Mycoscience 35: 187–189, 1994

Short Communication

Fruiting of *Lyophyllum tylicolor* in plate culture on Soytone-glucose agar and urea-treated soil extract agar

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Accepted for publication 1 April 1994

***Lyophyllum tylicolor*, which forms mycelial basidia (and basidiospores), produced fruit-bodies when cultivated at 20°C under continuous illumination of 400–700 lux on agar plates containing Bacto-Soytone and glucose or an extract from urea-treated soil. Under these conditions, mycelial basidia were also observed on the Soytone-glucose agar, but not on the soil extract agar. In darkness, fruit-bodies and mycelial basidia were not observed on either medium. In culture on the soil extract agar, fruit-body primordia were produced at the position of the margin of the colony when it was transferred from darkness to continuous light; stipes did not elongate under illumination of ca. 2000 lux; and mycelial basidia and basidiospores, but not fruit-bodies, developed when glucose concentration in the medium was as high as 1% (w/v).**

Key Words——fruit in culture; glucose; light treatment; *Lyophyllum tylicolor*; mycelial basidia; urea-treated soil.

Lyophyllum tylicolor (Fr.: Fr.) Lange & Sivertsen (syn. *Tephrocybe tesquorum* (Fr.) Moser) forms "mycelial basidia" in culture (Yamanaka and Sagara, 1990). Namely, it produces basidia and basidiospores on mycelia without forming fruit-bodies. Formation of fruit-bodies by this fungus in culture has been briefly noted, but the conditions were not specified (Sagara, 1976). I have previously tried to acquire fruit-bodies of this fungus in culture and found two agar media that favor constant fruit-body formation. In this paper I describe the media and conditions for this fungus to fruit. And I note the effects of light treatment on the fruiting.

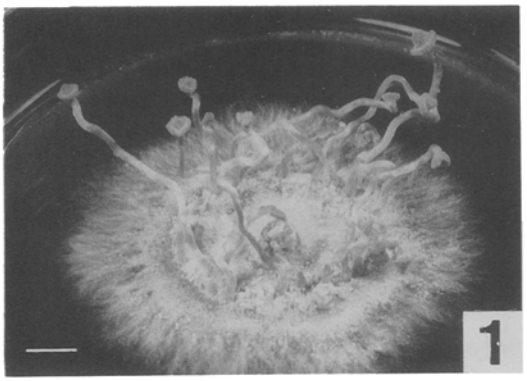
The strain of *L. tylicolor* used in this study was obtained by cultivation of basidiospores discharged from fruit bodies, which had been collected from urea-treated ground in a *Pinus densiflora* Sieb. & Zucc. forest of Kyoto University Forest, Kyoto City, on 12 June 1990.

Two media prepared to obtain fruit-bodies: Soytone-glucose agar (SGA) and urea-treated soil extract agar (USEA). SGA was made from 10 g of Bacto-Soytone (Difco), 10 g of glucose and 15 g of agar, and the pH was adjustd to 6.4 before autoclaving for 20 min at 120°C. USEA was made by modifying the procedure of Safar and Cooke (1988) for dung extract-cellulose agar. First, 200 g of raw humus (ca. 70% water content) collected from the litter layer (A_0 horizon) in the above forest was put in a 1/5000 are plastic pot. This soil was treated with 4 g of urea and 200 ml of distilled water and kept in the laboratory. Fruit-bodies of *L. tylicolor* developed ca. 3 weeks after this treatment (Yamanaka, unpublished data). Five days after the treatment, the soil was suspended in 1 litre of tap water at 80°C for 1 h. The suspension was filtered, and 15 g of agar was added to the filtrate. The pH of the medium was adjusted to 8.0 with 1 N HCl and 1 N NaOH. After autoclaving the medium at 120°C for 20 min, glucose solution was aseptically added to the molten medium by passage through a Millipore filter (pore size 0.45 μm). The final concentration of glucose in the USEA medium was adjusted to 0, 0.01, 0.1 or 1% (w/v)

Mycelial inocula were placed on 15 ml of the media in 9-cm petri dishes. Cultures were incubated at 20°C under continuous illumination of 400–700 lux (at plate surface) from day-light fluorescent tubes (Hitachi, FL 20 SSD/18-G). Most observations were recorded 3–4 weeks after inoculation, but the plates were kept for several weeks longer if fruit-bodies were not formed. Mycelial basidium formation was checked under a microscope after staining with acetocarmine (Yamanaka and Sagara, 1990).

On SGA, many fruit-bodies as well as mycelial basidia occurred 2–3 weeks after inoculation (Fig. 1). Stipes developed abnormally, and the pilei were recurvate. Although gills were formed in the pilei, basidiospores were not discharged. In darkness, neither fruit-bodies nor mycelial basidia were formed.

On USEA, one or two fruit-bodies appeared on each plate with 0, 0.01 or 0.1% (w/v) glucose (Fig. 2). The fruit-bodies developed normally and discharged basidiospores 7–8 days after inoculation. Slightly larger fruit-bodies were formed on the plates with 0.1% glucose than those with 0 and 0.01% glucose. The time of initiation of fruiting and the number of fruit-bodies were the same at these glucose concentration. Mycelial basidia were not formed under these conditions. The plates with 1% glucose did not produce normal fruit-bodies, but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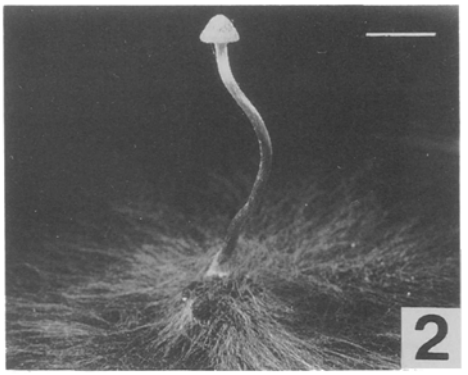
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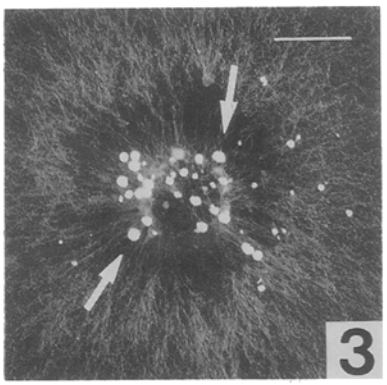
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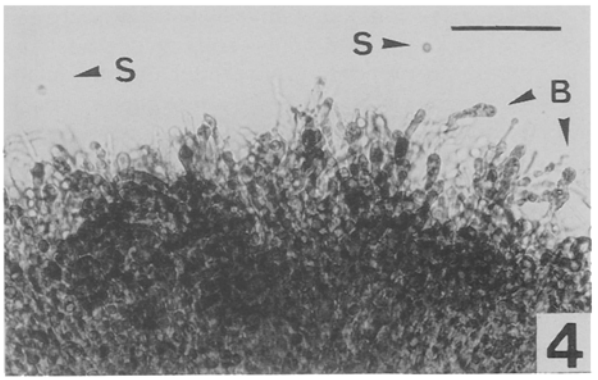

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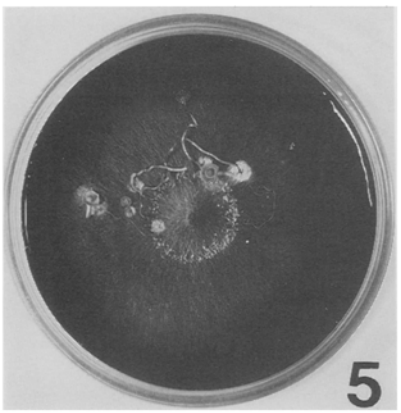
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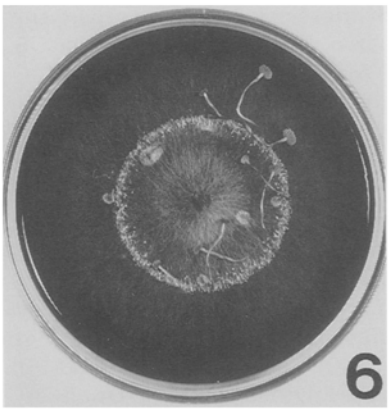
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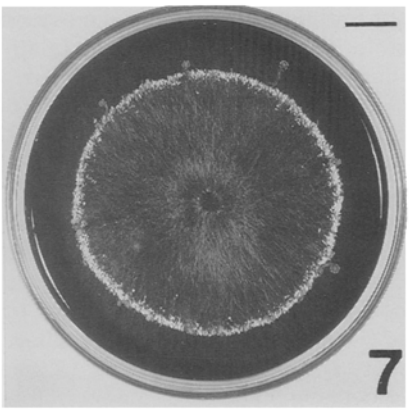
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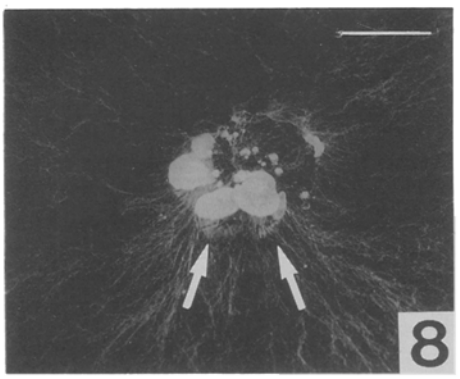
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primordium-like bodies which bore basidia and basidiospores at their surfaces (Figs. 3 and 4). In darkness, neither fruit-bodies nor mycelial basidia appeared.

Fruit-bodies of this fungus developed normally on USEA and discharged basidiospores, but on SGA, fruit-body formation was inhibited. This inhibition may be due to accumulation of self-inhibitory substances in the medium during active vegetative growth (Fig. 1). Mycelial colonies on USEA were sparse (Fig. 2) and, therefore, such substances would not accumulate so much to the detriment of fruit-body development.

Glucose concentration in USEA determined whether fruit-bodies or mycelial basidia would be formed. Normal fruit-bodies were yielded on plates with low concentration of glucose, namely, 0, 0.01, and 0.1% (w/v). At the concentration of 1%, the fungus formed not fruit-bodies but mycelial basidia and basidiospores. This is similar to MacMeekin's (1991) description that *Asterophora lycoperdoides* (Bull.) Ditmar: Fr. formed fruit bodies or chlamydospores depending on the concentration of glucose in an agar medium. When the glucose concentration was 1 or 2% (w/v), *A. lycoperdoides* produced fruit-bodies on the medium. Conversely, a mass of chlamydospores was formed on plates with 3 or 4% glucose. These two species have similar restricted habitats under natural conditions. *Asterophora lycoperdoides* is well known to appear on rotting agarics and *L. tylicolor* appeard on "what apparently were the very decayed remains of some fleshy fungus" (Smith, 1941; under the name *Collybia olympiana*).

Effects of light treatment on fruiting were tested in culture on USEA with 0.1% (w/v) glucose. Unless otherwise stated, plate cultures were incubated under the same conditions as described above. Cultures were initially placed in darkness, then transferred to continuous light 3, 6, 9 or 12 days after inoculation. At the time of transfer, the perimeter of the colony was outlined with a marker pen on the bottom of the plate. Fruit-body formation was observed for 1-2 weeks after the transfer.

In all light treatments except for the transfer after 12 days, fruit-body primordia appeared on clearly defined rings, which coincided with the front edge of the colony at the time of exposure to light (Figs. 5-7). Some of the primordia developed into normal fruit-bodies. Such fruit-body formation was also observed in other basidiomycetes (Madelin, 1956; Lu, 1965; Kitamoto et al., 1968; Perkins, 1969; Morimoto and Oda, 1973). When the plates were transferred to the light 12 days after inoculation, the margin of the colony had already reached the edge of the plates and there was no zone in which the primordia could develop. Cultures placed under continuous light from the beginning produced primordia close to the inoculum (Fig. 2). These observations suggest that the hyphal tip of a growing colony is susceptible to photoinduction of fruiting in this fungus. Mycelial basidia were not observed on these plates, but they might have been formed later on the primordia that did not develop into normal fruit-bodies.

When the plates were placed under continuous illumination of ca. 2000 lux, the stipes did not normally elongate, but the pilei, lamellae and basidiospores matured (Fig. 8).

Thus, *L. tylicolor* formed fruit-bodies on SGA and USEA. These media contain relatively large amounts of nitrogenous substances: Bacto-Soytone (soybean protein) in the former and urea (and ammonia) in the latter. Sagara (1976) noted fruit-bodies of this fungus on glucose-dry yeast agar supplemented with urea. These observations match the pattern of occurrence of this fungus in the field: it fruits after addition of some ammonia-releasing materials to forest soil (Sagara, 1975).

Acknowledgements——I thank Prof Dr N. Sagara, Kyoto University, for critically reading the manuscript.

Literature cited

Kitamoto, Y., Takahashi, M. and Kasai, Z. 1968. Light-induced formation of fruit-bodies in a basidiomycete, *Favolus arcularius* (Fr.) Ames. Pl. Cell Physiol. **9**: 797-805.

Lu, B. C. 1965. The role of light in fructification of the basidiomycete *Cyathus stercoreus*. Amer. J. Bot. **52**: 432-437.

McMeekin, D. 1991. Basidiocarp formation in *Asterophora lycoperdoides*. Mycologia **83**: 220-223.

Madelin, M. F. 1956. The influence of light and temperature on fruiting of *Coprinus lagopus* Fr. in pure culture. Ann. Bot. **20**: 467-481.

Morimoto, N. and Oda, Y. 1973. Effect of light on fruit-body formation in a basidiomycete, *Coprinus macrorhizus*. Pl. Cell Physiol. **14**: 217-225.

Perkins, J. H. 1969. Morphogenesis in *Schizophyllum commune*. I. Effects of white light. Pl. Physiol. **44**: 1706-1711.

Safar, H. M. and Cooke, R. C. 1988. Interactions between bacteria and coprophilous Ascomycotina and *Coprinus* species on agar and in copromes. Trans. Br. Mycol. Soc. **91**: 73-80.

Sagara, N. 1975. Ammonia fungi—a chemoecological grouping of terrestrial fungi. Contr. Biol. Lab. Kyoto Univ. **24**: 205-276.

Sagara, N. 1976. Supplement to the studies of ammonia fungi (1). Trans. Mycol. Soc. Japan **17**: 418-428 (in Japanese).

Smith, A. H. 1941. Studies on North American Agaricales. 1. Contr. Mich. Univ. Herb. **5**: 1-73.

Yamanaka, T. and Sagara, N. 1990. Development of basidia and basidiospores from slide-cultured mycelia in *Lyophyllum tylicolor* (Agaricales). Mycol. Res. **94**: 847-850.

Figs. 1-8. *Lyophyllum tylicolor* in plate culture. 1. Fruiting on glucose-Soytone agar. Scale=5 mm. 2. Fruiting on urea-treated soil extract agar supplemented with 0.1% (w/v) glucose. Scale=5 mm. 3. Primordium-like bodies (arrows) on the soil extract agar with 1% glucose. Scale=5 mm. 4. Basidia (B) and basidiospores (S) formed on one of the bodies shown in Fig. 3. Scale=0.1 mm. 5-7. Fruit-body primordia formed on the front edges of the colony at the time of transfer from darkness to continuous light 3 days (5), 6 days (6) or 9 days (7) after inoculation. Note some of the primordia developed into fruit-bodies. Scale=10 mm. 8. Fruiting without stipe elongation; note the basidiospores fallen on the media (arrows). Scale=5 mm.