韓 國 菌 學 會 誌 第 17 卷 第 4 號
Kor. J. Mycol. Vol. 17, No. 4, 197~201, 1989

Fusion between Protoplasts of *Ganoderma applanatum* and Oidia of *Lyophyllum ulmarium*

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잔나비걸상버섯 原形質體와 만가닥버섯 分裂子의 融合

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ABSTRACT: The fusion between protoplasts of *Ganoderma applanatum* and oidia of *Lyophyllum ulmarium* (*Hypsizigus marmoreus*) was induced with polyethylene glycol and $CaCl_2$. When transferred to *Ganoderma* complete medium plates, fusants showed mixed morphologies both parents. During three times subcultivation the fusants were changed similar to those of *L. ulmarium* type. All fusants produced oidia, clamp connections and basidiocarps similar to those of *L. ulmarium*. Isozyme pattern of esterase of interorder fusants showed both parental and non-parental bands. Each individual fusant did not showed both parental and non-parental bands. Each individual fusant did not show any differences in mycelial growth rate, colony morphology, esterase band pattern and basidiocarp.

KEYWORDS: Protoplasts-Oidia Fusion, *Ganoderma applanatum*, *Lyophyllum ulmarium*, Basidiomycotina

Edible mushrooms can offer us an important opportunity for a nutritious food for human beings. The mushroom production would receive benefit by applying the new genetics and breeding method. Protoplast mediated gene transfer is a powerful tool for strain improvement in living things. In relation to the protoplast fusion, foreign cell and organelle transfer, and transformation have been studied in fungi.

In this investigation I report gene interaction of *Lyophyllum ulmarium* oidia of genetic informations in cells of *Ganoderma applanatum* after uptake of oidia by protoplasts. I have seen no reports of relevant these experiments.

Materials and Methods

Strains and growth conditions

Lyophyllum ulmarium (=*Hypsizigus marmoreus*) ASI 8007 (Wild; donor) and *Ganoderma applanatum* ASI 7-18 (cys met; recipient) were obtained from the Agricultural Sciences Institute, Suweon, Korea. The isolation of auxotrophic mutant was performed as described by Park *et al.*, (1987). The Strain of *G. applanatum* was induced by irradiation of the mycelial fragments of *G. applanatum* ASI 07031 to ultraviolet lights. The strain *L. ulmarium* was maintained on the yeast glucose (YG; Conney and Emmerson, 1964) containing (gl^{-1}) yeast extract 5.0, glucose 10.0 and agar 20.0. The strain *G. applanatum* was maintained on the *Ganoderma* complete medium (GCM; Choi *et al.*, 1987) containing (gl^{-1}) yeast extract 10.0, peptone 4.0, casamino acid 5.0, $MgSO_4 \cdot 7H_2O$ 0.5, KH_2PO_4 0.46, K_2HPO_4 1.0, glucose 30.0, sucrose 20.0 and agar 20.0. Fusant selection after oidia transfer into protoplasts was carried

out on osomtically stabilized mushroom minimal medium (MMM; Raper *et al.*, 1972). It consists of (gl^{-1}) $MgSO_4 \cdot 7H_2O$ 0.5, KH_2PO_4 0.46, K_2HPO_4 1.0, DL-asparagine 2.0, glucose 20.0, Bacto-agar 20.0 and was supplemented with 0.6 M sucrose. Bottom agar was of 2.0% while overlaying soft agar was of 0.75%.

Preparation of oidia

The mycelia of *L. ulmarium* were grown for 7 days prior to inoculation onto cellophane membrane in YG petri dishes. The mycelial disks were ready for oidia production when mycelia grown over the disks. Mycelial disks of the strain from 7-14 days culture at 25°C were removed to clean sterile petridishes and the distilled water was added. The petri dishes incubation on reciprocal shaker (120 rpm min^{-1}) at 28°C for 24 h. Oidia from the solution were separated from mycelia by filtration through sintared glass filter (porosity 1) and resuspended with 0.6 M sucrose.

Formation of protoplasts

Protoplasts of *G. applanatum* were prepared using a mixture of Novozym 234 (Novo Biolabs), Cellulase onozuka R-10 (Yakult) and β-Glucuronidase (Sigma) basically as described by Park *et al.*, (1987).

Uptake of oidia

The procedure of oidia transfer was based on protoplast fusion technique. The suspension of recipient protoplasts (8.0×10^7) and donor oidia (4.0×10^7) were combined in a fusion tube and centrifuged at 1,000g for 20 min. The pellet was resuspended in 1 m*l* of a solution 30% polyethylene glycol 3350 (PEG) containing 10 mM $CaCl_2 \cdot 2H_2O$ and 50 mM glycine, adjusted to pH 8.0 with 1 mM NaOH. After incubation for 15 min. at 30°C, the suspension was washed with 0.6 M sucrose by centrifugation, and resuspended in 5 m*l* osmotic stabilizer. Serial dilutions of treated protoplasts were plated on to 0.6 M sucrose stabilized MMM to select complemented colonies.

Preparation of mycelial extracts

Mycelia were grown in squat 1 conical flasks containing 300 m*l* MCM solution. The flasks were incubated for 15 days at 25°C. The harvested mycelium with liquid nitrogen (-196°C) was ground at 4°C in a pre-cooled mortar for 10-20 min. The mycelial fragments were removed by centrifugation at 13,000 g for 30 min.

Gel electrophoresis and enzyme staining procedure

The mycelium homogenates were anlalysed by the polyacrylamide gel and discontinuous buffer system. Electrophoresis was done at 5°C at constant by voltage (7-10 mA/cm. gel). Esterase was detected in the gels by immersion in a solution on of TRIZMAL 7.6buffer 50 m*l*; 1 part TRIZMAL 7.6 buffer concentrate (Sigma), 9 parts deionized water and 1 capsule fast blue RR salt. When salt is completely dissolved in buffer, α-naphthyl acetate solution (1 capsule α-naphthyl acetate in 2 m*l* ethylene glycol monomethyl ether) were added. The gel in staining solution was incubation at 37°C for 30 min.

Results and Discussion

After PEG solution treatment of mixture of donor oidia of *L. ulmarium* in agaricales and recipient protoplasts of *G. applanatum* in aphylloporales, small colonies appeared on the minimal agar plates. To check for back mutation of the protoplast of *G. applanatum* control plates were inoculated on minimal medium. After 5days incubation protoplasts reverted to mycelial colonies in such culture due to leaky mutant. When reversion colonies were transferred to GCM before oidia germination, some colonies of them showed mixed morphologies both parents as like segregants. The phenotype of these fusants was changed similar to those of *L. ulmarium* type after three times subcultivation on YG or GCM (Fig. 1). Hyphae of them did form true clamp connections and oidia (Table I). These results suggested that protoplasts and oidia walls disintegrated at the point of transfer into protoplasts, resulting in protoplasts-oidia fusion. After a larger number of fused protoplasts was reverted to normal hyphal growth, oidium was germinated in the cells. The oidium then donated its cellular contents to the hyphae cells. Fusion between reversion hyphae and germination hyphae appeared to result in plasmogamy between the two cells. All fusants induced primordia and developed basidiocarps similar to those of *L. ulmarium* under certain

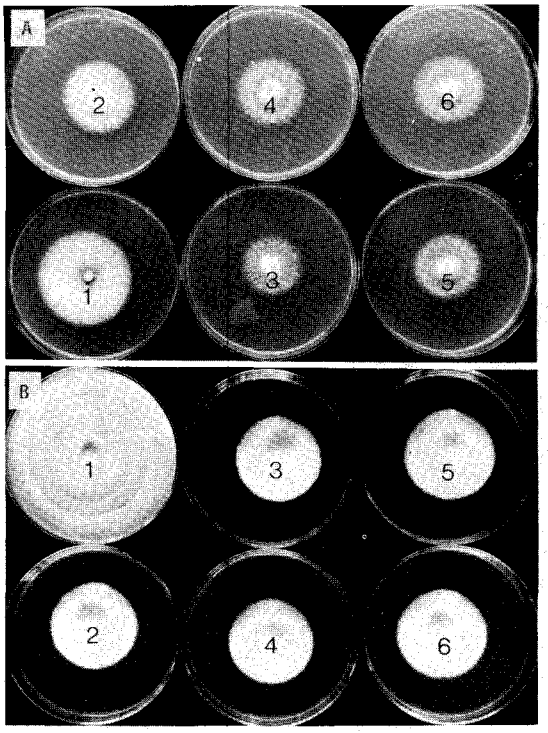

Fig. 1. Fusants between protoplasts of *G. applanatum* and oidia of *L. ulmarium* growing for fourteen days on (A) YG, and (B) GCM.
(1) *G. applanatum* ASI 7-18, (2) *L. ulmarium* ASI 8007, (3) Fusant P330, (4) Fusant P325, (5) Fusant P324, (6) Fusant P323,

conditions (Fig. 2).

A comparison of fusants between oidia and protoplasts was made using isozyme anlysis of esterase (Fig. 3). The banding Pattern of *L. ulmarium* extract contained one band at 0.07 R_f. The *G. applanatum* extract showed two bands at both 0.47 R_f and 0.75 R_f. Mycelial extracts of all fusants contained 5 bands, which were observed both parental bands and non-parental bands at 0.14 R_f and 0.35 R_f. Furthermore, in all fusants a different enzyme band was formed compare with transforment after uptake of isolated chromosomes of *L. ulmarium* by protoplasts of *G. applanatum* (Yoo *et al.*, 1988).

In the chance of fusion between hyphae and oidium, some researcher reported on intraspecies and interspecies fusion (Bistis, 1970; Fries 1981; Ingold, 1984). In this case, however, interorder fusion between reverted hyphae of *G. applanatum* and oidium of *L. ulmarium* could be impossible due to vegetative incompatibility.

Table I. Characteristics of fusion products between protoplasts of *G. applanatum* and oidia of *L. ulmarium*

Strain		Mycelial growth[1] GCM	YG	Clamp connection[2]	Fruiting[3]
Fusant	P323	MG	MG	+	F
Fusant	P324	MG	MG	+	F
Fusant	P325	MG	MG	+	F
Fusant	P330	MG	MG	+	F
G. applanatum ASI 7-18		FG	MG	−	S
L. ulmarium ASI 8007		MG	MG	+	F

1) FG: Fast growing
MG: Moderate growing
2) +: Presence of clamp connection
−: Absence of clamp connection
3) F: Fertile
S: Strile

Each individual fusant was not shown any differences in mycelial growth rate,colony morphology, esterase band pattern and basidiocarp. These results signified that fusants between protoplasts and oidia were extremely stable compare with fusion products by other gene transfer technology such as protoplast fusion, cell organelles transfer and transformation.

摘 要

野生形인 만가닥버섯 *Lyophyllum ulmarium* (=*Hypsizigus marmoreus*) ASI 8007에서 분리한 分裂子와 잔나비걸상버섯 영양요구주 *Ganoderma applanatum* ASI 7-18 (cys met)의 原形質體를 polyethylene glycol로 融合하여 융합주를 얻었다.

융합주는 GCM(Ganoderma complete medium)에서 양친균총이 혼합된 형태였으나 3회 계대배양되면서 만가닥버섯 균총으로 변하여 균사에는 클램프연결체와 분열자를 지녔고, 만가닥과 거의 유사한 자실체를 형성하였다. 균사체로 esterase 同位酵素를 분석하였는데 만가닥이 1개 밴드, 잔

Fig. 2. Basidiocarp of fusants between protoplasts of *G. applanatum* and oidia of *L. ulmarium on sawdust substrates. (1) L. ulmarium* ASI 8007, (2) Fusant P324, (3) Fusant P325, (4) Fusant P330,

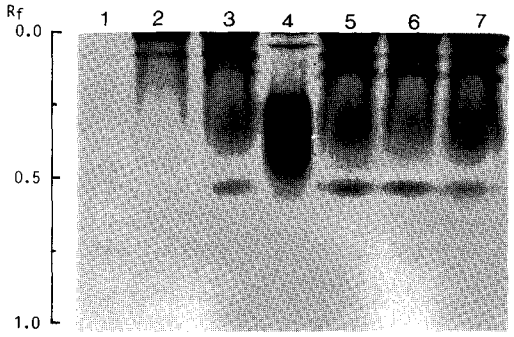

Fig. 3. Isozyme pattern of esterase of fusants between protoplasts of *G. applanatum* and oidia of *L. ulmarium* on polyacrylamide gel.
(1) *G. applanatum* ASI 7-18, (2) *L. ulmarium* ASI 8007, (3) Fusant P330, (4) Chromosome transfer product, (5) Fusant P323, (6) Fusant P324, (7) Fusant P325

나비길상이 2개의 밴드를 가진데 비하여 융합주는 양친의 3개와 새로운 2개의 밴드를 나타내었다. 이들 융합주 상호간에는 균총형태, 생장속도, esterase 동위효소, 자실체 특성에 있어서 거의 차이가 없어 아주 안정되게 양친의 유전물질을 보유하였다.

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Accepted for Publication 4 November 1989