Mycobiology 33(1): 30-34 (2005)
Copyright © 2005 by The Korean Society of Mycology

Changes of Enzyme Activities and Compositions of Abnormal Fruiting Bodies Grown under Artificial Environmental Conditions in *Pleurotus ostreatus*

Kab Yeul Jang*, Soo Muk Cho[1], Chang Sung June, Hang Yeon Weon, Jeong Sik Park, Sun Gyu Choi, Jong Chun Cheong and Jae Mo Sung[2]

Applied Microbiology Division, National Institute of Agricultural Science and Technology, R.D.A., Suwon 441-707, Korea
[1]Agriproduct Science Division, National Institute of Agricultural Science and Technology, R.D.A., Suwon 441-707, Korea
[2]Department of Environmental Biology, Kangwon National University, Chunchon 200-701, Korea

(Received November 29, 2004)

This study investigated the biochemical changes of abnormal fruiting bodies grown under artificial environmental conditions in *P. ostreatus*. Abnormal mushroom growth during cultivation damages the production of good quality mushroom. This study showed that different environmental conditions produced morphological changes in the fruiting bodies of *P. ostreatus*. The fruiting bodies with morphological changes were collected and examined for differences in biochemical properties, enzyme activities, and carbohydrates composition. The enzyme activities assay showed that glucanase and chitinase activities decreased when the temperature was below or above the optimum cultivation temperature for *P. ostreatus*. The biochemical compositions of the abnormal mushroom were significantly different from the normal fruiting bodies. It was suggested that the changes in the biochemical composition of abnormal mushroom were caused by the unfavorable environmental conditions during mushroom cultivation.

KEYWORDS: Abnormal mushroom, Amino acid, Carbohydrate, Enzyme, Fatty acid, *Pleurotus ostreatus*

Pleurotus ostreatus belongs to white rot fungi and is the prime commercial mushroom in Korea (Sanchez, 2004; Jhune *et al.*, 2000; Mansur *et al.*, 2003). It has been cultivated for many years since artificial cultivation methods were developed in the 1980's (Cha *et al.*, 1989). The demand for this mushroom has been increasing every year. Despite the increase in cultivation areas, the yields and quality are rather limited because of irregular environmental conditions and bacterial diseases in mushroom cultivation houses. There are no definite control methods yet to protect the cultivated oyster mushroom from the green mold disease (Jhune *et al.*, 2003, 2004). Waterlogging often occurs in oyster mushroom beds causing the development of bacterial diseases (Oh *et al.*, 2000). Although these diseases can be reduced by agricultural chemicals by arresting and preventing the pathogens, they cannot be completely controlled. Nevertheless, many abnormal fruiting bodies that grow during cultivation, are caused not by disease but by physiological disorders brought about by unfavorable environments for mushroom growth. Jang *et al.* (2003) reported that *P. ostreatus* cultivated under 20.5°C and 93.2% relative humidity (RH) produced a depressed or swollen cap center. Kinukawa and Takamatsu (1986) reported that in experiments on *P. ostreatus* treated at 6,000 ppm CO_2 developed abnormal fruiting bodies. Therefore, this experiment aimed to determine the factors causing changes in enzyme activities and compositions of abnormal fruiting bodies of *Pleurotus ostreatus* grown under artificial environmental conditions.

*Corresponding author <E-mail: gabriel@rda.go.kr>

Materials and Methods

Strain and sample analysis. Four different types of malformed or abnormal types of mushroom, *P. ostreatus*, ASI 2180, were investigated: (1) Normal shape (NS), typical form of ASI 2180 and with dark gray cap; (2) Drum-stick shape (DSS), with plump stipe and thick dark gray cap; (3) Elongated stipe shape (ESS), with long stipe and small cap; and (4) Trumpet like shape (TLS), with an overall shape like a trumpet and an upright margined gray cap (Fig. 1).

The samples were originated from the farm located in Youngweol, Kangwondo, which were collected by the National Institute of Agricultural Science and Technology, RDA. They were grown in different cultivation houses with different temperatures and relative humidity (Table 1). NS samples grew in 14.3°C and 83.3% RH; DSS in 10.2°C and 53% RH; ESS in 20.5°C and 93.2% RH; and TLS in 24.1°C and 67.6% RH. The changes in enzyme activities and compositions of the four types of abnormal mushrooms were analyzed.

Preparation of crude enzyme and assay. The fruiting body powders of *P. ostreatus* were put into cold, 10-fold (V/W) 1 mM acetate buffer (pH 4.2), and stirred well in a blender at high speed for a total of 5 min (1 min of blending with 2-min intervals on ice). The resultant suspension

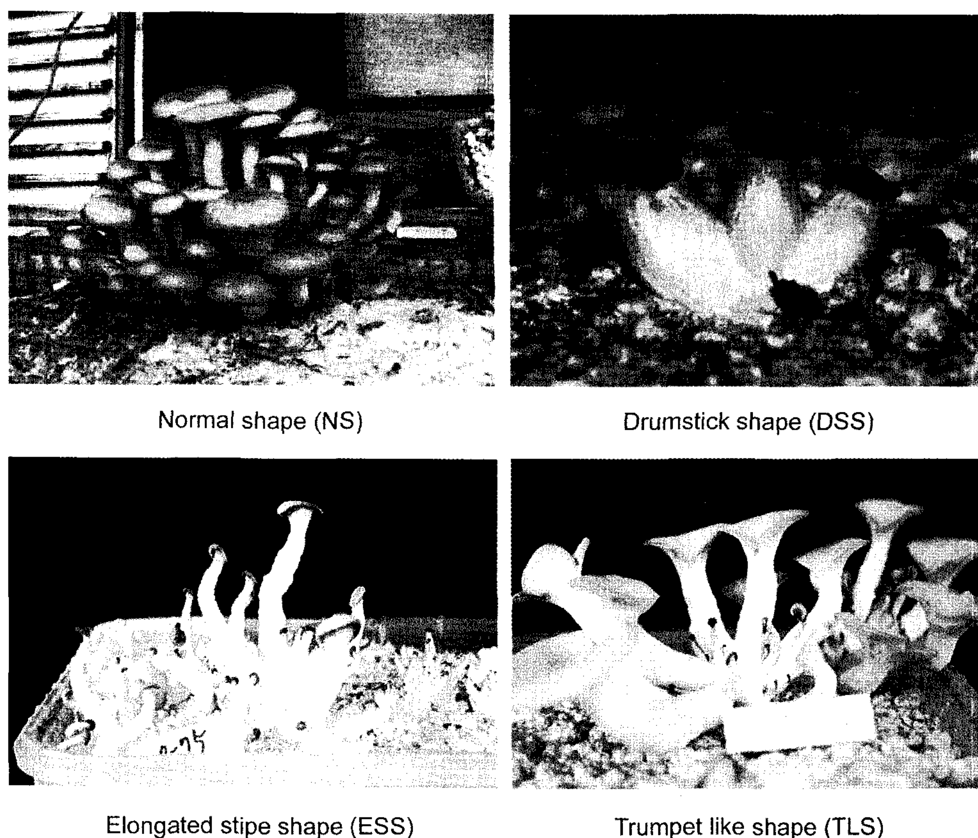

Fig. 1. The normal and malformed mushrooms used in this study.

Table 1. Environmental conditions and description of fruiting bodies used in this study

Code	Average temperature (°C)	Relative humidity (%)	Source	Descriptions
NS	14.3	83.3	NIAST*	Normal shape
DSS	10.2	53.0	"	Drumstick shape
ESS	20.5	93.2	"	Elongated stipe shape
TLS	24.1	67.6	Youngweol	Trumpet like shape

*The National Institute of Agricultural Science and Technology.

was centrifuged and the supernatant fraction was used as a crude enzyme. The substrates, 0.25% Laminarin and 0.25% colloidal chitin were used for detecting β-1,3-glucanase and chitinase, respectively (Tokimoto *et al.*, 1987). Glucanase activity, as indicated by the amount of reducing sugars released by the enzyme, was measured by the Somogyi-Nelson method (Somogyi, 1952). Chitinase activity, as indicated by the amount of N-acetylglucosamine, was measured by the Morgan-Elson method (Reissig *et al.*, 1955). Both methods were done through spectrophotometry by following changes in optical density at 395 nm and 500 nm, respectively. One unit of activity was defined as one • mol of released substance/min/mg protein. Protein content of the crude enzyme was measured by Lowrys method (1951).

Carbohydrate analysis of fruiting body. Total sugar content was measured by phenol-sulfuric acid method using D-glucose (Sigma) as the standard. Uronic acid content was measured by meta-hydroxydiphenyl reagent using D-glucuronic acid (Sigma) as the standard. The monosaccharide component was identified by placing 2 mg of each polysaccharide in 1 *ml* of 2 M trifluroacetic acid (TFA) and heated for 1 h at 121°C. After the acid was removed by repeated evaporation, the monosaccharide was hydrolyzed to sugar alditol acetate by reduction using sodium borohydride, and by acetylation using pyridine and acetic anhydrous. The sugar alditol acetates were analyzed by gas chromatography (HP 5890,USA) with sp-2380 fused silica capillary column (• 0.32 mm x 30 m, 0.2 μm film, Supelco, Bellefonte, BA). The column temperature program was set at 230°C for the first 5 min, then at 10°C/min up to 270°C, and held for 10 min. The temperature of injector and detector was held at 300°C. The flame-ionization detector (FID) was used as a detector.

Amino acid analysis of fruiting body. A 1-mg protein sample was dissolved in 1 *ml* distilled water. Aliquots of 0.5~1 μg sample (about 200 pmol protein) were taken from the sample solution and dried under vacuum. Dried sample was treated with constant boiling HCl at 100°C for 24 h. HCl was removed by repeated evaporation to dryness at 35°C. The remaining HCl was removed by adding MeOH : Water : trimethylamine (2 : 2 : 1, v/v) and the hydrolyzate was dried completely under vacuum. Analysis of free amino acids was done by derivatization with phenylisothiocyanate and analyzed with Pico-Tag column (3.9 × 300 nm, Waters, USA). The amino acid was identified by comparing the retention time and the amount, which was determined by peak heights, with those of amino acid standard (Pierce, 2.5• moles/*ml*).

Fatty acid analysis of fruiting body. A 100-mg sample was weighed and lipid was extracted by the modified Folch's method (Hamilton and Hamilton, 1992). The lipid was methylated by BF3-methanol to form fatty acid methyl esters (FAMEs). The 100 mg/*ml* FAMEs were analyzed by gas chromatography (GC) using capillary column, SP-2560 (100 m × 0.25 mm ID, 0.20 μm film, Supelco co.), with a helium carrier gas flow rate of 10 cm/sec, in HP Model 5890 with flame ionization detector (FID), split-splitless injector. The oven temperature was set at 140°C for the first 5 min, then at 4°C/min up to 240°C held for 15 min. The temperature of injector and detector was held at 260°C.

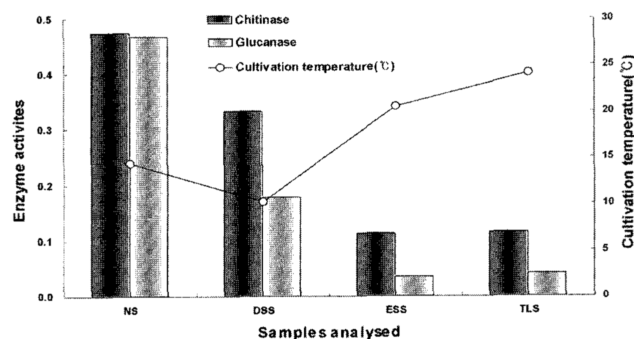

Fig. 2. Enzyme activities of *P. ostreatus* on the different cultivation temperature. NS: Normal shape, DSS: Drumstick shape, ESS: Elongated-stipe shape, TLS: Trumpet-like shape.

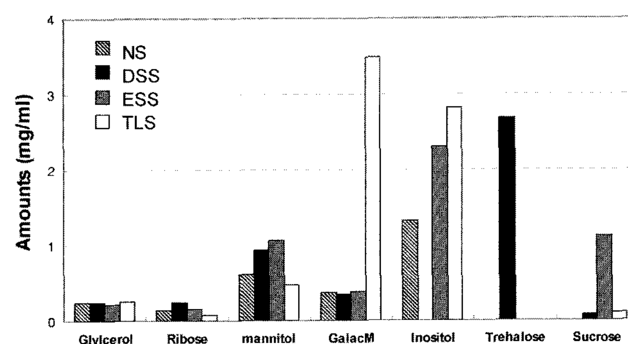

Fig. 3. The saccharides contents of the various shapes of fruiting bodies of *P. osreatus*.

Results and Discussion

Enzyme activities. Glucanase and chitinase activities were assayed to investigate their difference among the samples. Enzyme activities were higher in the normal type than in the abnormal types. Among the abnormal types, enzyme activity of drumstick type was higher. According to Jang *et al.* (2003), the optimum temperature range for cultivating *P. ostreatus* is 13~16°C. The average temperature (14.3°C) from where the normal samples were obtained matched the optimum temperature. Temperatures higher or lower than the optimum temperature caused a decrease in glucanase and chitinase activities, as indicated in Fig. 2. The decrease in enzyme acivities, particularly β-1,3-glucanase and chitinase, were thought to degrade the cell walls because of lack of β-1,3-glucan and chitin, the main structural components of cell walls (Tokimoto *et al.*, 1987), thus, the resulting malformed fruiting bodies.

Sugar contents. The saccharides contents varied among the various shapes of *P. ostreatus* fruiting bodies. Although relatively higher in DSS and ESS, mannitol content was generally similar in all the different shapes since mannitol metabolism plays a major role in the development of mushroom fruiting bodies (Kulkarni, 1990). Trehalose was higher in DSS. Trehalose and mannitol are major soluble sugars in mushroom fruiting bodies (Harada *et al.*, 2004). The glycogen and trehalose serve as storage of carbohydrates, which are degraded under specific circumstances, e.g. stress or fructification. Some evidences showed that glycogen and trehalose supply a significant proportion of the carbon for growth of fruiting bodies (Wannet *et al.*, 1998). Wakita (1958) reported that trehalose disappeared from the *Flammulina* mycelium after the fruiting bodies started to grow. Galactosamine and inositol were higher in TLS. The inositol content of abnormal fruiting body TLS, with 2.754 mg g^{-1}, was higher than that of the normal fruiting body, with 1.315 mg g^{-1}. Inositol has been shown to alter other membrane associated phenomena such as temperature tolerance (Hayashi *et al.*, 1978) and osmotic relations (Atkinson *et al.*, 1977). It was suggested that the significant difference in inositol as well as trehalose contents between malformed and normal fruiting body was due to the different cultivation conditions during development of fruiting body.

Free amino acids contents. The malformed mushrooms included all kinds of amino acids like the normal mush-

Table 3. Free amino acids contents of normal and abnormal fruiting bodies

Amino acid	Content (mg g^{-1} dry wt.)			
	NS	DSS	ESS	TLS
L-Aspartic acid	3.012	0.782	1.025	1.569
L-Glutamic acid	2.400	tr	tr	tr
L-Serine	2.185	0.534	0.598	0.831
Glycine	6.789	0.211	0.244	0.199
L-Histidine	3.214	tr	tr	tr
L-Arginine	3.841	0.156	0.145	0.456
L-Threonine	3.322	tr	tr	tr
L-Alanine	3.321	tr	0.111	1.123
Proline	1.711	tr	tr	tr
L-Valine	1.444	0.245	0.233	0.201
L-Methionine	1.031	0.201	tr	0.214
Cysteine	0.944	tr	tr	0.132
L-Leucine	tr	tr	0.111	tr
L-Phenylalanine	0.801	0.522	0.742	0.698
L-Lysine	0.741	0.537	0.541	0.522

NS: Normal shape, DSS: Drum stick shape, ESS: Elongated stipe shape, TLS: Trumpet like shape, tr: trace.

room, but the contents levels were much lower than the normal mushroom (Table 3). It was evident that the unfavorable environmental conditions caused physiological disorders in the fruiting bodies. In TLS, it is possible that the amino acids of the fruiting bodies have been rapidly used up for the spore formation. In DSS, the flow of nutrients from the medium to the hyphae might have been prevented because of cell damage.

Fatty acid contents. The fatty acid analysis of the fruiting bodies showed that linoleic acid ($C^{18:2}$) was high in all the fruiting bodies. However, palmitic acid was not detected in ESS and TLS mushrooms. Alterthum and Rose (1973) reported that in *Saccharomyces cerevisia*,

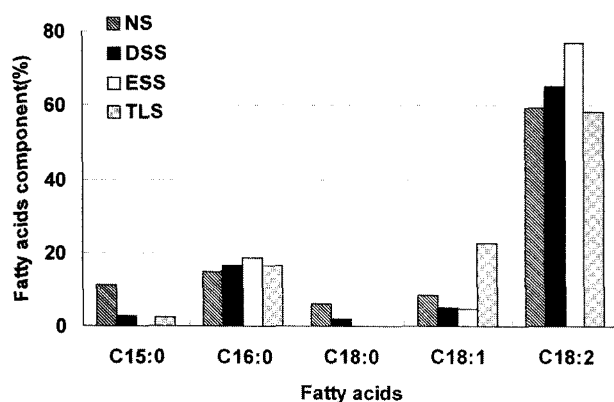

Fig. 4. Fatty acid composition (%) of the various types of fruiting bodies. 15 : 0, pentadecanoic acid; 16 : 0, palmitic acid; 18 : 0, stearic acid; 18 : 1, octadecanoic acid; 18 : 2, linoleic acid.

linoleic acid was required as a growth factor under anaerobic conditions, but not under aerobic conditions. Other external environmental factors such as nutrition also played a selective role as growth factor in mushroom growth.

References

Altherthum, F. and Rose, A. H. 1973. Osmotic lysis of spheroplasts from *Saccharomyces cerevisia* grown anaerobically in media containing different unsaturated fatty acids. *J. Gen. Microbiol.* **77**: 371-382.

Atkinson, K. D., Kolat, A. I. and Henry, S. A. 1977. Osmotic imbalance in inositol starved sphaeroplasts of *Saccharomyces cerevisiae. J. Bacteriol.* **132**: 806-807.

Cha, D. Y., You, C. H. and Kim, K. P. 1989. New technology of mushroom cultivation. Sangrok press. Pp. 1-19.

Hamilton, R. J. and Hamilton, S. 1992. Lipid analysis. Oxford University Press. 22-24, 47-48.

Harada, A., Gisusi, S., Yoneyama, S. and Aoyama, M. 2004. Effects of strain and cultivation medium on the chemical composition of the taste components in fruit body of *Hypsizygus marmoreus. Food Chem.* **84**(2): 265-270.

Hayashi, E., Hasegawa, R. and Tomita, T. 1978. The fluctuation of various enzyme activities due to myo inositol deficiency in *Saccharomyces carlsbergensis. Biochem. Biophys. Acta* **540**: 231-237.

Jang, K. Y., Jhune, C. S., Park, J. S., Cho, S. M., Weon, H. Y., Cheong, J. C., Choi, S. G. and Sung, J. M. 2003. Characterization of fruitbody morphology on various environmental conditions in *Pleurotus ostreatus. Mycobiology* **31**(3): 145-150.

Jhune, C. S., Jang, K. Y., Kong, W. S., Cho, Y. H., Do, E. S. and Park, S. B. 2003. Additive effect of tobacco waste in substrates on yield and mycelial growth of oyster mushroom, *Pleurotus* spp. and *Trichoderma* disease. *J. Mushroom Sci. Product.* **1**(1): 48-53.

_____, Kim, G. P. and Shin, C. W. 2000. Effect of rice bran added at spawn-making on the cultivation of oyster mushroom, *Pleurotus* spp. *Kor. J. Mycol.* **28**(1) :1-5.

_____, Kong, W. S., Jang, K. Y., Yoo, Y. B., Do, E. S. and Chun, S. C. 2004. Effect of $CaCO_3$ treatment on cultivation of oyster mushroom. *J. Mushroom Sci. Product.* **2**(2): 69-75.

Kinukawa, K. and Takamatsu, Y. 1986. Effect of concentrated carbon dioxide on the fruiting cultivated basidiomycetes (I). *Trans. mycol. Soc. Japan* **27**: 327-340.

Kulkarni, R. K. 1990. Mannitol metabolism in *Lentinus edodes*, the shiitake mushroom. *Appl. Environ. Microbiol.* **56**(1): 250-253.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-273.

Mansur, M., Arias, M. E., Copa-Patino, J. L., Flardh, M. and Gonzalez, A. E. 2003. The white-rot fungus *Pleurotus ostreatus* secretes laccase isozymes with different substrate specificities. *Mycologia* **95**: 1013-1020.

Ministry of Agriculture & Forestry. 2003. The actual production of industrial crop. Pp. 8-9.

Oh, S. J., Kim, H. K., Kim, H. K. and Fermor, T. R. 2000. Effect of sodium hypochlorite for controlling bacterial blotch on *Pleurotus ostreatus. Mycobiology* **28**(3): 123-123.

Reissig, J. L., Strominger, J. L. and Leloir, L. F. 1955. A modi-

fied colorimetric method for the estimation of N-acetylamino sugars. *J. Biol. Chem.* **217**: 959-966.

Sanchez, C. 2004. Modern aspects of mushroom culture technology. *Appl. Microbiol. Biotechnol.* **64**: 756-762.

Somogyi, M. 1952. Notes on sugar determination. *J. Biol. Chem.* **195**: 19-23.

Tokimoto, K., Fukuda, M., Kishomoto, H. and Koshitani, H. 1987. Activities of enzymes in bedlogs of *Lentinus edodes* during body development. *Rept. Tottori Mycol. Inst.* **25**: 24-35

Wakita, S. 1958. Biochemical studies on *Collybia velutipes*. Part IV. Relation between the growth and the fructification of fungus. *J. Agric. Chem. Soc. Jap.* **32**: 562-566.

Wannet, W. J. B., Camp, H. J. M. O., Wisselink, H. W., Drift, C. V. D., Van Griensven, L. J. L. D. and Vogels, G. D. 1998. Purification and characterization of trehalose phosphorylase from the commercial mushroom *Agaricus bisporus*. *Biochimica et Biophysica Acta* **1425**: 177-188.