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Cultural properties of a luminous mushroom, *Mycena chlorophos*

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Cultural conditions on mycelial growth and fruit-body formation of *Mycena chlorophos* were studied. The optimum temperature of the mycelial growth was 27°C and the optimum initial pH of medium was 4.0. Peptone agar medium was suitable for the spawn culture. Compost medium containing rice bran at 10% (fw/fw) was appropriate for fruit-body formation in the Petri dish. Light was essential for initiation of primordia, and low-temperature treatment induced fruit-body formation effectively. The optimum conditions for fruit-body formation were found to be the cultivation at 27°C for 4 wk and continued cultivation for 3 wk under illumination at an intensity above 0.2 lx and at 21°C after casing with moist compost powder. In the fruit-bodies obtained, the maximum photosensitive wavelength of luminescence was 522 nm and the optimum temperature for emission was 27°C. The luminescence of a fruit-body was observed for about 3 d consecutively at 21°C.

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

Mycena chlorophos (Berk. et Curt.) Sacc. (*yakoh-take*, "night-light mushroom" in Japanese) is a luminous mushroom distributed in Southeast Asia that emits a strong, pale green luminescence (Kobayasi, 1937). In Japan, *Lampteromyces japonicus* (Kawam.) Sing. is a well-known luminous mushroom and detailed studies have been conducted on its luminescent substances (Isobe et al., 1987, 1994). The mechanism of luminescence of *M. chlorophos* is considered to be based on the oxidation of a luminescent substance, as in the other luminous mushrooms (Shimomura, 1992), but the details have not yet been elucidated. Since the fruit-body is small and develops only in a limited season and a limited area in Japan, it is difficult to collect samples in large amounts. Establishment of the large-scale cultivation of the fruit-body is necessary for further study of its bioluminescence. In the present report, the cultural conditions of *M. chlorophos* were examined and the luminescence of the fruit-body was observed.

Materials and Methods

Strain The fruit-bodies of *M. chlorophos* were collected from the rotten petioles of a palm, *Phoenix roebelenii* O'Brien, on Hachijo Island in July 1990. Basidiospores were isolated from them and germinated on yeast extract-malt extract agar medium. A strand of mycelium was picked up from the periphery of the colony and cultivated on fresh agar medium. The fruiting mycelium, strain H-113, cultivated at 25°C was used for experiments.

Composition of agar media The following media were used for the experiment of mycelial growth: Czapek's medium (3% glucose, 0.2% $NaNO_3$, 0.1% $KH_2PO_4 \cdot 7H_2O$, 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, 0.001% $FeSO_4 \cdot 7H_2O$, 2% agar), Waksman's medium (1% glucose, 0.5% peptone, 0.1% $KH_2PO_4 \cdot 7H_2O$, 0.05% $MgSO_4 \cdot 7H_2O$, 2.5% agar), potato-dextrose agar medium (PDA; 20% potato extract, 2% glucose, 1.5% agar), yeast extract-malt extract agar medium (YMA; 1% glucose, 0.5% peptone, 0.3% yeast extract, 0.3% malt extract, 2% agar), peptone agar medium (PA; 2% glucose, 0.5% peptone, 0.2% yeast extract, 0.1% $KH_2PO_4 \cdot 7H_2O$, 0.05% $MgSO_4 \cdot 7H_2O$, 2% agar), Hamada's medium (2% glucose, 0.5% dry yeast, 1.5% agar, 1 N HCl 1.6 ml/l) and malt extract agar medium (MA; 2% glucose, 2% malt extract, 0.1% peptone, 2% agar). The media were autoclaved at 121°C for 15 min, then 20-ml portions were dispensed into Petri dishes, 15 mm deep and 90 mm in diam.

To examine the optimum initial pH, YMA medium was adjusted to values in the range of pH 2.0–8.5 with 1 N HCl or 1 N NaOH. For pH below 5.0, agar solution and the other components were sterilized separately, then mixed to prepare the media. In other cultivation experiments, pH was not adjusted.

PDA medium was the product of Difco, and the other reagents were produced by Wako Pure Chemical Indus-

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Fig. 1. Fruit-bodies of *Mycena chlorophos* growing on the rotten petioles of a palm, *Phoenix roebelenii*, on Hachijo Island in July 1990.

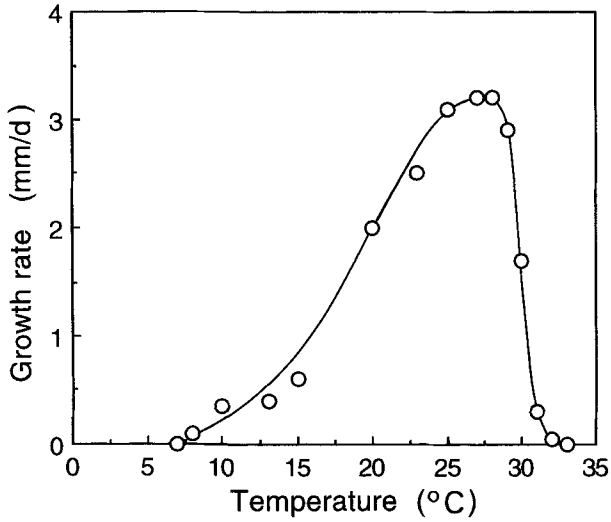

Fig. 3. Effect of cultivation temperature on mycelial growth. Mycelia were cultivated at various temperatures on MA medium. The growth rate is expressed as elongation of mycelia per day in the linear growth phase.

tries.

Composition of solid media For the experiment on mycelial growth, the following media A–H were used. The basal components were the powdered stems of *Oryza sativa* L. (rice) for the medium A, sawdust of *Quercus acutissima* Carr. (Japanese oak) for B, sawdust of *Cryptomeria japonica* L. (Japanese cedar) for C, decomposed petioles of *P. roebelenii* for G, and four kinds of commercial composts for horticulture, i.e., decomposed sawdust of Japanese cedar for D (Hitachi Farm product), decomposed bark of Japanese cedar for E (Green Tec product), and decomposed leaves of Japanese oaks (*Q. acutissima* Carr. and *Q. serrata* Murr.) for F and H. (Esta product and JT Agris product). The composts were made by heaping up the raw materials and allowing them to decompose in open air for more than 12 mo. These basal components were air-dried indoors for 3 wk, ground with a Wiley's mill, then sieved to get a powder having particles of 0.25 mm to 2.0 mm in diam, ca. 15% in moisture content. Commercial rice bran, ca. 10% in moisture content, was added to each powdered basal component in a portion of 10% (fw/fw), then the moisture content was adjusted to 70% (w/w) on wet basis by adding purified water. A 30-g portion of each medium was packed in a glass Petri dish of 40 mm in height and 90 mm in diam, then autoclaved at 121°C for 15 min. For the experiments on formation of primordia and fruit-bodies, compost medium H was used.

Casing To induce fruit-bodies, compost H powder adjusted to a moisture content of 75% (w/w) and autoclaved at 121°C for 15 min was used as the covering material (casing). Mycelia were cultivated for 4 wk at 27±0.5°C, then 5 g of casing was put on the mycelial mat with a sterilized spoon and pressed lightly on it. The casing layer was 1–3 mm thick. Similarly, 5 g of purified

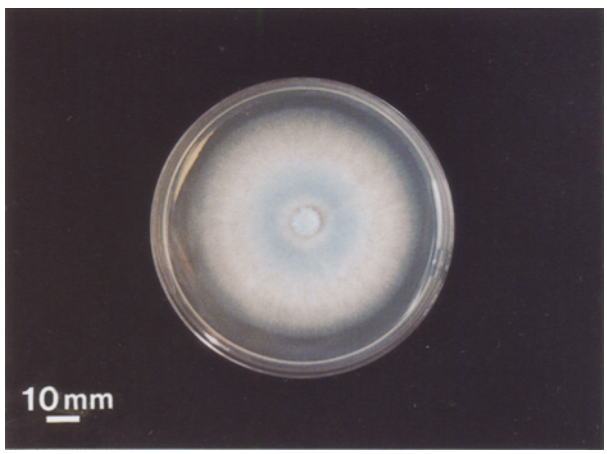

Fig. 2. A colony of strain H-113 on YMA medium. The cultivation period was 3 wk at 27°C.

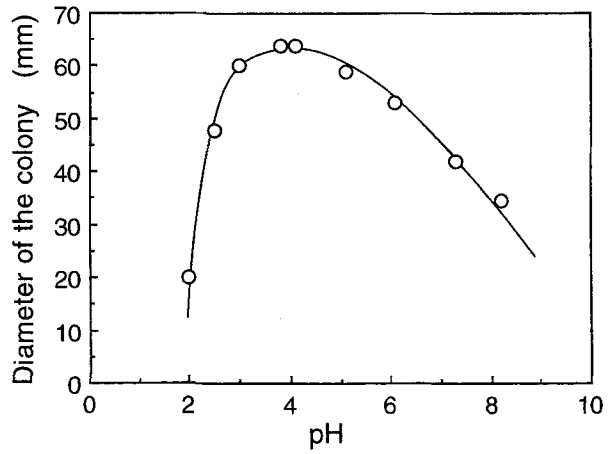

Fig. 4. Effect of initial pH of the medium on mycelial growth. Mycelia were cultivated at 27°C on YMA media of various pHs. The growth rate is expressed as colony diam after cultivation for 10 d.

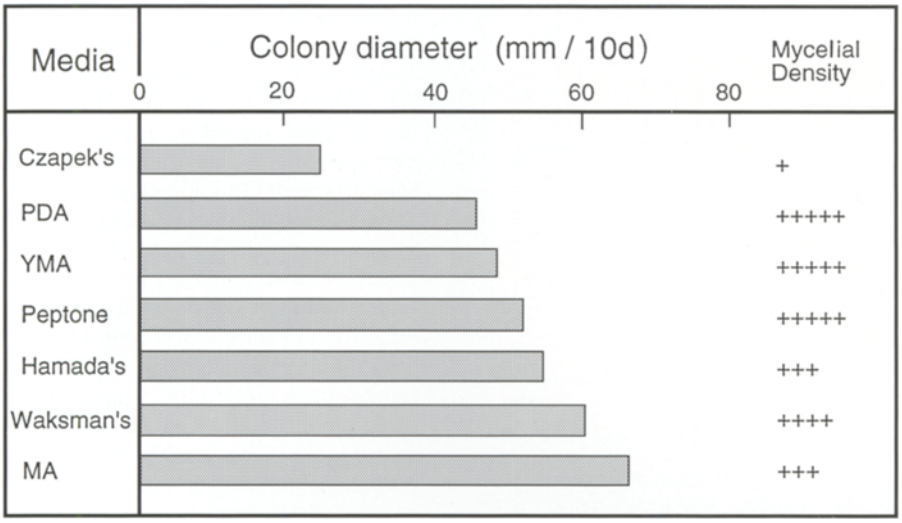

Fig. 5. Mycelial growth rate on various agar media.
The diam of colonies and mycelial densities were measured after cultivation for 10 d at 27°C. Mycelial density: thin (+), slightly thin (++), slightly dense (+++), dense (++++), very dense (+++++).

water-absorbed silica gel particles (2–3 mm in diam) were tested as the casing instead of moist compost powder.

Cultivation Spawn was cultured on the peptone agar medium at 27±0.5°C for about 2 wk. A disk (5 mm in diam) of mycelial mat was cut from the periphery of the colony with a sterilized corkborer and used as spawn. A spawn disk was inoculated at the center of each medium, which were subsequently cultivated at 27±0.5°C.

To examine the effect of temperature on mycelial growth, the mycelia were cultivated on MA medium at various temperatures from 5±0.5°C to 35±0.5°C. To examine the optimum initial pH of media, the mycelia were cultivated at 27±0.5°C. To examine the effect of temperature on primordium formation, the mycelial mat was cased with moistened compost powder after cultivation for 4 wk at 27±0.5°C. Then the Petri dishes were placed in incubators fitted with 20 W fluorescent lamps (MIR-151, MIR-251, MIR-552, Sanyo Electric Co. Japan) and cultivated at temperatures from 13±0.5°C to 27±0.5°C.

To examine the effect of light on primordium formation after casing, the mycelia in Petri dishes were placed and cultivated at 21±0.5°C in 28 positions illuminated with different intensities from 0.1 lx to 1000 lx in incubators. The light intensity at each position was measured with an illuminometer T-1 (Minolta Co. Japan).

Measurements of the mycelial growth To measure the growth rate of mycelium at various temperatures, the diam of mycelial colonies was measured in two directions for 15 d at intervals of 3 to 4 d. The average elongation

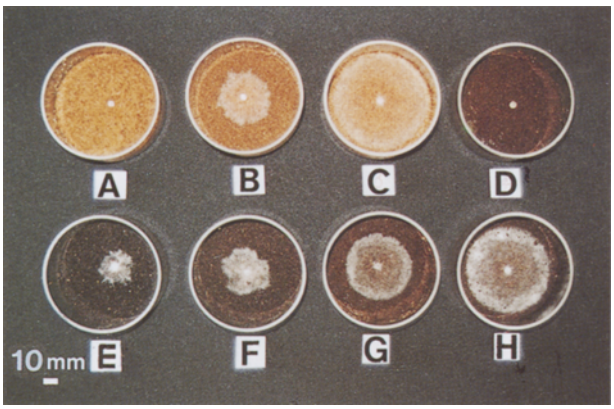

Fig. 6. Mycelial growth on various solid media.
Mycelial growth was observed after cultivation for 3 wk at 27°C. Basal component of the media; A: straw powder (*Oryza sativa*), B: sawdust (*Quercus acutissima*), C: sawdust (*Cryptomeria japonica*), G: compost (*Phoenix roeberenii*), D–H: compost (commercial).

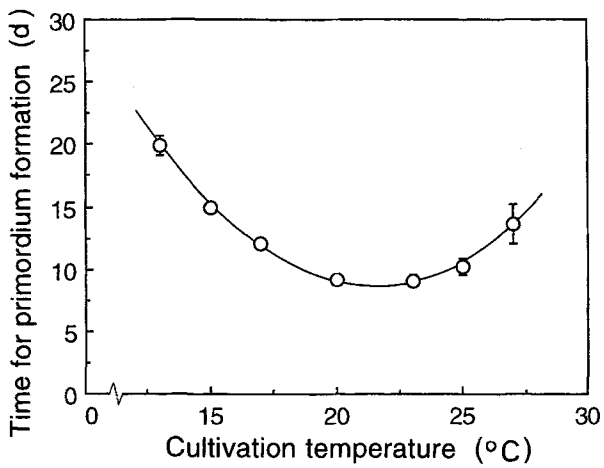

Fig. 7. Effect of cultivation temperature on primordium formation.
Days required for primordium formation at various temperatures were counted after casing. Vertical bars show standard errors.

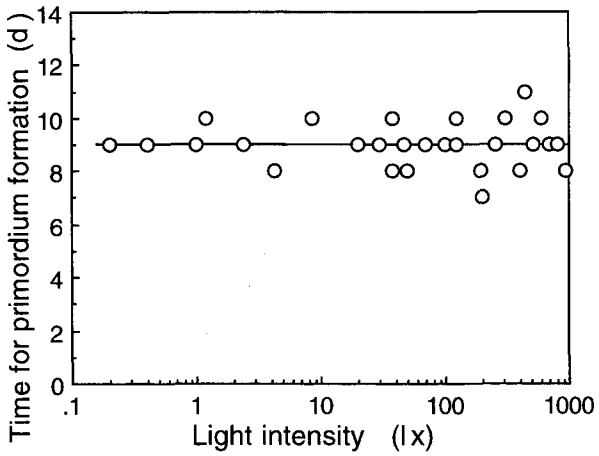

Fig. 8. Effect of light intensity on primordium formation. Mycelium was cultivated at various light intensities in the incubator. Days required for primordium formation were counted after casing.

of mycelium per day (mm/d) in the linear growth period was calculated from the measurement in five Petri dish cultures.

To compare various media for mycelial growth, the diam of colonies was measured in two directions in five Petri dishes after cultivation for 10 d at 27±0.5°C, and the average growth rate of mycelium (mm/10 d) was calculated. Mycelial density was assessed visually and expressed in five levels from + to +++++ in order of increasing density.

Factors influencing primordium formation After casing, the surface of the mycelial mat was observed daily under a 10-power stereoscopic microscope. The number of days required for primordium formation after casing was determined at various culture temperatures (13°C to 27°C) and light intensities (0.1 lx to 1000 lx). Ten Petri dishes were tested at each temperature, and one Petri dish was observed at each intensity of light.

Measurement of luminescence The intensity of luminescence emitted by a fruit-body was measured with a photodetector INC-7000 (Otsuka Electronic Co. Japan). The condenser of the photodetector was set vertically 4 mm above the upper surface of the pileus. In the examination of temperature effect, the lower part of a Petri dish cotaining a fruit-body was immersed in a constant temperature water bath regulated from 2°C to 28°C and the temperature of the medium was measured with a thermocouple inserted in it. After the temperature of medium reached a given value, the intensity of luminescence emitted from the pileus was measured.

Results

Appearance of the fungus Figure 1 shows fruit-bodies of *M. chlorophos* developed naturally on rotten petioles of *P. roebelenii*. Figure 2 shows a colony of strain H-113 on YMA medium. Colonies are normally white, becoming blue in age.

Effect of temperature, pH, and medium on mycelial growth Figure 3 shows the growth rates of mycelium at various cultivation temperatures. The mycelium grew in the range of 8°C to 32°C, and the maximum growth rate of 3.2 mm/d was seen at 27°C. Figure 4 shows the effect of initial pH of medium. The maximum colony diam of 63.8 mm was observed at pH 4.0. Figure 5

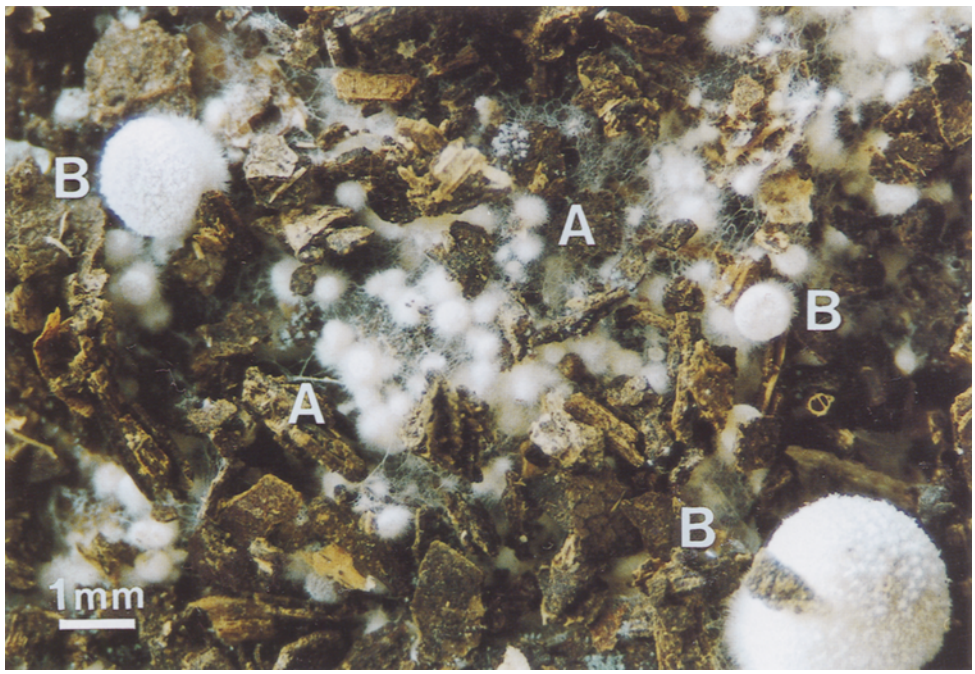

Fig. 9. Primordia and young fruit-bodies on the compost medium. Mycelium was cultivated for 12 d after casing. A: primordium, B: young fruit-body.

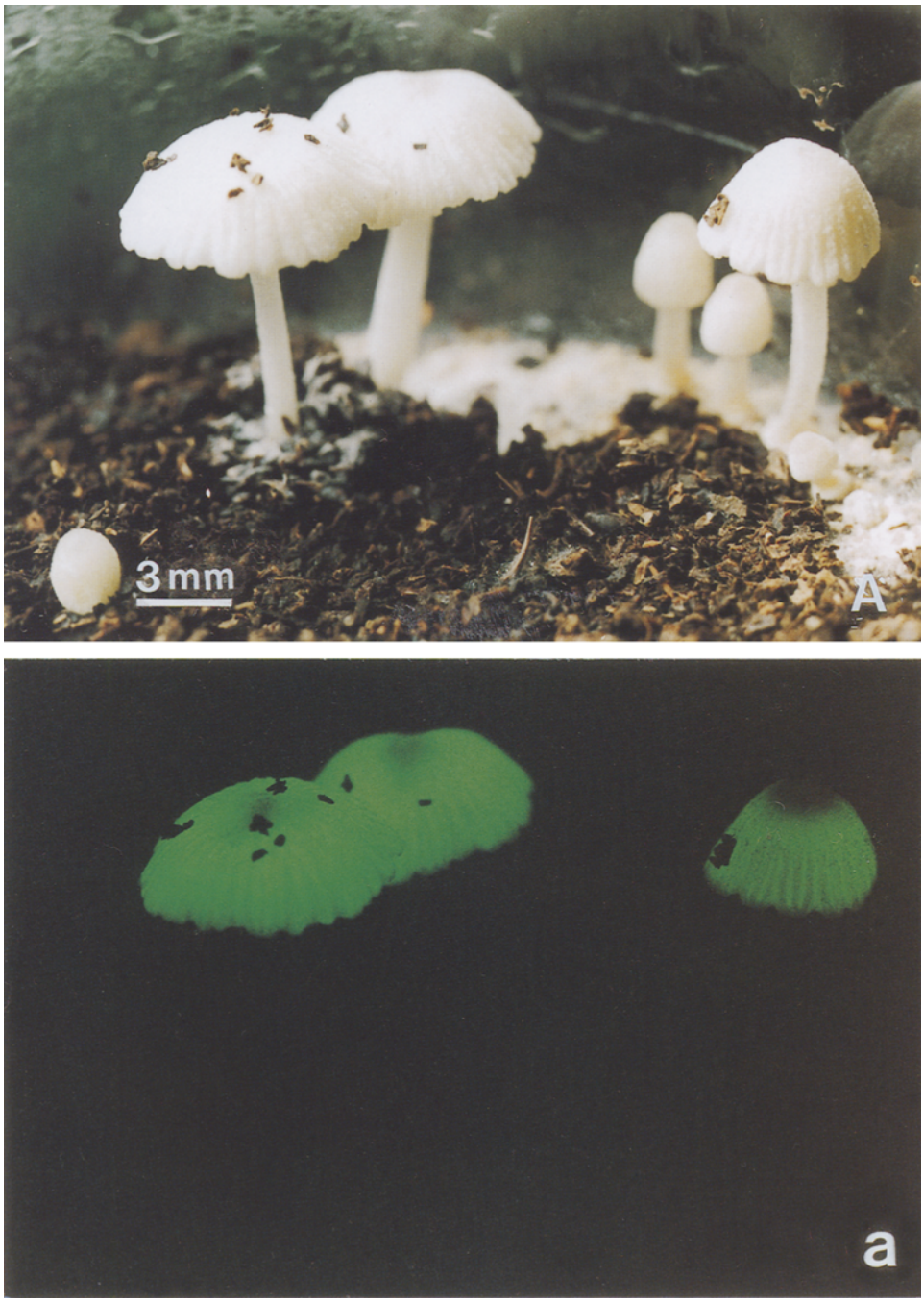

Fig. 10. Young fruit-bodies and mature fruit-bodies on the compost medium. Photographed in the light (A) and in the dark (a).

shows the evaluation of mycelial growth on the various agar media. The largest colony diam of 65 mm was attained on MA medium, while the mycelial density was highest on PDA, YMA and peptone agar media. Optimal growth in terms of both colony diam and mycelial density was observed on peptone agar medium.

Mycelial growth on the various solid media Figure 6 shows the growth of mycelium on eight solid media. Colony diam and mycelial density were both highest in the compost medium H, followed by medium G, which was made from the petioles of *P. roebelenii*. Media B and C, containing respectively the sawdust of Japanese oak and Japanese cedar, showed relatively low mycelial densities. In medium A containing rice straw and medium D containing sawdust compost, no mycelial growth was observed.

Effects of temperature and light on primordium formation Figure 7 shows the relation between the time required for

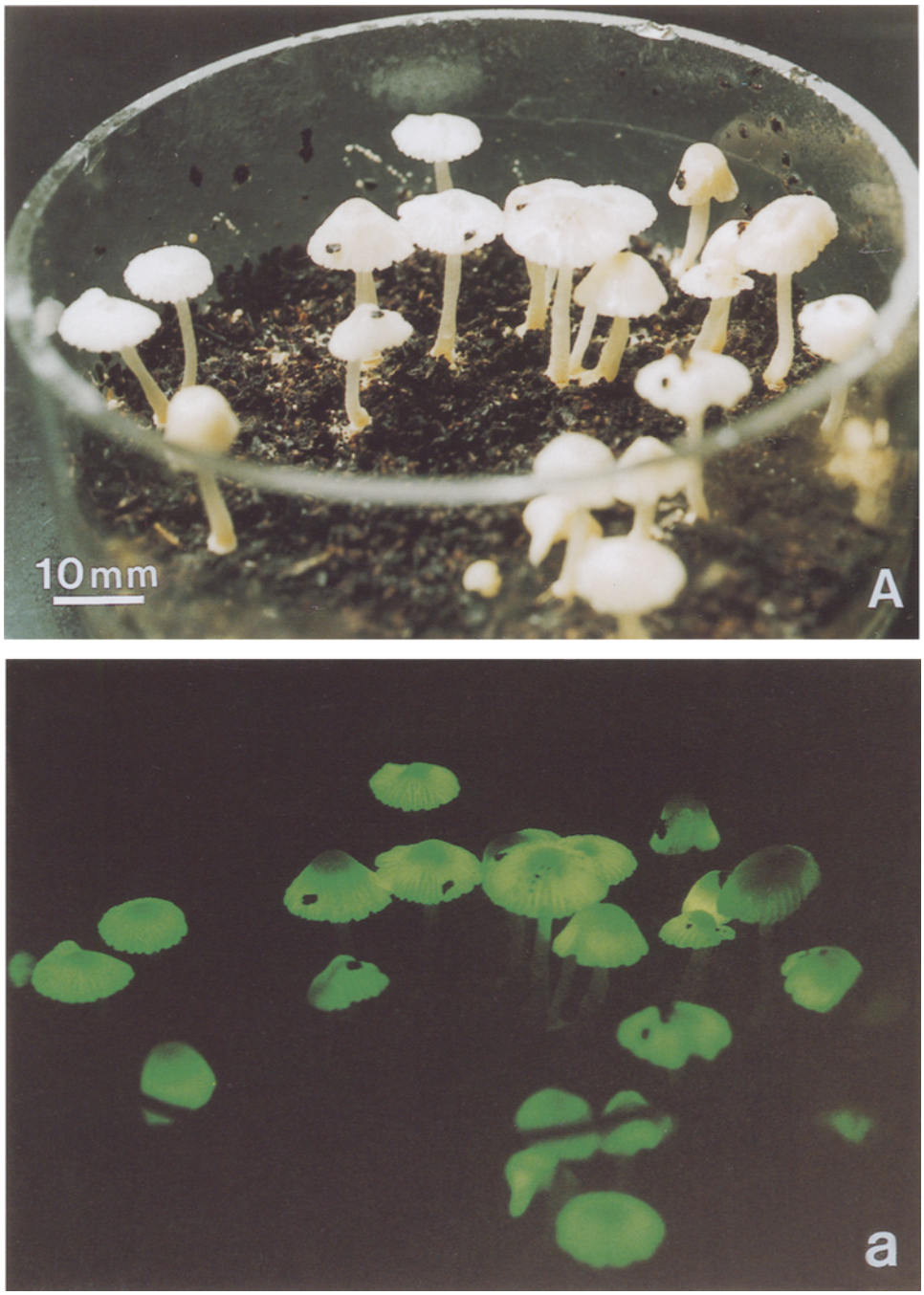

Fig. 11. Fruit-bodies growing in a Petri dish.
Mycelium was cultivated for 7 wk. Photographed in the light (A) and in the dark (a).

primordium formation after casing and culture temperature. At 21°C, primordia were formed after 9 d, the shortest time observed. At 13°C, the required time averaged 20 d, fruit-body formation was also delayed, and expansion of the pileus did not occur. At 27°C, the primordia were formed after 14 d on average, and the number of primordia was relatively small.

Figure 8 shows the relation between the time required for primordium formation and light intensity. Primordium formation was not observed in complete darkness or under illumination at 0.1 lx. At above 0.2 lx, primordium was developed, but the intensity of illumination had little effect on the time required for primordium formation.

Effect of casing Primordia developed scarcely without casing the mycelial mat. Casing with 5 g of moist compost powder effectively promoted the formation of primordia and fruit-bodies. Casing with water-absorbed

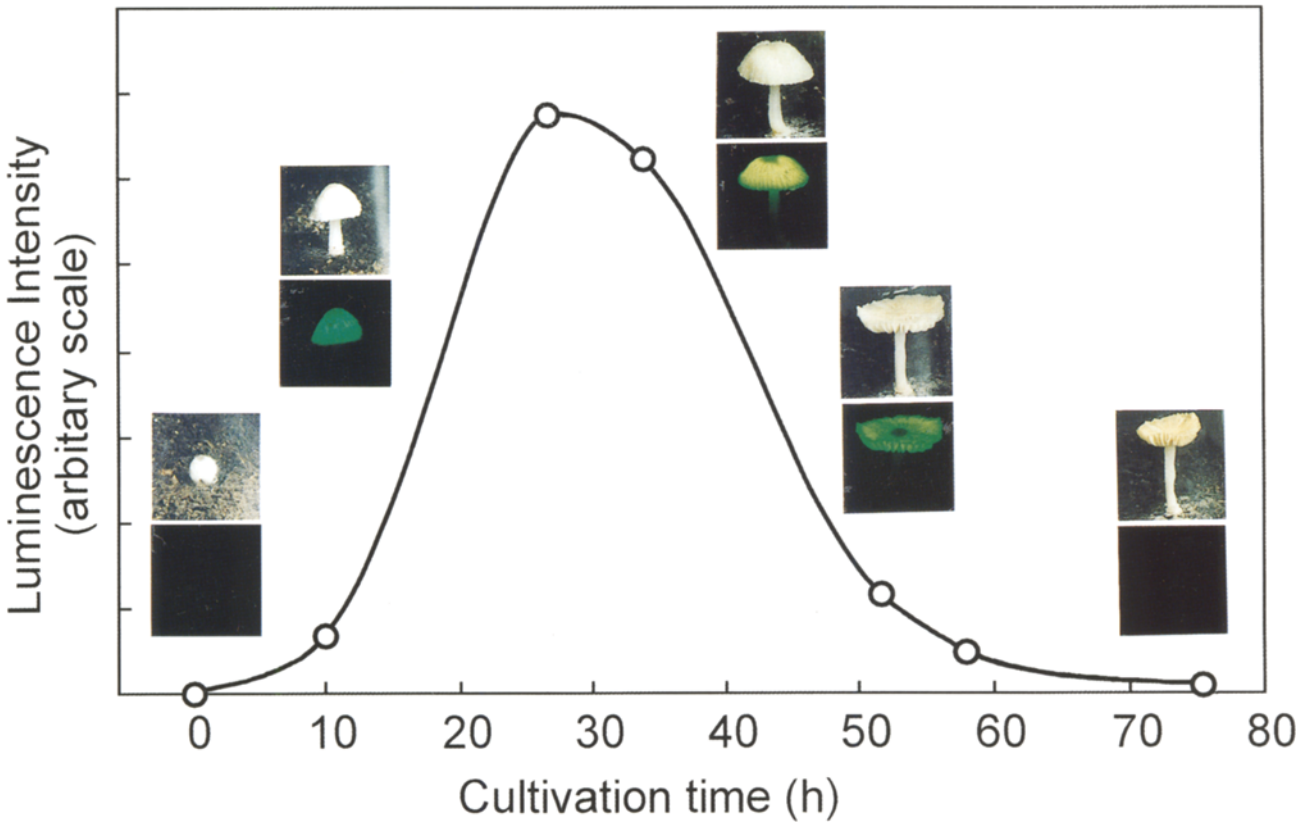

Fig. 12. Changes in luminescence intensity during the growth of the fruit-body.
The insets show the shapes and luminescence of a fruit-body cultivated between 19 d (0 h) and 21 d (72 h) after casing.

silica gel particles in place of moist compost powder scarcely promoted primordium formation.

The process of primordium and fruit-body formation Mycelial mat was cultivated at 21°C under photo-irradiation after casing. Around 5 d after casing, mycelia gathered together to form a number of star-shaped clusters. These developed into cotton-like masses after 7 d and minute balls after 9 d. The balls were primordia, which were covered with short white hairs, ca. 0.5 mm in diam (A in Fig. 9). A yellow-brown jelly-like substance was observed inside each primordium. After about

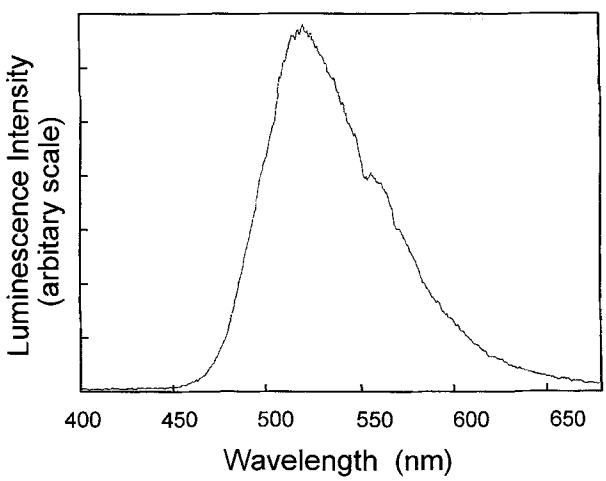

Fig. 13. Bioluminescence spectrum on the fruit-body.
The intensity of luminescence emitted from the upper surface of a pileus was measured.

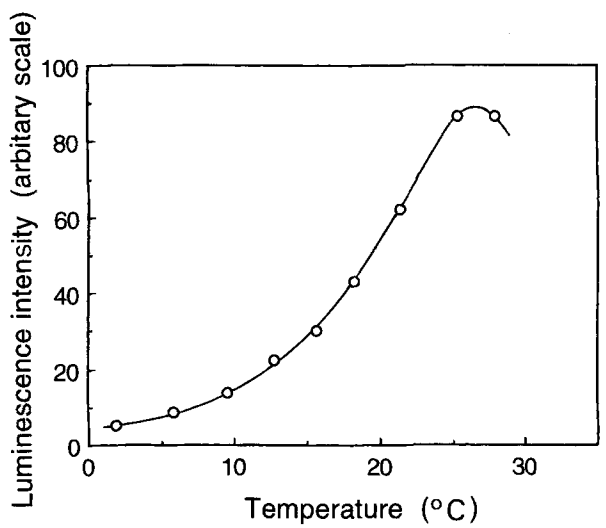

Fig. 14. Effect of temperature on luminescence intensity of the fruit-body.
The temperature of the fruit-body was regulated in a water bath and the luminescence emitted from the pileus was measured at each temperature.

12 d, the primordia became larger balls (young fruit-bodies), 1–2 mm in diam, and were covered with minute white particles (B in Fig. 9). After about 14 d, the pileus and stipe of young fruit-bodies were differentiated morphologically in the balls. After about 16 d, the spherical bodies became thinner and the top parts turned gray. After about 19 d, the stipes elongated and the pilei expanded, and mature fruit-bodies were formed about 21 d after casing.

The luminescent state of fruit-bodys Figures 10 and 11 show fruit-bodies formed on the compost medium in a Petri dish and their luminescent state. Figure 12 shows the changes in luminescence during the development of a fruit-body. No luminescence was observed in the young fruit-body around 19 d after casing (0 h in the figure). About 10 h later, the stipe elongated, the pileus expanded a little and luminescence was observed on the periphery of pileus. Luminescence reached maximum about 30 h later, when the pileus had expanded completely. About 50 h later, the luminescence decreased considerably, becaming invisible to the naked eye about 72 h after the begining of emission. The luminescence from a fruit-body normally persisted for about 3 d at 21°C.

The luminescence spectrum and temperature effect on the luminescence intensity. Figure 13 shows the wavelength distribution of luminescence of a fruit-body. The maximum photosensitive wavelength was found to be 522 nm. Figure 14 shows the relation between the intensity of luminescence of the fruit-body and its temperature from 2°C to 28°C. The optimum temperature for emission was about 27°C.

Discussion

Mycena chlorophos is a mushroom normally distributed in the tropics and the subtropics. The optimum temperatures for the mycelial growth and primordium formation of the strain used in the present experiments were found to be 27°C and 21°C respectively, generally similar to those of the other mesophile mushrooms. These properties are consistent with the climate of Hachijo Island, located in the Temperate zone. The average air temperature of the island in July has a daily maximum of 27.7°C and a minimum of 23.2°C, so the temperature difference of approximately 6°C between the two optima mentioned above appears reasonable.

Peptone agar medium was suitable for spawn culture, supporting a fine mycelial growth. Rice straw and sawdust were inadequate as the basal component of the solid culture medium. Several composts were found to be suitable for mycelial growth, though they differed depending on the kind of composted material and the degree of decomposition. Compost H was most practical for fruit-body production.

To induce primordium formation, it is necessary to cover the mycelial mat with a layer of soil or peat as casing in the commercial productin of the edible mushroom, *Agaricus bisporus* (Lange) Sing. In the present experiments using *M. chlorophos*, casing with compost powder was also essential to produce normal fruit-bodies. Why fruit-bodies develop spontaneously in nature without casing is unclear, although various physical, chemical, and microbial factors have been suggested to stimulate primordium formation (Urayama, 1961; Hayes et al., 1969). In the present experiments, casing was performed with the same sterilized compost as was used as a medium component. Primordium formation also occurred when the mycelial mat was covered with sterilized water-absorbed silica gel particles. So the participation of bacteria or physiologically active substances contained in the casing, such as that in the cultivation of *A. bisporus*, can be ruled out. The optimum thickness of casing layer in the present experiments was relatively thin, 1 mm to 3 mm, so the acceleration of gas exchange due to a chimney effect is unlikely to be significant. Thus, the effect of casing in this experiment may be the result of appropriate supply of water to the mycelial mat accompanied with good ventilation. Because this mushroom grows well on Hachijo Island only in the rainy season in June and July, moisture may be a large factor in the primordium and fruit-body formation.

The maximum wavelength of luminescence was 522 nm, almost the same as that of *L. japonicus* (524 nm). The optimum temperature for emission of *M. chlorophos* was about 27°C, about 8°C higher than that reported for *L. japonicus* (Isobe et al., 1987). The higher optimum temperature reflects the subtropical property of this strain of *M. chlorophos*.

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