Light-induced Synchronous Conidiation in the Fungus *Botrytis cinerea*

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SUMMARY

Botrytis cinerea Pers. ex Fr. in stationary liquid cultures conidiated asynchronously in darkness after 4 days' growth. Synchronous conidiation was induced by irradiating dark-grown cultures with near-ultraviolet light for 12 h. The number of conidia increased very rapidly 10 h after the end of the photo-induction period, and conidiation was completed by the 14th hour. Filter paper cultures of the fungus also showed synchronous conidiation upon irradiation with near-ultraviolet light, but the rapid increase in the number of conidia took place 2 h earlier, conidiation being completed by the 12th hour. Cultures irradiated with blue light, however, produced sterile mycelia and showed complete suppression of conidiation.

INTRODUCTION

Procedures for the induction of synchronous development are valuable for the study of the biochemical events that occur during differentiation. Although light is effective in inducing sporulation of fungi (Carlile, 1965, 1970; Leach, 1971), it has rarely been used to induce synchronous development, there being only one report of light-induced synchronous conidiation for the true fungi (Siegel, Matsuyama & Urey, 1968). Light is routinely used to induce synchronous sporulation in the Myxomycete *Physarum polycephalum* (Daniel, 1966; Sauer, Babcock & Rusch, 1969; Sauer, 1973), and the cellular slime mould *Dictyostelium discoideum* (Newell, Telser & Sussman, 1969; Newell & Sussman, 1970; Nestle & Sussman, 1972). This paper describes two procedures for obtaining synchronous conidiation by means of irradiation with near-ultraviolet (n.u.v.) light in *Botrytis cinerea* Pers. ex Fr., a species in which the physiology of sporulation has already been studied (Tan & Epton, 1973, 1974; Tan, 1974, 1975*a, b*).

METHODS

Organism. *Botrytis cinerea* Pers. ex Fr. was the isolate used by Tan & Epton (1973).

Conidium inoculum. Subcultures of the stock (Tan & Epton, 1973), which had been kept under continuous n.u.v. light for 6 to 7 days after 4 days of dark growth, were used for preparation of the conidium inoculum. A suspension was prepared by flooding the conidiating culture with distilled water and gently 'brushing' the mycelia with a glass rod. The suspension was then filtered through lens paper (Whatman No. 105), adjusted to the required concentration, and kept at 0 to 4 °C for 12 to 16 h before use for inoculation. Such a conidium inoculum was found to give more homogeneous mycelial mats in liquid cultures than did a freshly prepared suspension.

Medium. Malt extract-peptone (MEP) medium (malt extract, Oxoid L39, 30 g l⁻¹; mycological peptone, Oxoid L40, 5 g l⁻¹) was used. The medium was filtered after autoclaving, and dispensed into 9 cm diameter plastic Petri dishes.

Liquid culture. Liquid cultures were prepared by inoculating 0.2 ml of conidium suspension (concentration adjusted to about 10⁶ conidia/ml H₂O) into 20 ml MEP medium.

Filter paper culture. Filter paper cultures were prepared by spraying 2 ml of conidium suspension (adjusted to a concentration of 10^5 conidia/ml MEP medium) uniformly on to a 7 cm diameter Whatman No. 541 filter paper and then placing this filter paper circle on to a 7 cm diameter Whatman No. 17 filter paper pad soaked with 5 ml MEP medium.

Incubation of cultures and irradiation experiment. Incubation of cultures in the dark was carried out at 20 °C and irradiation experiments were carried out at 20 to 21 °C in 'light cabinets'. Philips 'black light' fluorescent tubes (TL 20W/08) were used as the source of n.u.v. radiation (300 to 400 nm) for inducing conidiation. Blue light (410 to 520 nm) was obtained by using a combination of Cinemoid filters under a bank of Philips 'daylight' No. 33 fluorescent tubes. Irradiances of 'black' light and blue light at culture level, measured by a YSI-Kettering radiometer (model 65) were 100 to 160 and 100 to 200 $\mu\text{W cm}^{-2}$, respectively.

Quantification of conidium production. The number of conidia produced was determined by counting in a haemocytometer. Liquid cultures were homogenized for 30 s using a Polytron homogenizer (type PT20 OD) at power setting 6, made up to a known volume, and homogenized again before counting. For filter paper cultures, the fungal mycelia were scraped from the filter paper with a razor blade and homogenized in about 15 ml distilled water at maximum speed, before making up to a known volume and counting. In cases where samples could not be counted immediately, the homogenate after the first homogenization was chilled in ice and left at 0 to 4 °C.

RESULTS

Growth and conidiation of B. cinerea in liquid culture

Experiments were carried out to study the general cultural characteristics of *B. cinerea* in liquid medium. Conidiation was observed to occur after 4 days' growth in darkness and it continued until the whole Petri dish was covered with conidia. This is in contrast to centrally inoculated agar cultures of the fungus where, in darkness, conidiation occurred only after about 7 days' growth (Tan & Epton, 1973) and was always very sparse, giving the culture a 'patchy' appearance.

In liquid culture the fungus could be photo-induced after 2 days dark growth and was most responsive when irradiated after 4 days' dark growth (Fig. 1). Sensitivity to light decreased after 5 days' dark growth, and after 10 days' growth in the dark, light treatment did not increase conidiation above the dark level.

Light-induced synchronous conidiation

Liquid culture. Cultures which have been grown in the dark for 4 days have just started dark conidiation. To develop an experimental system in which no conidiation had occurred at the time of photo-induction, cultures were grown in the dark for $3\frac{1}{2}$ days before being photo-induced for 12 h. Determination of conidium production during the dark period following photo-induction showed that there was a rapid increase in number of conidia from about the 10th to the 14th hour, when conidiation was completed (Fig. 2a). This sharp rise in number of conidia reflects the synchrony of the developmental process. Counts were also made of cultures kept in complete darkness and kept in continuous blue light after the initial growth period. Cultures kept in complete darkness showed a slow increase in number of conidia while those kept under blue light gave rise to sterile mycelia with the consequent suppression of conidiation (Fig. 2a). Morphological observations of the n.u.v.-induced cultures showed that conidiophores were initiated at about the 4th hour after photo-

Fig. 1

Fig. 2

Fig. 1. Conidium production of *B. cinerea* in liquid culture in response to 24 h. n.u.v. irradiation. ○, Conidium number 24 h after irradiation commenced on the day shown; ●, conidium count of dark-grown cultures of comparable age at the time of conidium count of irradiated cultures. Each point is the mean of six cultures (ten counts per culture) from two experiments.

Fig. 2. Time course of conidiation of *B. cinerea* in (a) liquid culture and (b) filter paper culture. Cultures were grown in darkness for (a) 3½ days or (b) 3 days, before irradiation. Conidium count of: (○) cultures irradiated with n.u.v. light, (●) cultures in complete darkness, and (△) cultures irradiated with blue light. Each point is the mean of 4 to 6 cultures (ten counts per culture) from two experiments.

induction, and branching of conidiophores occurred around the 6th hour. After the 8th hour, the apical regions of the conidiophore branches had swollen, and started to give rise to conidium initials. By the 10th hour, characteristic botryose-type conidia were clearly recognizable.

Filter paper culture. In initial experiments, cultures were grown for 3½ days in the dark before irradiation with n.u.v. light for 12 h. However, some conidiation was found to occur in such cultures at the time of transfer to light. So, in subsequent experiments, cultures were grown for only 3 days before irradiation. In the latter cultures, dark conidiation did not occur at the time of transfer to light, but by the end of the photo-induction period some had already begun. Variation of conidium production with time is shown in Fig. 2(b). A high degree of synchrony was obtained. Conidiation, however, began about 2 h earlier than for liquid cultures, and was completed by the 12th hour. Again, blue-light irradiated cultures showed complete suppression of conidiation whereas dark-grown cultures showed a slow increase in number of conidia.

DISCUSSION

Until recently, there were few reports of synchronous fungal development (Zeuthen, 1964; *Cell synchrony. Studies in Biosynthetic Regulation*, 1966). Methods for synchronous sporulation have now been developed for *Blastocladiella emersonii* (Lovett & Cantino, 1960; Goldstein & Cantino, 1962; Murphy & Lovett, 1966), *Achyla* sp. (Griffin & Breuker, 1969), *Neurospora crassa* (Stine & Clark, 1967; Siegel *et al.*, 1968), *Aspergillus niger* (Anderson & Smith, 1971), *Penicillium digitatum* (Zeidler & Margalith, 1972) and *Schizophyllum commune* (Wessels, 1965; Schwalb, 1971); synchrony was usually obtained by either nutritional

changes or cultural manipulation. In the present study, synchronous conidiation was obtained by the relatively simple technique of irradiating dark-grown cultures with n.u.v. light. A high degree of synchrony was obtained, as reflected by the sudden increase of spore number. Conidiation was completed about 4 h after conidium formation had started. In the light-induced system developed for *Neurospora crassa* by Siegel *et al.* (1968), conidiation was observed to start from the periphery of the mycelial mat and spread towards the centre—a lack of total synchrony.

The procedures for inducing synchronous conidiation in *B. cinerea* described here should be useful for biochemical and morphological study of fungal differentiation. Moreover, conidiation can be induced or inhibited by light depending on whether n.u.v. or blue light is applied, making the fungus useful for the study of the control of morphogenesis by light.

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