Short Communication

Intraspecific Protoplast Fusion of *Pleurotus ostreatus* using Auxotrophic Mutants

Masatake Ohmasa

*Division of Forest Protection, Forestry and Forest Products Research Institute, Kukisaki, Tsukuba, 305*

Hybrid strains of *Pleurotus ostreatus* were produced by intraspecific protoplast fusion of auxotrophic mutants derived from monokaryotic strains. Auxotrophic mutants were obtained by treating fragmented mycelia by N-methyl-N' -nitro-N-nitrosoguanidine. One of them obtained from 241 ss 8 strain required uracil for growth on minimal medium, and another one obtained from 235 psA strain required methionine. After fusion of mixed protoplasts of these two auxotrophic mutants by polyethylene glycol, many colonies were formed on a plate containing minimal agar medium. By isolating those colonies, several dikaryotic strains were obtained. They were cultured in sawdust-rice bran medium at 25°C. After transferring into a condition alternating in temperature and light condition in cycle, fruit bodies were formed. Color of the fruit body was gray which was the similar color to one of the parent FMC 241, while the temperature for primordia formation of those fruit bodies was similar to the other parent FMC 235. Thus, the hybrid strain had the characteristics of both parents. Analysis of esterase isozyme pattern also showed the hybrid nature.

KEY WORDS: *Pleurotus ostreatus*, mushrooms, protoplast fusion, auxotrophic mutant, intraspecific hybrid, fruit body, primordia formation.

Introduction

Intraspecific protoplast fusion will become a useful method in the mushroom breeding, because protoplasts can be easily prepared from mycelia of many mushrooms. It is also an important step in the process of the development of methods of interspecies protoplast fusion. Because, in general, fungal protoplasts are lacking in useful morphological markers for selection such as plastids for plant protoplasts, availability of selection methods of hybrid cells from fusion mixtures can be first examined in intraspecific protoplast fusion. Biochemical mutants were frequently used for the purpose of selection of hybrid cells of fungal strains (Perenczy, 1981). Intraspecific protoplast fusion is also a convenient method to examine proper fusion conditions for each fungal species (Anne and Peberdy, 1975) related to interspecies protoplast fusion. Hybridization of strains of *Coprinus macrorhizus* was performed by intraspecific protoplast fusion (Kimura, 1985). But for main cultivated mushrooms such as *Pleurotus ostreatus* (Fr.) Quel., hybridization by intraspecific protoplast fusion was not successful.

In the present paper, the author reports hybridization of *Pleurotus ostreatus* strains by intraspecific protoplast fusion. For the selection of hybrid cells, the author used auxotrophic mutants of each strains of *Pleurotus ostreatus*. The hybrid strains obtained by protoplast fusion were cultivated in a sawdust-rice bran medium to form fruit bodies.

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Materials and Methods

Monokaryotic strains, 241 ss 8 and 235 psA, were obtained from two dikaryotic strains of *Pleurotus ostreatus*, FMC 241 and FMC 235, respectively, both of which were stock cultures of Section of Mushrooms of Forestry and Forest Products Research Institute. Auxotrophic mutants were obtained from two of these monokaryotic strains by treatment of fragmented mycelia with 20 μg/ml or 40 μg/ml of N-methyl-N’-nitro-N-nitrosoguanidine (NTG).

Fragmented mycelia were cultured for 4~6 days at 25°C in PMY medium (peptone 1%, malt extracts 1%, yeast extracts 0.4%). Protoplasts were prepared from these mycelia by treating with Novozym 234 (Novo Industri A/S) 1% and chitinase (Sigma Chemical Co.) 0.1% as described previously (Ohmasa et al. 1986). In order to fuse protoplasts, protoplasts precipitated by centrifugation were suspended and incubated in 30% polyethylene glycol (PEG) 4000, 50 mM CaCl₂ in glycine–NaOH, pH 8.5, at 30°C for 30 minutes. Then, protoplasts were diluted with 0.5 M mannitol, 0.05 M maleic acid–NaOH, pH 6.5, sequentially, and then cultured in solid medium containing minimal medium (Takegawa, 1982) and agar. After culturing for about 2 weeks, colonies appeared and subcultured once on solid medium containing the minimal substances. Then, they were maintained on SMYA (sucrose 1%, malt extracts 1%, yeast extracts 0.4%, agar 1.5%).

For obtaining fruit bodies, dikaryotic mycelia were cultured at 25°C for 3~4 weeks in a sawdust–rice bran media contained in about 1,000 ml polypropylene vessels in a dark room. Then, they were transferred to either of the two conditions. Condition A: cycles of 12 hrs. at 16°C and light on (about 200 lx), 12 hrs. at 10°C and light off. Condition B: 12 hrs. at 13°C and light on, and 12 hrs. at 6°C and light off.

Isozyme patterns were analyzed using isoelectric focusing by a similar method described previously (Ohmasa and Furuwaka, 1986).

Results and Discussion

After treating fragmented mycelia of the monokaryotic strain, 241 ss 8, by NTG, about 500 colonies were recovered, and the growth on the minimal medium and subsequently growth factors were examined. An auxotrophic mutant which required uracil for growth on minimal medium, 241-8-22, was obtained. By the similar way, an auxotrophic mutant which required methionine, 235 psA-437, was obtained from the monokaryotic strain, 235 psA.

The two mutants were cultured in PMY medium and protoplasts were obtained from both mycelia respectively, as described in the Materials and Methods. Protoplasts obtained were treated with PEG in three combinations. In the first and the second combinations, protoplasts obtained from each mutants were treated with PEG separately. In the third combination, protoplasts of the both mutants were mixed approximately in same number and treated with PEG. In the control treatment, protoplasts obtained from both strains were mixed as in the third combination, but they were not treated with PEG solution. Protoplasts were then plated on the minimal medium agar and
Fig. 1. Fusion of protoplasts of auxotrophic mutants of *Pleurotus ostreatus* by polyethylene glycol.
Upper left and right: fusion of protoplasts of only one strain, left, 241-8-22 and right, 235-psi A-437.
Lower left: mixture of protoplasts of both mutants without polyethylene glycol treatment.
Lower right: fusion of protoplasts of both mutants by polyethylene glycol.

Fig. 2. Formation of primordia of fruit bodies in condition A. Primordia of fruit bodies are seen in bottles of FMC 235 and hybrid obtained by fusion, but not in the bottle of FMC 241.

incubated for about 2 weeks. A result is shown in Fig. 1. Upper two plates show results of the first and the second combinations. In these plates, colonies are not observed. The lower left plate shows the result of the control treatment. Also in this plate, colonies are not observed. The lower right plate shows the result of the third combination, and only in this plate, colonies are observed. This fact shows that colonies were formed by fusion of nutritionally complementing protoplasts.

The hybrid nature of colonies was verified by the intermediate character of strains isolated from the colonies obtained by fusion. These strains were dikaryon and cultured in sawdust-rice bran medium. FMC 235 produced white fruit bodies when cultures were transferred to the condition A as described in the Materials and Methods. FMC 241

Fig. 3. Fruit bodies of parent strains and a hybrid strain obtained by protoplast fusion of parent strains.
Middle: hybrids obtained by fusion. Right: FMC 235. Left: FMC 241
produced gray fruit bodies only when transferred to and cultured in condition B. When cultures of strains obtained by fusion were transferred to condition A, primordia of fruit bodies were formed like FMC 235, as shown in Fig. 2. But the color of fruit bodies was gray as shown in Fig. 3. Thus, the strains obtained by fusion show characters intermediate between the parents. Furthermore, esterase isozyme patterns of mycelia of these strains obtained by fusion showed both isozyme bands of those of parents (Fig. 4).

These data show that use of auxotrophic mutants is a powerful method to select and culture hybrid cells in protoplast fusion of cultivated mushrooms. In principle, this method is applicable to interspecies protoplast fusion of mushrooms, as in cases of Ascomycetes, such as Aspergillus (Ferenczy et al. 1977), Penicillium (Anbé and Peberdy, 1981) and yeasts (Peberdy, 1979).

Literature Cited


栄養要求性突然変異株を利用したヒタケの品種間細胞融合

大谷 正武
（林業試験場保護部、福岡県志賀町 〒 305）

ヒタケの品種間細胞融合に関する成功例はこれまで報告されていないので、雑種細胞の培養と選抜に栄養要求性の突然変異株を用いてヒタケの品種間細胞融合株の作出を試みた。

すなわち、ヒタケの二核系統 FMC 235, FMC 241 由来の一核系統 235 psA, および 241 ss 8 系統の菌糸断片を N-メチル-N'-エトロ-N-エトロソグアミジンで処理することにより、それぞれメチオニンおよびウラシルを要求する突然変異株をえた。これらの変異株の培養菌糸から、ノボザイム 234 とキチナーゼの処理によりプロトプラストを得た。これらのプロトプラストを混合してポリエチレングリコールで処理し、最少培地で培養することにより多数のコロニーを得た。一方、同じ株のプロトプラストのみをポリエチレングリコール処理した場合や、異なるプロトプラストを混合してもポリエチレングリコール処理をしなかったブレートではコロニーが見られなかった。得られたコロニーを分離して一度最少培地で培養後、再分離して菌株として保存した。

得られた雑種系統を錦屑-米糖培地で培養し、さらに低温の環境に移すことで子実体を得た。雑種系統の子実体の色は灰色で FMC 241 系統に近かった。一方、雑種系統の子実体の原基形成温度は比較的高く FMC 235 系統に近かった。このような雑種系統は両親の性質を併せ持っており、雑種であることが確認された。また、エステラーゼアイソザイムパターンも両親の特徴を備えていた。