Note

Protoplast Fusion of Incompatible Mating Type Combinations of *Lentinus edodes* (Shiitake) Auxotrophs

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In recent years, protoplast fusion has been useful in the basic and applied science of basidiomycetes.¹⁻⁴⁾ We have reported an efficient method for the formation and the regeneration of protoplasts of *Lentinus edodes*⁵⁾ which is industrially an important mushroom in Japan. In this note, intraspecific fusion between incompatible monokaryons of *Lentinus edodes* is described.

Four monokaryotic strains of *Lentinus edodes*, No. 23, 25, 17, and 47, used in this study were the same as those used in our previous paper.⁵⁾ Protoplast preparation with Cellulase Onozuka RS and Sigma chitinase, and fusion treatments with polyethyleneglycol 4000 were also described in that paper.⁵⁾ Auxotrophic mutants were isolated as described elsewhere.⁶⁾ The minimal medium composition used here was; 2 g of glucose, 0.2 g of ammonium tartrate, 0.05 g of $MgSO_4 \cdot 7H_2O$, 0.1 g of KH_2PO_4 , 0.112 g of Na_2CO_3 , 0.132 g of fumaric acid, 12.5 μg of

thiamine-HCl, 1.0 mg of $FeSO_4 \cdot 7H_2O$, 0.88 mg of $ZnSO_4 \cdot 7H_2O$, 0.72 mg of $MnCl_2 \cdot 4H_2O$, in 100 ml of deionized water (a modification of a reported medium⁷⁾), adding 0.4 g of Sanpearl CP and adjusting the pH to 4.5.

With UV irradiation for 50~70 sec of the protoplasts, more than 90% lethality was attained in every strain. Three auxotrophic mutants, 23-53A (lysine⁻), 25-555A (methionine⁻), and 47-42A (inositol⁻), were obtained by this method.

In the case of compatible mating type combinations of 23-53A (lys⁻, A1B1) and 47-42A (ino⁻, A2B2) protoplasts, regenerated colonies on the minimal medium plates were observed only with the fusion treatment. Clamp structures were detected in every colony regenerated, indicating that the mycelia were dikaryotic.

Table I showed the results of protoplast fusion between incompatible combinations of 23-53A (lys⁻, A1B1) and 25-555A (met⁻, A1B2). Regenerated colonies were also appeared on the minimal medium plates (frequency of 0.05% to 0.17%, Table I exp. No. 1~5) only with the fusion treatment. It was consistent with the case of *Coprinus cinereus* that fusion of incompatible monokaryons, as well as compatible combinations, resulted in auxotrophically complimented colonies on the minimal medium.²⁾ The mycelia of regenerated colonies obtained here had no clamp structures and could not differentiate fruit bodies.

Sixteen colonies, designated as F-1 to F-16, were picked up among the regenerated protoplasts on the minimal medium plates after fusion treatments of 23-53A and 25-555A. Fifteen colonies among the sixteen (94%), all except for F-12, were able to mate with both strains of No.47 and No. 17 which were compatible with 23-53A and 25-555A, respectively (Table II).

The mycelia of F-3, 6, and 9 were each re-protoplasted, and regenerated colonies without clamps were picked up.

TABLE I. PROTOPLAST FUSION BETWEEN INCOMPATIBLE COMBINATIONS OF *Lentinus edodes* MONOKARYONS, 23-53A AND 25-555A

Exp. No.	Treatments		Regenerated colonies (%)		Frequency of fusion (M/C, %)
			M ^a ($\times 10^{-3}$)	C ^b	($\times 10^{-1}$)
1	23-53A $\times$ 25-555A (fusion)	pH 5.5	2.6	1.6	1.6
2		pH 6.5	4.0	2.7	1.5
3		pH 7.5	0.8	1.6	0.5
4		pH 8.5	1.8	1.9	1.0
5		pH 9.0	1.6	2.3	0.7
6	23-53A + 25-555A (mix)		0	0.6	0
7		23-53A	0	7.5	0
8		25-555A	0	8.0	0

^a M, minimal medium. ^b C, complete medium.

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TABLE II. MATING EXPERIMENTS BETWEEN THE FUSANTS OF INCOMPATIBLE PROTOPLASTS AND OTHER MONOKARYONS OF *Lentinus edodes*

Strains		No. 23 (A1B1)	No. 47 (A2B2)	No. 25 (A1B2)	No. 17 (A2B1)
Prototrophs	No. 23 (A1B1)	— ^a	+ ^b	—	—
	No. 47 (A2B2)	+	—	—	—
	No. 25 (A1B2)	—	—	—	+
	No. 17 (A2B1)	—	—	+	—
Auxotrophs	23-53A (A1B1)	—	+	—	—
	25-555A (A1B2)	—	—	—	+
Fusants	F-1 to F-11 and F-13 to F-16	—	+	—	+
	F-12	—	—	—	+

^a —, no clamps. ^b +, clamp formation.

TABLE III. MATING TYPES AND NUTRITIONAL REQUIREMENTS OF *Lentinus edodes* MONOKARYONS GENERATED FROM THE PROTOPLASTS OF DIKARYONS WHICH WERE DERIVED FROM MATINGS OF INCOMPATIBLE PROTOPLAST FUSANTS AND OTHER MONOKARYONS

(1) Mating types

Mating	Number of monokaryons examined	Mating type		
		F-Type ^a	No. 17-Type	No. 47-Type
F-3 × No. 17	35	11	24	—
F-6 × No. 47	50	9	—	41

(2) Nutritional requirements

Mating	Number of monokaryons examined	Nutritional requirement		
		Lys	Met	None
F-6 × No. 47	52	0	0	52
F-9 × No. 47	63	0	0	63

^a F-type, the same mating type as fusants.

All the colonies tested were prototrophs, and showed the same mating types of F-3, 6, and 9, that is, they were compatible with both strains of No. 17 and No. 47. These results suggested that the fusant colonies were not heterokaryons of 23-53A and 25-555A.

Clamp-bearing colonies derived from the matings of F-3, 6, and 9 with No. 47 or No. 17, were protoplasted, and the mating types and nutritional requirements of the regenerated colonies with no clamps were analyzed (Table

III). The colonies with no clamps showed the mating types of either parent, and no nutritional requirements. From these results the fusants were shown to mate with other monokaryons apparently through the ordinary way to form heterokaryons.

B-common protoplast fusion of *Coprinus cinereus* monokaryons also formed fusants without clamps in some combinations, but formed clamp-bearing fusants in some combinations and even primordia in a combination in the recent experiments by Ohba, Kawasumi, and Yanagi. Meanwhile, similar results to these for *L. edodes* were obtained with incompatible protoplast fusions of *Pleurotus salmoneo-stramineus* by Iijima *et al.* (personal communication). In our previous report,⁵⁾ mating types remained stable through the procedure of protoplasting and fusion treatments. We were not able to conclude that this *L. edodes* protoplast fusion was linked recombination (or backmutation) of the genes for incompatibility and for nutritional requirements or to be partial polyploidy of the genomes as reported with yeasts⁸⁾ and filamentous fungi.⁹⁾ More detailed investigations are necessary. However, very rare or unusual phenomena could be screened for by the technique of protoplast fusion, and it may provide some of important information for studies on the complex physiology of basidiomycetes.

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