



**Research Paper**

**Interspecific Hybridization between *Pleurotus eous* and *Pleurotus flabellatus* by PEG – induced Protoplast Fusion**

**\*Parani K.<sup>1</sup> and Eyini M.<sup>2</sup>**

<sup>1</sup>Department of Botany, The Standard Fireworks Rajarathnam College for Women, Sivakasi, INDIA

<sup>2</sup>Research Center in Botany, Thiagarajar College (Autonomous), Madurai, Tamilnadu, INDIA

(Received 18<sup>th</sup> September 2012, Accepted 30<sup>th</sup> November 2012)

Available online at: [www.ijrce.org](http://www.ijrce.org)

**Abstract:** Biological efficiency of *Pleurotus eous* was 93.9% on paddy straw and 82.9% on coffee pulp, whereas the *Pleurotus flabellatus* on paddy straw was 86.97%, neither *Pleurotus flabellatus* nor fusant could produce fruiting bodies on coffee pulp.

**Keywords:** Biological efficiency, *Pleurotus eous*, *Pleurotus flabellatus*,

**Introduction**

Protoplast fusion is of current interest because of its applications in pure and applied genetics. Protoplast fusion technology is applied for developing interspecific, intraspecific and intragenetic suprahybrids with higher potentiality than their parental strains. Through protoplast fusion technique, improved strains with enhanced antagonistic potential, antibiotics, enzymes, useful mycoproducts, high yielding mushrooms, etc. could effectively be achieved<sup>[1]</sup>.

The basidiomycete, *Phanerochaete chrysosporium* and other white rot fungi have potential application in a variety of schemes for the commercial processing of lignocellulose. These organisms have already been used in numerous studies on lignin and cellulose degradation<sup>[2]</sup> and on lignocellulose bioprocessing applications<sup>[3]</sup>. Their potential would be enhanced, however if genetic methods for producing strains with superior capacities were available. Recently, considerable interest has been focused on the isolation of fungal protoplasts and their use in fusion and transformation experiments<sup>[4]</sup>.

Therefore the research work was designed to look for organisms, which are substrate specific and grow in association with a mushroom synergistically so that the resulting association is specifically suitable for the optimal hydrolysis of the targeted substrate *i.e.*, the coffee pulp. It was also expected that such associations could be improved further by genetic manipulation. The research plan was formulated with the following objectives to

reveal the development of a new strain through protoplast fusion.

**Material and Methods**

**Organisms:** Pure cultures of the oyster mushrooms *viz.*, *Pleurotus eous* (Berk.) Sacc. (APK-1) and *Pleurotus flabellatus* (Berk and Br.) Sacc. (MDU2) were obtained from TamilNadu Agricultural University, Coimbatore, India. The cultures were maintained on Potato Dextrose Agar (PDA) slants and stored at 4°C and the slants were subcultured once a month.

**Fruiting tests:** The protoplast fusant and their parental strains were subjected to fruiting tests. Paddy straw (Control) or coffee pulp (100 g with 60% moisture content) was used as the fruiting medium. The seed spawn and mushroom beds were prepared by the method of Sivaprakasam<sup>[5]</sup>. When mycelia had colonized the substrate completely, the bags were opened to stimulate fruiting body formation. Temperature was then maintained at 28°C and the relative humidity was adjusted to 90% ventilation and light were provided for healthy fruiting body development. The yield attributes of the two parental strains and the fusant strains in the two substrates were studied<sup>[6]</sup>. The data were analyzed statistically.

**Results and Discussion**

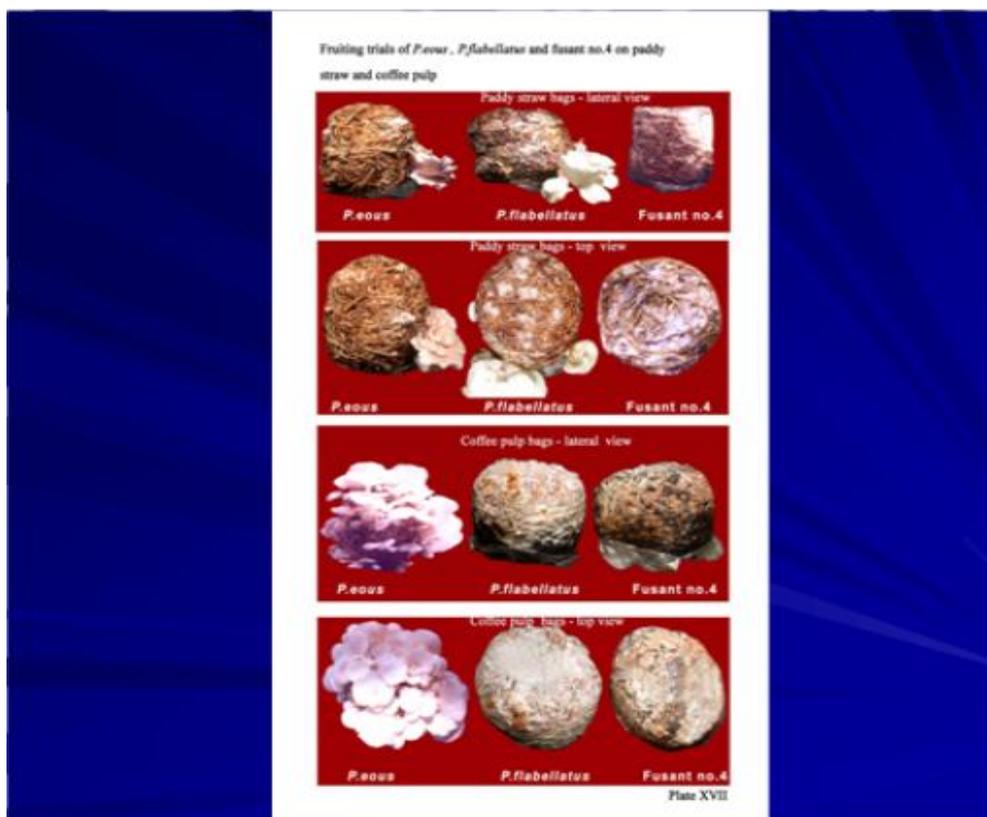
**Fruiting Tests:** The fusant of *Pleurotus eous* and *Pleurotus flabellatus* was subjected to fruiting tests on paddy straw and coffee pulp. It was observed that *Pleurotus eous* had a biological efficiency (B.E.) of 93.9%

in paddy straw and comparatively less 82.9% B.E. in coffee pulp. Interestingly, the yield in the I flush on coffee pulp was 56.27% more than that on paddy straw. The higher percentage of soluble carbohydrates in coffee fruits than in paddy straw would have helped higher and faster initial colonization and could explain this phenomenon.

No significant difference was observed between the sporophores of *P.eous* growing on paddy straw or coffee pulp with respect to colonization, pinhead appearance, number of pinheads, the stipe length and pileus diameter (Table 1, Plate I).

**Table 1**  
**Growth and yield attributes of the selected mushroom fungi and their fusant on paddy straw or coffee pulp**  
 Values of mean  $\pm$  S.E of three replicates

S. N.	Parameters	Paddy straw			Coffee pulp		
		<i>P. eous</i>	<i>P. flabellatus</i>	<i>Fusant</i>	<i>P.eous</i>	<i>P.flabellatus</i>	<i>Fusant</i>
1	Days taken for complete colonization of bag	7 $\pm$ 0.008	14 $\pm$ 0.03	12 $\pm$ 0.2	7 $\pm$ 0.005	30 $\pm$ 0.4	40 $\pm$ 0.6
2	Days taken for pinheads appearance after complete colonization	2 $\pm$ 0.006	5 $\pm$ 0.003	-	2 $\pm$ 0.006	-	-
3	No. of pinheads appeared	68 $\pm$ 0.17	65 $\pm$ 0.17	-	65 $\pm$ 0.17	-	-
4	Yield (g / bag)						
	a. First flush	45.43 $\pm$ 0.24	52.90 $\pm$ 0.34	-	56.27 $\pm$ 0.12	-	-
	b. Second flush	38.47 $\pm$ 0.12	20.37 $\pm$ 0.12	-	20.58 $\pm$ 0.04	-	-
	c. Third flush	11.03 $\pm$ 0.10	13.70 $\pm$ 0.12	-	6.07 $\pm$ 0.10	-	-
5	Total yield / biological efficiency (%)	93.93 $\pm$ 0.7	86.97 $\pm$ 0.9	-	82.92 $\pm$ 0.4	-	-



**Plate I**

*Pleurotus flabellatus* had a 86.97% B.E on paddy straw, but it could not produce fruiting bodies on coffee pulp. Since *Pleurotus flabellatus* had lower tannin and caffeine degradation potential as observed in the present study, the higher concentration of caffeine and tannin left in coffee pulp colonized by *P.flabellatus* could have affected the fruiting body formation (Table 1, Plate I). The protoplast fusant No.4 of *Pleurotus eous* and *Pleurotus flabellatus* could not produce fruiting bodies either on paddy straw or on coffee pulp even though it showed good colonization of the substrates (Plate I).

Identification of protoplast fusants which is the most difficult step, is carried out through a) observation of clamp connections b) isoenzymes analysis c) fruiting trials and d) through basidiospores analysis. However there are some imperfections in each of these methods. Even though the formation of fruiting body was the best evidence for fusion products, noted that monokaryotic fruiting could also be possible [7].

Further reported that the fusants from the compatible isolates produced normal fruiting bodies [8], while those from the incompatible isolates did not produce clamp connections and basidiocarps. These fusants were suggested to be heteroploids or aneuploids. As per their suggestion, it is derived that fusant No. 4 obtained in this study could be the fusion progeny of the incompatible species of *Pleurotus eous* and *Pleurotus flabellatus*. In the absence of fruiting, other strategies were undertaken to characterize the fusant of *P. eous* and *P. flabellatus* and to study the possibility of the fusant strain showing improved efficiency of coffee pulp degradation over its parents.

Monokaryotic isolates were raised from two *Pleurotus* species, namely *Pleurotus sajor-caju* and *Pleurotus sapidus* for study of the monokaryotic primordial formation [9]. The protoplast fusion technology could improve mushroom cultivation and productivity by intra and inter specific hybridization [10]. Stable and successful fusants developed through protoplast fusion should have the desirable characteristics of both the parents. They were selected by several morphological and biochemical marker characteristics.

Strain improvement by protoplasmic fusion for enhancement of xylanase production had been achieved through interspecific recombination of *Aspergillus indicus* and *A.wentii* [11]. Induced heterokaryosis via protoplast fusion to isolate fusants from mutants derived from *Trichoderma reesei* with different combinations of genetic markers like auxotrophic and cellulolytic characterization [12].

## References

1. Lalithakumari D., Fungal protoplast. A Biotechnological tool. Oxford and IBH Publishing Co. Pvt. Ltd., NewDelhi, 101-112 (2000)
2. Eriksson K, E. Cellulases of fungi. In: *Trends in the biology of fermentation for fuels and chemicals*. A.Hollaender and R.Rabson (eds.), Plenum Publishing Corp., New York, pp. 19 – 32 (1981)
3. Kirk T. K. and Chang H.M., Potential applications of bioligninolytic systems. *Enzyme Microbial. Technol.*, **3**, 189 – 196 (1981)
4. Peberdy J.F., Protoplast fusion, a tool for genetic manipulation and breeding in industrial microorganisms. *Enz. Microbial. Technol.*, **2**, 23 – 29 (1980)
5. Sivaprakasam K., *Oyster Mushroom Cultivation*, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, India, 22 – 50 (1985)
6. Chang S.T., Lan O.W. and Cho K.Y., The cultivation and nutritional value of *Pleurotussajor-caju*. *Eur.J.Appl.Microbiol.Biotechnol.*, **12**, 58 – 62 (1981)
7. Zhao, J. and S.T. Chang, Monokaryotization by protoplasting heterothallic species of edible mushrooms. *World J.Microbiol. Biotechnol.*, **9**, 538-543 (1993)
8. Zhao J. and Chang S.T., Intergeneric hybridization between *Pleurotus ostreatus* and *Schizophyllum commune* by PEG – induced protoplast fusion. *World J. Microbiol. Biotechnol.*, **12**, 573 – 578 (1996)
9. Thakur K. and Bandal M.S., Monosporous isolates and their intermating in *Pleurotus sapidus* and *Pleurotus sajor -caju*. *Mush. Res.*, **2**, 41 – 44 (1993)
10. Bhattiprolu G. R., Protoplast fusion of *Coprinus cinereus* auxotrophic mutants. *Mushroom Res.*, **4**, 69 – 72 (1995)
11. Thingamajig D. and Swaminathan K., Strain improvement by protoplasmic fusion for enhancement of xylanase production from intergeneric recombination. In: *International Conference on New Horizons in Biotechnology* (Abs), Trivandrum, India, April, 18-21, 84 (2001)
12. Bawa S. and Sandhu D. K., Parasexual analysis in *Trichoderma reesei* using protoplast fusion. <http://www.fgsc.net/fgn41/bawa.html>(2003).