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PROTOPLAST FUSION BETWEEN WHITE AND BROWN OYSTER MUSHROOMS

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ABSTRACT

Genetic crossing of white oyster mushroom (*Pleurotus floridae*) to introduce longer storage life trait can only be done within individuals in this particular species. However, longer storage life trait is possessed by brown oyster mushroom (*Pleurotus cystidiosus*). Therefore, a protoplast fusion experiment between white and brown oyster mushrooms was conducted to obtain an oyster mushroom strain showing high productivity and long storage life. The experiment was done at the biology laboratory of the University of Al Azhar Indonesia from May 2008 to August 2009. Protoplast fusion was done by isolating protoplast from 5-day old monokaryotic mycelia grown in potato dextrose broth (PDB). Around 3.15 x 10^5 protoplasts ml^{-1} were harvested using mixture of cellulase Onozuka R-10 (1%) and macerozyme R-10 (1%) from brown oyster mushroom with 80.61% viability. Similarly, 3.71 x 10^5 protoplasts ml^{-1} were harvested using lysing enzyme (2%) from white oyster mushroom with 83.68% viability. Protoplast fusions were conducted using 40% PEG_{6000} for 10 minutes. The candidate fusants were then screened using minimum regeneration media (MRM). There were 22 colonies grew on MRM media and four colonies (FS1, FS2, FS3, and FS4) showed clamp connection as well as primordia formation to be chosen as candidate fusants. However, isozyme studies using malate dehydrogenase and acid phosphatase as marker enzymes confirmed that only FS1 and FS2 were the hybridized products. The two colonies showed different mycelia growth patterns and hyphae sizes, fruit body morphology and productivity compared to their parents. These two fusants, however, did not indicate the presence of longer storage life trait as expected despite a higher productivity achieved by FS1. In this study, the protoplast fusion only yielded higher productivity strain of mushroom with different colors without any changes in storage life.

[***Keywords***: *Pleurotus floridae*, *Pleurotus cystidiosus*, protoplast fusion, storage life, productivity]

INTRODUCTION

White oyster mushroom (*Pleurotus floridae*) is a widely grown edible mushroom in Indonesia due to its nutritive as well as medicinal values (Gunde-Cimerman 1999). This particular mushroom has higher carbohydrate, protein, vitamins, and minerals compared to other edible mushrooms. It is not suprising that white oyster mushroom is the second most cultivated type of edible mushrooms in the world after button mushroom (*Agaricus bisporus*) (Ramirez *et al*. 2000). However, consumption of this tasty mushroom is restricted to the producing areas which is mainly in the high altitude regions. One of the reasons is that this particular mushroom has a relatively short storage life that prevents it from widely distributed accross Indonesia. Physiological disorders caused by slow handling of the products that leads to enhancement of opening and darkening of the gills, wilting as well as brown discoloration are the main problems in the postharvest handling of oyster mushroom (Kannaiyan and Ramasamy 1980; Barden *et al*. 1990).

Several efforts, chemically and physically, have been done to address these problems. Applying chitosan to freshly cut oyster mushroom was able to extend the shelf life to a certain degree (Hesham 2007). Other physical treatments such as keeping the mushroom at 0°C with 95% RH (Tano *et al*. 1999) or packaging it in a certain design of linear low density polyethylene (LLDPE) container with addition of 3 g magnesium oxide (Jayathunge and Illeperuma 2004) have been reported able to extend the shelf life. Unfortunately, all those treatments are complicated and difficult to aplly in the field especially by farmers. It is important, therefore, to introduce a genetic variation that may lead to a longer storage life by recombination.

Brown oyster mushroom (*P. cystidiosus*) has the longest storage life among the genus of *Pleurotus*. This species would be an ideal partner to be crossed with the white oyster mushroom to introduce longer storage life trait. Unfortunately, these two species are not compatible to be crossed conventionally. In this case protoplast fusion is the most appropriate method to cross these mushrooms.

Protoplast fusion has been used as a method to create mushroom hybrids especially when conventional method cannot be achieved. As conventional hybridization, protoplast fusion can be performed intraspecifically (Kiguchi and Yanagi 1985; Toyomatsu and Mori 1987), interspecifically (Takehara *et al*.

1993; Matsumoto *et al*. 1997), intergenerically (Eguchi *et al*. 1993; Zhao and Chang 1996) and even inter-heterogenerically (Eguchi and Higaki 1995; Toyomatsu and Mori 1987). However, for the same reason as that implied to the conventional method, the greater the distance in genetic relationship between the two mating isolates, the less successful protoplast fusion will be (Anne and Peberdy 1976).

In this study we performed protoplast fusion between white and brown oyster mushrooms in an attempt to obtain a highly productive as well as longer storage life mushroom strain. The aim of this study was to obtain a new strain of oyster mushroom that combine the superior traits of their parents, i.e. higher productivity from white oyster mushroom and longer storage life from brown oyster mushroom. Therefore, the highly productive and easily cultivated strains can be marketed all over the country to fulfill the domestic demand and perhaps can be exported to increase the Indonesian economic development.

MATERIALS AND METHODS

Strains and Media

The parental strains of white and brown oyster mushrooms were obtained from Bandung Institute of Technology (ITB) culture collection. Potato dextrose agar (PDA) and potato dextrose broth (PDB) media were used in this experiment to grow mycelia. All steps of the experiment were done at the biology laboratory of the University of Al Azhar Indonesia from May 2008 to August 2009.

Protoplast Isolation

Mycelia from white and brown oyster mushrooms were transferred onto 50 ml of PDB in a 250 ml Erlenmeyer flask placed on a shaker with the agitation speed of 200 rpm, at 25°C for 5 days. Protoplasts were separated from the mycelia using a method modified from Santiago (1982) by transferring 100 mg of brown oyster mushroom mycelia onto 1 ml of the sterilized lytic enzyme mixture containing cellulase Onozuka R-10 (1%) and macerozyme R-10 (1%) in a test tube and shaking it at 80 rpm at room temperature for 5 hours. While for the white oyster mushroom mycelia, the protoplasts were separated from the mycelia using a method modified from Santiago (1982) by transferring 100 mg of brown oyster mushroom mycelia onto 1 ml of the sterilized lysing enzyme (2%) solution in a test tube and shaking it at 80 rpm at room temperature for 1 hours.

The mycelia remnants of both white and brown oyster mushrooms were removed by filtration through layers of sterile filter papers and the suspended protoplasts were precipitated at 2000 rpm for 20 minutes. The protoplasts were then washed twice with the osmotic stabilizer containing 0.6 M $MgSO_4.7H_2O$ dissolved in 0.01 M phosphate buffer (pH 5.8) and were finally suspended in 5 ml of the osmotic stabilizer. Protoplasts obtained from both white and brown oyster mushrooms were counted using a haemocytometer. Protoplast viability was confirmed using methylene blue staining system and the viability percentage was calculated according to Kao (1991b).

Protoplast Fusion and Fusant Regeneration

One milliliter of each of the freshly prepared protoplasts (diluted to be $1x10^6$ protoplasts ml^{-1}) of white and brown oyster mushrooms were mixed in a test tube and centrifuged at 2500 rpm for 20 minutes. The supernatant was rinsed off and 1 ml of sterilized 40% PEG_{6000} (40 g PEG_{6000} in 100 ml 0.05 M $CaCl_2.2H_2O$) was added to the protoplasts in the test tube and incubated at room temperature for 10 minutes by gently shaking the tube every 2 minutes. The fusion process was examined under the microscope by taking 10 µl aliquot of fusion mixture and examined it using 10 x 40 magnification. Another 4 ml of the osmotic stabilizer were then added to the tube before centrifugation at 2000 rpm for 10 minutes. The supernatant was rinsed off and the mixed protoplasts were washed twice with the osmotic stabilizer.

The protoplast solution was then diluted to 1×10^4 protoplasts ml^{-1} and 1 ml of the suspension was used for protoplast regeneration by culturing it on a plate of minimum regeneration media (MRM) according to Kiguchi and Yanagi (1985). MRM contained 10 g dextrose, 2.8 g $(NH_4)_2SO_4$, 50 ml A solution (10 g KCl, 10 g $MgSO_4.7H_2O$ and 0.2 g $FeSO_4.7H_2O$), 50 ml B solution (20 g K_2HPO_4) and 15 g bacteriological agar followed by overlaying with the same kind of medium but with a concentration of agar of only 5 g. The plate was incubated at 25°C until colonies appeared. Each colony was isolated day by day onto a MRM slant. This experiment was done at the biology laboratory of the University of Al Azhar Indonesia from May 2008 to August 2008.

The presence of clamp connection was observed during the growth on MRM plates while the primordial

formation leading to fruiting bodies was observed when the mycelia were grown in baglogs at the glass house. Subsequently, fusants were selected on MRM. The screened colonies were selected by the presence of clamp connections and the ability to form fruiting bodies to be chosen as candidate fusants. The candidate fusants were then confirmed by their zymogram patterns before their mycelial growth, hyphal size, productivity, morphology of fruiting bodies as well as their storage life were analyzed.

Isozyme Analysis

Isozyme patterns of mycelia of all hybrids showing clamp connection and primordial formation along with their parental strains were studied following a method modified from that of Pasteur *et al*. (1988). This experiment was done at the biology laboratory of the University of Al Azhar Indonesia from August 2008 to September 2008. Mycelia of each strain were cultured on 100 ml PDB at 25°C for 20 days. The mycelia were filtered with two layers of muslin cloth which were then washed twice with sterilized distilled water followed by grinding with liquid nitrogen in a mortar. The mycelia were then picked up into a micro-centrifuge tube in which extraction buffer for enzyme extraction was added. The tube was then centrifuged at 10,000 rpm at 4°C for 30 minutes and the supernatant was kept at -20°C. Electrophoresis was performed by mixing 15 µl of the supernatant with 5 µl of the sample buffer (pH 6.8, 0.6 M Tris-HCl, 10% glycerol and 0.025% bromophenol blue) before loading sample solution of each strain into each slit on the acrylamide gel in the electrophoresis set. The electrophoresis was then conducted using standard starch gel electrophoresis (Pasteur *et al*. 1988). The gel was then picked up and stained with substrate solution of the two enzyme markers namely acid phosphatase and malate dehydrogenase. The gel was then photographed using a KODAK Polaroid camera.

Determination of Mycelial Growth and Hyphal Size

Innocula of mycelia from 22 confirmed fusants together with the parental strains were subcultured on PDA plates and incubated at room temperature for 10 days. Subsequently, experiment was continued by determining the diameter of the fusant colonies for at least 10 replications to represent mycelial growth, while hyphal width was measured microscopically as the hyphal size using a calibrated eyepiece micrometer for 10 replications. This experiment was designed using a completely randomized design. Data were then analyzed using analysis of variance (ANOVA) using ANOVA SAS G-13 followed by a Duncan's Multiple Range Test (DMRT) ($\alpha = 0.05$).

Cultivation for Fruiting Bodies to Analyze Productivity and Observation for Morphology of Fruiting Bodies

All four confirmed hybrids resulted from the previous experiment were subjected to fruiting tests. The fruiting compost or baglog used contained 30% (w/v) sawdust, 2% (w/v) lime, 2% (w/v) rice husks, and 66% water. When mycelium colonized the substrate completely, the bags were opened to stimulate fruiting body formation. The temperature was then maintained at 25°C and relative humidity at 90%. If a strain did not form any primodia in all triplicate bags after 25 days, it was considered to be sterile.

The shape and color of the fruiting bodies were determined and photographed. The same characteristics of fruiting bodies studied in their parental strains were determined in all fusants and the results were compared to those of the parents. The experiment was designed using a completely randomized design with 10 replications.

Quantitative data such as diameter and amount of fruiting bodies, fresh weight, and dry weight of harvest were replicated six times. All data were analyzed by ANOVA followed by DMRT ($\alpha = 0.05$).

Biological efficiency ratio (BER) was determined from each genotype by measuring the fresh weight harvest of fruiting bodies from each baglog dividing it with the weight of baglogs which are made uniformly of 1 kg and multiplying the value with 100% (Aryantha and Rachmat 1999).

Storage Life Analysis

Fruiting bodies from all four hybrids as well as their parents were subjected to shelf life analyses. Ten fruiting bodies per genotype were cleaned using dry tissue papers. These fruiting bodies were arranged in a styrofoam container covered with a plastic wrap. This is a common practice for edible mushroom packaging. The experiment was arranged using a completely randomized design with 10 replications. The styrofoam packages containing mushrooms were stored at air-conditioned room temperature (20°C and

70% RH) and symptoms of decaying were observed every 2 days for a 12-day storage period. Data were analyzed using ANOVA followed by a DMRT (α = 0.05).

RESULTS AND DISCUSSION

Protoplasts were isolated from 5-day old mycelia grown on PDB media. At this stage, both parents were at exponential growth phase. Higher protoplast yield and healthier protoplasts were obtained from mycelia harvested at that phase because of simpler cell wall structure that are easily degraded by lytic enzymes (Smith 1993) and a better protoplast regeneration was found due to actively dividing cells at that phase (Kao 1991a).

The counted number of protoplasts obtained in *P. floridae* and *P. cystidiosus* were 3.71 x 10^5 with 83.68% viability and 3.15 x 10^5 protoplasts ml^{-1} with 80.61% viability, respectively. Observation of Budiwati and Pudjiraharti (2001) demonstrated that it would take at least 10^5-10^6 viable protoplast ml^{-1} to undergo a successful protoplast fusion. Based on this study, the diameter of *P. floridae* and *P. cystidiosus* protoplasts were 3.04 and 3.24 μm, respectively.

Protoplast production, however, varied with the factors used in the isolation process, e.g. species and age of fungal mycelia (Yamada *et al.* 1983), type and condition of the lytic enzyme and osmotic stabilizer (Peberdy and Fox 1993). Preliminary experiments (Irawan 2004) indicated that the highest amount of protoplasts obtained from brown oyster mushroom using a mixture of 1% cellulase Onozuka R-10 and 1% macerozyme R-10 for 1 hour while 2% of lysing enzyme (isolated from *Trichoderma harzianum*) for 2 hours was able to yield the highest amount of white oyster mushroom protoplasts in laboratory condition. This discrepancy was probably caused by different cell wall compositions between those two parental genotypes.

Protoplast fusion between the two species of *Pleurotus* sp. was done using 40% PEG_{6000} (polyethyleneglycol) for 10 minutes (Fig. 1). There was no visible contact between protoplasts in the control (without PEG_{6000}) indicating the importance of PEG_{6000} as a fusion agent (data not shown). According to Endress (1994), higher molecular weight of PEG_{6000} will act as a bridge between two protoplast membranes which eventually induces the adhesion of those protoplasts. Preliminary experiment using 30% PEG_{6000} showed only a short time contact between protoplasts while using 50% PEG_{6000} showed a tendency of more than two protoplasts were fused that potentially formed a multinucleate fusant (data not shown). A multinucleate fusant cannot regenerate and therefore should be avoided (Kao 1991a).

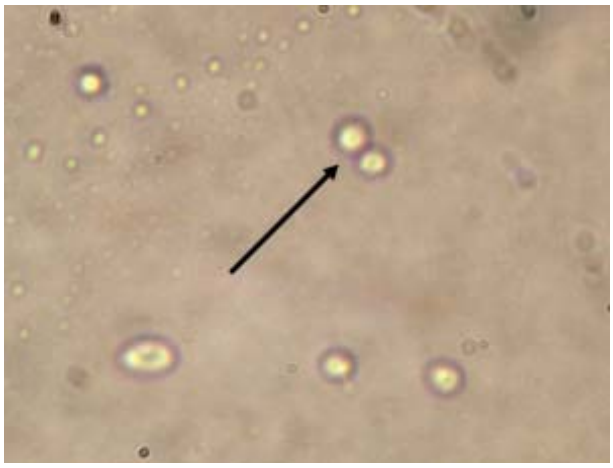

Fig. 1. Protoplast fusion between *Pleurotus floridae* and *P. cystidiosus*.

The number of colonies grown on MRM after protoplast fusion of *P. floridae* and *P. cystidiosus* was 22 colonies. No visible mycelial growth shown on MRM when parental genotypes were innoculated as controls.

Among these, only four possessed clamps and thus were selected as fusants and named as FS1, FS2, FS3, and FS4. Those fusant candidates appeared after 2-4 weeks grown on MRM. In fruiting test, all of the candidate fusants were able to form primordial within 3-week incubation on fruiting composts/baglogs. The fusants from the compatible isolates produced normal fruiting bodies, while those from the incompatible isolates did not produce clamp connections and fruiting bodies (Dhitapichit and Pornsuriya 2005). Furthermore, according to Dhitapichit and Pornsuriya (2005), these types of fusants were suggested to be heteroploids or aneuploids.

Determination of Isozyme Patterns

Isozymes are proteins thus they can directly reflect the alteration in the DNA sequence through changes in amino acid composition which provide an extremely useful method for evaluating genetic differences due to hybridization. Manifestations of changes due to hybridization were reflected by the combination of bands from their parents (de Charisey *et al*. 1985). In this experiment, enzyme specific stains used are

malate dehydrogenase (MDH) and acid phosphatase (ACP) which gave 3 and 2-4 bands of isozymes respectively (Fig. 2 and 3). According to de Charisey *et al*. (1985), subcellular localization of ACP is varied in the cell with 2-4 numbers of isozymes. On the other hand, subcellular localization of MDH is at the cytosol, mitochondria, and microbodies with three numbers of isozymes (Tyson *et al*. 1986).

For the two enzymes tested (Fig. 2 and 3), only FS1 and FS2 verified the hybridization relationship as the fusants showed band(s) common to both of their parents (Dhitapichit and Pornsuriya 2005). Furthermore, the non-parental new bands were also observed in the ACP isozyme patterns of the four fusants indicating that there was a new occurrence of interaction between the two parental genomes (Toyomatsu *et al*. 1986). Similarly, disappearance of a band in the FS3 observed in the MDH isozyme patterns as well as in the FS3 and FS4 of the ACH isozyme patterns also indicated an occurrence of interaction between the two parental genomes (Toyomatsu *et al*. 1986).

The same MDH and ACP isozyme patterns of FS1 and FS2 fusants also indicated that the two fusants coincidentally had the same MDH and ACP genes. However, more enzymes should have been tried and DNA fingerprinting should be further studied to obtain a more precise evidence of the hybridization.

Mycelial Growth and Hyphal Size

Mycelial growth shown by mycelial colony diameter and hyphal size of FS1 and FS2 was significantly different from those of the parental strains (Table 1). These results are consistent to the previous studies indicating that fusants which are dikaryotic (n+n) grow faster (Toyomatsu and Mori 1987) and have larger hyphae (Abe *et al*. 1982) than the monokaryotic parental strains. There were 10 replicates of mycelia taken from three different colonies of FS1 and FS2 fusants as well as their parents used in this experiment. Total samples analyzed were 120 mycelia. FS2 showed longer mycelial diameter but is not statistically different from the parental strains (Table 1).

Table 1. The colony diameter and hyphal width of the FS1 and FS2 fusants of *Pleurotus floridae* and *P. cystidiosus* together with their parents at 6 days after inoculation.

Genotype	Colony diameter (cm)	Hyphal width (μm)
Pleurotus floridae	4.50a	1.60a
Pleurotus cystidiosus	5.20a	1.85a
FS1	8.70b	3.65b
FS2	6.10ab	3.80b

Means followed by different letters within a column represent a significant difference ($\alpha = 0.05$) by DMRT.

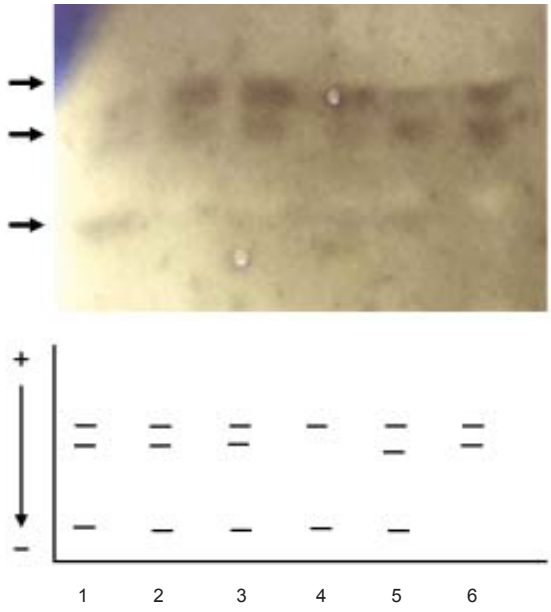

Fig. 2. Starch gel of fusants of *Pleurotus floridae and P. cystidiosus* and their parents assayed for malate dehydrogenase activity; lane no. 1 = *P. floridae*, 2 = FS1, 3 = FS2, 4 = FS3, 5 = FS4, 6 = *P. cystidiosus*. Arrows indicate the expected isozyme bands.

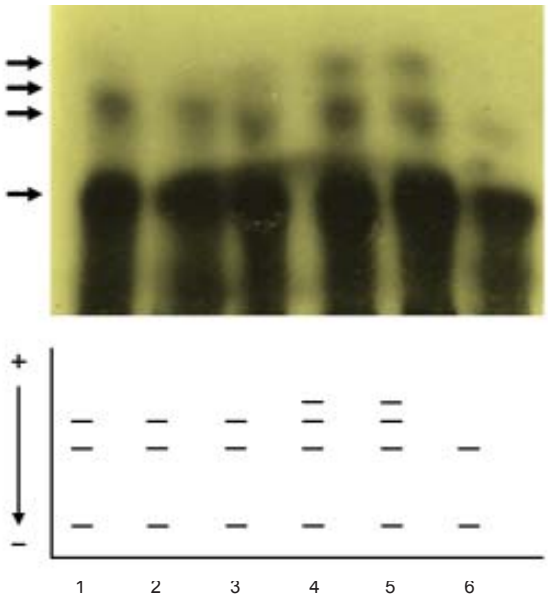

Fig. 3. Starch gel of fusants of *Pleurotus floridae and P. cystidiosus* and their parents assayed for acid phosphatase activity; lane no. 1= *P. floridae*, 2 = FS1, 3 = FS2, 4 = FS3, 5 = FS4, 6 = *P.cystidiosus*. Arrows indicate the expected isozyme bands.

Fruiting Body Morphology and Fusant Productivity

The morphology of fruiting bodies of both FS1 and FS2 (Fig. 4) was not different from their parents. There was no any deviation (curling, etc) in the morphology of fruiting bodies detected in these two fusants. Fruit bodies of FS1 were creamish white in color which were identical to that of *P. floridae*, while FS2 were light brownish. The color of FS2 fruiting bodies was between those of *P. floridae* and *P. cystidiosus*. These results suggested that the whole genomes of FS1 and FS2 were not identical and the differences in their morphology should be resulted from genome-wide gene recombinations. Therefore, the reasons that FS1 and FS2 were similar in MDH and ACP isozyme patterns (Fig. 2 and 3) but different in morphology (Table 2, Fig. 4) and productivity (Table 2) could be due to their whole genomes not being identical but having only some genes (e.g. MDH and ACP) in common.

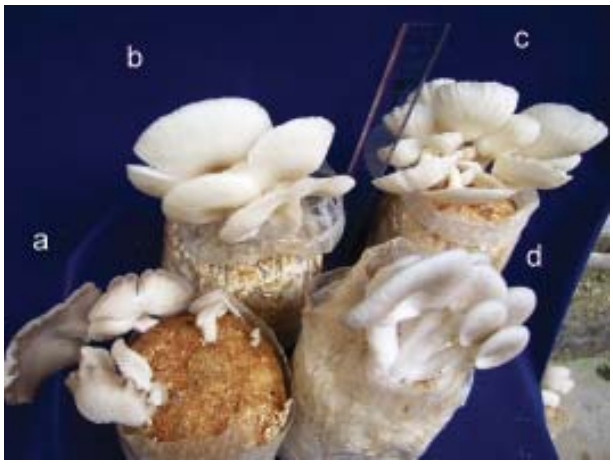

Fig. 4. Fruiting bodies of *Pleurotus cystidiosus* (a); *P. floridae* (b); FS1 (c); and FS2 (d).

The manifestation of the hybridization between the two fusant genomes was further investigated by the observations of growth and productivity. In addition, there was no difference in the flushing period between FS2 and its parents. However, FS1 showed faster flushing period (4 days after aeration) compared to FS2 and its parents (2 weeks after aeration). The harvest pattern of all the genotypes showed a typical pattern in which the first flush was the heaviest with later flushes becoming progressively smaller until further cropping becomes economically unfeasible.

Growth performance presented in Table 2 indicated that the FS1 fusant had a better growth performance compared to other genotypes tested. FS1 also produced more fruiting bodies compared to its parents and FS2 (Fig. 4). Fresh and dry weights of the FS1 were significantly higher than other three genotypes. The biological efficiency ratio (BER) of FS1 is around 17% which is higher than the other genotypes (approximately 9%). BER is a parameter which is expressed as the value of fresh weight harvest per baglog divided by the weight of the media (1 kg) that is often used by mushroom farmers to describe productivity. Aryantha and Rachmat (1999) reported that mushroom production should have BER values higher than 10% to be categorized as an economically feasible cultivation. Based on the production data, FS1 demonstrated superior productivity compared to the existing white oyster mushroom strains in

Table 2. Morphology of fruiting bodies and productivity of fusants of *Pleurotus floridae* and *P. cystidiosus* and their parents harvested at 12, 14, and 16 weeks post-inoculation into the baglogs.

Genotype	Morphology of fruiting bodies	Number of fruiting bodies/baglog			Fresh weight harvest (g)/kg baglog			Dry weight harvest (g)/kg baglog			Biological efficiency ratio (%)
		12 wpi	14 wpi	16 wpi	12 wpi	14 wpi	16 wpi	12 wpi	14 wpi	16 wpi	
Pleurotus floridae	Normal, white	28a	26a	12a	100a	90a	75a	13.0a	11.7a	9.8a	8.8
P. cystidiosus	Normal, brown	26a	29a	12a	95a	90a	68a	10.0a	11.0a	9.2a	8.4
FS1	Smaller than *P. floridae* but higher numbers of fruiting bodies, white	50b	42b	28b	220b	160b	125b	28.6b	20.8b	16.3b	17.0
FS2	Normal, light brown	23a	30a	10a	110a	84a	80a	13.5a	11.0a	11.2a	9.1

wpi = weeks post-inoculation to the baglogs.
Means followed by different letters within a column represent a significant difference ($\alpha = 0.05$) by DMRT.

Indonesia. It would then be very beneficial if this superior strain also demonstrated a longer storage life.

Storage Life Analysis

Several efforts to increase the storage life of mushrooms have been done in the past few years. Lowering storage temperature has been reported to be effective retarding deterioration and senescence of harvested mushrooms due to decreased rate of respiration. Further improvement of mushroom quality and thereby extended marketable life has been shown when stored at 0°C and 95% RH (Tano *et al*. 1999). Furthermore, Jayathunge and Illeperuma (2004) claimed to be able to increase storage life up to 12 days by packaging oyster mushrooms in a certain design of LLDPE container with addition of 3 g magnesium oxide. Jayathunge and Illeperuma (2004) stated that their achievement can be improved further by using a new strain of oyster mushroom that has a longer storage life trait.

In this experiment, our intention was to increase the storage life of white oyster mushroom (*P. floridae*) by introducing the longer storage life trait owned by brown oyster mushroom (*P. cystidiosus*) through protoplast fusion. Table 3 showed that brown oyster mushroom can be kept fresh up to 5 days in store room conditions (20°C, 70% RH) while white oyster mushroom only stands for 3 days. Unfortunately, both fusants did not show any improvement in storage life as expected. Despite its excellent performace in productivity, FS1 did not show better storage life compared to its parents. In this case, these fusants cannot solve the marketing problem of the oyster mushroom. Future direction would be increasing the number of candidate fusants for a higher probability in obtaining the desired economical traits such as longer storage life. Other experimental approaches (e.g. postharvest treatment with radiation) need to be explored.

Table 3. Storage life of fusants of *Pleurotus floridae* and *P. cystidiosus* and their parents.

Genotype	Storage period (day)				
	2	4	6	8	10
Pleurotus floridae	√	x	xx	xx	xx
P. cystidiosus	√	√	x	xx	xx
FS1	√	x	xx	xx	xx
FS2	√	x	xx	xx	xx

√ = fresh, x = mildly rotten, xx = heavily rotten.

CONCLUSION

Protoplast fusion between *P. floridae* and *P. cystidiosus* resulted 22 colonies grown on MRM media. Among those only four candidate fusants (FS1, FS2, FS3, and FS4) were confirmed to be fusants based on their ability to form clamp connections and primordia. Mycelial growth and hyphal size as well as isozyme analyses indicated that only FS1 and FS2 were confirmed to be fusants.

FS1 was able to form more fruiting bodies and higher productivity compared to the parent strains and FS2. Despite the excellent productivity of FS1, neither FS1 nor FS2 showed longer storage life. Future experiments would be directed to obtain higher number of candidate fusants and exploring other technical aspects of postharvest technology to improve their storage life ability.

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