Short Communication

Fruit Body Formation of the Fusion Products Obtained on Interspecific Protoplast Fusion between *Pleurotus* Species

Tetsuo TOYOMASU and Kan-ichi MORI

*The Mushroom Research Institute of Japan,
8-1 Hirai-cho, Kiryu, Gunma 376, Japan*

Received April 10, 1987

Recently, considerable attention has been paid to protoplast fusion and its application to strain improvement of edible mushrooms. However, little work has been performed to obtain interspecific hybrids of basidiomycetes, although the intraspecific fusion of *Phanerochaete chrysosporium*,¹⁾ *Coprinus macrorhizus*²⁾ and *Pleurotus ostreatus*³⁾ has been reported. In the present paper, we report the interspecific protoplast fusion between four *Pleurotus* species and subsequent fruit body formation of the fusion products.

The auxotrophy of the strains of *Pleurotus* species used is as follows: *P. ostreatus* 1qu-14A, ileu⁻; *P. columbinus* 4f-1A, ade⁻; *P. pulmonarius* 4x-3A, lys⁻; *P. sajor-caju* 3s-11A, met⁻. All these strains were monokaryotic, and the cross-incompatibility among the strains used here was described in a previous paper.⁴⁾ Briefly, sexual hybridization is possible between *P. ostreatus* 1qu-14A and *P. columbinus* 4f-1A, and between *P. pulmonarius* 4x-3A and *P. sajor-caju* 3s-11A. Protoplast formation and fusion treatment were performed according to the methods previously described.⁵⁾ The methods used for the preparation of cell-free extracts, gel electrophoresis and staining for the detection of isozymes were described elsewhere.^{5,6)} Nuclei were stained with pro-

pidium iodide (Sigma) as described by Suzuki *et al.*,⁷⁾ and were observed under a Nikon interference contrast microscope (XF-NT) equipped with an epifluorescence attachment, using a G excitation filter (510~560 nm) and a barrier filter (590 nm). For fruit body formation, mycelium grown on GMY agar (1% glucose, 1% malt extract and 0.4% yeast extract) was inoculated into sawdust medium (oakwood sawdust, 6 vol.; corn bran, 1 vol.; tap water 60%) in a 300-ml conical beaker. After 3 weeks incubation at 25°C, the cultures were transferred to natural light conditions at a temperature of 17°C. Basidiospores obtained from the fruit bodies were suspended and then spread on GMY agar plates to obtain approximately 100 germlings. Colonies derived from a single basidiospore were transferred to GMY slants to determine nutritional requirements.

In order to isolate the interspecific hybrids, protoplast fusion experiments were performed between four mutants of *Pleurotus* species. After PEG-treated protoplasts of each combination had been incubated for 2~3 weeks, prototrophic colonies that developed on MM⁵⁾ containing 0.65 M mannitol were isolated and transferred to MM slants. All of the fusion products obtained on interspecific fusion between 1qu-14A and 4f-1A possessed clamp connections, while in the other fusion products, 1qu-14A+3s-11A, 4x-3A+3s-11A and 4f-1A+3s-11A, no clamp connections were observed, as previously described.⁴⁾ Nuclear staining with propidium iodide of the mycelia of these fusion products showed they were uninucleate, as previously reported in the case of the fusion product of *P. ostreatus*+*P. salmoneo-stramineus*.⁵⁾ The fusion products isolated for compatible and incompatible combinations differed from both parental strains in colony morphology, and the latter fusion products, 4f-1A+3s-11A, showed sectors spontaneously. When the fusion products of 4f-1A+3s-11A were cultured on GMY agar plates with their parental strains, a barrage-

Abbreviations: ileu⁻, isoleucine; ade⁻, adenine; lys⁻, lysine; met⁻, methionine; MM, minimum medium; PEG, polyethylene glycol.

FIG. 1. Isozyme Patterns of the Interspecific Fusion Products between *P. ostreatus* 1qu-14A and *P. columbinus* 4f-1A.

1qu-14A $\times$ 4f-1A and 4f-1A $\times$ 1qu-14A, sexual hybrids obtained by reciprocal crossing. qf-1 ~ 17, fusion products.

The cell-free extracts were analyzed by electrophoresis in a 7.5% polyacrylamide slab gel by the method of Davis.⁶⁾ Electrophoresis was performed at 4°C and a constant voltage of 14 V/cm for 6 hr. After electrophoresis, the gels were incubated in the staining solutions for the detection of isozyme bands as previously described.⁵⁾

FIG. 2. Fruit Bodies of the Interspecific Fusion Products.

Left: qf-16, fusion product of *P. ostreatus* 1qu-14A + *P. columbinus* 4f-1A.

Right: fs-1 $\times$ 3s-3, fusion product (fs-1) of *P. columbinus* 4f-1A + *P. sajor-caju* 3s-11A was crossed with a monokaryotic strain of *P. sajor-caju* 3s-3.

like phenomenon was observed at the contact zone between the fusion products and 3s-11A, and a brown-zone line was formed between the fusion products and 4f-1A without exception.

To rule out the possibility that the prototrophic fusion products arose as a result of contamination, the isozyme patterns of esterase (EST), acid phosphatase (ACP) and succinate dehydrogenase (SDH) of the fusion products were compared with those of the parental strains. The results are shown in Fig. 1. The EST isozyme patterns of the fusion products of 1qu-14A+4f-1A showed bands common to both of the parental strains, apparently indicating that they are interspecific hybrids, although no major differences among the parental strains and their fusion products were observed in the ACP and SDH isozymes. On the contrary, the EST, ACP and SDH isozymes of the fusion products of 1qu-14A+3s-11A, 4x-3A+3s-11A and 4f-1A+3s-11A showed either patterns similar or identical to the other parental strain, 3s-11A, except that the fusion products of 4x-3A+3s-11A and 4f-1A+3s-11A were different from both of the parental strains in the ACP patterns (data not shown).

The auxotrophic mutants used as parents are unable to form fruit bodies. The interspecific fusion products of 1qu-14A+4f-1A formed fruit bodies on the sawdust medium

(Fig. 2). On the other hand, the fusion products of the other three combinations, 1qu-14A+3s-11A, 4x-3A+3s-11A and 4f-1A+3s-11A, did not develop fruit bodies. However, it should be noted that the fusion products of 4f-1A+3s-11A formed fruit bodies when they were cultured together with a monokaryotic strain derived from *P. sajor-caju* 3s. Fruit bodies produced by these fusion products were almost invariably abnormal in morphology, although the fruit bodies yielded viable basidiospores (germination, more than 85%). As shown in Table I, random basidiospore isolates obtained from fruit bodies of the fusion product, qf-16 (1qu-14A+4f-1A), were analyzed for auxotrophy. Forty-four out of 103 isolates were of the parental types, *ileu*⁻ and *ade*⁻, and the remaining 59 isolates were of recombinant types, *ileu*⁻*ade*⁻ and prototrophy. This clearly demonstrated that the interspecific hybridization between *P. ostreatus* and *P. columbinus* occurred through protoplast fusion. Meanwhile, the fusion product, fs-1 (4f-1A+3s-11A), was crossed with a monokaryotic strain, 3s-3, derived from a dikaryon of *P. sajor-caju* 3s, and then random basidiospore progenies were checked for auxotrophy. Seventy-two out of 98 isolates obtained from the cross between these two prototrophs, fs-1 and 3s-3, were prototrophs, and the remaining 26 isolates were all *met*⁻, as was the mutant of 3s-11A. This indi-

TABLE I. SEGREGATION FOR AUXOTROPHIC MARKERS IN BASIDIOSPORE ISOLATES FROM FRUIT BODIES OF THE INTERSPECIFIC FUSION PRODUCTS

Fruit bodies ^a	Number of basidiospore isolates			
	Parental type		Recombinant type	
Fusion product	<i>ileu</i> ⁻	<i>ade</i> ⁻	<i>ileu</i> ⁻ <i>ade</i> ⁻	prototrophy
qf-16	25	19	12	47

Cross	Parental type		Non-parental type	
	<i>met</i> ⁻		prototrophy	Total
fs-1 × 3s-3 ^b	26		72	98

^a Fruit bodies of qf-16 and fs-1 × 3s-3 were the same as in Fig. 2.

^b 3s-3, wild-type monokaryon derived from the dikaryon of *P. sajor-caju* 3s. See the text for auxotrophy of the parental strains.

cated that the fusion product, fs-1, arose as a result of protoplast fusion, and also suggested that the fusion product was allodiploid or aneuploid.

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