMA91 (Table 3). The D1 stocks mOW89 (×MA91) represent three genotypes: EST1(86) +EST1(100), EST1(93)+EST1(100), and EST1(100) +EST1(100). The former two genotypes were segregated in a 1 : 1 ratio (Table 3), which shows random mating of EST1(100) from MA91 with segregated EST1(86) or EST1(93) of mOW89.

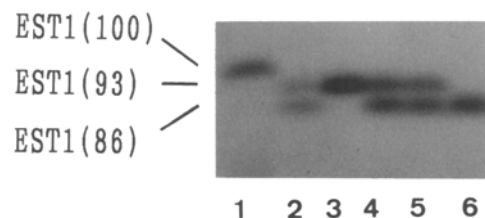**Comparison of quantitative traits between F1 and D1**

Fig. 2. Esterase isozyme pattern in free selfed S1 progenies of OW89.
Lane 1: MA91; lane 2: OW89;
lanes 3–6: S1 progenies of OW89.

Table 1. Segregation ratio of the esterase isozymes in monokaryotic progenies mMA91 and mOW89.

Strains	Isozymes			Deviations from expected ratio*
	EST1(86)	EST1(93)	EST1(100)	
mMA91	0	0	31	
mOW89	10	11	0	$\chi^2=0.048$, $0.9>P>0.8$

*: Segregation ratio of EST1(86) : EST1(93)=1 : 1 in mOW89.

Table 2. Segregation of genotypes at the EST1 locus in S1 dikaryotic progenies from free selfing of OW89.

	Genotype			Total
	EST1(86) +EST1(86)	EST1(86) +EST1(93)	EST1(93) +EST1(93)	
No. observed	4	7	4	15
No. expected	3.75	7.5	3.75	15

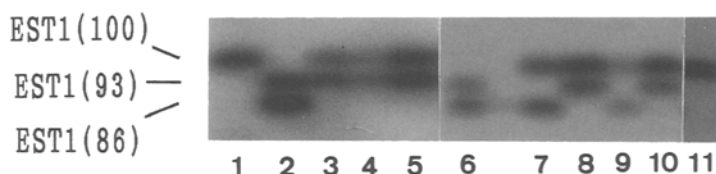Deviations from expected ratio (1 : 2 : 1): $\chi^2=0.067$, $P>0.9$.

Fig. 3.   Esterase isozyme pattern in D1 progenies between MA91 and OW89.
Lane 1: MA91; lanes 2 and 6: OW89; lanes 3-5: mMA91 (×OW89); lanes 7-11: mOW89 (×MA91).

progenies   Table 4 shows mycelial biomass, mycelial growth rate, and wood weight loss of F1 and D1 progenies.   No significant differences were detected in means and variances (F values) of quantitative traits between reciprocal F1 populations and between individual reciprocals.   By contrast, the variances of each trait in D1 stocks mMA91 (×OW89) were significantly smaller than those in their reciprocal population mOW89 (×MA91).

Discussion

Pleurotus ostreatus is known to be a tetrapolar fungus (Terakawa, 1960).   Monokaryotic strains derived from Japanese stock MA91 and from Thai stock OW89 were dikaryotized with each other in almost all combinations, indicating that these two geographically isolated stocks carry different mating type factors from each other.

Table 3.   Segregation at the EST1 locus in the D1 dikaryotic progenies from dikaryon-monokaryon matings.

D1 stocks	Genotype		
	EST1 (86) +EST1 (100)	EST1 (93) +EST1 (100)	EST1 (100) +EST1 (100)
mMA91 (×OW89)*			
observed	1	42	0
expected	21.5	21.5	0
mOW89 (×MA91)**			
observed	21	13	8
expected	17	17	0

* Deviations: $\chi^2 = 39.09$, $P < 0.01$.
** Deviations: $\chi^2 = 1.88$, $0.2 < P < 0.3$.

In fully compatible di-mon matings, selective nuclear conjugation has been observed in several fungi, including *Coprinus fimentarius* Fr. (Quintanilha, 1939), *C. macrorhizus* Rea f. *microsporus* Hongo (Kimura, 1958), and *Schizophyllum commune* (Papazian, 1950; Ellingboe and Raper, 1962b; Crowe, 1963).   Some derived dikaryons were found to have the same nuclear constitutions as the parent dikaryons (Papazian, 1950; Kimura, 1958).   In the crosses mMA91×OW89, if each of conjugate nucleus of OW89 can equally contribute to dikaryotization of mMA91, the genotypes EST1 (86)+EST1 (100) and EST1 (93)+EST1 (100) should occur in equal frequencies.   The observed ratio, however, did not agree with the expectation, as most D1 stocks represented the genotype EST1 (93)+EST1 (100).   This result suggests that nuclei carrying EST1 (93) selectively participated in dikaryotization of monokaryotic mMA91s.   Raper (1966) pointed out that in compatible di-mon mating, selective nuclear association appears to work between nuclei having a greater degree of genetic heterogenity.

Some D1 stocks of mOW89 (×MA91) represented genotype EST1 (100)+EST1 (100).   This result suggests that two nuclei of MA91 migrated together into monokaryotic mOW89 and replaced the nucleus of the latter.   This type of nuclear behavior was frequently reported in certain combinations of fully compatible di-mon matings in *C. macrorhizus* f. *microsporus* (Kimura, 1958), while rarely (Papazian, 1950; Ellingboe and Raper, 1962b) in *S. commune*.   Nuclear substitution was only detected in D1 stocks derived from mOW89× MA91 but not from the reciprocal mMA91×OW89, and the frequencies observed here using *P. ostreatus* were

Table 4.   Comparisons of quantitative traits of progenies derived from artificial crosses.

Stocks	Mycelial dry weight (mg)				Growth rate (mm/day)				Weight loss of wood (%)			
	m	sd	n	F	m	sd	n	F	m	sd	n	F
Parent dikaryons												
MA91	100.7				7.5				19.5			
OW89	55.0				6.4				30.0			
F1 stocks												
mMA91 (×mOW89)	69.2	9.15	25	1.14ns	6.0	0.77	24	1.73ns	21.9	6.15	76	1.37ns
mOW89 (×mMA91)	69.3	9.73	25		6.3	1.01	24		21.7	5.26	78	
D1 stocks												
mMA91 (×OW89)	69.3	8.85	58	3.03*	6.6	0.96	58	3.75*	22.3	3.72	43	5.30*
mOW89 (×MA91)	64.0	15.40	58		5.3	1.82	54		18.7	8.57	43	

m: mean.   sd: standard deviations   F: $(sd)^2/(sd)^2$ *: significant at the 5% level.   ns: not significant at the 5% level.

higher than those of previous studies using other species. It appears that there is some affinity between the recipient cytoplasm and the selected donor nucleus.

There were no significant differences in distributions of quantitative traits between reciprocal F1 populations or between pairs of reciprocals. Thus these traits would be mainly controled by nuclear genes rather than cytoplasmic inheritance. The greater variations in quantitative traits found in the D1 population derived from mOW89×MA91 (Table 4) are of interest in considering mushroom breeding. The smaller variations seen in the D1 population mMA91 (×OW89) are probably attributable to selective nuclear conjugation. These results suggest that the di-mon matings in breeding schedules of mushrooms carry the risk that genetic veriations large enough for breeding would not occur in fully compatible mating combinations of *P. ostreatus*.

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