Locus of Blue and Near Ultraviolet Reversible Photoreaction in the Stages of Conidial Development in *Botrytis cinerea*

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SUMMARY

The effect of the blue and near ultraviolet reversible photoreaction on conidial development in *Botrytis cinerea* was studied by observing microscopically selected conidiophores. Conidiophore development was divided into six stages: when the developing conidiophores from stage 2 (i.e. a mature conidiophore) to stage 5 (i.e. a conidiophore with conidium initial) were exposed to blue light for a short time, conidiation was suppressed; the conidiophores already formed de-differentiated to 'sterile' conidiophores with sharply pointed tips. The suppression of conidial development by blue light could be reversed by subsequent exposure to near ultraviolet light, and conidia then developed normally. This mycochrome system functioned reciprocally within the range of identified conidiophore developmental stages and near ultraviolet light acted only at the same developmental stage as was inhibited by blue light.

INTRODUCTION

Many studies have been made on the control of sporulation in fungi by light (Carlile, 1965; Leach, 1971). We have found that a new pigment system, mycochrome, which is involved in the blue and near ultraviolet (near u.v.) reversible photoreaction, plays an important role in the photocontrol of conidial development in *Helminthosporium oryzae* (Honda, Sakamoto & Oda, 1968). This system has since been found during conidiation in *Alternaria tomato* (Kumagai & Oda, 1969, 1973; Kumagai, Yoshioka & Oda, 1976) and *Botrytis cinerea* (Tan & Epton, 1974). We suggested that the developmental stage in which the photosystem participates might be the maturation of the conidiophore. This led us to question, with particular reference to the morphological changes in conidial development, what developmental stage of the conidiophore is controlled by the blue and near u.v. reversible photoreaction.

In the isolate of *Botrytis cinerea* used in this study, six distinct developmental stages of the conidiophores from vegetative hyphae to conidium formation are recognized. In this paper, the action of the blue and near u.v. reversible photoreaction is studied in relation to morphological changes during conidial development.

METHODS

Organism and growth. *Botrytis cinerea* Pers. ex Fr. was isolated from a diseased rose calyx in the botanical garden of Tohoku University in Sendai. It was grown on a modified potato dextrose agar (initial pH 5.0) prepared as follows. Potato blocks (200 g) were steamed at 100 °C for 60 min in 1 l distilled water, filtered through gauze and then diluted to 2 l. Dextrose (5 g) and agar (20 g) were added to 1 l potato extract, the medium was autoclaved

Fig. 1. Developmental stages of conidiophores of *B. cinerea* in darkness. Stage 1, initial conidiophore; stage 2, mature conidiophore; stage 3, spherical ampulla at tip of conidiophore; stage 4, denticles blown out from the ampulla; stage 5, conidium initials at the tips of the denticles; stage 6, mature conidia.

at 110 °C for 15 min and 20 ml was used per Petri dish (9 cm diam.). A mycelial agar disc (3 mm diam.) from the margin of a colony grown in darkness for 4 days at 20 °C was placed on the centre of the agar plate. Experimental cultures were grown in darkness for 3 days at 26 ± 1 °C and then exposed to various light treatments.

Photoreactivity. Black light lamps (FL-BLB, Toshiba Electric, Tokyo, Japan) were used as the source of near u.v. radiation for inducing sporulation, the intensity being about 300×10^{-6} J cm⁻² s⁻¹. When it was necessary to remove the blue region spectrum, near u.v. (320 to 400 nm, mainly 360 nm) was filtered through a glass filter (UV-DIA, Toshiba Kasei Industry, Tokyo, Japan). The intensity of this light was about 30×10^{-6} J cm⁻² s⁻¹. Blue light was from coloured fluorescent lamps (20 B-F, Mitsubishi Electric, Tokyo, Japan) filtered through a blue plastic filter (Mitsubishi Jushi, Tokyo, Japan). The intensity of this light was about 150×10^{-6} J cm⁻² s⁻¹. Radiation intensities were measured with a compensated thermopile (model E1; Kipp & Zonen, Delft, The Netherlands) and a microvoltmeter (Towa Denpa, Tokyo, Japan).

Sporulation was measured microscopically by counting the mean number of conidia-bearing conidiophores in five areas (each 1 × 1 mm) per colony for each replicate. The values for several replicates were then averaged.

Conidial development was observed in the dark as follows. The culture plate was fixed on the slide holder of a microscope at the beginning of darkness following inductive irradiation with the black light lamp. A single conidiophore was chosen 3 h after the start of darkness and it was examined microscopically at 30 min intervals for the next 13 h. The culture plates fixed on the slide holder were used for light treatments. Since red light had no effect on the development of conidiophores in our preliminary experiments, a red cut filter (R-60, Hoya Glass Works, Tokyo, Japan) was inserted under the condenser lens to obtain a safe light for observation.

RESULTS

Relation of blue light inhibition to conidiophore developmental stages

When cultures of the fungus were kept in darkness, no conidiophores or conidia were formed. However, when they were exposed to near u.v. light followed by darkness, conidiation did occur. In cultures exposed to blue light after inductive exposure to black light, the conidiophores already formed de-differentiated to those with sharply pointed needles

Fig. 2. Sterile conidiophores de-differentiated by blue light irradiation at various stages of conidiophore development. Cultures with selected conidiophores at various stages, following inductive exposure to black light for 12 h, were irradiated with blue light (B) for 1 h. D, darkness.

rather than conidia. Furthermore, when blue light was shone for 2 h at various times during the dark period following inductive irradiation, the phase sensitive to blue light was between 5 and 8 h after the beginning of darkness.

The following experiments were designed to determine the developmental stage of the conidiophore which corresponded to the phase sensitive to blue light. The six developmental stages (Fig. 1) of conidiophores during darkness after inductive irradiation are:

Stage 1, initial conidiophores with slightly pointed tips and thick, smooth hyphae distinguishable from vegetative hyphae.

Stage 2, conidiophores slightly thicker, about 1 mm long; darker green than at stage 1.

Stage 3, elongation ceases, conidiophore tips become round, then swell to produce a spherical ampulla about $13 \mu\text{m}$ diam.

Stage 4, ampulla at maximum size; denticles are produced over its surface.

Stage 5, conidia begin to develop at the tip of each denticle.

Stage 6, conidia $10 \mu\text{m}$ diam. and completely matured, having developed a septum in the stalk of the conidium.

In the preliminary morphological experiment in which a selected conidiophore was observed, 1 h exposure to blue light 5 h (i.e. stage 2) after the beginning of darkness completely inhibited conidiation. Cultures with a selected conidiophore at one of the numbered stages were exposed to blue light for 1 h and the development of this conidiophore was observed. The results are summarized in Figs 2 and 3. At stage 1 (3 h), conidiation was not inhibited by blue light but was completed normally 10 h after the beginning of darkness following inductive irradiation. However, conidiophores from stages 2 to 5 were inhibited by blue light exposure. Conidiophores at stage 2 that had been exposed to blue light showed a thinner pointed tip 7 h after exposure and developed into slender hyphae about $100 \mu\text{m}$

Fig. 3. De-differentiation of conidiophores in various stages of development by blue light irradiation. (a) Stage 2, conidiophore de-differentiated into sterile hypha; the conidiophore has become a sharply pointed, long hypha. (b) Stage 3, ampulla de-differentiated into sterile hypha (arrowed) without denticles. (c) Stage 4, radially de-differentiated denticles. (d) Stage 5, de-differentiated conidium initials; note hypha developing from conidium initial (arrowed).

long during a 20 h dark period following exposure. When the ampullae in stage 3 were exposed to blue light, normal denticles did not develop on the surface. Instead, swellings were observed on the ampullae that elongated into sterile hyphae. Blue light exposure of the denticles in stage 4 caused tubular outgrowths that grew radially into long, slender hyphae. Similarly, blue light prevented the maturation of conidium initials in stage 5, causing them to develop into sterile hyphae. However, after stage 6, the development of conidiophores was no longer influenced by blue light and conidiation occurred normally.

Thus, it was concluded that conidial development from stages 2 to 5 was inhibited by blue light and conidiophores already formed in these stages de-differentiated into long, slender hyphae.

Locus of blue and near ultraviolet reversible photoreaction in the stages of conidial development

In colonies exposed to continuous black-light irradiation, following initial growth in the dark, conidiophores, but not conidia, formed in a narrow region of the colony that was growing just before irradiation. All conidiophores remained at stage 2 under continuous irradiation with black light. The fact that they remained at stage 2 and did not proceed to the next stage seemed to be due to the antagonism of blue and near u.v. regions in the black light. Therefore, the effect of near u.v. light on the inhibition of conidiation by blue light was investigated. As shown in Table 1, the suppression of conidial development by blue light

Table 1. *Production of fertile conidiophores by B. cinerea after exposure to blue and near u.v. light in sequence*

Five hours after the beginning of darkness following inductive irradiation for 12 h with black light, conidiophores were irradiated alternately with blue light for 1 h and near u.v. light for 1 h.

Irradiation programme	Conidia-bearing conidiophores (% of control)
24D (control)	100*
N	107
B	28
B+N	95
B+N+B	52
B+N+B+N	81
B+N+B+N+B	55

D, Dark; B, blue light; N, near u.v. light.

* Equivalent to 103 mm^{-2} .

Fig. 4. Diagram showing the blue and near u.v. reversible photoreaction at various stages in the development of the conidiophore. Exposure to blue light (B) and near u.v. light (N) was for 60 min and 30 min respectively. D, darkness.

could be reversed by subsequent exposure to near u.v. light. This reversal was nullified by further exposure to blue light immediately following irradiation with near u.v. light.

To determine whether the blue and near u.v. photoreactions act at the same stage in conidial development, the effect of alternate irradiation with blue and near u.v. light was examined using selected conidiophores at various stages (Fig. 4). When conidiophores were exposed to near u.v. light immediately after exposure to blue light at stage 2, conidiation proceeded normally with no blue light inhibition. Similar results were obtained with near u.v. light after exposure to blue light at stages 3, 4 and 5. The reaction of blue and near u.v. radiation was repeatedly reversible at stage 2, but was less so at stages 3, 4 and 5. The most effective recovery from blue light inhibition was achieved by immediate near u.v. treatment, indicating that the longer the dark period after irradiation with blue light, the weaker the reversal effect by near u.v. light.

DISCUSSION

Tan (1974) reported that blue and near u.v. reversible photoreactions participate in the conidial development of *B. cinerea* and that a longer term irradiation with blue light (at least 4 h) is required to suppress conidiation. However our results show that short exposures to blue light of 30 min and 60 min inhibit conidiation by about 50 % and 100 % respectively. Aragaki (1961) and Cohen (1975) showed that the inhibitory effect of blue light depends on the temperature. In our experiments, 80 % inhibition was observed at 26 °C and virtually no inhibition at 20 °C. The difference between these two results seems to be due to temperature sensitivity.

From our results we conclude that the blue and near u.v. reversible photoreactions function at the same stage in conidial development.

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