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Fruit-body production of a luminous mushroom, *Mycena chlorophos*

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Cultural conditions for fruit-body production of *Mycena chlorophos* were investigated with the aim of using the mushroom for study of bioluminescence, scientific exhibition, and ecological conservation. A small glass jar having a cap with a microfilter was used as a culture vessel. A compost powder mixed with rice bran in proportion of 20% (fw/fw) and adjusted to 70% (w/w) in moisture content was used as production medium. Casing with 2 g/jar of moistened compost powder was necessary for fruit-body formation. Mycelium was grown in a culture chamber at 27°C, relative humidity (RH) of 80% for 4 wk, then transfered to a culture chamberat 21°C, 90% RH and light intensity of 300–800 lx after casing its, and incubated for 3 wk to produce fruit-bodies. The mean yield was 31 fruitbodies, i.e., 150 mg dry weight per jar.

Key Words——biological luminescence; fruit-body production; luminous fungus; mushroom cultivation; *Mycena chlorophos*.

Mycena chlorophos (Berk. et Curt.) Sacc. is a luminous mushroom which grows naturally on Hachijo Island and in the Bonin Islands, Japan (Kobayasi, 1937). Light emission from this fungus is much stronger than that from *Lampteromyces japonicus* (Kawam.) Sing. and is a beautiful pale green in colour. In the Bonin Islands, *M. chlorophos* is nicknamed "Green Pepe" and regarded as a unique object of sightseeing after sunset. However, the naturally occurring number of this species is decreasing with the loss of habitats. It will become necessary to ecologically preserve the species in the near future. The mushroom is also useful as a new experimental subject to study biological luminescence.

Recently, air conditioning techniques for cultivation of edible mushrooms have advanced, and large-scale commercial production is now progressing. As far as luminous mushrooms are concerned, however only a short report has been presented on the laboratory cultivation of *L. japonicus* to study its fluorescent constituents (Endo et al., 1970). We aim to produce fruit-bodies of *M. chlorophos* on a large scale and utilize them as an experimental material for studying bioluminescence, as a subject for scientific exhibition, and to study breeding for its ecological preservation.

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Materials and Methods

Strain *Mycena chlorophos* H-113 from Hachijo Island was used, as in our previous report (Niitsu et al., 2000).
Agar medium Peptone agar medium containing 2% glucose, 0.5% peptone, 0.2% yeast extract, 0.1% $KH_2PO_4 \cdot 7H_2O$, 0.05% $MgSO_4 \cdot 7H_2O$ and 2% agar was used for the culture medium to produce spawn. This medium was autoclaved at 121°C for 15 min and a 20-ml aliquot was put into a Petri dish, 90 mm in diam, and 15 mm in depth. All reagents were purchased from Wako Pure Chemical Industries.
Compost medium A commercial compost (JT Agris Co.) for gardening was used as the basal component of the medium to produce fruit-bodies. The compost was made by heaping up fallen leaves of Japanese oaks (*Quercus acutissima* Carr. and *Quercus serrata* Murr.) in the open air. The compost was used after air-drying, grinding, and sieving into particles of 0.25–2.0 mm in size and ca. 15% in moisture content. Commercial rice bran, ca. 10% in moisture content, was mixed with this compost powder in a portion of 20% (fw/fw), and the water content of the mix was adjusted to 70% on wet basis by adding purified water. A 30-g aliquot of medium was placed in a culture jar. A hollow ca. 3 mm in depth was made by pressing the central part of the medium surface with an aluminium rod (14 mm in diam and 138 mm in length) to use as an inoculating hollow. The medium was autoclaved at 121°C for 15 min before use.

To determine the optimum content of rice bran in the medium, 10, 15, 20, 25, or 30% (fw/fw) of rice bran was

added to compost powder at a fixed water content (70%). To determine the optimum water content for fruit-body production, the water content in the medium was changed in 5 steps: 55, 60, 65, 70 and 75%, while the content of the compost and rice bran were fixed at 8 and 2 g per jar, respectively. Ten jars were used for each set condition and examined three times.

Culture jar A glass jar, 50 mm in diam, 95 mm in height (commercial mayonnaise jar) was used as the culture vessel. A hole of 10, 16, 20, or 25 mm in diam was made in the plastic cap of each jar and covered with a sheet of microfilter (Dupont, Tyvek 1073B) using a heat-resistant adhesive in the inside of the cap. This closed-type cultivating jar was used to investigate the effect of filter size on the fruit-body pruduction. Ten jars were used for each filter size and the experiment was done twice. The cap was replaced by translucent polyethylene cap having a hole of ca. 2 mm in diam when the emitting mushroom was exhibited.

Casing The same compost powder as in the culture medium was used at 75% water content as a casing after autoclaving at 121°C for 15 min. Different amounts of the casing, 0.5, 1, 2, 3, 4, or 8 g per jar, were used to determine the optimum size of casing. The casing was put on the mycelial mat in the jar with a sterilized spoon and pressed down softly using a sterilized L-shaped spatula. Ten jars were used for each casing amount and were examined three times.

Cultivation A portion of mycelial mat (ca. 8×8 mm) cut from the colony grown on the peptone agar plate was inoculated into a hollow on the medium. After capping and covering with aluminium foil (ca. 100×100 mm), the jar was placed in a cultivation chamber at 27±2°C and 80±10% RH in the dark. When the mycelium spread throughout the medium after 4 wk of cultivation, the mycelial mat was cased, and the jar was placed in a cultivation chamber equipped with a 40 W fluorescent lamp (Sanyo Electric Co. Japan, BSM-TICG-0012-Z0) and incubate at 21±2°C, 90±10% RH, and 300–800 lx for 3 wk. The foil cover was not used in the cultivation after casing.

Spraying Spraying water directly on the mycelial mat was tested as a moisturing treatment instead of casing. One-half ml of purified water was sprayed on the mycelial mat using a handsprayer after 4 wk of cultivation. Around the 16 d after casing, which corresponds to the growth period of young fruit-bodies, 0.5 ml of purified water was sprayed in the jar to prevent drying of pilei.

Measurement of yield The yield of fruit-bodies was expressed as dry weight (mg) per jar after drying to constant weight at 95°C.

Results

Effect of filter size on fruit-body production As shown in Figure 1, the maximum yield of fruit-bodies, 149.5 mg/jar, was obtained with a filter of more than 20 mm in diam. Thus, a jar with a 25 mm filter in the cap was used for the following experiments.

Effect of the amount of casing on the fruit-body production As shown in Figure 2, the yield of fruit-bodies was the maximum when 2 g/jar of the casing was used. With 0.5 g/jar of casing, the mycelial mat dried out and fruit-bodies were not formed. When 8 g of casing was used, the time required for fruit-body formation increased to 30 d or more and resulted in a decrease in yield.

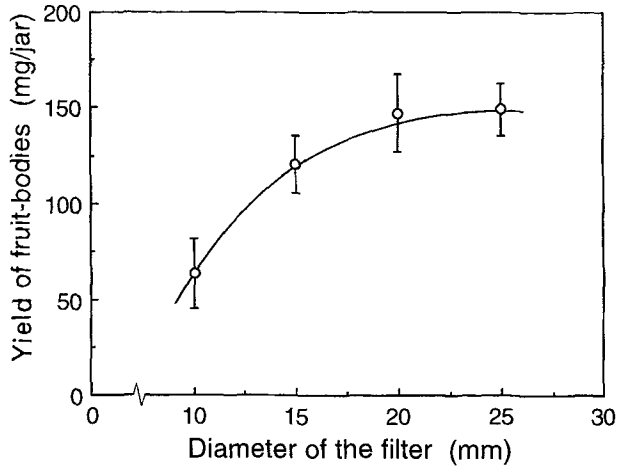

Fig. 1. Effect of filter size on the fruit-body production. Jars with filters of four different sizes were used as culture vessels under the same culture conditions. Vertical bars show standard errors.

Effect of water content of the medium on fruit-body production Figure 3 shows the number of days required for mycelial growth and yield of fruit-bodies in media of different water contents. For water content of 75%, the mycelium spread throughout the medium within 22.9 d on average, the shortest time observed. The highest yield of the fruit-bodies were harvested 120.6 mg/jar in dry weight, when water content was 70%.

Effect of rice bran content of the medium on fruit-body production Figure 4 shows the number of days required

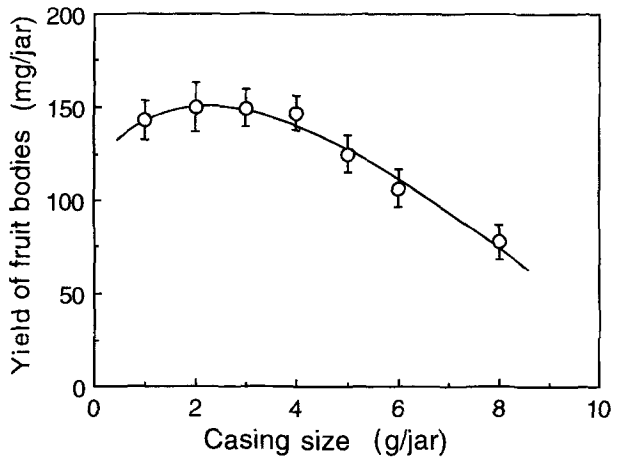

Fig. 2. Effect of casing size on the fruit-body production. Different amounts of moist compost powder were used for casing of mycelial mat. Vertical bars show standard errors.

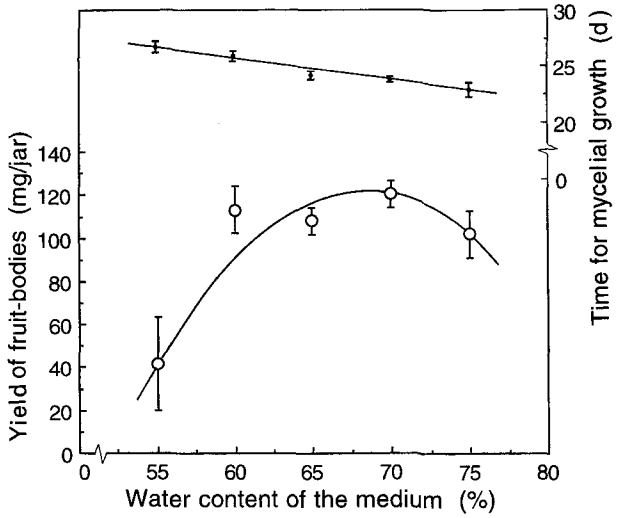

Fig. 3. Effect of water content of the medium on the mycelial growth and fruit-body production.
Different water contents of the medium were tested in same culture conditions. The number of day which the mycelia spread throughout in the medium and the yield of fruit-bodies were measured.
—●—: time of mycelial growth, —○—: yield of fruit-bodies. Vertical bars show standard errors.

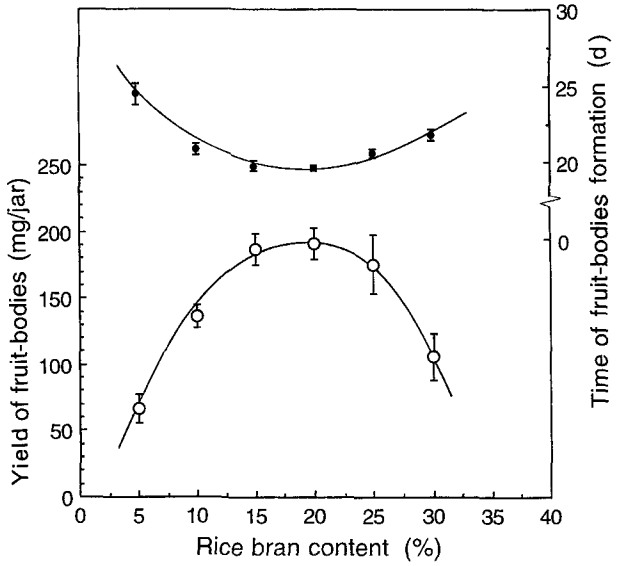

Fig. 4. Effect of rice bran content of the medium on the fruit-body production.
Media with different rice bran contents were tested under the same culture conditions. Water content of the medium was fixed at 70 %.
—●—: time of fruit-body formation, —○—: yield of fruit-bodies. Vertical bars show standard errors.

for fruit-body formation and the yield of fruit-bodies in media of various rice bran contents. When the content was 20%, the fruit-bodies were formed in the shortest period of 19.7 d on average and the yield (191.5 mg/jar) was also the highest. As the content decreased or increased, the period increased and the yield decreased. The mean number of fruit-bodies produced per jar was highest, 31, when the rice bran content was 20%.

Effect of spraying Spraying of water directly onto the mycelial mat had almost no promotive effect on primordium formation. Over moistening of primordia resulted in deformation of fruit-bodies. On the other hand, when the fruit-bodies were dried, the growth was delayed and

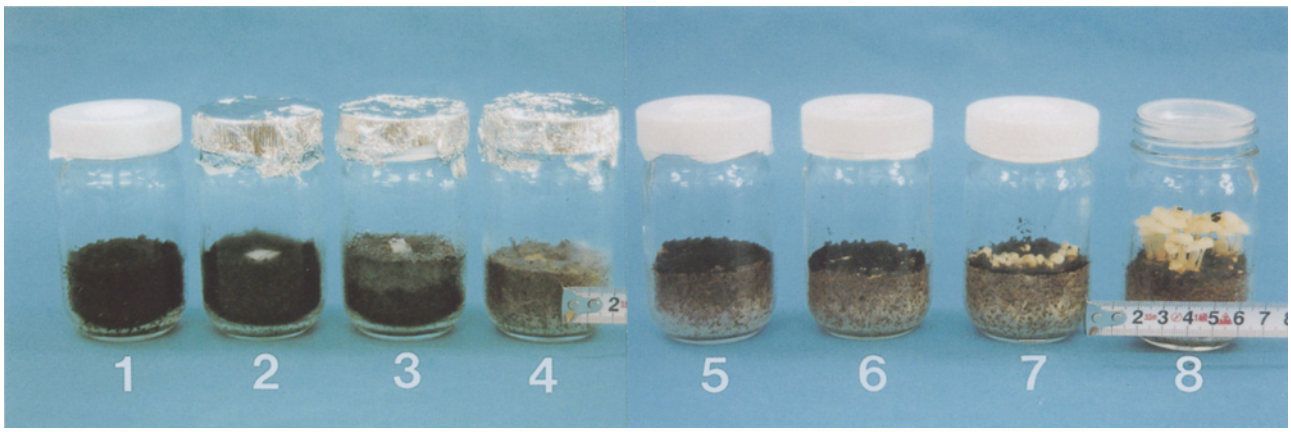

Fig. 5. Time course of the jar culture.
1. Prepared culture jar filled with compost medium.
2. Culture jar covered with a sheet of aluminium foil after inoculation.
3. Growing mycelia in the jar after 2 wk of cultivation.
4. Mycelia filling the medium after 4 wk of cultivation.
5. Cased mycelia after 4 wk of cultivation. The aluminium foil cover was removed.
6. Primordia produced on the mycelial mat 9 d after casing.
7. Young fruit-bodies 16 d after casing.
8. Mature fruit-bodies 3 wk after casing. The cap was replaced by a translucent cap with a hole.

the pilei were often warped and broken because of shrinkage of the gelatinous membrane. To prevent such breakage, it was effective to spray 0.5 ml of purified water on the young fruit-bodies or developing pilei.

Growth process of mycelia, primordia, and fruit-bodies

The appearance of *M. chlorophos* at different stages of growth in the jar are shown in Figure 5. The autoclaved jar packed with the culture medium is shown in Fig. 5-1. The jar was covered with aluminium foil after inoculation (Fig. 5-2). The mycelium spread over almost half of the medium volume after incubation for 2 wk (Fig. 5-3) and all over the medium after 4 wk (Fig. 5-4). The foil cover was removed after casing, and incubation was resumed at the lower temperature under illumination (Fig. 5-5). Primordia were formed 9 d after casing (Fig. 5-6). Young fruit-bodies were observed 16 d after casing (Fig. 5-7) and fruit-body formation was complete 21 d after casing (Fig. 5-8).

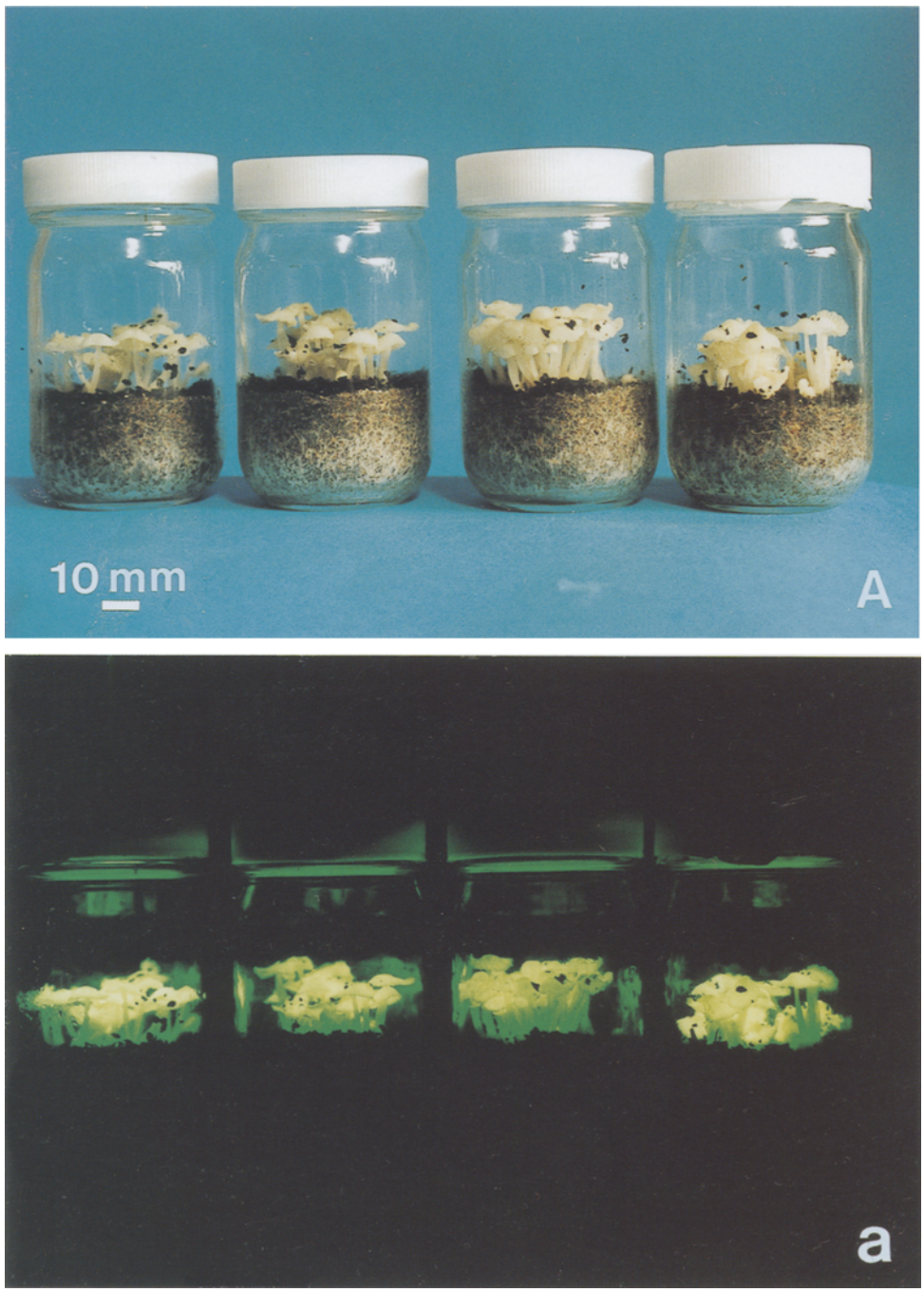

Fig. 6. Fruit-bodies growing in the culture jars after 7 wk of cultivation. Photographed in the light (A) and in the dark (a).

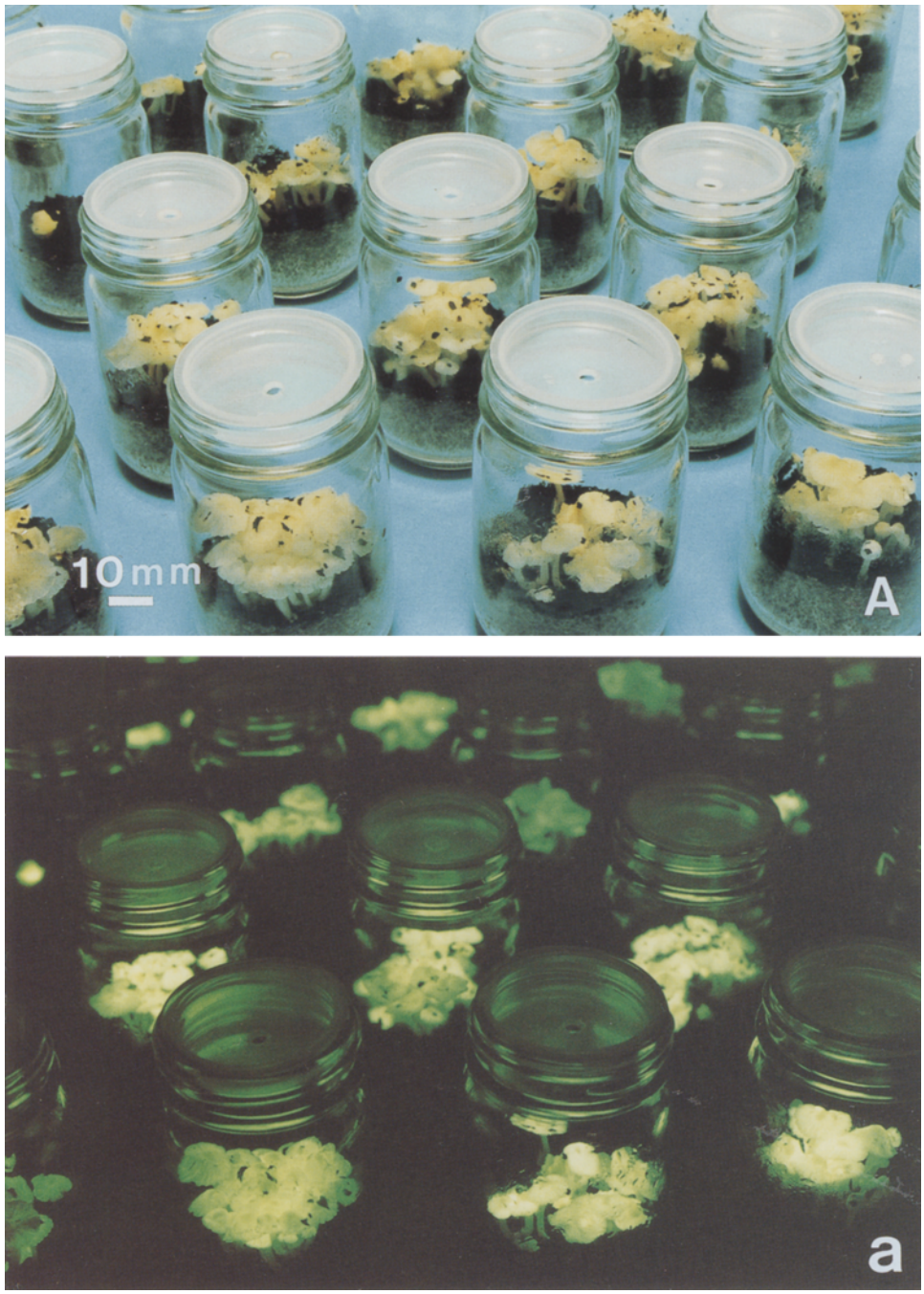

Fig. 7. Diagonal view of culture jars with translucent caps for better viewing. Photographed in the light (A) and in the dark (a).

Light emission from fruit-bodies Figure 6 shows a side view of fruit-bodies and their light emitted in the jar with filter-cap. As shown in Figure 7, after light emission started, the filter-cap was replaced by a translucent cap with a small hole. This was effective for preventing drying of fruit- bodies and for better exhibition. Figure 8 shows a view from above of the pilei in the jar. The yield under appropriate conditions was 31 fruit-bodies, i.e., 150 mg dry weight per jar on the average and 72 fruit-bodies per jar at maximum.

Discussion

Air conditioning cultivation of edible mushrooms is normally carried out in open culture bottles, which allows fruit-bodies to grow out of the bottles. In this study, it was necessary to use a closed culture jar having a cap with a microfilter in order to maintain appropriate

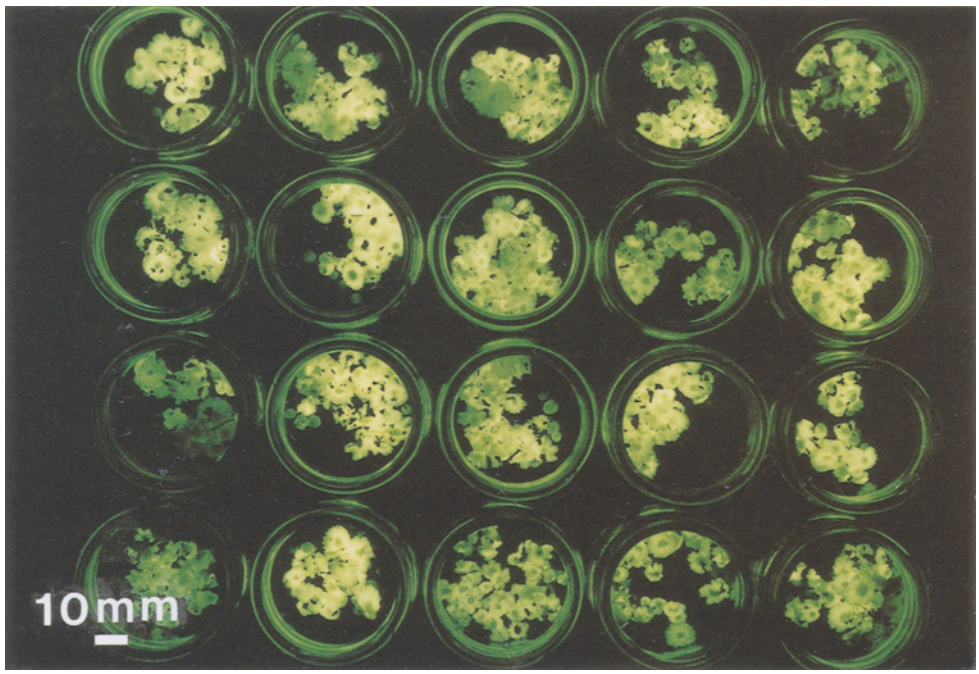

Fig. 8. View from above of emitting pilei in the jars.
The mean yield was 31 fruit-bodies per jar under suitable culture conditions.

moisture conditions around the mycelial mat. The fungus grew inside the jar throughtout its cultivation period.

Optimum water content of the medium for fruit-body production was 70%, slightly higher than for conventional cultivation of other edible mushrooms (65%).

Casing with moist compost powder was effective for primordium formation and essential to produce good fruit-bodies. The yield of fruit-bodies varied depending on the casing size. A thin casing layer allows permeation of air and light to the mycelium, but might also allow drying of the mycelial mat because its moisturizing effect is small. A thick casing layer is less permeable to air and light, and might cause over-moisturizing of the mycelial mat. In both cases, the number of days required for the fruit-body formation increased and resulted in a decrease in yield. Most favorable results were obtained by casing with 2 g of compost powder per jar. Spraying water directly on the mycelial mat was easier than casing but not practical for fruit-body production.

Although the relative humidity in the cultivating chamber was kept at 90±10%, it was not enough to prevent drying of mycelium and fruit-bodies in the jar. It was necessary to cover the cap with aluminium foil in the mycelial growth phase. Spraying of water onto the young fruit-bodies was effective to prevent their breakage. In addition, it was desirable to replace the filter-cap of the jar by a holed cap to prevent drying of mushrooms exhibited in the outside of cultivation chamber.

It was concluded that the moisture conditions around the mycelial mat were an important factor for the production of this mushroom. *M. chlorophos* is a hygrophylous fungus and requires sufficient moisture for fruiting. On Hachijo Island, the fruit-bodies of *M. chlorophos* develop naturally only in the rainy season. The island's average air humidity in June and July is 88% (RH). It is suggested that the present culture conditions meet the proper hydration requirement to some extent.

The cultured fruit-bodies emit a beautiful pale green luminescence when the pileus opens. *Mycena chlorophos* is an attractive mushroom. The dried fruit-bodies of *Ganoderma lucidum* (Fr.) Karst. have been enjoyed as an ornament in Japan and China since the Middle Ages (Kobayasi, 1983). Cultivated fruit-bodies of *M. chlorophos* have potential for scientific exhibition due to their fantastic luminescence. The mass production of fruit-bodies by the above method will also provide large amounts of experimental material for study on bioluminescence and ecological conservation.

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