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# Fruiting body development and genetic analysis of somatic hybrids by protoplast fusion in edible fungi

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# 식용버섯의 원형질체 융합체의 자실체 발생 및 유전분석

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**ABSTRACT : Somatic hybrids of inter-compatible and inter-incompatible strains were obtained by protoplast fusion. The fusion products between compatible strains, *Pleurotus ostreatus* and *P. florida*, formed heterokaryons, while fusants between incompatible strains such as *P. cornucopiae* + *P. florida* , *P. ostreatus* + *Ganoderma applanatum*, *P. florida* + *Ganoderma lucidum*, and *P. ostreatus* + *Flammulina velutipes* formed synkaryons that retained genes from both parents. The heterokaryons showed the same level of basidioma development. In contrast, the synkaryons showed unique characteristics including clamp connection formation at mitosis, either partner basidioma development, and abnormal segregation and recombination compared with inter-compatible strains. Synkaryons can be classified into homokaryoyic and heterokaryotic type. A comparison of somatic hybrids with compatible and incompatible strains was made using random amplified polymorphic DNA (RAPD) analysis. The heterokaryons between compatible species showed the same level of variability and contained both parental RAPD bands. In contrast, most of the synkaryons between incompatible species showed similarity to those of either parental bands and non-parental RAPD bands. Synkaryons can be classified into microgenome insertion type and macrogenome insertion type. A tetrapolar mating system was found among monospore isolates in somatic hybrids and wild type *P. ostreatus*. Homokaryons from each somatic hybrid combination were paired with tester homokaryons of the initial wild type of *P. ostreatus*. The changed mating types were identified in progenies. The pattern of mating type switching in somatic hybrids depends on compatibility of fusion partner. There are several factors related to the mechanism of clamp connection formation and fruiting body development of synkaryons. Of these,the major factor may be associated with self-fertility and mating type switching such as homokaryotic fruiting of wild type *P. ostreatus*. This review will discuss these aspects.**

**KEYWORDS : Basidiomycota, Fruit body development, Molecular genetic analysis, Protoplast fusion, *Pleurotus, Ganoderma, Flammulina velutipes*. RAPD-PCR, Somatic hybrids**

## INTRODUCTION

There is a long history of mankind's use of mushrooms as a food and for medicinal or tonic purposes. Mushrooms lack chlorophyll and are nongreen organisms. They can produce extensive enzymes that can degrade lignocellulosic materials for their growth and fruiting. Mushrooms not only can become nutritious protein rich food, but also can provide nutriceutical and pharmaceutical products. The most significant challenge is to recycle the byproducts in the course of each stage of mushroom production and to retain a pollution-free environment. In 1994, world production of cultivated edible mushrooms was estimated to be

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4,909,300 tons, which was valued at about 9.8 billion US dollars. The ten most popular species of cultivated edible mushrooms in 1994 were *Agaricus* (37.6%), *Lentinula edodes* (16.8%), *Pleurotus* (16.2%), *Auricularia* (8.6%), *Volvariella volvacea* (6.1%), *Flammulina velutipes* (4.7%), *Tremella fuciformis* (3.2%), *Hypsizygus marmoreus* (1.1%), *Pholiota nameko* (0.5%), *Grifola frodosa* (0.3%), and others (4.9%). In recent years, several new species of mushrooms such as *Hericium erinaceus, Dictyophora indusata, Stropharia rugosoannulata, Lepista nuda, Agrocybe aegerita* and *Pleurotus citrinopileatus* have also been commercially cultivated (Chang, 1999). The production of oyster mushrooms has increased significantly during the last 10 years, especially in Oriental countries where they are a popular edible mushroom.

The second major attribute of mushrooms, their medicinal properties, has long been recognized in China, Japan, and Korea. Traditional medicine attributed medicinal properties to *Pleurotus* species. Scientific evidence supports their importance as producers of substances with antibiotic, antiviral, anticarcinogenic, antiinflammatory, and hypocholesterolemic activities (Gunde-Cimerman, 1999). *Ganoderma lucidum* has been considered as a panacea for all types of diseases as described 2000 years ago in *Shen Nung's Herbal*. The traditional medicinal propertites of the fungus have been utilized as treatments for hepatopathy, nephritis, hypertension, hyperlipemia, arthritis, neurasthenia, insomnia, bronchitis, asthma, gastric ulcer, arteriosclerosis, and diabetes. Some of these efficacies, such as cytotoxicity, inhibition of platelet aggregation, inhibition of histamin release, antihypertensive, hypocholesterolemic, and anti-HIV activities were scientifically proven by isolation of the corresponding active components. Although various compounds have been reported as components, it appears that triterpenoids are mainly responsible for the biological activities (Kim and Kim, 1999).

The *Pleurotus* species have a reproductive pattern of bifactorial heterothallism. Single basidiospore isolates are homokaryotic and self-sterile (Terakawa 1960; Eugenio and Anderson 1968; Eger 1974). However, homokaryotic fruiting has been reported in some species of *Pleurotus* including *P. flabellatus* (Samsudin and Graham 1984), *P. eous* (Elliott 1985), and *P. sajor-caju* (Go and Shin 1986).

Protoplast fusion has been developed to break down the barrier of gene exchange imposed by conventional breeding systems. In recent years, intraspecies, interspecies and intergenus protoplast fusion have been achieved in higher fungi (Liang and Chang 1989; Liu et al. 1991; Park et al. 1991; Sonnenberg et al. 1991; Pan et al. 1992; Sunagawa and Miura 1992; Wang et al. 1992; Bok et al. 1994; Kim et al. 1997).

Determination of DNA polymorphism is used widely to distinguish individuals or to study phylogenetic relationships. Polymerase chain reaction (PCR) methods to detect gene transfer events after protoplast fusion and transformation have been described and reported for various fungi (Aufauvre-Brown et al. 1993; Foster et al. 1993; Broda et al. 1995). Here, we describe the characterization of the formation of fruiting bodies, genetic recombination, and molecular genetic analysis of somatic hybrids in pairings of *Pleurotus ostreatus* + *P. florida, P. ostreatus* + *P. sajor-caju, P. citrinopileatus* + *P. florida, P. ostreatus* + *Ganoderma applanatum, P. florida* + *Ganoderma lucidum and P. ostreatus* + *Flammulina velutipes*. We also describe studies on the formation of clamp connections in initial fusion colonies, basidiocarps, and progenies. This review will describe and discuss these aspects.

## DEVELOPMENT OF FRUITING BODIES

The somatic hybrids between compatible strains produced fruiting bodies rapidly and abundantly. Fruiting bodies of the heterokaryons showed intermediated or mixed characters between parents. The fruit body yield indices of somatic hybrids ranged between 27-155, while those of their parents, *P. ostreatus* 2018 and *P. florida* 2016 were 100 and 138, respectively (Yoo et al, 1993; Yoo & Cha, 1993).

Fusion products between incompatible species were derived from auxotrophic mutants of *Pleurotus* and *Ganoderma* after protoplast fusion. The fusion colonies were produced after 10-30 days of incubation on MM plates. When transferred to MM, all fusion colonies exhibited slow growth rate. Fusion products 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.

When two different protoplasts were fused, the fused cell first produced a heterokaryon that contained

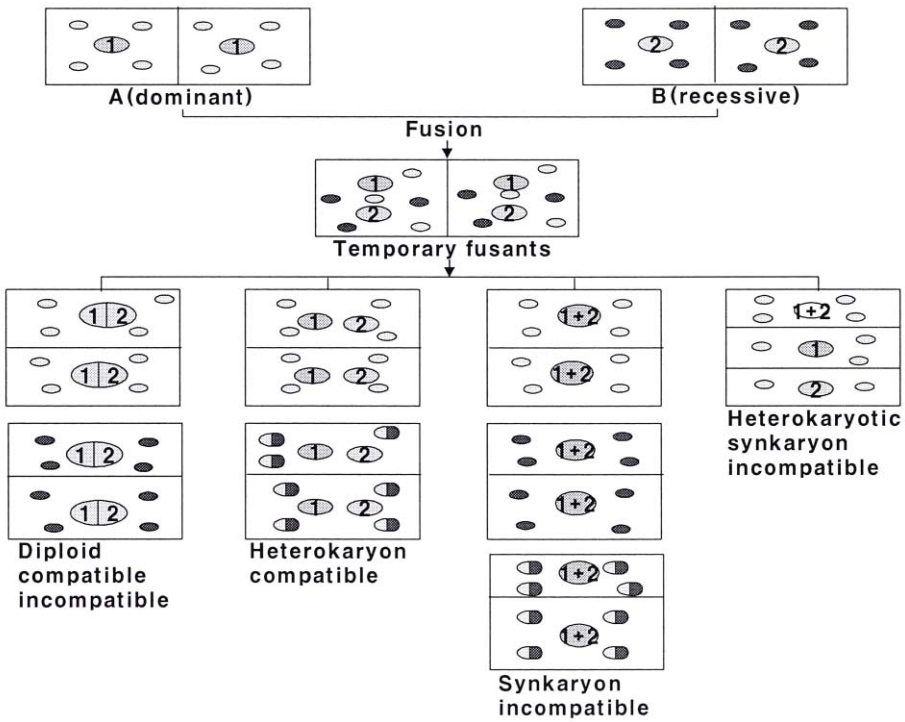


**Fig. 1.** The different kinds of protoplast fusion products which can be obtained in heterothallic basidiomycetes (Yoo et al., 1999).

both nuclei and both cytoplasms. However, most of them did not keep both nuclei. Eventually the two nuclei fused to form a mononuclear hybrid known as a synkaryon (Primrose 1987). The cytoplasms (mitochondria) also did not stay together; eventually only one species predominated although some mitochondria fused together (Mellon et al. 1983; Sonnenberg et al. 1991; Yoo 1994; Fukuda et al. 1995). After fusion, the nuclear and mitochondrial genomes reassorted and recombined, resulting in a wide array of gene recombination that cannot be obtained through hyphal anastomosis. The interspecific and intergeneric fusants between vegetatively incompatible species in *Pleurotus* and yeast were revealed to be uninucleate in a cell (Spencer et al. 1983; Toyomasu and Mori 1989). Intergeneric somatic hybrids were not real heterokaryons because of asynchronous fruiting body development of the two partners (Liang and Chang 1989). Several kinds of somatic hybrids can be obtained by protoplast fusion (Fig. 1).

Out of 377 synkaryons, 35 somatic hybrids induced fruiting bodies (Table 1). Phenotype of fruiting bodies in synkaryons could be similar to either one of the fusion partners. Only some genetic characters including fruiting body morphology, and pileus colour might be different from parental species. All of the basidiocarps of synkaryon produced clamp connections except the combination of *P. cornucopiae* and *P. florida*. The amount of the basidiospores from these two strains was small or even zero. None of these clampless primordia could produce mature basidiocarps. A certain part of the hyphae of clamped primordia in sawdust medium also formed clamp

**Table 1.** Frequency of fertile somatic hybrids of protoplast fusion between incompatible species (Y.B.Yoo unpublished)

| Fusion combination | NUMBER examined | NUMBER fruiting (%) |
|---|---|---|
| Inter-Compatible species | | |
| *Pleurotus ostreatus* + *P. florida* | 78 | 78 (100) |
| Inter-Incompatible species | | |
| *Pleurotus citrinopileatus* + *P. florida* | 73 | 13 (17.8) |
| *P. ostreatus* + *P. sajor-caju* | 70 | 7 (10.0) |
| *P. ostreatus* + *Ganoderma applanatum* | 36 | 5 (13.9) |
| *P. florida* + *Ganoderma lucidum* | 46 | 8 (17.4) |
| *P. ostreatus* + *Ganoderma lucidum* | 60 | 0 (0) |
| *P. ostreatus* + *Flammulina velutipes* | 92 | 2 (2.17) |
| Total | 377 | 35 (9.3) |

connections. When small tissues of stipe from primordium or basidiocarp were cultured on CM plates, mycelial colonies grew more vigorously than those of the initial fusants and, then produced clamp connections. Basidiocarp characters could be similar to either one of the fusion partners except in the case of compatible fusion products between closely related species (Cha abd Yoo, 1997; Yoo et al., 1984; Liang and Chang, 1989; Yoo, 1992, 1994 ; Ogawa, 1993; Yoo and Lee, 1994).

The major species of *Pleurotus* are all bifactorial heterothallism. Single basidiospore isolates from fruiting bodies are homokaryotic and self-sterile. However, homokaryotic fruiting has been reported in some species of *Pleurotus*. We found that homokaryons derived from one strain (WT-2029) could develop fruiting bodies of three different types. First group had not only mature or sporulate fruiting bodies but also clamp connections, although the initial isolate did not present clamp connections (Abortive homokaryotic fruiting, AHF). Second group had developed fruiting bodies with clamp connections even though THE initial homokaryotic colony did not form clamp connections (Pseudo- homokaryotic fruiting, PHF). The mycelial colonies derived from AHF and PHF by tissue culture formed clamp connections, while mycelial colonies of AHF lacked them (Y. B. Yoo unpublished). However, monokaryotic auxotrophs of *Pleurotus* and *Ganoderma* in these experiments were self-sterile. The clampless fusant did not produce fruiting bodies on complete agar medium or complete liquid medium in flasks. Clamp connections of initial fusion products did not form in the phase of vegetative mycelial growth on sawdust substrates. Light and low temperature were The main factors to initiate the emergence of clamp connections in hyphae from clampless initial fusion colonies. When clamped mycelia were grown completely, fruiting bodies developed on sawdust substrates. These results indicateD that formation of clamp connections and development of fruiting bodies are influenced by a number of factors such as light, temperature, nutrition, mycelial age, and physical state of culture media. Fruiting body inducing genes were silent in vegetative mycelial growth but became active when aerial hyphae were exposed to light at low temperature. An essential prerequisite for fruiting in heterothallic basidiomycetes is heterokarysis derived from two intercompatible nuclei. By the way, synkaryon contains one nucleus synthesized by fusion of two inter-incompatible nuclei in each hyphal compartment.

Monokaryotic fruiting has been observed in species of six genera of the poriales and 12 genera of the

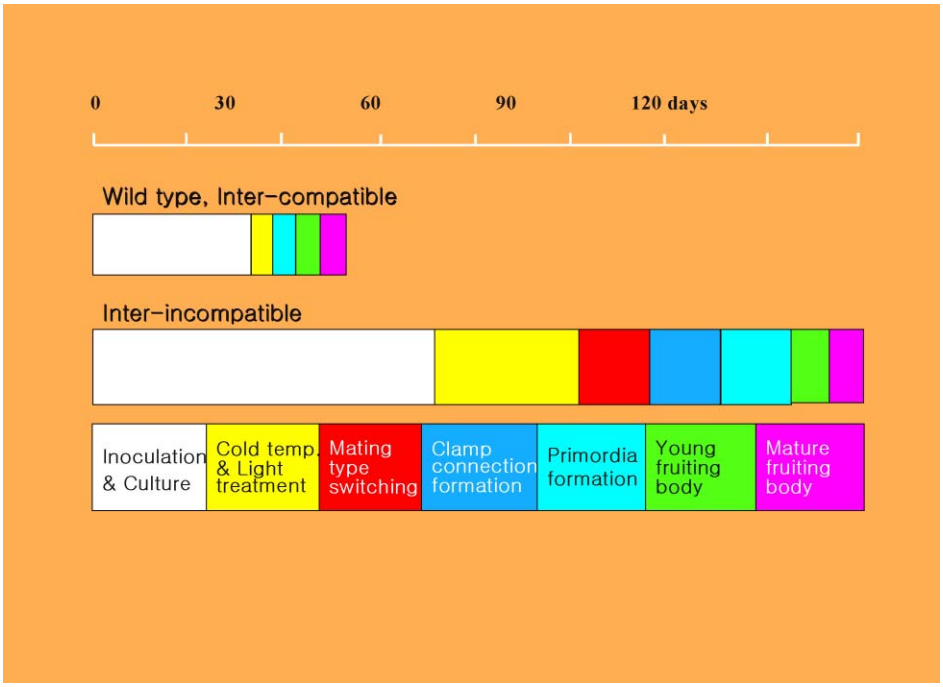


**Fig. 2.** Comparison of THE basidioma development processES OF inter-compatible and inter-incompatible species in heterothallic basidiomycetes (Y. B. Yoo unpublished).

agaricales (Esser 1979). Esser suggested that there are at least two genes that control the potential for monokaryotic fruiting. In the presence of allele *fi*+ only fruiting initiation occurred; the additional presence of allele *fb*+ led to production of normal fruiting bodies (Stahl and Esser 1976; Esser and Meinhardt 1977).

There are several factors related to the mechanism of clamp connection formation and fruiting body development of synkaryons between incompatible species. The major factor may be associated with self-fertility and mating type switching as reported earlier (Labarere and Noel, 1992; Schmidt and Gutz, 1994). Gene expression during emergence of clamp connections in synkaryons may be linked to the mating type switching. This is related to the function of heterokaryotization of synkaryons without transfer of mating type genes or nuclei, and eventually leads to the differentiation and development of mature fruiting bodies (Fig. 2).

Development of fruitbodies in synkaryons dependS on self-fertility of initial wild fusion partners. We found that synkaryons could develop fruitbodies of two different types. First group had developed fruitbodies with clamp connections (Pseudo-homokaryotic fruiting). Second group did not have mature fruit bodies (Abortive homokaryotic fruiting). There are several factors related to the mechanism of fruitbody development in synkaryons by protoplast fusion.* The most important of these factors may be associated with self-fertility and mating type switching (Fig. 3). Gene expression during emergence of clamp connections in synkaryons may be linked to the mating type switching+. This is related to the function of heterokaryotization of synkaryons without transfer of mating type genes or nuclei, and eventually leads to the differentiation and development of mature fruiting bodies. These results indicate that basidioma development and mating system pattern of somatic hybrids between incompatible species were similar to those of self-fertile homokaryons of wild type *P. ostreatus*.

## GENETIC RECOMBINATION

Somatic hybridization was carried out in pairings of *P. ostreatus* + *P. florida* (OF), *P. citrinopileatus* + *P. florida* (CF), *P. ostreatus* + *G. applanatum* (OA), *and P. florida* + *G. lucidum* (FL) by protoplast fusion. Somatic hybrids of inter-compatible and inter-incompatible strains were analysed for the segregation and recombination of progenies by random spore analysis (Table 2). The genetic markers were shown to segregate and recombine in the first generation of

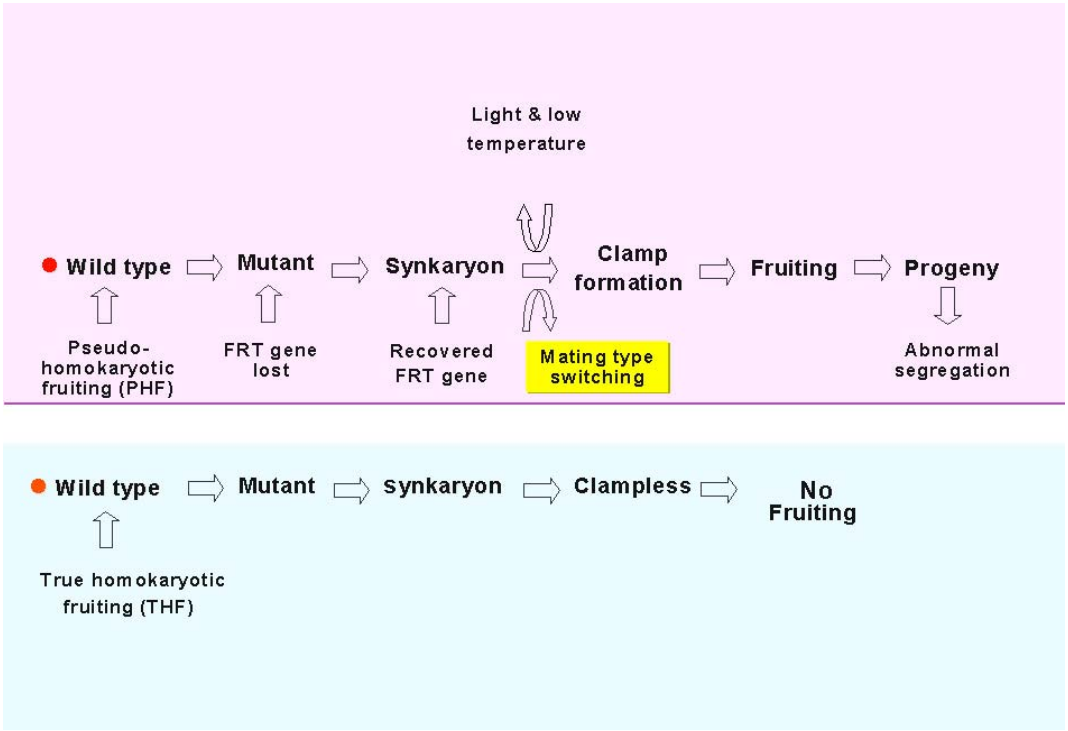


**Fig. 3.** Comparision of basidioma development of fertile and sterile synkaryons in *Pleurotus* (Y.B.Yoo unpublished).

**Table 2.** Segregation and recombination of genetic markers in progenies of somatic hybrids (Yoo et al., 2000)

| Strain combination[a] | | Fusant | Parentals | | Recombinant | | % Recombi-nant |
|---|---|---|---|---|---|---|---|
| A | B | | A | B | Prototroph | Auxotroph | |
| O 2-1-arg | + F 2-3-rib | OF 5 | 178 | 39 | 212 | 33 | 53.03 |
| O 2-2-gly ser | + F 2-3-rib | OF 34 | 168 | 0 | 105 | 1 | 38.68 |
| O 2-1-arg | + F 2-4-rib | OF 22 | 98 | 9 | 94 | 43 | 56.14 |
| O 2-2-gly ser | + F 2-4-rib | OF 15 | 227 | 0 | 240 | 11 | 52.51 |
| C 2-29-ade | + F 2-3-ura | CF549 | 22 | 0 | 51 | 29 | 68.64 |
| | | CF564 | 34 | 3 | 70 | 11 | 78.34 |
| C 2-28-gln arg | + F 2-73-ura | CF863 | 8 | 9 | 191 | 164 | 95.43 |
| | | CF864 | 6 | 7 | 229 | 115 | 96.32 |
| C 2-39-cit pan | + F 2-3-rib | CF842 | 0 | 0 | 320 | 0 | 100 |
| O 2-1-arg | + A 7-18-cyn met | OA382 | 13 | 0 | 290 | 0 | 95.70 |
| | | OA386 | 1 | 0 | 204 | 91 | 99.66 |
| | | OA399 | 5 | 0 | 230 | 65 | 98.33 |
| F 2-3-rib | + L 7-20-cyt | FL938 | 123 | 1 | 164 | 2 | 57.24 |
| | | FL964 | 60 | 3 | 175 | 24 | 75.95 |
| F 2-3-rib | + L 7-35-paba | FL917 | 160 | 3 | 192 | 44 | 59.15 |

[a] Abbreviations used : A, *G. applanatum* ; C, *P. cornucopiae* ; F, *P. florida* ; L, *G.. lucidum* ; O, *P. ostreatus* ade, adenine ; arg, arginine ; cit, citrulline ; cyn, cystine ; cyt, cytosine ; gln, glutamine ; gly, glycine ; paba, para-aminobenzoic acid ; pan, pantothenic acid ; rib, riboflavine ; ser, serine ; ura, uracile

monospore isolate. Progenies of the somatic hybrids can be classified into four genotypes: auxotrophs of one parental type, auxotrophs of the other parental type, prototrophs, and auxotrophic recombinants. Parental markers could not be clearly detected in some fusants containing three or four factor pairings. In all heterokaryons from pairing of *P. ostreatus* and *P. florida*, there was no evidence of linkage between the genetic markers. This genetic analysis provides not only proof of heterokaryosis, but also evidence for haploidy of vegetative nuclei, and sexual reproduction. In all somatic hybrids, however, prototrophic recombinants were recovered in large numbers against auxotrophic characters. Several somatic hybrids did not segregate with one parental genetic marker. Some were shown to be segregated into non-parental auxotrophs in the progenies of CF and OA pairings (data not shown). In the somatic hybrids of dikaryotic *P. florida* and monokaryotic *P. citrinopileatus* strains, all the genetic characters from parents were segregated and recombined in the progenies.

Since basidiospores were derived exclusively from four-spored basidia and were heterokaryotic, independent assortment of the marker loci and segregation of their alleles with random distribution of meiotic nuclei to the spores should produce progenies of four phenotypes in a Mendelian ratio of 1:1:1:1 for prototrophs, auxotrophs of one parental type, auxotrophs of the other parental type, and auxotrophic recombinants. Several fusion products between incompatible species did not segregate parental genetic markers. The genetic markers in a large number of auxotrophic progenies were not found in the CF and OA pairings. In these cases, abnormal non-parental auxotrophs were shown, indicating that donor chromosome fragments were integrated into random sites of the recipient chromosomes, causing gene disruption. Most of the parental genotypes were recovered, except from some fusants. Comparatively large numbers of prototrophic recombinants were recovered from most types of fusant. This phenomenon may be related to the amphithallism and germination frequency of basidiospores derived from particular genotypes. Similar results have been obtained in the heterokaryon of basidiomycete fungi (Raper et al. 1972; Alic and Gold 1985; Yoo et al. 1986; Yanagi et al. 1988; Toyomasu and Mori 1989). A somatic hybrid between protoplast fusant P188 (*P. ostreatus -arg* + *P. sajor-caju -rib ane*) and *P. florida-rib* by triple cross produced fruiting bodies similar to those of the fusant

between *P. ostreatus* and *P. florida*. All the genetic markers from the three strains were shown to be segregated and recombined (Yoo and Lee 1994). In the triple cross between a non-fertile fusant from *P. columbinus-ade* + *P. sajor-caju-met* and *P. sajor-caju* wild typeX however, the *P. columbinus-ade* genotype was not shown to segregate and recombine (Toyomasu and Mori 1989).

For heterokaryotic synkaryons, somatic hybrids showed an unequal distribution of different nuclei in the hyphae even though they are mononucleate in a cell. Because most genetic markers of the two fusion partners are segregated and recombined in the progeny, a more frequent distribution of one nuclear type over the other will lead to the phenotype. Characteristics of only one parental phenotype after genome reassortment can be explained by an unequal contribution of both nuclei to the fusion products. If heterokaryons occur in the incompatible pairings and one of the nuclear combinations exhibits a dominant, only one parental phenotype will be detected in the heterokaryotic synkaryon hyphae.

## MOLECULAR GENETIC ANALYSIS WITH RAPD MARKERS

Genetic variations within or among inter-compatible and inter-incompatible somatic hybrids of Pleurotus were assessed using RAPD markers. PCR amplification of DNA from 15 strains of *P. ostreatus* + *P. florida*, 18 strains of *P. citrinopileatus* + *P. florida*, 15 strains of *P. ostreatus* + *G. applanatum*, and 21 strains of *P. florida* + *G. lucidum* was carried out using 39 primers (Fig. 4 and Fig. 5). A specific DNA band pattern resulted for somatic hybrids with each amplimer. To determine genetic relationships among parental strains and somatic hybrids, a dendrogram was generated from the similarity matrix using UPGMA cluster analysis (Fig. 6).

RAPD-PCR patterns of 11 inter-compatible heterokaryons in pairing of *P. ostreatus* and *P. florida* by hyphal anastomosis and protoplast fusion were compared with the parental strains. No polymorphism was found among 11 heterokaryons, which did not contain non-parental RAPD bands.

The inter-compatible somatic hybrids were classified into three major groups using the 75 RAPD bands obtained. The first group comprised parental type *P. florida*, the second comprised parental type *P. ostreatus*, and the third comprised all somatic hybrids. The third group had slightly higher affinity to parental *P. ostreatus* than to *P. florida* strains.

To assess the genetic variability of inter-incompatible somatic hybrids between *P. citrinopileatus* and *P. florida*, RAPD analysis was performed with 10 different primers. RAPD profiles of CF hybrids

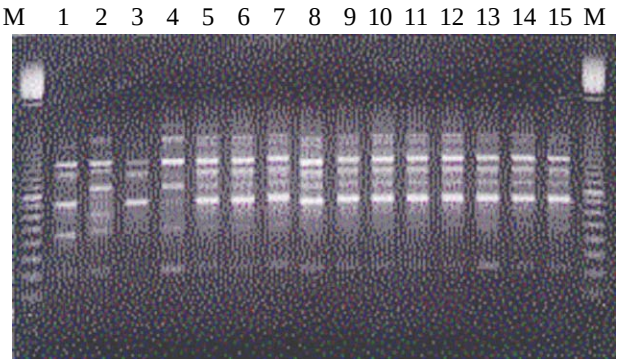


**Fig. 4.** RAPD profiles of parentals and somatic hybrids between *Pleurotus ostreatus* and *P. florida* with primer OPA 19 (Yoo et al., 2002) : *Lanes M*, marker 100bp ladder; *1*, *P. florida* 2016-wild; *2*, *P. ostreatus* 2018-wild *3*, *P. florida* 2-3-*rib*; *4*, *P. ostreatus* 2-1-*arg*; *5-6*, OF973 and OF974 by hyphal fusion; *7-15*, OF5, OF624, OF625, OF627, OF633, OF640, OF642, OF650 and OF658 by protoplast fusion

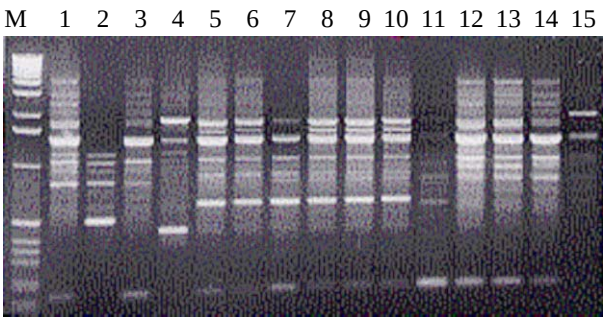


**Fig. 5.** RAPD profiles of parentals and somatic hybrids between *Pleurotus ostreatus* and *G. applanatum* with primer OPA 01 (Yoo et al., 2002). *Lanes M*, marker 1Kb DNA ladder; *1*, *P. ostreatus* 2018-wild; *2*, *G. applanatum* 7031-wild; *3*, *P. ostreatus* 2-1-*arg*; *4*, *G. applanatum* 7-18-*cyn met*; *5*, OA399; *6*, OA399-TC (tissue culture isolate); *7*, OA399-MS (multispore germination isolate); *8*, OA386; *9*, OA386-TC; *10*, OA386-MS; *11*, OA409; *12*, OA380; *13*, OA380-TC; *14*, OA380-MS; *15*, OA394

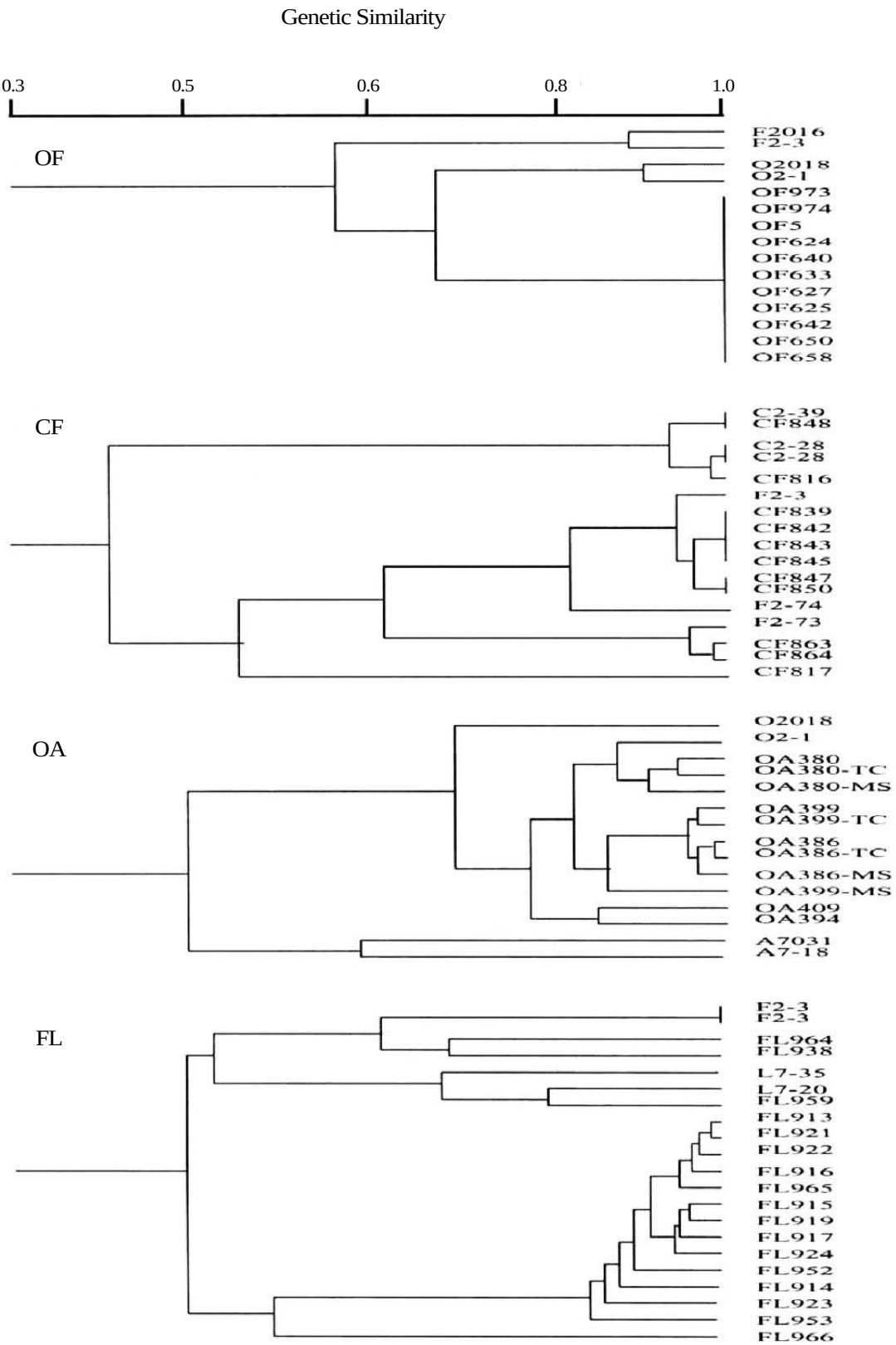


**Fig. 6.** Relationships of parentals and somatic hybrids in pairings of (OF) *Pleurotus ostreatus* + *P. florida*, (CF) *P. cornucopiae* + *P. florida*, (OA) *P. ostreatus* + *G. applanatum*, and (FL) *P. florida* + *G. lucidum*. The dendrogram was generated from genetic similarity coefficients obtained from 75, 108, 127, and 109 DNA bands, respectively, using UPGMA cluster analysis (Yoo et al. 2002).

revealed a high polymorphism of DNA fragments compared with inter-compatible species. The major bands were identical to those of *P. florida*, but minor bands were different. Band patterns of *P. citrinopileatus* were not distinct, but they showed non-parental bands.

To examine banding pattern similarity, DNA from the 11 somatic hybrids between *P. ostreatus* and *G. applanatum* and four parental strains were amplified with nine different primers. In two clamped somatic hybrids and related strains OA399 (lane 5), OA399-TC (lane 6), OA399-MS (lane 7), OA386, OA386-TC and OA386-MS, the primer OPA-01 produced one unique intense band at 0.85kb. Primers OPA-08, OPF-12, and FGL-12 showed slightly different banding patterns among the initial fusants (lane 5 and 12), tissue culture isolates (lane 6 and 13), and multi-spore isolates (lane 7 and 14). From 15 strains, 127 RAPD bands of interorder somatic hybrids were generated. Two main groups of OA hybrids were observed. All somatic hybrids and parental type *P. ostreatus* formed a tight cluster, indicating a high genetic affinity. The similarity coefficient within the first group was 0.76. The other group was formed by parental *G. applanatum*.

Somatic hybrids of inter-incompatible species showed unique genetic characteristics compared with those of inter-compatible species, such as clamp connection formation at mitosis, asynchronous fruiting body, non-parental RAPD bands, and abnormal segregation and recombination. From these results, we classified synkaryons into microgenome insertion type, macrogenome insertion type, and heterokaryotic synkaryon.

We did not detect a change in the major RAPD banding patterns of microgenome insertion type when compared with the recipient (dominant) partner. In this case, most of the genome of one fusion partner is lost (donor or recessive). However, some genetic materials remain in the chromosomes of the other fusion partner. Despite the fact that the donor genetic material consists of full-size chromosomes from nuclei, most somatic hybrids in CF and OA pairings receive small subchromosome fragments. In most cases, the genetic similarity between the somatic hybrids and the recipient parent was over 80%. However, most genetic markers of two fusion partners were segregated in the progenies.

Macrogenome insertion types were recognized by the appearance of intense non-parental RAPD bands. A minority of CF and OA, and all FL hybrids contained detectable major non-parental banding patterns. Some progeny from both microgenome and macrogenome insertion types segregated non-parental auxotrophs due to gene disruption (Yoo and Lee 1994; Yoo, 1994).

## FRUITING BODY PRODUCTION BETWEEN COMPATIBLE SPECIES

The 40 somatic hybrids of inter-compatible species between Pleurotus florida and *P. ostreatus* were examined for the yield on fermented and pasteurised rice straw in a tray. The fruit body yield indices of *P. florida-ostreatus* hybrids ranged between 27-155 compared with parental values of 100 (ASI 2018) and 138 (ASI 2016). The pilei of fusants between orange white *P. florida* and dark grey *P. ostreatus* had mixed colors in the young fruiting stage. Other breeding programmes were performed to improve commercial strains with high yields of good quality fruiting bodies. Two kinds of new oyster commercial strains, Wonhyeong-neutari (P72), and Wonhyeong-neutari #2 (P49) were developed at the National Institute of Agricultural Sciences, Rural Development Administration in 1990 (Yoo and Cha, 1993; Yoo et al., 1993). These somatic hybrids were selected from 38 protoplast fusion products between *P. florida-ostreatus* recombinant P5-M43-arg rib and *P. ostreatus* ASI 2-13-o 2001-19-pro orn. The yield indices of of the 38 somatic hybrids ranged between 41-153 compared with the parental values of 100 (ASI 2018), 108 (ASI 2001), and 130 (ASI 2016) respectively. Hybrids P72 and P49 were characterized by the large fruiting bundle of semispherical shape with long stipe and by the small and circular pileus, resulting in lower harvesting cost. A significant increase in carpophore production was observed in somatic hybrids of protoplast. This phenomenon may be associated with heterosis due to gene interaction of nucleus and / or mitochondria.

## MATING SYSTEM ANALYSIS

To determine the mating system of somatic hybrids OF650, OA386, and OA399, 16 single-spore isolates from each basidioma were paired in all possible combinations. A tetrapolar mating system was found among monospore isolates in all somatic hybrids. Four

mating types were found in a single basidioma and pairings between them yielded 25% fertility, similar to normal wild type *P. ostreatus* strain. Colony growth of these progenies is faster compared with initial fusion colonies and tissue culture isolates of basidioma in all somatic hybrids.

Some somatic hybrids from the OF, CF, OA, and FL pairings demonstrated that germlings of a single basidiospore were able to form clamp connections. Four auxotrophic recombinants in FL964 hybrids also formed clamp connections (Table 3). These clamped progenies from the CF, OA, and FL pairings produced basidiomata similar to those of *P. florida*. The pileus colour of clamped progenies from the of pairing differed somewhat from that of somatic hybrids and wild parental strains.

**Table 3.** Frequency of clamp connection formation of single-basidiospore isolates from wild parental and somatic hybrid strains (Yoo et al., 2000)

| Strain a | Isolate type | NUMBER examined | NUMBER OF clamped isolateS (%) |
|---|---|---|---|
| O 2018 | Wild | 200 | 2(1.0) |
| F 2016 | Wild | 200 | 1(0.5) |
| C 2011 | Wild | 200 | 0(0) |
| OF2 | Prototroph | 55 | 26(47.3) |
| OF5 | Prototroph | 65 | 51(78.5) |
| OF6 | Prototroph | 30 | 15(50.0) |
| OF7 | Prototroph | 50 | 18(36.0) |
| CF864 | Prototroph | 19 | 5(26.3) |
| OA399 | Prototroph | 28 | 5(17.9) |
| FL917 | Prototroph | 13 | 5(38.5) |
| FL938 | Prototroph | 20 | 2(10.0) |
| FL964 | Prototroph | 87 | 76(87.4) |
| FL964 | Auxotrophic recombinant (rib cyt) | 5 | 4(80.0) |

[a] Abbreviations used : C, *P. cornucopiae* ; F, *P. florida* ; O, *P. ostreatus* ; CF, *P. cornucopiae* + *P. florida* ; FL, *P. florida* + *G. lucidum* ; OA, *P. ostreatus* + *G. applanatum* ; OF, *P. ostreatus* + *P. florida*

Four auxotrophic recombinants had clamp connections, suggesting that single-basidiospore isolates did not mate between compatible homokaryons. Amphithallic behaviour has been observed in the wild species of *Anthracophyllum lateritius, Hypsizygus tessulatus, Omphalotus illudens, O. mexicana,* and *Oudemansiella canarii*. Amphithallism in which basidiospores were plurinucleate or binucleate results in A varied arrangement of mating type within the spores. Percentages of amphithallic spores in some species such as *A. lateritius* is influenced by host and climate (Petersen 1995).

## CONCLUDING

Somatic hybrids of inter-compatible species formed heterokaryons, but somatic hybrids of inter-incompatible species formed synkaryons by protoplast fusion. Development of fruit bodies in synkaryons dependS on self-fertility of initial wild fusion partners. We would note that inter-incompatible fusion products produced poor fruiting bodies compared with wild parental strains. However, recombinant progeny derived from such fruiting bodies may provide many possibilities for strain improvement in edible mushrooms. Somatic hybridization systems using protoplasts open up many possibilities for mushroom production and mushroom products. Genetic recombination using protoplast fusion could be used to alter growth rate, fertility, sporulation, fruiting body yields, and substrate utilization efficiencies in edible mushrooms.

## 적 요

원형질체 융합에 의한 화합성 및 불화합성 종간 체세포 잡종을 얻었다. 화합성 종간인 *Pleurotus ostreatus* 와 *P. florida* 의 융합체는 이질핵체 (heterokaryon) 를 형성하였고, 불화합성 종간인 *P. cornucopiae* + *P. florida* , *P. ostreatus* + *Ganoderma applanatum, P. florida* + *Ganoderma lucidum,* 그리고 *P. ostreatus* + *Flammulina velutipes* 는 합핵체(synkaryon) 를 형성하였다. 이질이핵체는 동일한 양상의 자실체를 형성하는데 비해 합핵체는 유사분열상의 꺽쇠연결체 형성, 한쪽 친과 유사한 자실체 형성, 비정상적 유전형질 분리 및 유전자재조합 현상을 나타내었다. 화합성 및 불화합성 계통간 융합체의 RAPD 분석결과 화합성 종간 융합체는 동일한 DNA 패턴을 나타내었고, 불화합성 종간 융합체는 한쪽 친과 유사한 DNA 양상이면서 비양친 DNA 밴드도 형성하였다. 합핵체의 패턴은 microgenome insertion type 과 macrogenome insertion type 으로 구분되었다. 합핵체의 자실체 발생은 융합 모균주 양친의 자가임성에 의존하는데 이는 느타리의 동형핵체 자가임성과 유사한 양상이었고, 교배형 전환과 관련이 있는 것으로 사료된다. 여기서는 이러한 관점에서 논할 것이다.

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