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## Note

# Changes in low molecular weight carbohydrates composition and chitin throughout the cultivation stages of *Pleurotus ostreatus*

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**Changes in contents of soluble low molecular weight carbohydrates and chitin in a sawdust-rice bran medium during mycelial growth of *Pleurotus ostreatus* in bottle cultivation were examined in relation to fruit-body yield of nine stocks. Glucose, mannitol, inositol, sucrose, and trehalose were detected in cultures after mycelial spreading. No significant correlation was observed between contents of soluble low molecular weight carbohydrate during mycelial growth and the fruit-body yield. Negative correlation was found between trehalose content in post-harvest cultures and the fruit-body yield. Chitin content in cultures decreased in the fruiting stage. Positive correlation was detected between chitin content of fruit-bodies and the decrement of chitin in post-harvest culture caused by fruit-body growth.**

Key Words——bottle cultivation; chitin; *Pleurotus ostreatus*; sawdust culture; soluble low molecular weight carbohydrate.

Hiratake mushroom, *Pleurotus ostreatus* (Jacq.: Fr.) Kummer, is mainly produced by bottle culture using sawdust mixed with nutritional supplements, for example, rice bran, as substrate. The many stocks of this fungus show various characteristics on commercial cultivation. Previous studies on the physiology of *P. ostreatus* focused on nutritional requirements (Hashimoto and Takahashi, 1974), chemical composition of mycelia (Yoshida et al., 1986, 1987), and chemical composition of fruit-bodies during post-harvest (Kazuno and Miura, 1985; Yamashita et al., 1983). However, these studies were conducted in vitro using a single stock. Although it is essential in screening for stocks with high productivity to determine their characteristics on commercial cultivation, no investigation into the differences in physiological characteristics among stocks in relation to their fruiting abilities on bottle cultivation has been reported. In this study, the differences in metabolism of chitin and soluble low molecular weight carbohydrates in culture among nine stocks were examined in relation to fruit-body formation.

Nine stocks of *P. ostreatus* were used for bottle cultivation, as listed in Table 1. Nos.1-4 are from the collections of Nara Prefectural Forest Experiment Station and Nos.5-9 are commercial stocks.

The medium per bottle was composed of sugi (*Cryptomeria japonica* (Linn. fil.) D. Don) sawdust, 84.2 g (dry weight); rice bran, 83.8 g (dry weight); and tap water. The moisture content of the medium was adjusted to 65% on wet basis. Portions of 480 g of the medium

Table 1. Origin of stocks of *Pleurotus ostreatus*.

| No. | Stock | Origin |
|---|---|---|
| 1 | NPO-631 | Nara Prefectural Forest Experiment Station |
| 2 | NPO-636 | Nara Prefectural Forest Experiment Station |
| 3 | NPO-003 | Nara Prefectural Forest Experiment Station |
| 4 | NPO-014 | Nara Prefectural Forest Experiment Station |
| 5 | Kawamura K10 | Kawamurashiki Shukin Kenkyusho, Inc. |
| 6 | Kawamura KH3 | Kawamura Shokuyoukin Kenkyusho, Inc. |
| 7 | Mori M39 | Mori Industry, Inc. |
| 8 | Kinkou Fuyushimeji | Kinkou Shiitake Co. |
| 9 | Hiratake No. 5 | Yamato Mycological Institute |

were dispensed into 800-ml polypropylene bottles, which were plugged with a cap (with non-woven fabric filter). Bottles were autoclaved at 118°C for 12 min, then allowed to cool to room temperature. Each bottle was inoculated with 10–15 g (fresh weight) of spawn which had colonized sawdust basal medium supplemented with rice bran. The inoculated media were incubated at 20±2°C and 75±5% relative humidity (R.H.) in the dark for 22 d. To induce primordia of fruit-bodies, the top surface of the culture was removed (*kinkaki* in Japanese), water was added to the bared surface, and excess water was drained 1 h later. The cultures were then placed in a fruiting room controlled at 15±2°C, 95±5% R.H. and 200 lx continuous illumination by fluorescent lamp. The cultivation process was divided into six stages, defined as follows: stage 0, start of incubation; stage 1, when mycelia spread over the whole culture (18th d after inoculation); stage 2, when the surface of culture was removed (*kinkaki*) for fruiting (22nd d after inoculation); stage 3, when primordia appeared; stage 4, when caps of fruit-bodies grew to 1–2 mm in diam and darkened (young fruit-body); stage 5, when the largest cap grew to ca. 2.5–3.5 cm in diam and was harvested. Days needed from inoculation to stages 3, 4, and 5 varied with stocks. At each stage, two bottles per stock were subjected to chemical analyses. Samples of the culture medium were removed, their fresh weights were measured, then they were lyophilized. Fruit-body samples at the stages 4 and 5 were harvested and also lyophilized. The lyophilized samples were ground with a mill (Heiko Seisakusho, TI-100) into powder and passed through 40 mesh sieve. Dry weights of the cultures and fruit-bodies were determined by drying at 105°C for 48 h. Soluble low molecular weight carbohydrates were extracted from the lyophilized samples with 80% (v/v) ethanol solution at 90°C for 15 min. This treatment was carried out twice. Extract solutions were centrifuged at 3,000×*g* and passed through a filter paper, and the filtrates were neutralized with 0.1 N NaOH and reduced in volume to 50 ml in a rotary evaporator. The solutions were centrifuged at 15,000×*g* for 10 min, and the supernatant was filtered through an Ultra filter (Advantec Toyo, 10,000 molecular weight cut-off). A known quantity of *n*-docosane was added to the filtrate as an internal standard, and trimethylsilyl ester of the mixtures were made by the method of Sweely et al. (1963). TMS derivatives were injected into a gas chromatograph (Shimadzu GC-14A) with a flame ionization detector (FID) at 270°C and a fused-silica capillary column (GL Science, FS-WCOT BONDED, 30 m× 0.32 mm). Helium was used as the carrier gas at a flow rate of 1.44 ml $min^{-1}$. The oven temperature was programmed to rise at 7°C $min^{-1}$ from 150°C to 270°C, then remain at 270°C for 15 min. Peaks were identified by comparison of the retention time with authentic samples, and quantitative analyses were made after calibration with standards against the *n*-docosane internal standard. Chitin was determined by the method of Braid and Line (1981). The lyophilized samples were hydrolyzed in 5 N HCl at 80°C for 20 h. The hydrolyzed samples were filtered (Advantec Toyo No. 5A), then added to glass columns containing Dowex 50W-X8, 100–200 mesh

Table 2. Fruit-body yield in dry weight, and contents of low molecular weight carbohydrates and chitin in fruit-bodies.

| Stage of fruit-body development | Stock No. | g/100 g dry weight[a] | | | | | Fruit-body yield in dry weight (g)[b] |
|---|---|---|---|---|---|---|---|
| | | Glucose | Mannitol | Inositol | Trehalose | Chitin | |
| Young fruit-body (Stage 4) | 1 | 0.15 | 1.75 | 0.89 | 4.50 | 1.74 | 2.7 |
| | 2 | 0.06 | 1.81 | 0.60 | 3.93 | 1.89 | 1.8 |
| | 3 | 0.06 | 2.62 | 0.92 | 6.28 | 1.96 | 1.6 |
| | 4 | <0.01 | 2.21 | 0.57 | 5.25 | 1.90 | 2.2 |
| | 5 | 0.10 | 2.76 | 0.59 | 5.24 | 2.02 | 3.4 |
| | 6 | 0.12 | 2.77 | 0.60 | 6.30 | 1.85 | 4.1 |
| | 7 | 0.06 | 1.60 | 0.63 | 5.17 | 2.00 | 2.1 |
| | 8 | 0.11 | 2.46 | 0.73 | 5.49 | 2.18 | 4.3 |
| | 9 | 0.05 | 1.24 | 0.79 | 8.21 | 1.87 | 3.7 |
| Mature fruit-body (Stage 5) | 1 | 0.17 | 1.44 | 0.81 | 4.32 | 2.26 | 9.9 |
| | 2 | 0.06 | 1.26 | 0.76 | 6.09 | 2.13 | 9.1 |
| | 3 | 0.11 | 1.57 | 1.02 | 10.22 | 2.00 | 8.0 |
| | 4 | 0.11 | 1.26 | 0.38 | 7.61 | 1.91 | 5.8 |
| | 5 | 0.06 | 1.49 | 0.71 | 6.02 | 2.48 | 10.3 |
| | 6 | 0.06 | 2.04 | 0.60 | 7.86 | 2.22 | 10.5 |
| | 7 | <0.01 | 1.57 | 0.79 | 8.32 | 2.14 | 9.3 |
| | 8 | <0.01 | 1.51 | 0.74 | 6.51 | 2.74 | 10.0 |
| | 9 | 0.10 | 1.94 | 0.72 | 6.92 | 2.76 | 8.4 |

a) Data are means of 2 samples.
b) Data are means of 5 samples.

strongly-acid cation-exchange resin. Colorimetric assays were carried out by the method of Tsuji et al. (1969).

Fruit-body yield, contents of soluble low molecular weight carbohydrates and chitin of fruit-bodies during bottle cultivation on sawdust-rice bran medium are shown in Table 2. Significant difference in fruit-body yield among stocks was revealed by ANOVA ($P<0.01$). Fructose, glucose, mannitol, inositol, and trehalose were detected in young fruit-bodies at stage 4 and in mature fruit-bodies at stage 5. The fructose content was too low to be shown (less than 0.01 g/100 g). Trehalose was the major soluble low molecular weight carbohydrate in both young and mature fruit-bodies, and constituted about 60–80% and 64–81% of total soluble low molecular weight carbohydrates, respectively. The chitin contents were 1.7–2.2% in young fruit-bodies and 1.9–2.8% in mature fruit-bodies. The chitin content increased slightly in mature fruit-bodies.

In the culture medium at stage 0, fructose, glucose, sucrose and maltose were present. Fructose and maltose contents were 0.39 and 0.34 g/100 g at stage 0, respectively. Sucrose was the major soluble low molecular weight carbohydrate in substrate. At stage 1, mannitol, inositol, and trehalose were present in the culture with the same sugars detected in medium at stage 0. Fructose and maltose disappeared from the culture after stage 1. Inositol content in the culture during the cultivation was in the range of 0.10–0.45 g/100 g. After stage 1, soluble low molecular weight carbohydrates detected in the culture were similar to those reported by Yoshida et al. (1986) in the composition of sugar and sugar alcohol of the mycelium in liquid culture. This indicated that the soluble low molecular weight carbohydrates of the culture detected after stage 1 were derived from the mycelium in the culture.

Figure 1 shows the changes in contents of soluble low molecular weight carbohydrates in the cultures during bottle cultivation. Total soluble low molecular weight carbohydrates decreased during the cultivation of all stocks except No. 4, in which they increased at stage 1 and then decreased. Glucose content varied among the stocks at stage 1. After stage 1, glucose content changed during the cultivation and decreased at stage 5 for all stocks. In general, trehalose and mannitol are known as translocate carbohydrates in Basidiomycetes and decrease in mycelium during the fruit-body formation (Wessels, 1961). Contents of mannitol and trehalose

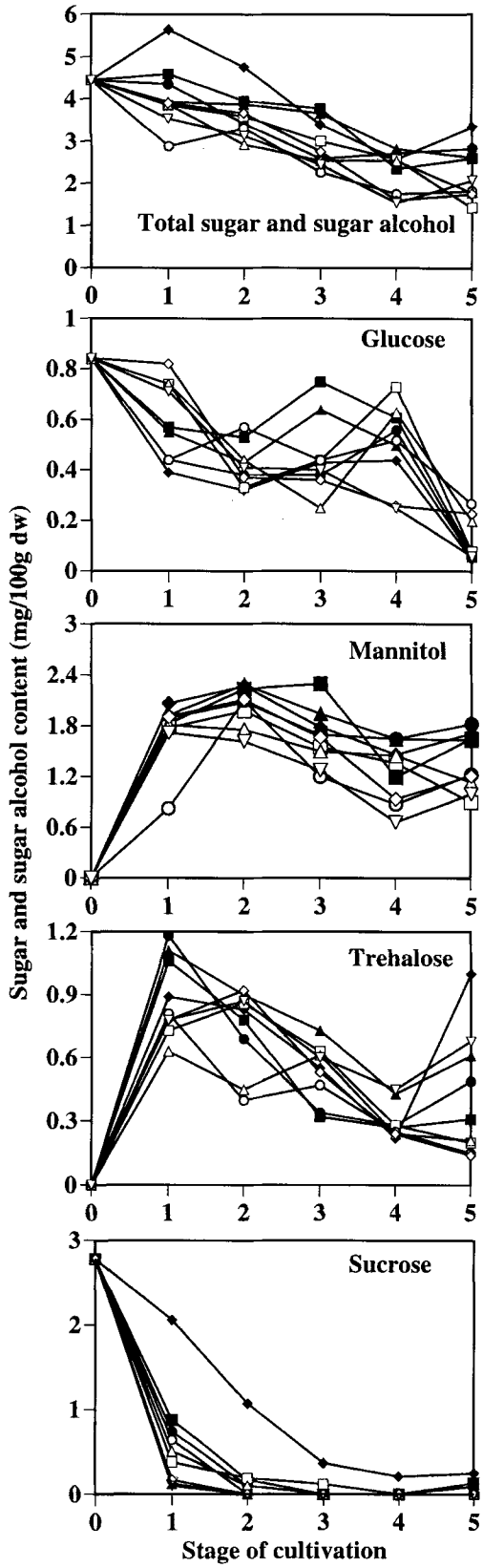


Fig. 1. Changes in the contents of soluble low molecular weight carbohydrates in culture during the bottle cultivation of *P. ostreatus*.
No. 1 (■),No. 2 (●), No. 3 (▲), No. 4 (◆), No. 5 (□), No. 6 (○), No. 7 (△), No. 8 (◇), No. 9 (▽). Stage 0, incubation started; stage 1, 18th d after inoculation; stage 2, removing culture surface (22nd d after inoculation); stage 3, primordia appear (26th–28th d after inoculation); stage 4, pileus 1–2 mm in diam (28th–30th d after inoculation); stage 5, pileus 2.5–3.5 mm in diam, harvesting (30th–33rd d after inoculation).

reached maximum at stages 1 or 2, and then decreased. Sucrose content decreased rapidly at stage 1 and nearly disappeared after stage 2 for all stocks except for No. 4, in which it gradually decreased during the cultivation. In liquid media, mannose, glucose, fructose, maltose, sucrose, and starch were effective carbon sources for mycelial growth of *P. ostreatus* (Hashimoto and Takahashi, 1974). In the sawdust media supplemented with rice bran, sucrose and maltose were found to be a major soluble low molecular weight carbohydrates at the initial stage after the medium was autoclaved. These sugars disappeared as soon as mycelia spread over the medium, and glucose content in the culture decreased in stage 2. Iwahara et al. (1981) reported that extracellular cellulase activity in *P. ostreatus* was low until fruiting started, then rapidly increased as the fruit-body developed following the consumption of soluble glucose on wood meal and rice bran medium. Obatake (1994) demonstrated using 15 stocks of *P. ostreatus* that the activities of $\alpha$-glucosidase, $\beta$-glucosidase and xylanase were low until fruiting began and increased at the fruiting stage on sawdust-rice bran medium. These findings indicate that the mycelial growth of *P. ostreatus* does not require polysaccharides such as cellulose or starch, but soluble glucose and sucrose in the sawdust-rice bran medium during the early stage of the cultivation.

No significant correlation was observed between soluble low molecular weight carbohydrate content at stages 1 to 4 and fruit-body yield. Translocation and consumption of the nutrient in mycelium were caused by development of primordia and growth of fruit-body, but nutrient storage of soluble low molecular weight carbohydrates in the mycelium at the end of incubation did not relate to fruit-body yield. Furthermore, the total content of soluble low molecular weight carbohydrates in the culture after harvesting did not relate to that contained in the fruit-body. However, there was a negative correlation between the content of trehalose in the post-harvest culture and the fruit-body yield (Fig. 2, $r=-0.952$, $P<0.001$).

Changes in the content of chitin in the cultures are shown in Fig. 3. The chitin content gradually increased until stage 4. At stage 5, chitin contents strikingly decreased to 35–93% of those at stage 4 for all stocks except No. 3. Chitin is contained in mycelia and fruit-bodies as the cell wall skeleton, and utilized as a substrate for fruit-body formation in *P. ostreatus* (Yoshida et

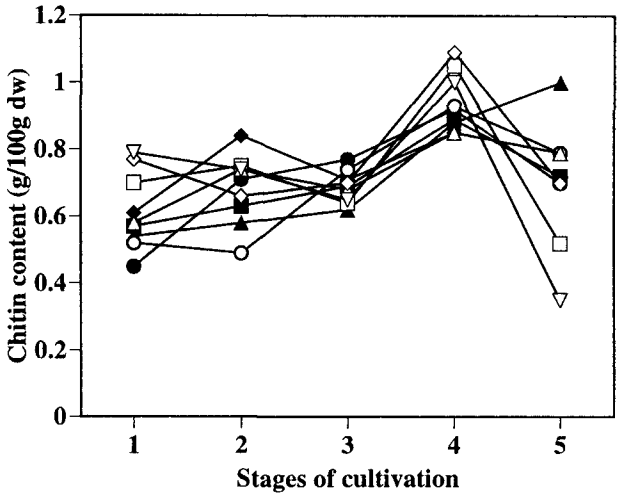


Fig. 3. Changes in content of chitin in cultures during mycelial growth and fruit-body development in the bottle cultivation of *P. ostreatus*.
Symbols are the same as in Fig. 1.

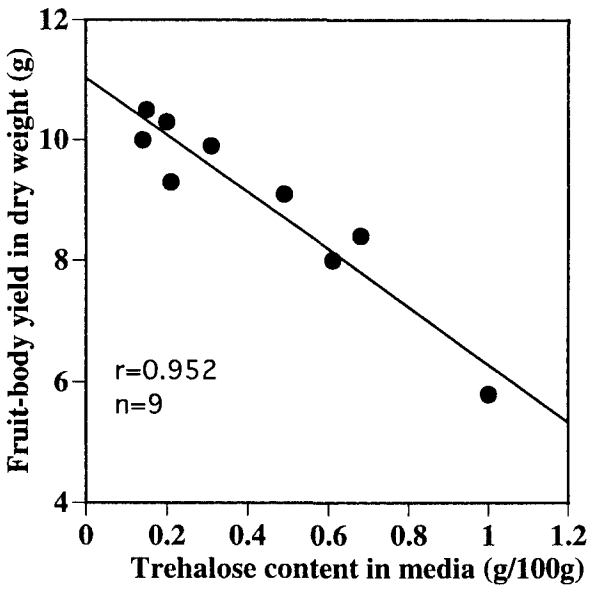


Fig. 2. The relationship between fruit-body yield in dry weight and trehalose content in culture after harvest of fruit-body of *P. ostreatus*.

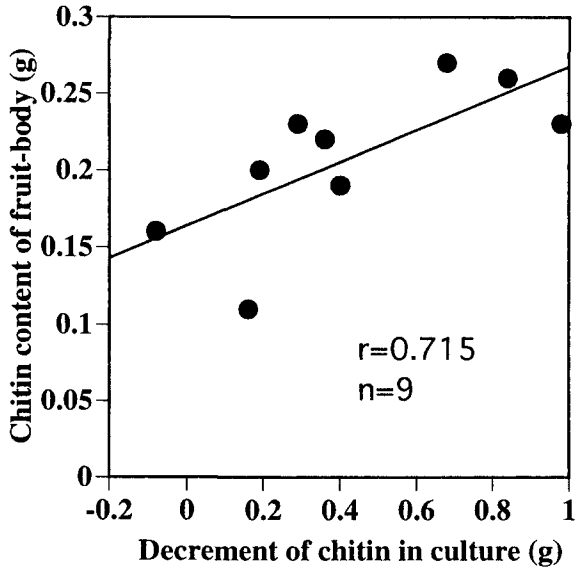


Fig. 4. The relationship between chitin content of fruit-bodies and decrement of chitin in culture during fruit-body growth. The decrement of chitin in the culture was obtained from the contents at stages 4 and 5.

al., 1986) and *Flammulina velutipes* (Curtis: Fr.) Singer (Terashita et al., 1991). Tokimoto and Fukuda (1981) reported that glucosamine content in a bed-log with *Lentinula edodes* (Berk.) Pegler correlated positively with the yield of fruit-bodies. Iwamoto et al. (1990) reported with *P. ostreatus* that chitinase and β-*N*-acetylglucosaminidase activity increased at fruiting and that these enzymes might serve to supply *N*-acetylglucosaimne as a material and an energy source for fruit-body growth. In the present study, chitin decrement from the culture did not correlate with the fruit-body yield, but positively correlated with chitin content in fruit-bodies (Fig. 4, $r=0.715$, $P<0.05$).

On the bottle cultivation using sawdust-rice bran medium, the results obtained in this study indicate that trehalose and chitin metabolisms have the major role for fruit-body formation of *P. ostreatus*.

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