THE KOREAN JOURNAL OF MYCOLOGY
Vol. 22, No 4, p378-385 December 1994
Printed in S. KOREA

Interspecific Hybridization between *Pleurotus ostreatus* and *Pleurotus sajor-caju* by Protoplast Fusion

Young-Bok Yoo* and Haing-Sook Lee
Genetics Division, Agricultural Biotechnology Institute, RDA, Suweon 441-707, Korea

原形質體 融合에 의한 느타리와 여름느타리버섯의 種間 交雜

劉英福* · 李幸淑
農村振興廳 農業遺傳工學研究所 細胞遺傳科

ABSTRACT: Interspecific somatic hybrids were obtained by protoplast fusion between *Pleurotus ostreatus* and *Pleurotus sajor-caju*. The fusion products between incompatible strains did not form clamp connections. Fruiting body of the clampless fusants was induced by light-dark cycle on sawdust-rice bran substrate in glass bottles. Out of them, seven somatic hybrids produced fruiting bodies of intermediate morphology of the two species. Light and low temperature were the initiating factors for the development of clamped hyphae from the clampless mycelial colonies. All of these basidiocarps had clamp connections. Eight fusants from the six crosses were analysed with the segregation of genetic characters by random spore isolates. In the three combinations, unexpected alleles were shown. Somatic hybrid between P188 (*P. ostreatus* 2-1+*P. sajor-caju* 2-53) and *P. florida* 2-3 by triple cross produced fruiting bodies similar to those of fusant between *P. ostreatus* and *P. florida*. All the genetic charaters from the three strains were shown to segregate and recombine.

KEYWORDS: Interspecific protoplast fusion, genetic analysis, *Pleurotus ostreatus*, *Pleurotus sajor-caju*, Basidiomycotina

Introduction

The consumption of oyster mushroom has increased significantly during the last several years in Korea. The *Pleurotus* species accounted for about 60% of the total mushroom production. The mushroom, *Pleurotus* spp., were cultivated on the rice straw, cotton waste, sawdust or various substrates. The major species of *Pleurotus* are tetrapolar mechanism of incompatibility. Single spore isolates from basidiocarps are homokaryotic and self-sterile. However, homokaryotic fruiting has been reported in some species of *Pleurotus* including *P. ostreatus* and *P. sajor-caju*.

Protoplast manipulation can be a potentially valuable tool for mushroom genetics and breeding. Yoo *et al.* (1993) showed that some somatic hybrids of protoplasts between *P. ostreatus* and *P. florida* yielded 55.2% increase in fruit body weight. Interspecific protoplast fusion have been obtained in the genus *Pleurotus* (Yoo *et al.* 1984; 1986; Toyomasu *et al.*, 1986; Toyomasu and Mori, 1987a; 1987b; 1989; Go *et al.*, 1989; Yoo, 1992; Ogawa, 1993; Yoo and Cha, 1993).

This investigation described interspeific hybridization and genetic recombination between vegetatively compatible and incompatible strains by protoplast fusion.

Materials and Methods

Strains and Growth Conditions

The isolation of auxotrophic mutants was perfomed as described by Yoo *et al.* (1988). The strains used in these experiments were listed in Table 1. They were maintained on the mushroom complete medium (CM) containing (per liter) $MgSO_4 \cdot 7H_2O$ 0.5 g, KH_2PO_4 0.46 g, K_2HPO_4 1.0 g, peptone

*Corresponding author

Table 1. List of strains used

Species	Code no.	Genetic marker[1)]	Origin
P. ostreatus	ASI 2- 1- 0 2018 - 83	arg	ASI 2018 from Korea
	ASI 2- 2- 0 2018 - 51	gly ser	
P. sajor-caju	ASI 2-44- S 2070 - 16	lys	ASI 2070 from India
	2-45- S 2070 - 11	lys	
	2-47- S 2070 - 84	orn ala	
	2-49- S 2070 - 23	pan	
	2-51- S 2070 -131	ane pn	
	2-52- S 2070 - 8	ane nia	
	2-53- S 2070 -148	rib ane	
	2-55- S 2070 -300	an pn asn	

[1)]Mutant symbols: ala (alanine), ane (aneurine), arg (arginine), asn (asparagine), gly (glycine), lys (lysine), nia (nicotinic acid), orn (ornithine), pn (pyridoxine), rib (riboflavine), ser (serine)

2.0 g, yeast extract 2.0 g, glucose 20.0 g and agar 20.0 g. Heterokaryon selection after protoplast fusion was carried out on osmotically stabilized mushroom minimal medium (MM). It consists of (per liter) $MgSO_4 \cdot 7H_2O$ 0.5 g, KH_2PO_4 0.46 g, K_2HPO_4 1.0 g, DL-asparagine 2.0 g, thiamine-HCL 120 μg, glucose 20.0 g, Bactoagar 20.0 g and was supplemented with 0.6 M sucrose. The concentration of bottom agar was 2.0% while that of overlaying soft agar was 0.75%.

Protoplast Formation and Fusion

Disks of sterile cellophane sheets were placed on the surface of CM in Petri dishes. When the colonies of *P. ostreatus* and *P. sajor-caju* on the cellophane sheets grew enough for protoplast isolation, the cellophan sheets were transfered to sterile Petri dishes. Protoplasts of *P. ostreatus* and *P. sajor-caju* were obtained using a mixture of Novozym 234 (Novo Biolabs), β-Glucanase(BDH), and β-Glucuronidase (Sigma) basically as described by Yoo *et al.* (1984).

The procedure of protoplast fusion was based on that of Anne and Peberdy (1976) and Yoo *et al.* (1984). Approximately $10^7 \sim 10^8$ protoplasts of each strain were combined in a fusion tube and centrifused at 500 g for 10 min. The pellet of protoplasts was resuspended in 1 m*l* of a solution of 30% polyethylene glycol (PEG) 8000 containing 10 mM $CaCl_2 \cdot 2H_2O$ and 50 mM glycine, adjusted to pH 8.0 with 10 mM NaOH. After incubation for 10 min. at 30℃, the suspension was diluted with 0.6 M sucrose, washed once by centrifugation, and resuspended in 5 m*l* osmotic stabilizer. Serial dilutions of the treated protoplasts were plated onto CM stabilized with 0.6 M sucrose for viability and onto MM for selection of fusion products. The fusion frequency (F_f) was expressed as the number of colonies on MM to the number of colonies reverted on CM after 10~20 days incubation at 27℃ or

$$F_f = \frac{\text{number of colonies on MM}}{\text{number of colonies on CM}} \times 100$$

Basidiocarp Production

Induction of carpophores was attempted one the 570 g sawdust substrates containing poplar tree plus 20% rice bran in 1,000 m*l* glass bottle. For cultivation under sterile conditions, the media was autoclaved at 121℃ for 90 min. When cooled, the media was inoculated with spawn. The bottle was plugged with cottton. The cultures were incubated at 27℃ for 25~50 days under low intensity light. When mycelia grew on sawdust media, the bottle was transferred to a light room. In order to get primordia, the mycelia in a bottle with cotton plug were exposed to high intensity white light for

20~60 days at 5~15℃. They were illuminated by lamp for 14 hours per day. When primordia initiation formed, the bottle which removed cotton plug was transferred to a light room.

Basidiospore Germination and Genetic Character Identification

Basidiospore prints obtained from the carpophores of fusion products were stored at 4℃ for the analysis of progeny. The procedure of genetic analysis was based on those of Yoo *et al.* (1986). Spores were spread on mushroom complete agar medium in Petri dish and incubated for 5~10 days at 27℃. Sporelings were individually transferred from the germination medium to complete medium and incubated for a week at 27℃. All colonies or sectors were transferred to minimal medium 12 colonies per plate. After 7~20 days incubation, the latter were identified by testing, again in replicate sets of 12 inocula, on the appropriate screening media.

Results and Discussion

Fusant Characterization and Basidiocarp Formation

Interspecific heterokaryons were derived from auxotrophic mutants of *P. ostreatus* and *P. sajor-caju* after protoplast fusion. The fusion colonies were produced after 10~20 days of incubation on MM plates. When transferred to MM, all fusion colonies exhibited slow growth rate. Fusion frequency of protoplasts was 0.002~4.0%. Heterokaryons between incompatible strains grew less vigorously than those between compatible strains. The interspecific somatic hybrids between incompatible pairs of strains did not form clamp connections. The clamped fusants between compatible strains in pairings of ASI 2-2+ASI 2-55 produced fruit bodies rapidly and abundantly on sawdust substrates. Fruitbody formation from clampless fusion colonies was induced by light-dark cycles on a medium containing 570 g poplar tree sawdust plus 20% rice bran in an 1000 m*l* glass bottle.

Out of 70 fusants between incompatible strains, 7 somatic hybrids induced fruiting bodies (Table 2). Monokanyotic auxotrophs of *P. ostreatus* and *P. sajor-caju* in these experiments were self-sterile except one strain of *P. sajor-caju* ASI 2-45-lys. The clampless fusant did not produce fruiting bodies on complete agar medium or complete liquid medium in flasks. Clamp connections did not form in the phase of vegetative mycelial growth on sawdust substrates. Light and low temperature were an initiating factors for the development of clamped hyphae from clampless mycelial colonies in glass bottles. When clamped mycelia from clampless mycelial colonies were grown completely, ma-

Table 2. Characteristics of fusion products of protoplasts between *Pleurotus ostreatus* and *P. sajor-caju*

Fusion combination	Compatibility[1]	No. of isolate	No. of fertile	Fruiting[2]		Designation of fusant
				Type	Clamp	
ASI 2-1+ASI 2-44	−	4	0	non-fertile		P184-P187
2-1+ 2-45	−	8	2	intermediate	+	P176-P183
2-1+ 2-49	−	5	0	non-fertile		P223-P227
2-1+ 2-51	−	4	0	non-fertile		P172-P175
2-1+ 2-53	−	35	0	non-fertile		P188-P222
2-2+ 2-45	−	1	1	intermediate	+	P171
2-2+ 2-47	−	6	2	intermediate	+	P165-P170
2-2+ 2-49	−	4	2	intermediate	+	P151-P154
2-2+ 2-52	−	3	0	non-fertile		P162-P164
2-2+ 2-55	+	7	7	*P. ostreatus*	+	P155-P161

[1] +(Vegetatively compatible), −(Vegetatively incompatible). [2] +(Present clamp connections)

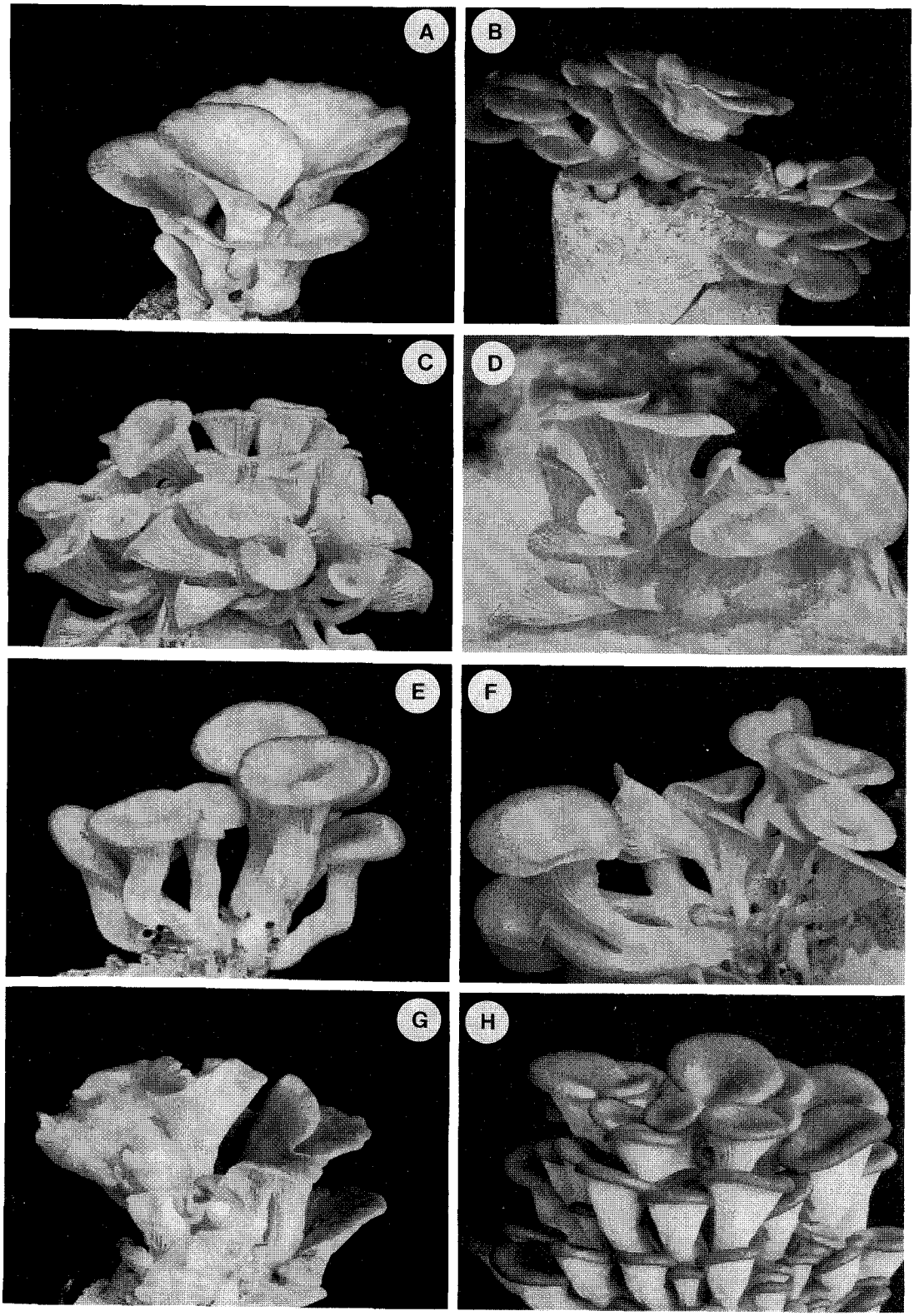
A
B
C
D
E
F
G
H

Fig. 1. Basidiocarps of somatic hybrids between *Pleurotus ostreatus* and *P. sajor-caju* following protoplast fusion.
(A) *P. ostreatus* ASI 2018
(B) *P. sajor-caju* ASI 2070
(C) Fusant P152 of *P. ostreatus* ASI 2-2+*P. sajor-caju* ASI 2-49
(D) Fusant P156 of *P. ostreatus* ASI 2-2+*P. sajor-caju* ASI 2-55
(E) Fusant P171 of *P. ostreatus* ASI 2-2+*P. sajor-caju* ASI 2-45
(F) Fusant P168 of *P. ostreatus* ASI 2-2+*P. sajor-caju* ASI 2-47
(G) Fusant P154 of *P. ostreatus* ASI 2-2+*P. sajor-caju* ASI 2-49
(H) Fusant P166 of *P. ostreatus* ASI 2-2+*P. sajor-caju* ASI 2-47

ture fruiting bodies developed on sawdust substrates in glass bottles. All hyphae or small tissues from the basidiocarps present clamp connections. When small tissues taken from stipe of fruiting bodies were cultured on agar plate, mycelial colonies grew more vigorously than original fusion products.

Somatic hybrids between compatible strains in pairings of *P. ostreatus* ASI 2-2+*P. sajor-caju* ASI 2-55 produced *P. ostreatus* type. Seven fusion products between incompatible strains formed intermediate morphology of fruit bodies of the two species. However, some fruiting characters of fusants such as hymenium, stipe, and growth habit were similar to those of *P. ostreatus*. Growth habit of *P. ostreatus* is caespitose whereas *P. sajor-caju* is closely scattered (Fig. 1).

Interspecific somatic hybrids between *P. cornucopiae* and *P. florida* were similar to *P. florida* in the morphology of fruit body (Yoo, 1992). In the intergenus and interorder hybrids, basidiocarp phenotype is similar to either of the fusion partners (Liang and Chang, 1989; Ogawa, 1993; Yoo and Cha, 1993; Yoo, 1994).

Fruit body development from vegetative mycelia of fusion products was influenced by some major factors such as light, temperature and culture medium. Fruit body inducing genes are silent during vegetative mycelial growth but become active when aerial hyphae are exposed to light and low temperature.

Table 3. Characteristics of basidiocarps of somatic hybrids of protoplasts between *Pleurotus ostreatus* and *P. sajor-caju*

Strain	Clamp	Color of mature pileus
ASI 2-2+ P152 ASI 2-49	+	orange grey
ASI 2-2+ P157 ASI 2-55	+	brownish grey
ASI 2-2+ P168 ASI 2-47	+	brownish grey
ASI 2-2+ P171 ASI 2-45	+	brownish grey
P. ostreatus ASI 2018	+	bluish grey
P. sajor-caju ASI 2070	+	dark brown

Genetic Recombination

Basidiospore prints of the eight protoplast fusants in six crosses described above served as a source of materials for the analysis of progeny. The genetic characters were shown to segregate and recombine in the first segregation of monosporus isolates of somatic hybrids. If the basidiospores were derived exclusively from 4-spored basidia and heterokaryotic, independent assortment of the marker loci and segregation of their alleles with random distribution of meiotic nuclei to the spores should yield progeny of four phenotyes in a Mendelian ratio of 1 : 1 : 1 : 1 for prototrophs, auxotrophs of one parental type, auxotrophs of the other parental type, and auxotrophic recombinant, respectively. In all somatic hybrids, however, prototrophic recombinants were recovered in large numbers against auxotrophic characters. Parentals and auxotrophic recombinants were not recovered in the cross *P. ostreatus* 2-1-arg and *P. sajor-caju* 2-45-lys, and *P. ostreatus* 2-2-gly ser and *P. sajor-caju* 2-55-ane asn pn (Table 4, 5, 6, 7, 8 and 9).

The markers of a large number of auxotrophic

Table 4. Frequency distribution of progenies of somatic hybrid between *Pleurotus ostreatus* ASI 2-1-arg and *P. sajor-caju* ASI 2-45-lys (P179)

Phenotype	No. of individual
prototroph	209
arg	1
lys	0
arg lys	0
others	110

Table 5. Freqneney distribution of progenies of somatic hybrid between *Pleurotus ostreatus* ASI 2-1-arg and *P. sajor-caju* ASI 2-53-rib ane (P207)

Phenotype	No. of individual
prototroph	226
rib ane	1
rib	89
rib ane arg	4

Table 6. Frequency distribution of progenies of somatic hybrid between *Pleurotus ostreatus* ASI 2-2-gly ser and *P. sajor-caju* ASI 2-45-lys (P171)

Phenotype	No. of individual
prototroph	303
gly ser	12
lys	2
gly	1
ser	7
gly ser lys	5
others	1

Table 7. Frequency distribution of progenies of somatic hybrids between *Pleurotus ostreatus* ASI 2-2-gly ser and *P. sajor-caju* ASI 2-47-orn ala (P166, P168)

Phenotype	No. of individual	
	P166	P168
prototroph	273	273
gly ser	1	0
orn ala	13	2
orn	17	24
ala	5	0
gly ser orn	1	0
gly ser ala	1	0
gly orn ala	0	1
gly ser orn ala	5	0
others	4	4

Table 8. Frequency distribution of progenies of somatic hybrids between *Pleurotus ostreatus* ASI 2-2-gly ser and *P. sajor-caju* ASI 2-47-pan (P152, P154)

Phenotype	No. of individual	
	P152	P154
prototroph	273	273
gly ser	12	4
pan	3	2
gly	5	1
ser	3	1
gly pan	2	0
gly pan	3	1
gly ser pan	14	0
others	4	41

progenies were not identified in the three combination of ASI 2-1-arg+ASI 2-45-lys, 2-2-gly ser +ASI 2-47-orn ala and ASI 2-2-gly ser+2-49-pan. These genetic characters were shown abnormally unexpected alleles. The modified Holliday method was used for identification of abnormal progenies derived from somatic hybrids P154 and P179. In order to identify genetic characters, the auxotrophic progenies were tested for growth on minimal medium supplemented with yeast extracts, casamino acids, and nucleic acid bases and related compounds, respectively. They were able to grow on minimal medium plus yeast extracts only. Therefore,these unexpected progenies required vita-

Table 9. Frequency distribution of progenies of somatic hybrid of protoplasts between *Pleurotus ostreatus* ASI 2-2-gly ser and *P. sajor-caju* ASI 2-55-ane asn pn (P157)

Phenotype	No. of individual
prototroph	104
gly asn	1
ane pn	1
ser asn	1
gly ser ane	1
gly ser pn	1
gly ser ane asn pn	11

Table 10. Frequency distribution of progenies of somatic hybrid between protoplast fusant P 188 (*P. ostreatus* ASI 2-1-arg+*P. sajor-caju* ASI 2-53-ane) and *P. florida* ASI 2-3-rib obtained by hyphal anastomosis

Phenotype	No. of individual
prototroph	30
arg	18
ane	0
rib	46
ane rib	5
ane arg	2
arg rib	6
arg ane rib	1

mins. They were tested on minimal medium supplemented with twelve kinds of vitamins such as aneurine, ascorbic acid, biotin,cholin, cyanocobalamin, folic acid, inositol, nicotinic acid, pantothenic acid, para-aminobenzoic acid, pyridoxine, and riboflavine, respectively, but they were non-viable. Therefore they could be multi-auxotrophic progenies.

For induction of fruiting bodies, Fusant P188 crossed with *P. florida* ASI 2-3-rib by hyphal anastomosis. In 15 days after hyphal fusion, the fusant formed clamp connections. The pilei of somatic hybrid was brownish grey. Most of the character of produced fruiting body were similar to those of the interspecific fusant between *P. ostreatus* ASI 2-1-arg and *P. florida* ASI 2-3-rib. Strain ASI 2-3-rib was compatible with ASI 2-1-arg, but incompatible with ASI 2-53-ane. Parental type arg and rib were recovered in large numbers compared to prototroph type. All the genetic characters of the three strains were shown to segregate and recombine. Parental phenotypes except *P. sajor-caju-ane* were recovered with the recombinant progeny amounting to 40.74% (Table 10).

The genetic analysis of intraspecific fusion products of protoplast in *Coprinus cinereus* have been described. Prototrophic recombinants were not recovered in large numbers against auxotrophic characters (Yanagi *et al.*, 1988). In the triple cross between non-fertile fusant (*P. columbinus*-ade+*P. sajor-caju*-met) and *P. sajor-caju* wild type, *P. columbinus*-ade genotype were not shown to segregate and recombine (Toyomasu and Mori, 1989). No abnormal metabolism were shown in the intraspecific hyphal anastomosis (Raper *et al.*, 1972; Alic and Gold, 1985), intraspecific protoplast fusion (Yanagi *et al.*, 1988) and compatible interspecific protoplast fusion (Yoo *et al.*, 1986). Distribution and recombination of unexpected alleles could be associated with gene interactions between differant genome structure as reported earlier (Yoo, 1992; 1994).

Interspecific somatic hybrid between vegetatively incompatible strains developed very slowly. Some fusants of them produced fruiting bodies slowly and poorly on sawdust substrates. However, recombinant strains derived from basidiocarps are useful material for other breeding programmes.

摘 要

느타리 *Pleurotus ostreatus*와 여름느타리 *Pleurotus sajor-caju*의 영양요구주로부터 분리한 원형질체를 polyethylene glycol로 융합하여 종간 이질핵체 heterokaryon를 획득하였다. 불화합 균주간에는 9 융합조합에서 70개 융합주를 획득하여 7 융합주의 자실체를 유도하였으며 화합 균주간에는 1조합에서 모두 자실체를 형성하였다. 화합 균주간 융합주를 제외하고는 불화합성간 융합체는 격쇠연결체 clamp connections가 없었고 한천배지나 액체배지에서는 자실체를 형성하지 않았다. 그러나 활엽수톱밥과 미강이 혼합된 배지에서 균사가 완전히 성장한후

일정한 광과 온도를 처리한 결과 꺽쇠연결체 있는 균사가 다시 성장하였으며 이들 균사에서 버섯자실체가 형성되었다. 버섯자실체는 양친의 중간형태로 갓 색깔도 양친이 혼합된 orange grey-brownish grey로 나타났다. 그러나 다소의 자실체 형질인 자실층 hymenium, 버섯대, 생장습성은 느타리를 닮았는데 특히 여름느타리가 단생 closely scattered인데 비해 느타리는 속생 caespitose으로 느타리와 유사하였다. 자실체는 모두 꺽쇠연결체를 가졌으며 조직배양한 결과 균사가 모두 꺽쇠연결체를 가져 본래 융합주와는 다른 형태로 변하였다.

6융합조합 8균주의 F^2에서의 유전형질 분리와 유전자 재조합을 분석한 결과 원영양형 prototroph이 양친 느타리형, 여름느타리형, 영양요구성 유전자 재조합형에 비해 많이 분리되었으며 3개조합에서 양친에 없는 유전자좌가 나타났다. 자실체가 형성되지 않는 융합주 P188과 사철느타리 ASI 2-3-rib와 균사접합 한 후 임성을 유도하여 유전분석한 결과 원영양형이 적게 분리되었고 여름느타리 양친형이 분리되지 않았으며 3친주 형질이 모두 유전자 재조합형으로 분리되었다.

References

Alic, M. and Gold, M. H. 1985. Genetic recombination in the ligin-degrading basidiomycete *Phanerochaete chrysosporium*. *Appl. Environ. Microbiol.* **50**(1): 27-30.

Anne, J. and Peberdy, J. F. 1976. Induced fusion of fungal protoplasts following treatment with polyethylene glycol. *Gen. Microbiol.* **92**: 413-417.

Go, S. J. You, C. H. and Shin, G. C. 1989. Effects of incompatibility on protoplast fusion between intra- and interspecies in basidiomycete, *Pleurotus* spp. *Korean J. Mycol.* **17**: 137-144.

Liang, Z. R. and Chang, S. T. 1989. A study on intergeneric hybridization between *Pleurotus sajor-caju* and *Schizophyllum commune* by protoplast fusion. *Mushroom Science* **12**: 125-137.

Ogawa, K. 1993. Interspecific and intergeneric hybridization of edible mushrooms by protoplast fusion. In *Genetics and Breeding of Edible Mushrooms*, pp 193-206. Edited by S. T. Chang, J. A. Buswell and P. G. Miles, U.S.A.: Gordon and Breach Science Publishers.

Raper, C. A., Raper, J. R. and Miller, R. E. 1972. Genetic analysis of the life cycle of *Agaricus bisporus*. *Mycologia* **64**: 1088-1117.

Toyomasu, T. Matsumoto, T. and Mori, K. 1986. Interspecific protoplast fusion between *Pleurotus ostreatus* and *Pleurotus salmoneostramineus*. *Agri. Biol. Chem.* **50**: 223-225.

Toyomasu, T. and Mori, K. 1987a. Fruit body formation of the fusion products obtained by interspecific protoplast fusion between *Pleurotus* species. *Agric. Biol. Chem.* **51**: 2037-2040.

Toyomasu, T. and Mori, K. 1987b. Intra- and interspecific protoplast fusion between some *Pleurotus species*. *Agric. Biol. Chem* **51**: 35-37.

Toyomasu, Y. and Mori, K. 1989. Characterics of the fusion products obtained by intra- and interspecific protoplast fusion between *Pleurotus* species. Mushroom Sci. **12**(1): 151-159.

Yanagi, S. O., Kawasumi, T., Takebe, I. and Takemaru, T. 1988. Genetic analyses of *Coprinus cinereus* strains derived through intraspecific protoplast fusion. *Agric. Biol. Chem.* **52**: 281-284.

Yoo, Y. B. 1992. Interspecific hybridization between *Pleurotus cornucopia* and *Pleurotus florida* following protoplast fusion. *Korean J. Mycol.* **20**(2): 118-126.

Yoo, Y. B. 1994. Interorder hybridization between *Pleurotus ostreatus* and *Elfvingia applanata* by protoplast fusion. *Korean J. Mycol.* **22**(1): 107-116.

Yoo, Y. B., Byun, M. O., Go, S, J., You, C. H., Park, Y. H. and Peberdy., J. F. 1984. Characteristics of fusion products between *Pleurotus ostreatus* and *Pleurotus florida* following interspecific protoplast fusion. *Korean J. Mycol.* **12**: 164-169.

Yoo, Y. B. and Cha, D. Y. 1993. Gene transfer in edible fungi using protoplasts. In *Genetics and Breeding of Edible Mushrooms*, pp 157-192. Edited by. S. T. Chang, J. A. Buswell and P. G. Miles. U.S.A.: Gordon and Breach Science Publishers.

Yoo, Y. B., Park, Y. H. and Chang, K. Y. 1988. Induction of auxotrophic mutants and back mutation in *Pleurotus*. *Res. Rep. Rural Development Administration (F.P.U. & M.) (Korea)* **30**: 133-140.

Yoo, Y. B., You, C. H. and Cha, D. Y. 1993. Strain improvement of the genus *Pleurotus* by protoplast fusion. *Korean J. Mycol.* **21**(3): 200-211.

Yoo, Y. B., You, C. H. Park, Y. H. and Peberdy, J. F. 1986. Genetic analysis of the life cycle in interspecific hybrids of *Pleurotus ostreatus* and *Pleurotus florida* following protoplast fusion. *Korean J. Mycol.* **14**: 9-15.