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https://doi.org/10.5109/24034

出版情報：九州大学大学院農学研究院紀要. 38 (1/2), pp.19-33, 1993-12. Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., 38(1・2), 19-33 (1993)

Sawdust-Based Cultivation and Changes of the Culture Mature Degree of the Edible Mushroom, *Pleurotus abalonus*

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(Received April 6, 1993)

The effects of physical and chemical agents on grown of ***Pleurotus abalonus,*** and change in mature degree of sawdust-based cultures in relationship to fruiting capabilities were studied. The optimal temperature and moisture content were somewhat high. Exposure of the culture under the light resulted in earlier initiation of fruit body. Laminarinase activity increased rapidly in the fruiting stage. FDA hydrolytic activity was influenced by temperature and light. Ergosterol content was correlated with production quantity of fruit body.

INTRODUCTION

Pleurotus ***abalonus*** is an edible mushroom that is made part of an increase in diversification in agriculture in Japan recently. The commercially cultivated mushroom ***P.*** *abalonus* is grown on the sawdust mixed with various ingredients (Fig. 1). Little is known about the sawdust-based cultivation of *P. abalonus.* The initiation of sporophores in edible mushroom is often manipulated by a combination of

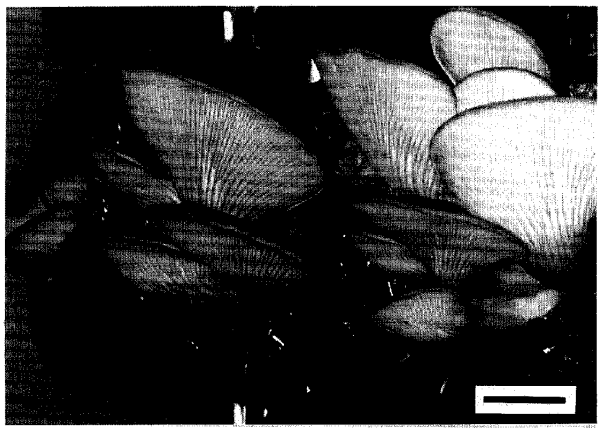

Fig. 1. Fruit bodies of ***P. abalonus*** cultivated on the sawdust-based cultures. Bar=3 cm

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environmental factors such as light, relative humidity and temperature (Flegg and Wood, 1985). In also the case of *P. abalonus,* a decrease in environmental temperature and a increase in relative humidity carried out. It is essential for increase the yield of fruit body of cultivated mushrooms that the manipulation is performed at the mature stage (Yamanaka, 1991).

The present study was undertaken to find the effects of physical and chemical agents such as temperature, composition of media, moisture content and pH on the grown of *P. abalonus,* and on the change of enzyme activity and ergosterol content. Correlation of those parameters with production of fruit body has been studied as well.

MATERIALS AND METHODS

Organism

Strains isolated from fruit bodies of *P. abalonus* Han. Chen et Chang were used in this study. The stock cultures were maintained on a potato dextrose agar (PDA, Difco) medium. A 7.5 mm diameter plug from agar plate was used as inoculum for all experiments.

Growth characterization

Media

P. abalonus was grown on five media which are given in Table 2 at 25°C in the dark. The colony diameter on the PDA medium was measured after 13 days of incubation. Brightness measurement of a culture surface was done with a photoelectric colorimeter Minolta CR-200 (Ohga, 1992a).

Temperature

Diameter of colonies on the PDA medium in 90 mm Petri dishes at different temperature was measured after 7 days of incubation.

Initial pH

The pH of the liquid potato dextrose medium (PD) was adjusted by 0.1 N HCl or 0.1 N NaOH. The experiments were conducted in a 100 ml Erlenmeyer flasks containing 20 ml medium. The medium was incubated at 25°C for 122 days and dry weight of mycelium were determined.

Moisture content

Growth studies were conducted in a 90 mm Petri dish filled with beech (*Fagus crenata*) sawdust supplemented with 10 % (dry weight basis) wheat bran. Thirty grams of the substrate were autoclaved at 121°C for 20 min, and inoculated with a 7.5 mm diameter plug. The cultures were incubated at 25°C for 16 days and the colony diameter was measured.

Effect of sawdust extracts

Beech and sugi (*Cryptomeria japonica*) were used in this study. Sapwood and heartwood of sugi sawdust (fresh weight 500 g) were refluxed with 11 deionized water

or methanol for 2h. Each evaporated extracts were dissolved in 100 ml deionized water or 50 ml methanol. One hundred gram of beech sawdust, each charged with 10 or 20 ml of solvent-dissolved sample, were dried at 90°C and supplemented with 10 % wheat bran. Moisture content was adjusted to 65 % with deionized water. After autoclaving at 121°C for 20 min, the medium was inoculated with spawn and incubated at 25°C for 13 days. Mycelial growth was measured with their colony diameter.

The extract from 2 g of sawdust was dissolved in 0.33 ml methanol and resuspended in 20 ml PD medium. The inoculated liquid cultures were grown in 100 ml Erlenmyer flasks at 25°C for 20 days and dry weight of their mycelium was determined.

Sawdust-based cultivation

Culture procedure

Experimental scale

Fungi were grown on the beech sawdust substrate supplemented with 10 % wheat bran and 5% corn powder. A preparation consisted of **15 g** of dry substrate in a test tube (2.5 cm ID X **20** cm) moistened 35 ml of deionized water. The preparations were sealed with silicon plugs, and autoclaved at 121" C for 20 min, then cooled prior to inoculation with a 7.5 mm diameter plug. The diagram of incubation conditions is illustrated in Fig. 2. The cultures were incubated at 25°C and 30°C in the dark for 55

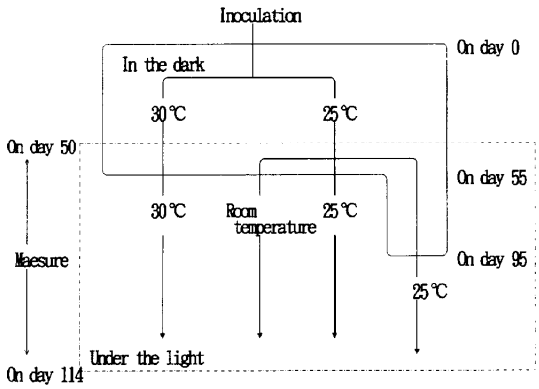

Fig. 2. Diagram of incubation condition on the sawdust-based cultures.

days. After the surfaces of the media were covered fully with mycelium, a part of them was exposed under the light. The remainder was further cultivated in the dark until the day 95.

Large industrial scale

Substrate was filled in a polypropylene bag (12 cm ID X **20** cm), and sterilized by autoclaving at 121°C for 3 h, then inoculated with solid spawn of 10 g. They were incubated at 25" C under the continuous light illumination (500 - 1000 lux). Relative humidity was maintained SO-85 % with an atomizer, Akimist (Ikeuchi Co.>.

Estimation of culture mature degree

Preparation of enzyme extracts

Samples of mycelium-colonized cultures (5g fresh weight) were taken from different positions. The samples were homogenized in cold, 20 ml of 0.1 N acetate-acetic acid buffer (pH 5.3) with 2.5 g polyvinylpyrrolidone in a Waring blender at high speed for a total 4 min (20 sec blending with 15 min intervals). The resultant suspensions were centrifuged at 14,000 rpm for 10 min at 0" C. The supernatant was used for the assay of enzyme activities.

Enzyme assay

All enzyme activities were measured with different substrates (Table 1). Cellulase and laminarinase activities were measured by a modified method of Samezima (1990). To 0.25 % carboxymethylcellulose or laminarin solution containing 0.1 N acetate-acetic acid buffer, pH 5.2 (1 ml), was added 0.1 ml of enzyme solution at 37" C. After incubation for 60 min, the reaction was stopped by the addition of 0.5 ml of 0.05 N NaOH. Carboxymethylcellulase and laminarinase were assayed by determining the amounts of reducing sugars liberated from the several substrates. Reducing sugars were measured by Nelson-Somogyi procedure (1952). Increased absorbance at 660 nm were measured by using n-glucose as a standard.

The acid and neutral protease activities were measured based on the method of Ohga (1992b) as follows. To 3 ml of 0.5 % substrate solution containing Mcilvain buffer at each optimum pH added 0.3 ml of enzyme solution at 30°C, and incubated for 20 min. The reaction was stopped by the addition of 0.6 ml of 48 % trichloro-acetic acid, followed by filtration. Amino acid were determined by Lowry procedure. Tyrosine was used as a standard.

Laccase activity was measured at 30°C in a mixture containing 2.0 ml of 0.1 N acetate-acetic acid buffer (pH 5.31, 0.2 ml of 0.5 mM syringaldazin in ethanol, and 0.4 ml of enzyme solution (Samezima, 1990). A change in absorbance at 525 nm of 0.001 per minute was calculated as one unit of enzyme activity.

Determination of fluorescein diacetate (FDA) hydrosis.

Hydrolytic activity was measured by using a modified Schnurer-Rosswall method (1982). FDA was dissolved in acetone (40 μ g/ml). The amount of FDA hydrolyzed was measured as absorbance at 490 nm. The sample was added with FDA (final concentration, 10 μg/ml) to 60 mM sodium phosphate buffer, pH 7.6, and the mixture

Table 1. Substrates and techniques used the enzyme assay.

Probable enzymes	Assay substrates	Reaction conditoins
Cellulase	0.25% CMC-Na	37°C,60min, pH5.3
Laminarinase	0.25% Laminarin	37°C,60min, pH5.3
Acid protease	0.5% Hemoglobin	30°C, 20min, pH3.0
Neutral protease	0.5 % Casein	30°C, 20min, pH7.0
Laccase	0.1mM Syringaldazine	30°C,10min, pH5.3
FDA hydrase	24 μM FDA	30°C, 30min, pH7.6

was incubated at 30°C. A change in absorbance of 0.001 per minute was defined as one unit of enzyme activity.

Ergosterol extraction and analysis.

The ergosterol determination was performed by a modified method of Desgranges et al. (1991). The saponification of a 3 g sample was carried out with 50 ml methanol, 25 ml ethanol and 10 g KOH. The solution was diluted with 40 ml deionized water and 40 ml hexane and mixed for 20 min. The mixture was centrifuged for 10 min at 10,000 rpm. A 20 ml of the hexan extract was evaporated. The dry residue was dissolved in 5 ml of methanol. The ergosterol was measured by HPLC using a μ Bondaspher 5 μC18-100 Å column (Waters Associates Inc.) and 5 % water in methanol. At a 1.0 ml per min flow rate, ergosterol was eluted at 18 min.

RESULTS AND DISCUSSION

Growth characterization

Media

Mycelial growth was estimated by the colony diameter and the surface brightness. The longest colony diameter was obtained in Czapek* medium (Fig. 3). But maximal density of mycelia was observed in Leonian and minimal in Czapek* judging from the surface brightness (Table 2). Leonian medium might be best composition for the mycelial growth of *P. abalonus*.

Temperature

As shown in Fig. 4, the maximum growth was shown at 25°C except one strain.

Initial pH

Best mycelial growth was obtained on the medium adjusted to initial pH 6.8. At lower initial pH values the growth was limited (Fig. 5). No significant changes in pH values were observed on the liquid media during the vegetative growth of 22 days cultivation.

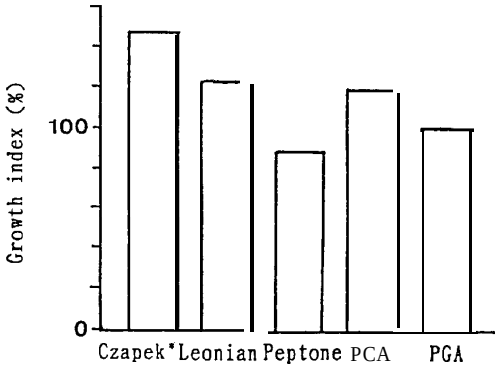

Fig. 3. Effects of various media on growth of mycelium.

Table 2. Compositions of various media and changes in the brightness of the culture surfaces.

	Composition (g/l)	Brightness
Czapek*	sucrose 30 $NaNO_3$ 2.0 K_2HPO_4 1.0 $MgSO_4 \cdot 7H_2O$ 0.5 KCl 0.5 $FeSO_4$ 0.01	11.5
Leonian	dextrose 6.0 malt extracts 6.0 peptone 0.6 KH_2PO_4 1.0 $MgSO_4 \cdot 7HzO$ 0.6	23.0
Peptone	peptone 10 NaCl 5.0	12.5
PCA	extracts of potato (20) and carrot (20)	13.0
PGA	glucose 20 extracts of potato (200)	12.8

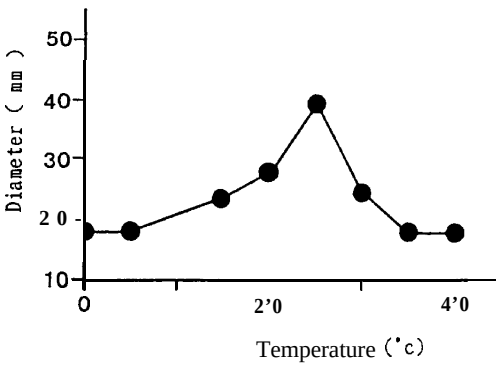

Fig. 4. Influence of temperature on mycelial growth.

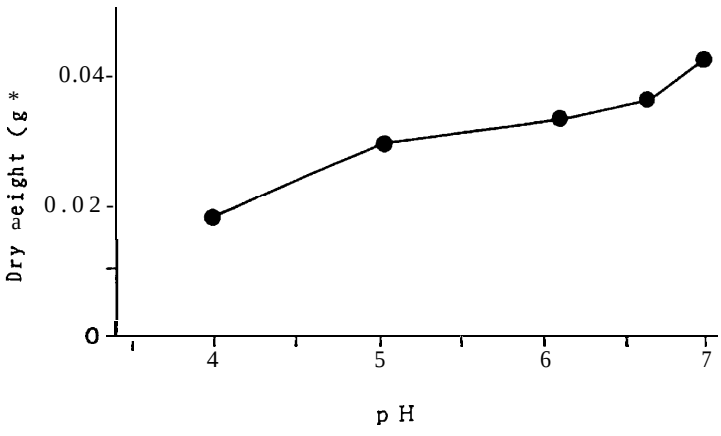

Fig. 5. Influence of initial pH on the growth of mycelium.

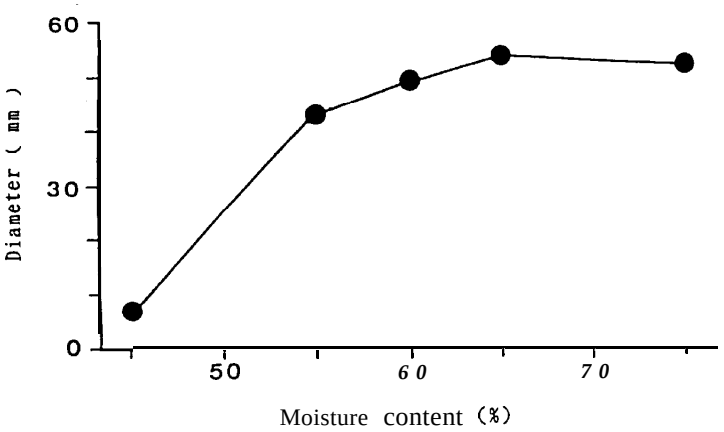

Fig. 6. Effect of moisture content of sawdust-based culture medium on the mycelial growth.

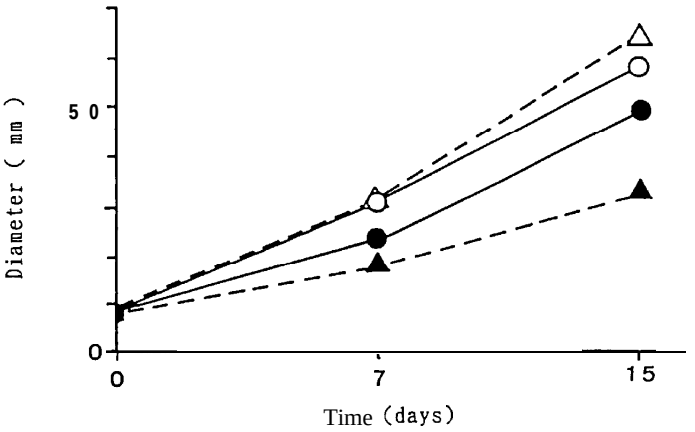

Fig. 7. Growth of mycelium on sawdust-based culture medium.

● : Buna, no addition of wheat-bran.
A: Sapwood of Sugi, no addition of wheat-bran.
○: Buna, wheat-bran ratio 9:1.
△: Sapwood of Sugi, wheat-bran ratio 9:1.

Moisture content

As shown in Fig 6, the mycelial growth took place well within moisture contents 65 to 70 %.

Effect of sawdust extracts

The mycelial growth on different species of wood are shown in Fig. 7. The effects of sawdust extracts on the mycelial growth are given in Figs 8, 9 and 10. The mycelial growth was limited on the culture containing methanol extracts of Sugi heartwood. Heartwood extracts were more inhibitory to the mycelial growth than sapwood

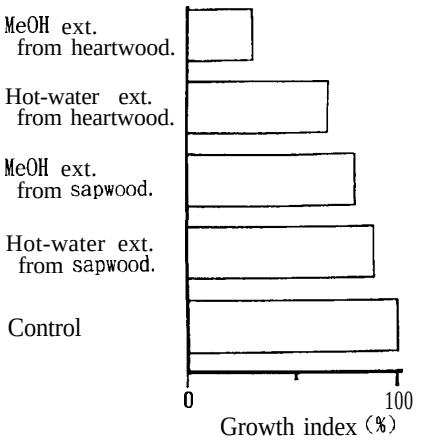

Fig. 8. Effect of extracts from Sugi on growth of mycelium on solid culture media (Buna, wheat-bran ratio 9:1).

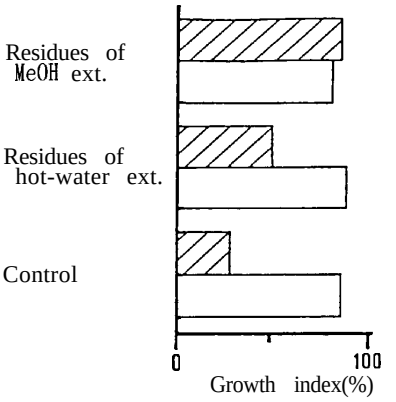

Fig. 9. Effect of residues of extraction on growth of mycelium on solid culture media (Sugi, wheat-bran ratio 9:1).
▨ : sapwood, □ : heartwood

extracts.

Residue of methanol extract from heartwood supported better mycelial growth than the residue of hot-water extracts. No significant differences were observed between Sugi sapwood and the residue of its extraction.

The liquid medium containing the hot-water extracts from Sugi sapwood showed the better growth than any other. Relatively good growth was observed on the medium containing the hot-water extracts from heartwood and the methanol extracts from sapwood. The growth of mycelium was very poor on the medium containing methanol extract from heartwood.

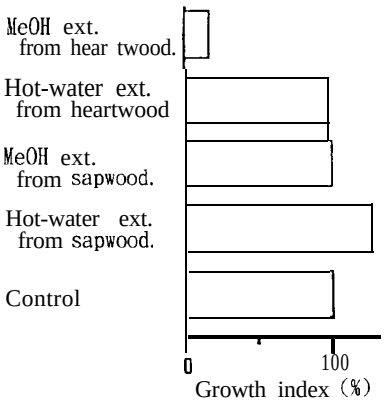

Fig. 10. Effect of extracts on growth of mycelium in liquid culture media (PD).

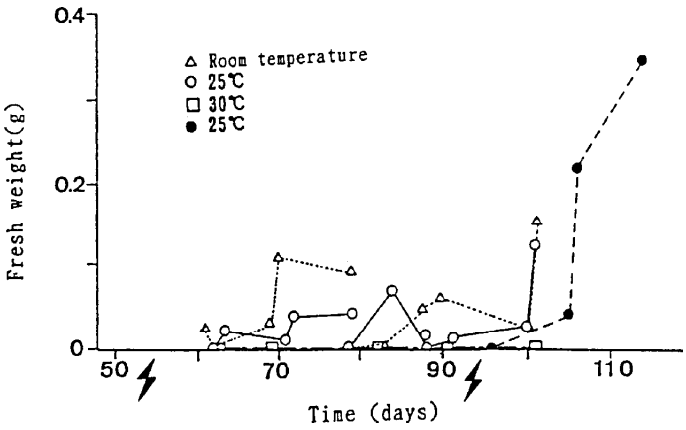

Fig. 11. Influence of temperature and light conditions on the production of fruit bodies.

Legend: ○: were incubated at 25℃, □: at 30℃, △: at room temperature (15-23℃) under the dark until the day 55. ● : were incubated at 25°C under the dark until the day 95.

based cultivation

Experimental-scale

Figure 11 illustrates the fresh weights of fruit bodies produced on the sawdust-based medium. No fruit body was observed on the medium growing at 30°C. The fruit body initiation was recognized on 60-75 days of incubation at optimum temperatures. Then fruit bodies were observed on the 100th day. The cultures incubated at 25°C in the dark until the 95th day after inoculation showed the primordia flushed on the 100th day and the fruit bodies formed on the 105th day. This long term incubation in the dark resulted high quantity production of fruit body.

Enzyme activity

Cellulase

Much higher cellulase activities were recorded for both temperatures (Fig. 12), reaching a maximum of 8,000 U per dry weight on the 30th day and then decreasing to 1,000 U per dry weight on the 50th day. Cellulase activities remained at low levels during the last.65 days of the experiment.

Laminarinase

The rapid increases in laminarinase were found on the fruiting culture incubated in the dark for 95 days (Fig. 13). Whereas, the slight increases in its activity were observed at 25°C and room temperature on about the 100th day, of with fruit body formation quantity was low. Therefore it is suggested that laminarinase activity is correlated with the formation of fruit body.

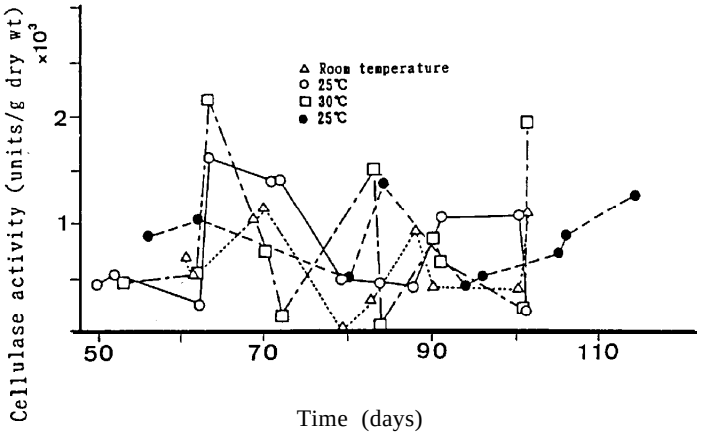

Fig. 12. Changes in cellulase activities on sawdust-based cultures until the fruiting. Note: For symbols refer to Fig. 11.

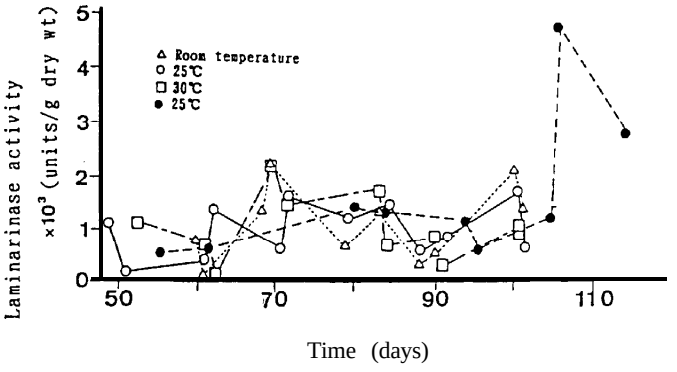

Fig. 13. Changes in laminarinase activities on sawdust-based cultures until the fruiting. Note: For symbols refer to Fig. 11.

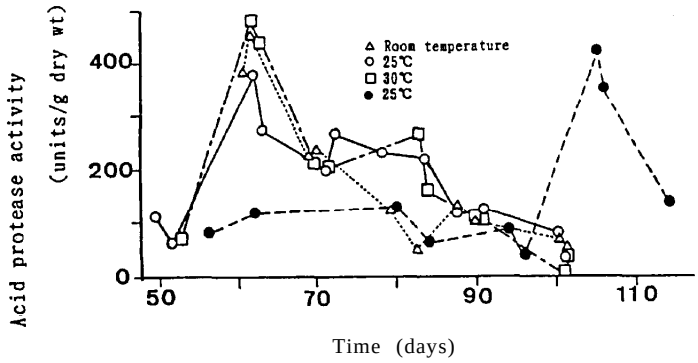

Fig. 14. Changes in acid protease activities on sawdust-based cultures until the fruiting. Note: For symbols refer to Fig. 11.

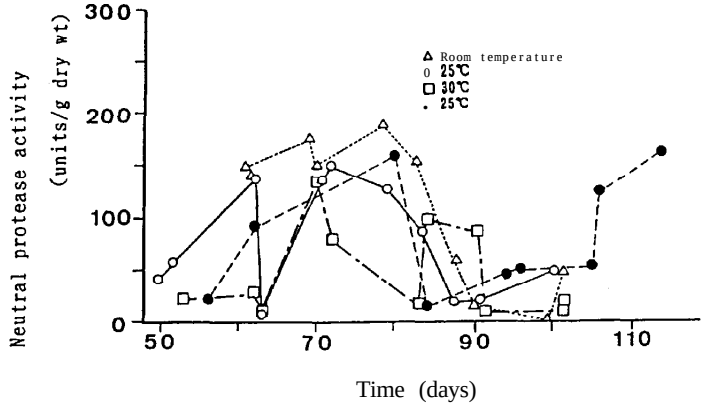

Fig. 15. Changes in neutral protease activities on sawdust-based cultures until the fruiting. Note: For symbols refer to Fig. 11.

Acid protease

Determination of acid protease showed a sharp increase of acid protease activity in the short term incubation in the dark on day 60 just after exposed under the light and then a decrease on day 70 (Fig. 14). In the long term incubation in the dark, acid protease activity increased on day 100. It seems that the increase in acid protease activity was caused by exposed under the light.

Neutral protease

Most of the neutral protease activity was detected between days 70 and 80 (Fig. 15). Activity decreased faster at 30°C than the other temperatures. The production of fruit body may be impeded decrease of neutral protease at 30°C (Terashita et *al.*, 1978).

Laccase

On the 30th day laccase activity was observed, but its activity could not be detected between days 50 and 114 after inoculation.

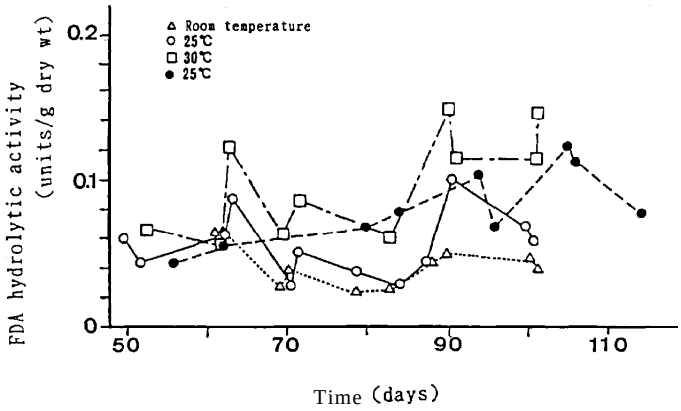

Fig. 16. Changes in FDA hydrolytic activities on sawdust-based cultures until the fruiting. Note: For symbols refer to Fig. 11.

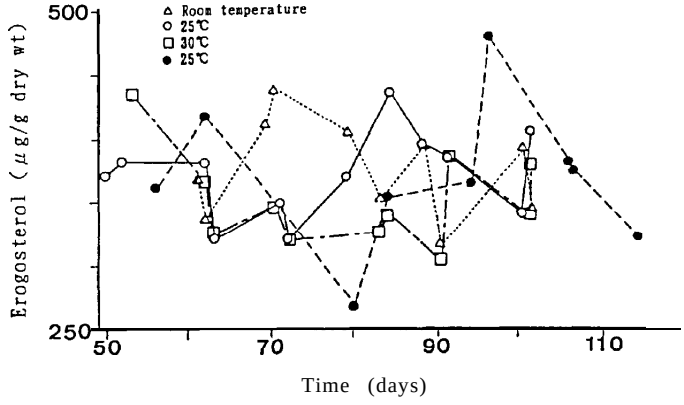

Fig. 17. Changes in ergosterol contents on sawdust-based cultures until the fruiting. Note: For symbols refer to Fig. 11.

FDA hydrase

FDA hydrolysis activity increased slightly until the end of the incubation on the cultures were exposed under the light on the 95th day (Fig. 16). At room temperature and 25" C FDA hydrolysis activity decreased slightly from days 60 to 70 and then increased until day 90. While, its activity gradually increased at 30" C until day 100. FDA hydrolysis activity was used to determine mycelial activity. The timing of fruit body formation may be related to FDA hydrolysis activity leveled off.

Ergosterol

Much higher ergosterol content was showed in long term incubation in the dark.The cultures were incubated at room temperature and 25" C recorded at similar content levels (Fig. 17). At 30°C, ergosterol contents decreased slightly, remaining at low levels during the end of experiment. It is the high ergosterol content that lead to the observed high quantity production of fruit body.

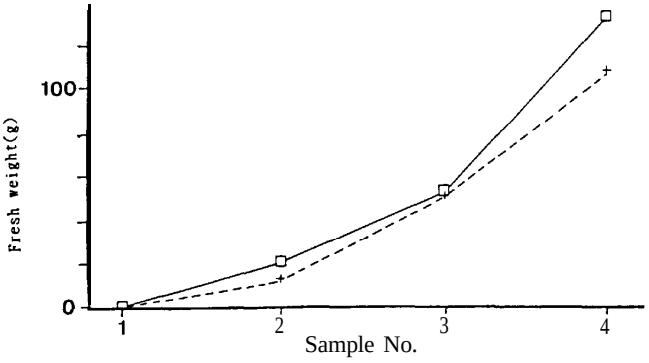

Fig. 18. Production of fruit body on the commercial cultures.
❑ :A +:B

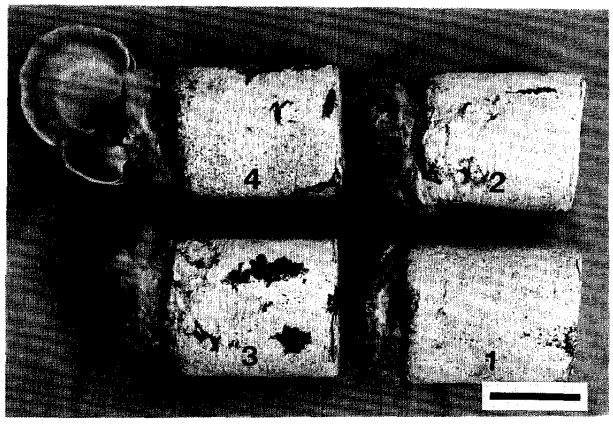

Fig. 19. Four measuring stages of *P.abalonus* substrates.
Bar = 10 cm

Large industrial scale

The vegetative mycelial growth was completely established until 30 days incubation. Cap was removed from the polypropylene bag containing fully colonized substrate. Then fruit body flush occurred spontaneously on the upper site of the substrate after 7 to 10 days. Average production quantity per 2.5 Kg substrate was 130 g for fresh weight.

Some enzyme activities and ergosterol contents were measured on the four stages of fruit body formation (Fig. 18 and 19). As shown in Fig. 20, laminarinase activityincrease was coincided with fruit body expansion. Ergosterol contents increased with fruit body formation and rapidly declined after maturation (Fig. 21). Similar positive correlations were observed for the laminarinase activity or ergosterol contents and fruit body formation showing in the experimental scale tests as described above.

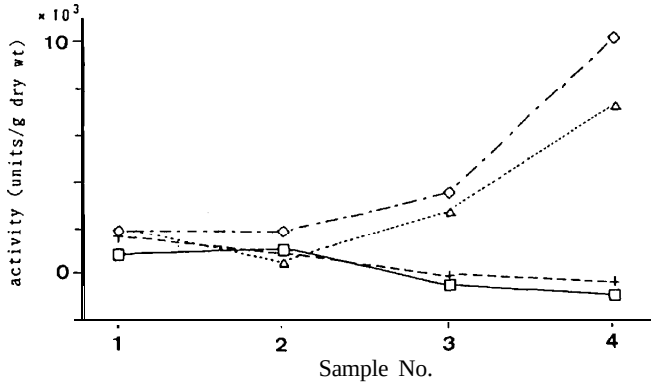

Fig. 20. Changes in cellulase and laminarinase activities on commercial cultures during the fruiting.
□ : A, + : B are cellulase activity.
◇ : A, △ : B are laminarinase activity.

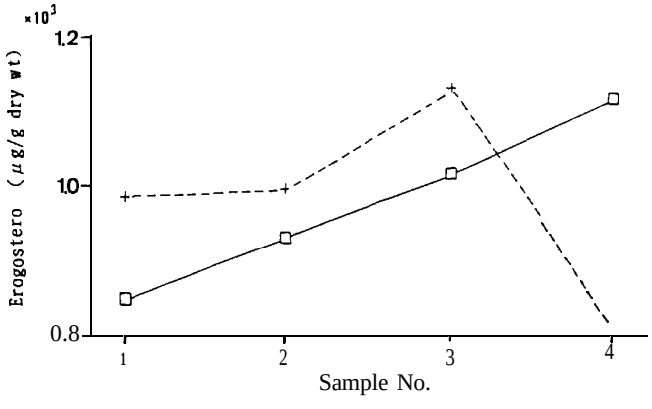

Fig. 21. Changes in ergosterol contents on commercial cultures during the fruiting.
❑ l:A,+:B

ACKNOWLEDGEMENTS

The authors thank Dr. H. Neda, Forestry and Forest Products Research Institute, for providing strain. We also thank professor Y. Kitamoto, Tottori University for valuable suggestions during the experiments.

REFERENCES

Desgranges, C., C. Vergoignan, M. Georges and A. Durand 1991 Biomass estimation in solid state fermentation, *Appl. Microbiol. Biotechnol., 35: 200-205*

Flegg, P. B. and D. A. Wood 1985 Growth and fruiting. *In* "The biology and technology of the

cultivated mushroom", ed. by P. B. Flegg, D. M. Spencer and D. A. Wood, John Wiley & Sons, Chichester, England, pp. 141-177

Lowry, 0. H., N. J. Rosebrough and R. J. Randall 1951 Protein measurement with the Folin phenol reagent. *J. Biol. Chem.,* **193:** 265-275

Ohga, S. 1992a Adaptability of *Lentinus edodes* strains to a sawdust-based cultivating procedure. *Mokuzai Gakkaishi, 38: 301-309*

Ohga, S. 1992b Comparison of extracellular enzyme activities among different strains of *Lentinus edodes* grown on sawdust -based culture in relationship to their fruiting abilities. *Mokuzai Gukkaishi., 38: 310-316*

Samezima, M. 1990 Measurement of enzyme activity. *In* "Methods in Wood Science, Vol II Chemistry", ed. by M. Usuda, H. Mizumachi, K. Iiyama, N. Morohosi and A. Yamaguchi, Chugaisangyo, Tokyo, pp. 342-348 (in Japanese)

Schniirer, J. and T. Rosswall 1982 Fluorescein .diacetate hydrolysis as a measure of total microbial activity in soil and litter. *Appl.* Environ. *Microbiol,* 43: 1256-1261

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

Terashita, T., M. Kono and S. Murao 1978 Effect of pepsin-inhibitor, Streptomyces-PI, on the fruit-body formation of a few basidiomycetes. *Hakkokogaku Kaishi, 56: 175-181* (in Japanese)

Yamanaka, K. 1991 Nutrimental conditions and environmental control on the mushroom cultivation. In "The fundamental science and modern technology ofmushroom", ed. by T. Takemaru *et al.,* Nousonbunkasya, Tokyo, pp. 158-167 (in Japanese)