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Short Communication

Light-induced fruit body formation of an entomogenous fungus *Paecilomyces tenuipes*

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Asexual fruit body formation of an entomogenous fungus, *Paecilomyces tenuipes*, on an artificial culture medium was investigated. For the fruit body induction, light/dark condition was important. Illumination by white light in a particular critical period preceded by a dark period induced the fruit bodies. The critical period was identified to be around 6–14 d after inoculation at 20°C.

Key Words——*Cordyceps* sp.; fruit body; induction by light; *Isaria japonica*; *Paecilomyces tenuipes*.

Paecilomyces tenuipes (Peck) Samson (Samson, 1974), sometimes referred to as *Isaria japonica* Yasuda or other synonyms, is a fungus parasitic to various lepidopteran insects. This species is thought to be the anamorph of a *Cordyceps* sp. (Fukatsu et al., 1997), probably *C. takaomontana* Yakushiji et Kumazawa (Shimizu, 1994) or *C. polyarthrea* Möller (Chen and Xu, 1989). It infects pupae or larvae of moths, forming yellow asexual fruit bodies (synnemata), which are simple, furcate, or irregularly branched, usually 1.5–4.7 cm in length, and with a densely branched and swollen conidiogenous structure on the tip region (Samson, 1974). *Paecilomyces tenuipes* has been isolated and cultivated on artificial media on which the fruit bodies can develop (Samson, 1974; Fukatsu et al., 1997; Yamanaka et al., 1998). In addition, the conidia of *P. tenuipes* can artificially be inoculated to moth pupae to produce fruit bodies like those occurring in natural conditions (Fukatsu et al., 1997; Yamanaka et al., 1998). Therefore, this fungus can be a useful model to investigate the mechanisms of fruit body formation. In attempts to control pest insects, entomogenous fungi such as *Beauveria*, *Metarrhizium*, and *Hirsutella* have been studied, and some of them have been commercialized as microbial insecticides (Burges, 1981). Several species of *Cordyceps* have been reported to possess pharmacological effects (Hobbs, 1995). *Paecilomyces tenuipes* has a similar potential for use. Thus, it is important to investigate the conditions under which the fruit bodies of *P. tenuipes* can be produced easily and efficiently on artificial media. Recently, Yamanaka et al. (1998) systematically examined such culture conditions as temperature, pH, medium type, CO_2 concentration, light intensity, etc., and established a system for mass-production of fruit bodies of *P. tenuipes*. In these works, however, the effect of the illumination regimen on fruit body formation was not examined in detail.

In the present paper, we investigated the effect of light/dark profiles on the fruit body development of *P. tenuipes* on an artificial medium, and identified the critical period when the fruit bodies are induced in response to white light illumination.

An unidentified moth pupa infected by *P. tenuipes* was collected on 9 June 1997 at Amami-Ohshima Is., Kagoshima Pref., Japan, and was kindly provided by K. Fujimoto. In this study, we used a strain of *P. tenuipes* isolated from the conidia formed on the fruit bodies of the specimen. The fungus was cultured and maintained with standard YMPD medium (3 g/l yeast extract, 3 g/l malt extract, 5 g/l Bactopeptone, 10 g/l glucose, with or without 1.5% agar).

We examined the fruit body formation using the following culture system. Mycelia of *P. tenuipes* were inoculated on thin YMPD agar plates and precultured at 20°C for a week in the dark. Then, agar discs of about 1.5 cm in diameter on which mycelial mat had formed were cut out of the plates. The discs were placed on 2 ml of liquid YMPD medium in 50 ml plastic tubes (25 mm in diameter × 115 mm in height, Greiner Labortechnik), and incubated at 20°C with the lid loosened for 20 days under various light/dark profiles using a BioMulti incubator LH-30-8CTS (NK System). Illumination was provided by a standard 8 watt fluorescent light installed in the incubator chambers.

Figure 1 shows the effect of simple light-dark or dark-light profiles of different durations. It was suggest-

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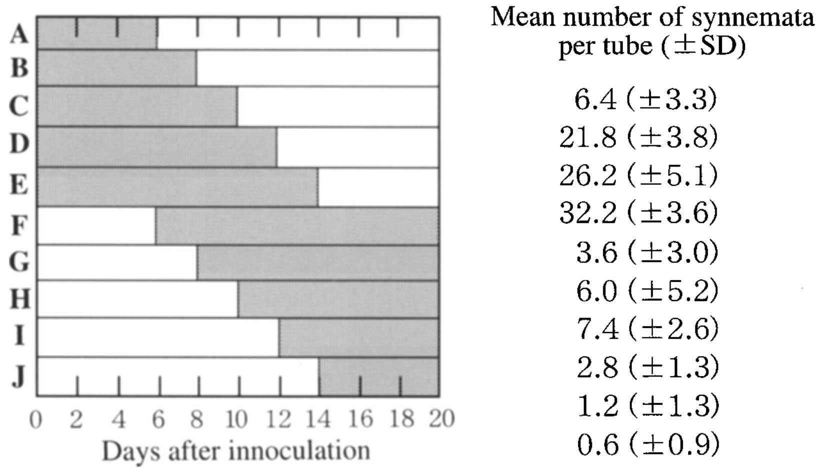

Fig. 1. Effect of simple light-dark or dark-light profiles of different durations on the fruit body formation of *P. tenuipes*. During the cultivation period of 20 d, illumination is indicated by the white area and darkness by the dark area. Five cultures were used for each treatment (A–J). The values on the right side indicate the mean number of synnemata ± standard deviation per tube for each treatment.

ed that illumination between day 8 and day 14 is important for the fruit body formation (A–E in Fig. 1). Illumination after day 14 or before day 8 had little effect. However, illumination between days 8 and 14 alone is not sufficient because fruit body formation was not induced (H–J in Fig. 1). It appears that illumination between days 8 and 14 should be preceded by a dark period in order to induce fruit body formation.

Figure 2 represents the effect of a transient light period of 2 d in an otherwise dark period of 20 d. This experiment showed that the illumination between days 6 and 12 induces the fruit bodies. It also showed that illumination for 2 d is enough for the induction.

These results clearly indicate that 1) the fruit body formation of *P. tenuipes* on YMPD medium is induced by a light stimulus of at least 2 d in a particular critical period, 2) the critical period is around 6–14 d after inoculation under the culture conditions adopted here, and 3) the light stimulus should be preceded by a dark period of a considerable length.

When the mycelial growth of *P. tenuipes* was measured on YMPD agar plates in the light and dark, we found a striking difference. After a week of incubation at 20°C, the average diameter of the colonies derived from a conidium was less than 1 cm in the constant light condition but about 3 cm in the constant dark (data not shown). This inhibitory action of light on the mycelial growth may be responsible for the requirement of the

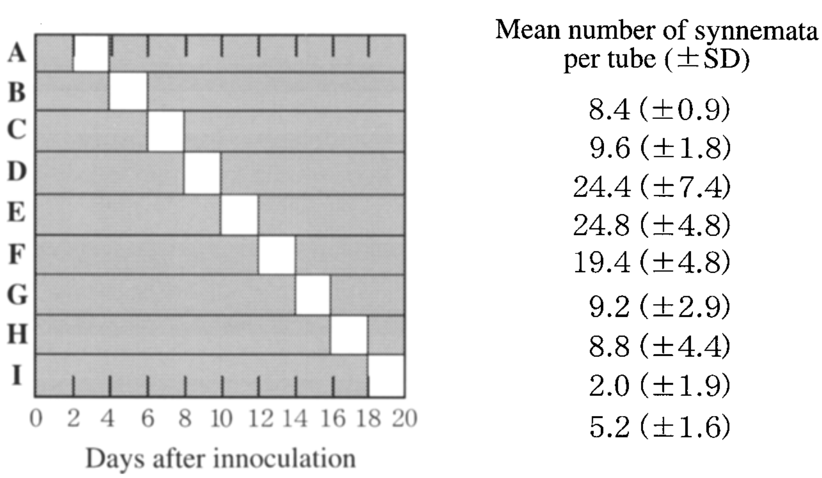

Fig. 2. Effect of a transient light period of 2 d in an otherwise dark period of 20 d. Illumination is indicated by the white area and darkness by the dark area. Five cultures were used for each treatment (A–I). The values on the right side indicate the mean number of synnemata ± standard deviation per tube for each treatment.

preceding dark period to obtain many fruit bodies.

It is not clear why the critical period identified in Fig. 1 differed slightly from that in Fig. 2. The difference in the light/dark profiles might result in the shift of the critical period in some way. Other uncontrollable parameters between independent experiments (e.g., condition of the preculture, slight variation in medium composition, etc.) might affect the results.

To further improve the yield of fruit bodies of *P. tenuipes*, many parameters remain to be examined. It has been reported that the intensity and wavelength of the illumination are also important for the fruit body induction in various fungi (Tsusue, 1969; Chapman and Fergus, 1972; Mooney and Yager, 1990). Various types of culture media should be tested in combination with the light/dark profiles. Isolation and selection of "good" fungal strains will also be important. It is possible that the system for mass-production of the fruit body of *P. tenuipes* (Yamanaka et al., 1998) might be improved if the critical period for light stimulus is properly considered.

For basic research, the results presented in this paper will be useful to investigate the mechanisms of fruit body formation in entomogenous fungi. For the applied aspects, the results indicate a way to produce fruit bodies and conidia in quantity for the control of insect pests. Since some *Cordyceps* fungi have long been utilized in oriental medicine, production of the fruit bodies of *P. tenuipes*, an anamorph of a *Cordyceps* species, would be of a pharmaceutical value.

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