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Short Communication

Activation of intracellular and extracellular phenol oxidases in photoinduced fruit-body formation of *Favolus arcularius*

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The activation of intracellular phenol oxidase (PO) was associated with the photoinduced fruit-body initiation in *Favolus arcularius*. The second activation of PO activity was also photoinduced in the rapidly developing stipe after the formation of pileus primordium under light exposure. However, the activity levels in the pileus and the mycelium remained low even in the cultures exposed to light. The extracellular PO activity in the culture filtrate also appears to be developmentally regulated: it rose sharply after primordium formation in the light, then decreased rapidly during further development of the fruit-body.

Key Words——*Favolus arcularius*; fruit-body development; fruit-body initiation; phenol oxidase; photomorphogenesis.

The initiation of fruit-body formation in *Favolus arcularius* (Fr.) Ames Fr.: Fr. is induced by blue light (Kitamoto et al., 1968, 1972). Light is also required for the initiation of pileus formation in fruit-body of *F. arcularius* (Horikoshi et al., 1974; Kitamoto et al., 1974, 1999). If the dark grown vegetative mycelium was exposed to light, the buff-colored mycelium was produced on a part of colony, at which fruit-body primordium was subsequently formed (Kitamoto et al., 1968). The mycelium-color development in fruit-body initiation suggests the involvement of the activation of intracellular phenol oxidase (PO: EC 1.14.18.1, tyrosinase: *o*-diphenol: oxygen oxidoreductase) to produce phenolic color compound(s), and the regulation of PO was closely associated with the induction and developmental processes of fruit-body formation in this mushrooms.

This paper describes a study on the photoinduced activation of intracellular and extracellular PO activities during the initiation and development of fruit-bodies in *F. arcularius*.

The dikaryotic strain (ATCC24461/69B) of *F. arcularius* was used in the present study. The static cultures by using a semi-synthetic liquid medium (Kitamoto et al., 1972) was used for all experiments presented here. The culture vessels were 50-ml Erlenmeyer flasks, in which 8 ml of the liquid medium was poured into the vessels. Cultures were autoclaved at 105°C for 10 min. For preparing inoculum agar blocks, 16 ml of agar plate medium, which consisted of glucose 20 g, the extract from 200 g of diced potatoes, agar 15 g and distilled water 1 L, at pH 5.6. After autoclaving at 115°C for 5 min, it was inoculated with the mycelium of the stock culture. The plate cultures were incubated at 25°C in darkness for 7 d. The mycelial colony of the plate cultures was cut by c.a. $3 \times 3 \times 3$ mm for the inoculum agar blocks, and an agar block was placed onto each liquid culture. The static cultures were preincubated at 25°C to allow mycelial growth for 6 d in the dark, and they were then moved into a culture room at 25°C under continuous illumination from daylight fluorescent tubes at about 200 lx for inducing fruit-body initiation and subsequent development of fruit-bodies.

For preparing the experimental samples, the vegetative mycelium and fruit-bodies at different culture ages (c.a. 1 g in fresh weight) were separately harvested, and washed sufficiently with distilled water, and then with 0.1 M potassium phosphate buffer (pH 6.5). Excess water in the samples was removed by using filter paper. The fungal materials were put into a glass homogenizer with 6 ml of 0.1 M potassium phosphate (pH 6.5), and homogenized for 3 min at 1,500 rpm. The resulting homogenate was centrifuged at 15,000 rpm for 30 min, and the supernatant solution was applied as the cell extract for determining PO activities. All procedures described as above were conducted at 0~5°C. Protein content was determined by the method of Lowry et al. (1951).

The PO activity was determined by measuring with DOPA (3,4-dihydroxyphenylalanine) as the substrate. The reaction mixture composed of 1.5 ml of 10 mM DOPA and 1 ml of 0.5 M acetate buffer (pH 5.4), and an appropriate amount of the cell extract in the total volume

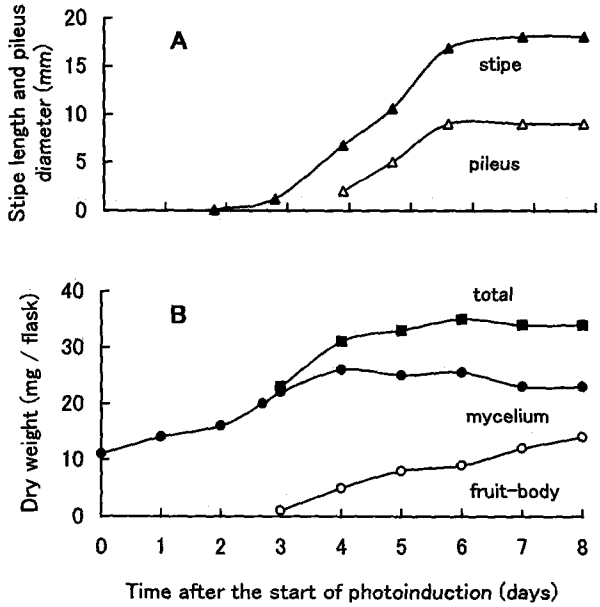

Fig. 1. Time course of fruit-body formation in *Favolus arcularius*.

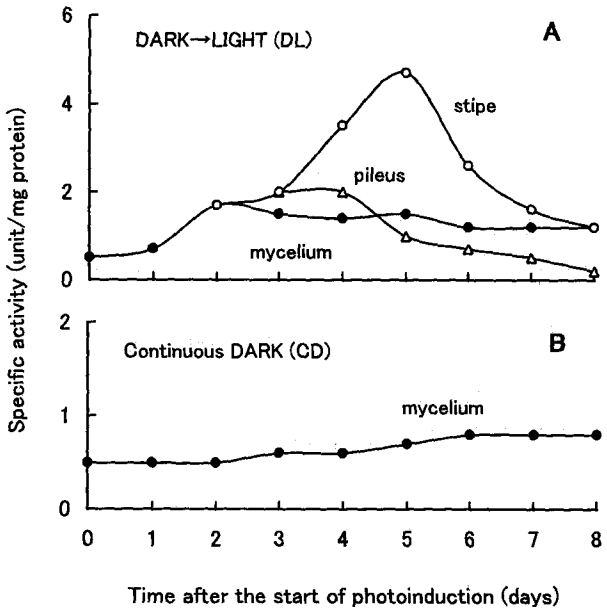

Fig. 2. Changes in specific activities of intracellular phenol oxidase in the mycelium and fruit-bodies of *Favolus arcularius* incubated in the DL and the continuous dark regimens.

of 3.5 ml. The enzyme reaction was carried out at 30°C by starting the addition of the cell extract, and the increase of absorbance at 475 nm by the formation of dopachrome from DOPA was determined by a spectrophotometer with a recorder (Shimazu model UV-2100S). One unit of PO is defined as an increase of absorbance of 0.001 per min by the enzyme reaction.

Time course of photoinduced fruit-body formation in the strain ATCC24461/69B was shown in Fig. 1. When the dark grown colony of vegetative mycelium was exposed to light, the buff-colored mycelium appeared on a part of the colony on the first day after the start of light exposure. The formation of fruit-body primordium, 0.5 mm in height, occurred in almost of the colonies on the 2nd day. Primordium developed into cylindrical stipe, and then pileus primordium was produced under light exposure on the apex of the stipe on the 4th day (cf. Horikoshi et al., 1974; Kitamoto et al., 1999). They matured on the 8th day, and the maximum dry weight of the fruit-body was attained on the same day.

The light requirements for the photoinduction of primordium and pileus primordium formations have been established in *Favolus arcularius* (Kitamoto et al., 1968; 1972; 1974; Horikoshi et al., 1974). A similar requirement of light in fruit-body formation of *Schizophyllum commune* Fr.: Fr. has been demonstrated by Perkins and Gordon (1969). Leonard (1971) and Leonard and Phillips (1973) reported that the intracellular PO activity was associated with fruit-body formation in *S. commune*. A variety of wild and mutant homokaryons and dikaryons were examined, and only those mycelia that produced fruiting structures were found to exhibit PO activity in *S. commune* (Leonard, 1972). In order to examine the correlation between photoinduced primordium and pileus primordium formations in *F. arcularius* and the activation of intracellular PO in the course of primordium and pileus-primordium formations, the changes in the enzyme activities were determined under the different light-programmed culture conditions for the *Favolus* colonies. The results are shown in Figs. 2 and 3.

When the 6 d-old dark grown cultures were exposed to continuous light (the DL regimen), the activity of PO was activated in the mycelium immediately after the start of light exposure (Fig. 2A). Fruit-body formation of *F. arcularius* was necessarily preceded by the production of melanin-buff-colored pigment in a corresponding part of the colony, in which fruiting might be initiated. The buff-colored mycelia might appear on the colony immediately after the initial increase in PO activity. Further, the PO level was increased by about four times on the second

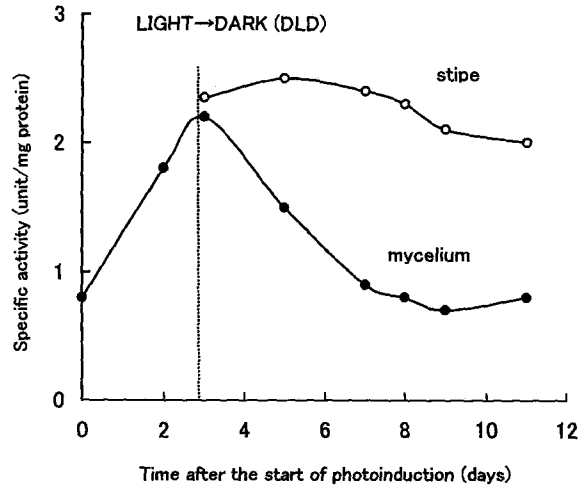

Fig. 3. Changes in specific activities of intracellular phenol oxidases in the mycelium and the fruit-bodies of *Favolus arcularius* incubated in the DLD regimen.

to the third day as compared with that in the dark grown mycelium. However, the PO activity in the vegetative mycelium during the period of vigorous mycelial growth in the dark remained at low level and it might not be significantly varied during their incubation for another 8 d (Fig. 2B). From these results, it is suggested that the activation of the PO activity in *F. arcularius* may be regulated by a mechanism of the photoinduction of primordium formation. Further, it is supposed that the PO should mediate the buff-color development of a part of mycelial colony where the primordium rises.

The activity of PO in the stipe seems to be also associated with the photoinduced pileus formation in *F. arcularius*. The specific activities of PO in the light grown cultures were stepped up, and maintained the same level until the completion of primordium formation. Subsequently, the second stage of increase of activity in the stipe occurred with pileus primordium formation and the further development, in which rapid elongation of stipe preceded (Fig. 2A). The activity level in the pileate stipe was attained at the maximum level on the 5th day, at which the value about 7 times as compared to that in the dark grown mycelia of the same culture age, and then decreased thereafter. On the other hand, if the culture with fruit-body primordium was moved into the dark chamber at the 3rd day after the start of photoinduction (the DLD regimen), it elongated in the dark, but it did not form pileus primordium. In this case, the PO activity gradually decreased after the slight increase in the activity at the early phase of epileate stipe growth (Fig. 3). However, the PO activity in the mycelium incubated in the dark only decreased to the basal level during the following days.

A relationship between morphogenesis and extracellular PO (laccase) activity in fungi has been investigated for several basidiomycetes, such as *Agaricus bisporus* (Lange) Imbach (Turner, 1974; Wood and Goodenough, 1977), *Phanerochaete chrysosporium* (Ander and Eriksson, 1976), *Lentinula edodes* (Berk.) Pegler (Leatham and Stahmann, 1981; Ohga, 1992), *Coprinus cinereus* (Schaeff.:Fr.) S. F. Gray (Vnenchak and Schwalb, 1989). Figure 4 shows the changes in extracellular PO activities of the *Favolus* cultures under different light-programmed experiments. The PO activity in the culture filtrate under the 6-d dark-continuous light (the DL regimen) raised radically up to the maximum level on the 2nd day after the start of light exposure, and the activity suddenly decreased to the basal level on the 4th day. The maximum value of the specific activity was reached at 34 units/mg protein. On the other hand, the corresponding value in the culture filtrate of the dark grown cultures remained at very low level, and the pattern of the enzyme activities did not show any significant changes during the incubation period. In conclusion, the extracellular PO activity in *Favolus arcularius* also appears to be developmentally regulated enzyme, as well as those as described in the above articles. Leatham and Stahmann (1981) have postulated that the PO activity found in fruit-bodies of *L. edodes* and other higher fungi may produce extracellular pigment, which aids in oxidation polymerization of cell wall components. *Coprinus cinereus* produced a developmentally regulated PO, which appeared to be responsible for the black pigmentation of fruit-bodies (Vnenchak and Schwalb, 1989). However, the role of extracellular PO in *Favolus arcularius* is still unclear.

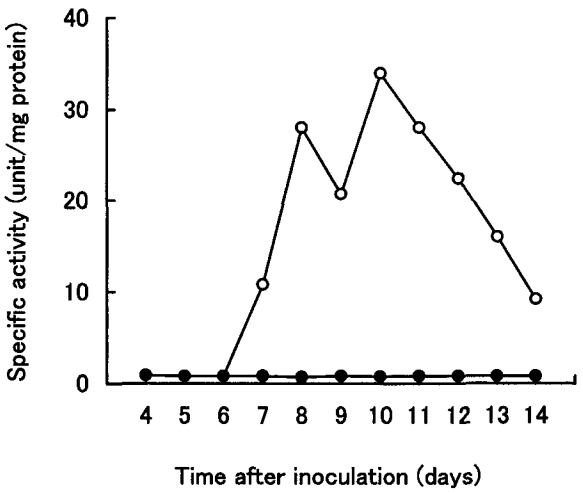

Fig. 4. Changes in specific activity of extracellular phenol oxidases in the mycelium and the culture filtrates of *Favolus arcularius* incubated in the DL and the continuous dark regimens.

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