Effect of Storage of Spawn on Mycelial Growth, Fruit-Body Formation and Mycelial Constituents of the Shiitake Mushroom (*Lentinus edodes*)

Takao TERASHITA, Saeko HIRATA, Kentaro YOSHIKAWA and Jiko SHISHIYAMA

Faculty of Agriculture, Kinki University, Nakamachi 3327–204, Nara 631, Japan

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The effects of short periods (15–200 days) of either refrigerated or room temperature spawn storage on the mycelial growth, fruit-body yield, chemical constituents and enzyme activities of the mycelia of three strains of *Lentinus edodes* were investigated. No significant decrease in the mycelial growth and fruit-body yield was observed under storage for 200 days at 4°C, 160 days at 15°C or 60 days at 24°C. Mycelial growth increased upon spawn storage for 60–110 days at 4°C or 15°C. These effects were observed in all strains tested. The fruit-body yield increased on storing the spawn for 30 days at 15°C. However, storage of the spawn for relatively longer periods (160–200 days) at low temperature (4°C, 15°C) decreased the formation of morphologically normal fruit-bodies. In contrast, normal fruit-body formation increased upon storage for shorter periods at 24°C compared with the not-stored spawn (control). Total sugar in mycelia after incubating the stored spawn decreased significantly, whereas the protein and free amino acid content did not show any decrease. Proteinase activity in mycelia after incubating the stored spawn did not differ significantly. However, amylase, β-1,3-glucanase and trehalase activity decreased markedly upon storage of the spawn.

INTRODUCTION

Mushroom production is an important utilization of the abundant forest resources available in Japan. In particular, based on production scale, the commercial production of *Lentinus edodes* (Berk.) Sing. (Shiitake mushroom) plays a pivotal role.

Although modern breeding technology has enabled the development of superior strains of *L. edodes*, many problems due to the storage of spawn have arisen, such as delays in mycelial growth, inadequate development of fruit-body, appearance of altered fruit-body, and contamination by other microbes (Yoshitomi *et al.*, 1983; Furukawa, 1992).

Kneebone *et al.* (1974) reported the successful cryopreservation of *Agaricus bisporus* (Lange) Sing. using liquid nitrogen (−196°C). Jodon *et al.* (1982) also reported that storage of the strain of *A. brunnescens* Peck without any significant change in mycelial growth is possible using liquid nitrogen. In a study of *Pleurotus ostreatus* (Jacquin: Fries) Kummer, Ohmasa *et al.* (1992) reported that no significant difference existed between the subcultured strain and cryopreserved strain. In addition, many reports on longer period storage of various mushroom strains may be found in the literature (Kneebone *et al.*, 1974; Jodon *et al.*, 1982; Ohmasa *et al.*, 1992, 1996).

In contrast, very few reports detail the effect of shorter storage (15–200 days) on the
growth of mushroom strains. Because storage for shorter periods of time in refrigerators or at
room temperature is a practice commonly employed by some researchers and mushroom
growers for strain preservation, it is very important to understand the effect of such storage on
mycelial growth and fruit-body formation. However, except for the reports of You et al.
(1982) and Suman and Jandaik (1992), who reported a decrease in fruit-body formation in
stored strains, no reports have described the changes in chemical constituents and enzymatic
activity of stored spawn.

We examined the effect of storing spawn at various temperatures for defined periods on the
mycelial growth, fruit-body formation, changes in the chemical constituents of mycelia and
activities of several mycelial hydrolytic enzymes.

**MATERIALS ANS METHODS**

**Fungal strains**

Three different *L. edodes* strains [Mori 465, Kinko 241 (TMI 563), and an isolate of a
Chinese commercial strain imported from Shanghai, China] were used in the preliminary
study (Fig. 1). Based on these results, only the Mori 465 strain was examined in further detail,
because among the three strains, this strain showed the best mycelial growth and development
of fruit-body formation in liquid medium. These strains were subcultured in potato dextrose
agar (PDA, Nissui Co.) medium.

**Storage preservation of spawn**

In the preliminary experiment, the mycelial spawn of the three strains was grown on filter
disks (0.6 cm in diameter, $D$) arranged in a concentric pattern at a distance of
2.5 cm from the center of a Petri plate ($D = 11$ cm) that contained PDA medium, for 10 days
at 24°C under a fluorescent lamp of 100-200 lx. The mycelial mat that formed on each paper
disk was stored as spawn for further use as the inocula in subsequent experiments. Ten paper
disks with mycelial mats (spawn) were put on a glass slide in a moist chamber Petri plate ($D =
11$ cm) and sealed with vinyl tape to prevent drying of the mycelial mat. The plates were then
stored for 15-200 days at 4, 15 or 24°C.

**Culturing of spawn after storage**

After various storage periods, a paper disk with stored spawn of each three strains was
inoculated separately into polycarbonate vessels, 250 ml, 8.0 ($d$) × 7.0 cm ($h$), Iwaki Glass,
Japan, containing 50 ml of glucose peptone yeast extract (GPY) liquid medium [2.5 g polype-
tone, 50 g glucose, 2.5 g yeast extract, 1.0 g $\text{KH}_2\text{PO}_4$, 0.5 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.5 g $\text{CaCl}_2$, 0.01 g
thiamine hydrochloride, 20 ml of trace salt solution (0.5 g $\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$, 0.36 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.5 g $\text{ZnCl}_2$, 0.05 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ per liter, initial pH 5.8 adjusted with 1 M HCl) in 1 L of
distilled water] sterilized at 119°C for 8 min. The effect of storage of spawn on mycelial
growth, composition of mycelial chemical constituents and activity of different hydrolytic
enzymes was investigated after incubating the stored spawn for 20 days at 24°C under a
fluorescent lamp of 100-200 lx. The effect of storage of Mori 465 spawn on fruit-body
formation was investigated by incubation of the stored spawn for 30 days at 24°C, after which
the temperature was altered to 15°C to induce fruit-body formation for an additional 30 days
under a fluorescent light (100-200 lx). All results represent averages of triplicate experiments
for each strain examined with 5 polycarbonate vessels per experiment.

**Measurement of mycelial growth and Mori 465 fruit-body formation**

Mycelial growth was measured using the dry weight of cultured mycelia. The culture
media was filtered and the mycelial mat that developed on the inoculated paper disk after 20
days incubation was separated, then washed thoroughly with distilled water. Excess water
was removed by aspiration, followed by hot air drying at 85°C. Fruit-body formation was
estimated using the ratio of normal fruit-bodies (stipe and pileus completely separated) to that of anomalous fruit-bodies (stipe and pileus not completely separated). Dry weight of mycelia and fruit-bodies was measured after hot air drying as described above.

**Preparation of samples for analysis of constituents of mycelia and enzyme activity assay**

Analysis of mycelial constituents was performed using lyophilized mycelia of the Mori 465 strain. After 20 days incubation of the spawn, the culture media was filtered and the mycelial mat was separated, washed thoroughly with distilled water and lyophilized after excess water had been removed by aspiration. The protein, free amino acid, and total sugar constituents in mycelia were determined using this lyophilized mycelia.

Enzyme activity was determined using the supernatant solution prepared from the mycelial homogenates of the Mori 465 strain. After 20 days incubation of the spawn, the culture media was filtered and the mycelial mat was separated. After washing with distilled water, the excess water was removed by desiccation. This mycelial mass was suspended in a buffer solution (0.1 M Költhoff buffer, pH 6.5) at a 1:3 ratio (w/v) and was homogenized in a glass homogenizer (Ikemoto Riken Co., Tokyo) at 0°C. The homogenized suspension was then centrifuged at 15,000×g (15 min, 0°C) and the supernatant was evaluated for hydrolytic enzyme activity.

**Analysis of protein, free amino acid and total sugar content**

The protein content in Mori 465 mycelia was determined by Lowry's method. The lyophilized mycelial mat was suspended in 0.1 M NaOH and was homogenized in a glass homogenizer. The homogenized suspension was then heated at 100°C for 10 min and was centrifuged (15,000×g, 15 min, 0°C). Trichloroacetic acid (TCA) was added to the supernatant to a final concentration of 20%, followed by recentrifugation to precipitate protein. The precipitate was dissolved in 0.1 M NaOH and total protein content was measured by Lowry's method using bovine serum albumin as the standard (Kitamoto et al., 1980).

The free amino acid content in mycelia was determined by the ninhydrin method using leucine as the standard (Kitamoto et al., 1980). The homogenized suspension of the lyophilized mycelial mat in 80% ethanol (1:100, w/v) was centrifuged (15,000×g, 15 min) and the solution was then subjected to ion exchange chromatography (Amberlite IR-120). The basic fraction which contained amino acids was collected.

Total sugar in mycelia was measured by the Anthrone method using glucose as the standard (Kitamoto et al., 1978). The homogenized suspension of lyophilized mycelial mass in 80% ethanol was centrifuged (15,000×g, 15 min) and the total sugar in the supernatant solution was determined.

Data obtained represent averages of triplicate experiments with 5 polycarbonate vessels per experiment.

**Enzyme activity on the Mori 465 strain**

Proteinase activity was measured by the Casein-Folin method with slight modification (Terashita et al., 1995) using Hammarsten casein (Wako Pure Chemical Industry Co.) and hemoglobin (Difco Co.) dissolved to 1.33% in 0.1 M McIlvaine buffer (pH 2.8) and Költhoff buffer (pH 7.2), respectively, as the substrate. One unit of activity (U) was defined as the quantity of enzyme that liberated 1 μg of tyrosine per ml of reaction mixture per min. Amylase activity was measured by the Wholegemuth method (Kawai, 1973) using soluble starch (Wako Pure Chemical Industry Co.) dissolved to 1.5% in 0.1 M McIlvaine buffer (pH 7.0), by heating, as the substrate. The reaction was stopped by addition of 0.5 M acetic acid and iodine and potassium iodide reagents were added to develop color. Then the difference in the absorbance at 600 nm was measured. One unit of activity (U) was defined as the quantity of enzyme that digested 1 μg of starch per min. β-1,3-Glucanase activity was measured using laminarin (Nacalai tesque Co.) as the substrate. Laminarin was dissolved in
0.1 M McIlvaine buffer (pH 5.0) at a concentration of 2 mg/ml and the reaction was allowed to proceed for 60 min at 37°C. Trehalase activity was measured by using trehalose (Wako Pure Chemical Industry Co.) as the substrate (Murao et al., 1989). Trehalose was dissolved in 0.1 M McIlvaine buffer (pH 6.0) at a concentration of 2 mM and the enzymatic reaction was carried out at 37°C for 100 min. The reaction was stopped by cooling on ice. The activity (U) of β-1,3-glucanase and trehalase were estimated by determining the liberated amount of reducing sugar by the method of Somogyi-Nelson using D-glucose as the standard (Terashita et al., 1995).

Data represent averages of triplicate experiments with 5 polycarbonate vessels per experiment.

RESULTS

The effect of storage of spawn on mycelial growth

Figure 1 shows the results of 20 days culture of the three different L. edodes spawn, stored for 50 days at 4°C, 15°C and 24°C, in GPY medium. On storing the spawn at 15°C, the mycelial dry weight increased about 1.2 (Mori 465)-1.7 (Chinese strain) times more than the not-stored strains (control). However, no significant difference was found between storing spawn at 4 and 24°C. Under all the conditions examined in the present study, the Mori 465 strain showed the best mycelial growth while the Chinese strain showed the least mycelial growth of the three strains. Based on this preliminary result, the Mori 465 strain was examined in further detail.

Using the Mori 465 strain, the effect of spawn storage temperature and duration on mycelial growth was investigated by storing the spawn at 4°C for 200 days, at 15°C for 160 days and at 24°C for 60 days. The results are shown in Fig. 2. Active mycelial growth was observed upon storing spawn for 60-110 days at 4°C and 15°C. In particular, upon storing spawn for 60 days at 15°C, mycelial growth increased to about 160% (0.59±0.04 g) of not-stored spawn (0.37±0.05 g). On the other hand, storing the spawn at 24°C for a short period (15 days) caused mycelial growth to decrease to about 68% of the control (0.23±0.02 g). However, growth was restored to the control level by increasing the storage period to 30 days or more.

Fig. 1  Effect of storage of spawn on the mycelial growth of Lentinus edodes.

<table>
<thead>
<tr>
<th>Storage temperature</th>
<th>Mycelial dry weight (g/task)</th>
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<tbody>
<tr>
<td>4°C</td>
<td>0.2 ± 0.05</td>
</tr>
<tr>
<td>15°C</td>
<td>0.4 ± 0.05</td>
</tr>
<tr>
<td>24°C</td>
<td>0.6 ± 0.05</td>
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</tbody>
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a): Control (not-stored)
Mycelial disks were stored at 4°C, 15°C and 24°C for 50 days, inoculated in glucose peptone yeast extract (GPY) liquid medium and incubated at 24°C. The dry weight of mycelium was measured 20 days after inoculation.
Vertical bars represent standard errors. Data represent averages of triplicate experiments with 5 polycarbonate vessels per experiment.
Fig. 2 Effect of storage of spawn on the mycelial growth of *L. edodes* Mori 465 strain.

- 4°C, -- O --: 15°C, —: 24°C

Mycelial disks were stored at 4°C, 15°C and 24°C for 15, 30, 60, 110, 160 and 200 days, inoculated in GPY liquid medium and incubated at 24°C for 20 days.

Vertical bars represent standard errors. Data represent averages of triplicate experiments with 5 polycarbonate vessels per experiment.

Fig. 3 Effect of storage of spawn on the fruit-body yield and formation rate of normal fruit-bodies of *L. edodes* Mori 465 strain.

- 4°C, -- O --: 15°C, —: 24°C

Mycelial disks were stored at each temperature for 200 days, inoculated in GPY liquid medium and incubated at 24°C for 30 days.

The induction of fruit-body formation was carried out by adjusting the temperature to 15°C under about 100-200 lx light.

Vertical bars represent standard errors. Data represent averages of triplicate experiments with 5 polycarbonate vessels per experiment.

**Effect of storage of spawn on fruit-body formation**

Figure 3 shows the effect of spawn storage of the Mori 465 strain on fruit-body formation and on the formation rate of normal fruit-bodies (the ratio of normal to anomalous fruit-bodies). A slight decrease in fruit-body formation was observed upon storing the spawn for 200 days at 4°C compared to not-stored spawn (control). No significant difference in fruit-body formation was observed between storing the spawn for 160 days at 15°C and for 60 days at 24°C. On the other hand, the dry weight of fruit-bodies was about 140% (1.58 ± 0.21 g) greater in spawn stored for 30 days at 15°C. This effect was not observed when the spawn were stored at 4°C or 24°C. The ratio of normal to anomalous fruit-body formation is shown in Fig. 3 (lower). Storage of the spawn for longer periods (160 days or more) at low temperature (4°C and 15°C) caused a decrease in normal fruit-body formation. However,
storing spawn for shorter periods (30 or 60 days) at 24°C increased the normal fruit-body formation compared to the control.

**Effect of storage duration of spawn on protein, free amino acid and total sugar content in mycelia**

The protein, free amino acid and total sugar content in mycelia was determined after 20 days culture of Mori 465 strain spawn stored at different temperatures for 50 days. These results are shown in Fig. 4. Compared to the not-stored spawn, the protein content of stored spawn at all temperatures tested decreased slightly. On storing spawn at 24°C, the content of free amino acids was lower than that of the not-stored spawn. However, no significant difference in the content of protein and free amino acids was found in this experiment.

The total sugar content in the mycelia stored at any tested temperature was markedly lower than that of the not-stored spawn. In the not-stored spawn, the total sugar content was 50.1 ± 6.52 mg/g dry weight of mycelia compared to 30.3 ± 4.31 mg/g dry weight of mycelia (60% of control) for storage at 4°C, 20.4 ± 4.55 mg/g dry weight of mycelia (41% of control) for storage at 15°C, and 10.8 ± 2.89 mg/g dry weight of mycelia (31% of control) for storage at 24°C.

**Fig. 4** Effect of storage of spawn on protein, free amino acid and total sugar content in the vegetative mycelia of *L. edodes* Mori 465 strain.

- a): Control (not-stored)
- Mycelial disks were stored at each temperature for 50 days, inoculated in GPY liquid medium and incubated at 24°C for 20 days.
- Chemical components (mg) in the mycelia are shown per gram dry weight of vegetative mycelium. Vertical bars represent standard errors. Data represent averages of triplicate experiments with 5 polycarbonate vessels per experiment.

**Fig. 5** Effect of storage of spawn on proteinase activity in the vegetative mycelia of *L. edodes* Mori 465 strain.

- ··: 4°C, --: 15°C, ▲·: 24°C
- A: carboxyl proteinase activity, B: metal proteinase activity
- Mycelial disks were stored at each temperature for 15, 30, 60, 120 and 200 days, inoculated in GPY liquid medium and incubated at 24°C for 20 days.
- Data represent averages of triplicate experiments with 5 polycarbonate vessels per experiment.
Fig. 6  Effect of storage of spawn on enzyme activity in the vegetative mycelia of L. edodes Mori 465 strain.

A : Amylase activity, B : β-1,3-Glucanase activity, C : Trehalase activity

Data represent averages of triplicate experiments with 5 polycarbonate vessels per experiment.

Effects of storage duration on proteinase, amylase, β-1,3-glucanase and trehalase activity

Figures 5 and 6 show the results of differences in the hydrolytic enzyme activity in the mycelia of the Mori 465 strain after culturing spawn stored at different temperatures. The carboxyl proteinase activity (Fig. 5A) of this strain was 2 times greater than the metal proteinase activity (Fig. 5B). The activity of both of the proteinases did not differ significantly between spawn stored at 4°C and 15°C up to 200 days and spawn stored at 24°C up to 60 days.

On storing the spawn, a marked decrease in amylase activity (Fig. 6A), β-1,3-glucanase activity (Fig. 6B) and trehalase activity (Fig. 6C) in the mycelia was observed. All three enzyme activities showed a similar pattern of decrease. Compared to the not-stored spawn, on storing the spawn for 200 days at 4°C and at 15°C, amylase activity was found to decrease to 30.4% and 39.1%, respectively, whereas β-1,3-glucanase activity decreased to 53.5% and 29.1%, respectively, and trehalase activity decreased to 10.9% and 22.8%, respectively. The activity of these enzymes decreased almost linearly with respect to the number of storage days.

DISCUSSION

Lentinus edodes is one of the genetically stable mushroom fungi, and the viability and fruit-body formation ability of this species do not markedly decrease even on subculturing for many years (Nakamura, 1982). Low temperature preservation and suppression of mycelial growth during storage allows preservation of a fungal strain for long periods (Tominaga, 1982). Furthermore, the lower concentration of the constituents of the storage media and culturing at a slightly lower temperature than the optimum culture temperature enables better preservation (Takada, 1983). During storage, cells in the log phase are more viable than cells in the lag phase (Takada, 1983). Although these phenomena explain the effect of mycelial
storage on growth and fruit-body formation, they cannot account for changes in the chemical constituents or enzymatic activity of the mycelia upon storage of the spawn.

Thus, the present study was designed to explain the effect of storage of spawn on the growth, chemical constituents and enzyme activity of the mycelia of a mushroom fungus under storage conditions generally employed by researchers or mushroom growers (refrigeration or room temperature storage for short periods). Under the storage conditions of the present study, mycelial growth did not decrease upon storage of the spawns compared to the not-stored spawn. In contrast, upon storage at 15°C, mycelial growth (dry weight) was promoted (Fig. 1). Further investigation revealed that storing the spawn of Mori 465 for 60–110 days at 4°C or 15°C resulted in promotion of mycelial growth (Fig. 2). Although the exact reason for this effect is not clear, it may be due to the increase in the amount of inocula in the mycelial mat that resulted from gradual mycelial growth during spawn storage. This explanation is supported by the fact that the spawn stored at 15°C showed better mycelial growth than the spawn stored at 4°C.

Storing the spawn at 24°C for 15 days retarded mycelial growth but increasing the number of storage days to 30 or more, resulted in growth returning to normal values (Fig. 2). This may have been due to as yet unidentified physiological changes, as different carbohydrate metabolizing enzyme activities were found to be altered. Increased amylase activity on storing the spawn at 24°C for 15 days decreased as the number of storage days increased while decreased trehalase and β-1,3-glucanase activity on storing the strain at 24°C for 15 days was unaltered even on increasing the storage days (Fig. 6).

In the present study, storage of L. edodes spawn did not result in a significant decrease in dry weight of fruit-bodies. In contrast, the dry weight of fruit-bodies increased (1.4 times) when spawn were stored at 15°C for 30 days. You et al. (1982) reported that for better fruit-body yield, the spawn of A. bisporus should be stored for less than 30 days below 5°C and the moisture content should be around 47%. In another report, Suman and Jandaik (1992) found that on storing A. bisporus for 6–24 months at 2–6°C, the fruit-body yield decreased as the storage period increased. According to that report, compared to the not-stored control spawn, when A. bisporus was stored for 6 months, the fruit-body yield decreased to 59.8% and on storing for 24 months, the yield decreased to 14.3%. Morphological changes such as elongation of stipe (maximum of 146%) and increase in the pileus area (maximum of 123%) were also observed.

Studying the effect of preservation on the growth of Flammulina velutipes (Curt.: Fr.) Sing. cryopreserved for 7 years, Ohmasa et al. (1996) reported that retardation of mycelial growth and variation in fruit-body yield depended upon differences in the preservation conditions and storage temperatures. In that study, retardation of mycelial growth was observed in the first mycelial growth but reverted to normal growth on second mycelial growth induced using the mycelia of the first mycelial growth. On the other hand, variation in fruit-body yield was observed even among cultures of the same strain stored using different cryoprotectant and this variation was most obvious in the strains with retarded mycelial growth.

Compared to these mushrooms, the mycelial growth and ability to form fruit-bodies of L. edodes, which is genetically a more stable mushroom, is undamaged on storing in agar medium for 2–6 months (Nakamura, 1982). Similarly, in the present study, no significant adverse effect on mycelial growth and fruit-body formation was observed when spawn were stored at low temperatures (4°C or 15°C) for up to 200 days. However, storage of the spawn for longer periods (160 days or more) at low temperature caused a decrease in normal fruit-body formation.

The total sugar content in the mycelia of the stored spawn was only half that of the
not-stored spawn (Fig. 4). Trehalose, a sugar necessary for the formation of fruit-bodies and a substrate of respiratory metabolism, is the most important sugar among mushroom fungi and present in high quantity (Kitamoto et al., 1978; Yoshida et al., 1987). Although storage did not affect morphological aspects of the mushroom, such as mycelial growth and fruit-body formation, the results of total sugar content analysis suggest that the fungus may have been considerably damaged during the long storage period. In contrast, storage of spawn did not affect protein and free amino acid content, which are nitrogenous substrates necessary for mushroom growth (Kitamoto et al., 1980).

We also studied the changes in enzyme activity that have a direct impact on mushroom growth. As shown in Figs. 5 and 6, proteinase activity did not change although activity of carbohydrate hydrolyzing enzymes such as amylase, β-1,3-glucanase, and trehalase decreased as the number of storage days increased. These results coincide with those of the investigation of the chemical constituents of the mycelia shown in Fig. 4. Ohga (1992) has reported that cellulase, xylanase, and proteinase are the main enzymes responsible for the degradation of substrates necessary for mushroom growth. In addition to these enzymes, Matsumoto (1988) reported that β-1,3-glucanase, amylase and chitinase are also necessary enzymes for the degradation of growth substrates. We believe that the amount or quality of hydrolytic enzyme necessary for degradation of a growth substrate may differ due to differences in the culture medium, because in the present study GPY medium was used for culturing, whereas in the reports mentioned above, sawdust rice bran medium was used.

Muto (1975) has reported that storage of ‘Tanegoma’ (chip spawn, spawn developed on pieces of wood), which is believed to maintain the degradation of ‘Komazai’ (material on which the spawn is run), in culture vessels for 47 months at 5°C, has no significant effect on the mycelial growth. On the other hand, room temperature storage has been reported to be better than low temperature storage for the formation of normal fruit-bodies in mushrooms (Takada, 1989). We believe that a detailed microscopic cytochemical investigation in addition to analysis of chemical constituents and enzyme activity in the mycelia is necessary to avoid confusion between studies.

REFERENCES


<和文抄録>

シイタケの菌糸生育，子実体形成および菌糸体成分に及ぼす接種菌保存の影響

寺 下 隆 夫・平 田 彩 子・吉川 賢 太 郎・獅 山 慈 孝

近畿大学農学部

冷蔵庫や室温での接種菌の短時間（15～200日）の保存が，シイタケ菌の菌糸生育や子実体の形成，菌糸中の化学成分および酵素活性に及ぼす影響を検討した。その結果，本実験の範囲（4℃，200日：15℃，160日：24℃，60日）では菌糸生育および子実体収量の低下は認められなかった。

4℃および15℃での50～100日の保存はむしろ菌糸生育を促進した。これらの傾向は供試3菌株に共通であった。子実体収量は接種菌を15℃で30日間保存すると対照の1.4倍に増加した。しかし，低温（4℃，15℃）での接種菌の保存は異常子実体の発生を増加させ，正常な子実体発生には24℃の保存が良好であった。保存後に培養した菌糸中の糖質含量は約半分に減少した。しかし，蛋白質および遊離アミノ酸含量には変化がなかった。また，菌糸保存後に培養した菌糸中のアミラーゼ，β-1,3-グルカナーーゼおよびトヘラーゼ活性は菌糸の保存によって著しく低下したが，プロテインーゼ活性は低下しなかった。

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