Studies on Basidiospore Development in *Schizophyllum commune*

By SUSAN K. BROMBERG* AND MARVIN N. SCHWALB

Department of Microbiology, College of Medicine and Dentistry of New Jersey,
Graduate School of Biomedical Sciences, Newark, New Jersey 07103, U.S.A.

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SUMMARY

The time required for synthesis of the spore components and the effect of different environmental conditions on basidiospore production were studied in the basidiomycete *Schizophyllum commune*. Both exogenous glucose and storage materials were used in the synthesis of spore components, which took 40 to 45 h to complete.

A temperature of 30 °C, the presence of 5 % CO₂, a continuous supply of glucose, or a lack of exogenous glucose, had no effect on the rate of spore production. Light, however, was required for sporulation. Darkness inhibited sporulation between karyogamy and the initiation of meiosis; complete inhibition occurred after 48 h in the dark. Spores were produced 5 h after release from dark inhibition.

INTRODUCTION

Many of the environmental and nutritional conditions which affect sporulation in fungi (Turian, 1974) also affect fruit-body formation in the basidiomycete *Schizophyllum commune* (Niederpruem & Wessels, 1969). The presence of 5 % carbon dioxide completely inhibits fruiting at the earlier stages of fruit-body development (Niederpruem, 1963). The effect of light on fruiting depends on the culture medium used. On complete medium, light is required for only a brief period at stage II, an early stage of fruit-body development when the stipe has formed but no hymenium is present (Leonard & Dick, 1968). However, continuous light is required for normal fruiting on minimal medium (Perkins, 1969), unless the cultures are placed over a carbon dioxide absorbant while in the dark (Bromberg, unpublished results). On all media, fruiting is enhanced by light (Raper & Krongelb, 1958). *Schizophyllum* also shows a phototropic and geotropic response (Schwalb & Shanler, 1974).

In some fungi sporulation is regulated by the concentration of glucose in the medium. According to Turian (1974), high glucose concentrations inhibit sporulation in *Schizophyllum*, but he gave no basis for this statement. Growth at 30 °C has little effect on the morphology of the fruit body (Schwalb, 1971).

We have examined some environmental conditions which may affect sporulation in *S. commune* in order to describe the system for further developmental studies.

METHODS

Strains, medium and growth. *Schizophyllum commune* strains 699c (thin) and 822-4 (wild type) were mated using a synchronous technique (Schwalb, 1971). The medium contained (per litre): 10 g glucose, 2 g L-asparagine, 1 g K₂HPO₄, 0.46 KH₂PO₄, 0.5 g MgSO₄·7H₂O, 0.12 mg thiamine hydrochloride, 20 g agar. Cultures were grown in 15 × 100 mm Petri

* Present address: Department of Biology, University of Rochester, Rochester, New York 14627, U.S.A.

Table 1. *Effect of environmental and nutritional conditions on spore production in Schizophyllum commune*

Condition	10 ⁻⁶ × No. of spores/plate in 1 h		
	Expt 1	Expt 2	Expt 3
20 °C, light	1.8	3.1	0.8
20 °C, dark	0	—	0
30 °C, light	0.5	3.8	0.2
5 % CO ₂ , light	4.6	3.5	0.4
No glucose	3.3	2.8	0.8
Fresh glucose	—	3.0	1.0

dishes on a sterile cellophane membrane placed on the agar surface before inoculation, unless otherwise noted.

Measurement of spore synthesis time. Cultures were grown in 60 × 15 mm Petri dishes on medium containing half the normal glucose concentration. When the fruit bodies were mature, the cellophane membranes bearing them were transferred to similar medium containing 1 μ Ci [¹⁴C]glucose/Petri dish. Spore samples were collected in water from the lids of the Petri dishes at various times after transfer. The number of spores was estimated using a Petroff-Hauser bacterial counter. The radioactivity was determined by suspending whole spores in a gel of 77 % Aquasol (New England Nuclear, Boston, Massachusetts, U.S.A.) and counting in a Beckman liquid scintillation counter, model 250. The radioactivity incorporated, expressed as c.p.m./10⁷ spores, was plotted against the time of collection after transfer to radioactive medium. The reverse experiment was also performed, starting the cultures on radioactive medium and transferring them to non-radioactive medium. Fresh medium was provided every 48 h.

Environmental studies. Fully-developed cultures were transferred to different environmental conditions. After 48 h or more in a closed Petri dish, a 1 h spore sample was taken. The spores were collected in 0.5 ml water from four or five Petri dishes, counted in a Petroff-Hauser bacterial counter, and the number of spores produced per plate in 1 h was calculated. Control experiments were conducted at 20 °C in continuous fluorescent light at approximately 700 lux with no change of medium. Other conditions, tested by changing one of the control conditions, were: the presence of 5 % CO₂; a temperature of 30 °C; transfer to medium containing no glucose; transfer to fresh medium; and wrapping the Petri dish in several layers of aluminium foil to obtain total darkness. For some studies, cultures were placed in the dark just before the fruit bodies reached the stage of hymenium formation: these cultures were kept inverted over a watch glass containing 5 % KOH and Malcosorb (Schwalb, 1971) and both Petri dish and watch glass were wrapped in foil.

RESULTS

Before determining the effects of various environmental conditions, it was necessary to determine the time required for the synthesis of a spore. If mature, actively sporulating fruit bodies are transferred to radioactive medium, the time required for maximum incorporation of radioactivity into the spores should give the maximum time for spore formation. The reverse experiment should give the minimum time. The results of three such experiments (Fig. 1) showed plateaus at 42 h and 44 h after transfer to radioactive medium, and at 41 h after transfer to non-radioactive medium. The time required for the synthesis of the spore components is therefore approximately 40 to 45 h.

Fig. 1

Fig. 2

Fig. 1. Time for synthesis of spore components in *Schizophyllum commune*. Mature fruit bodies were transferred from non-radioactive to radioactive medium (○, ●), or from radioactive to non-radioactive medium (■), and spore samples were collected at various times after transfer.

Fig. 2. Length of time for dark inhibition of spore production in *Schizophyllum commune*. Mature fruit bodies were placed in the dark and spore samples were collected and counted at the times indicated.

Table 2. Length of recovery time from dark inhibition of spore production in *Schizophyllum commune*

Time interval after removal from dark (h)	$10^{-5} \times$ Average no. of spores/plate in 1 h	Plates with no spores (%)
0-1	0.05	80
1-4	0.10	20
4-5	1.10	20
5-6	1.45	0
6-7	2.0	20

Thus plates were kept in the different environmental conditions for 48 h before 1 h spore samples were collected, so that all basidia beyond a point of inhibition would have completed sporulation. The results are presented in Table 1. A temperature of 30 °C, the presence of 5 % CO₂, and the presence or lack of glucose had no effect on sporulation. The process was, however, inhibited in the dark.

If cultures were placed in the dark just before hymenium formation, the fruit bodies continued to develop although the size and number of them were greatly reduced. Since the basidia were not exposed to light, most of those produced by the time the fruit body reached maturity should have ceased to develop at the earliest stage for which light is required. When basidia from cultures treated this way were observed cytologically, almost all of them were in the one-nucleus stage. Thus, the earliest point at which darkness inhibits development appears to be between karyogamy and the initiation of meiosis.

To determine the length of time that cultures must be kept in the dark to inhibit sporulation, spore samples were taken every 6 h after mature fruit bodies had been placed in the dark. There was a sharp drop in the number of spores produced during the first 12 to 18 h and then a much slower decrease over the next 30 h (Fig. 2).

The recovery from dark inhibition was examined using cultures placed in the dark before hymenium formation and exposed to light when the fruit bodies were fully developed.

Table 3. *Length of light period necessary for recovery from dark inhibition of spore production in Schizophyllum commune*

Length of exposure to light (h)	$10^{-5} \times$ Average no. of spores/plate in 1 h	Plates with no spores (%)
0.17	0	100
1.0	0.19	50
2.0	0.40	50
3.5	0.25	33
5.3	2.33	33

Using this technique, none of the basidia developed beyond the point of inhibition. The recovery time from dark inhibition, tested by taking spore samples from inhibited cultures that were exposed to light, was approximately 4 to 5 h (Table 2). By exposing dark-inhibited cultures to light for various periods, the length of the light period necessary for recovery was found to be greater than 3.5 h (Table 3). The spore counts in Tables 2 and 3 are low compared with those in Table 1 because the number and size of fruit bodies produced in dark cultures were much smaller than those which developed in the light.

Cultures from nine to 20 days old were tested: in this range the age of the culture did not affect the dark inhibition. Also, stage V cultures could remain in the dark for at least 96 h with no release of inhibition.

DISCUSSION

Light is required for the initiation of meiosis in *S. commune*. The time required for recovery from dark inhibition is 4 to 5 h, which represents the time from the beginning of meiosis to ejection of the spore. Yet cultures must be kept in the dark for 12 to 18 h to inhibit sporulation. This time difference could be a result of the accumulation of a photo-induced product. Such a product has been proposed by Lu for the light initiation of fruit-body formation in *Coprinus* (Lu, 1974). He proposed that a photoreceptive precursor is produced which is converted to an effector by light. The effector then allows initiation of primordia. If an excess of such an effector accumulated in *S. commune* during exposure to light, it might be sufficient to allow basidia, which had not yet reached karyogamy, to complete sporulation. The length of time for inhibition would then appear longer than the 5 h predicted.

The variation both between and within individual experiments in the environmental studies (Table 1), was caused by variations in the ages of the cultures used as well as in the number and size of the fruit bodies on individual plates.

The results indicate that exogenous glucose is rapidly taken up and utilized in spore synthesis. Sporulation occurs normally whether or not exogenous glucose is present, implying that storage products may be utilized when there is a low endogenous supply of nutrients. Also, a continuous supply of glucose does not inhibit sporulation. Although at 30 °C the vegetative growth rate increases, the number of spores produced per unit time does not increase.

The 40 to 45 h required for the synthesis of the spore components seems rather long considering that only 4 to 5 h is required from the initiation of meiosis to the final product. However, the time required for formation of subhymenial cells may also be included in this period if some of the products of these cells are incorporated into the spore.

When cultures were transferred from [^{14}C]glucose medium, the amount of exogenously derived non-radioactive material in newly forming spores increased until a plateau was reached (Fig. 1): this may represent incorporation of components derived from storage

material. Although the radioactivity must eventually drop to zero, we did not observe any further decrease over the 70 h of the experiment, so the pre-existing material must be turned over very slowly. These observations also suggest that the 40 to 45 h period of synthesis starts with the basidial or subhymental cells.

Our results should be useful in studying the regulation of sporulation. The dark effect provides a mechanism for inhibiting the process at a specific stage in the development of the spore, the one-nucleus stage. In addition, on recovery from the dark effect, there seems to be a burst of synthesis at 4 to 5 h, which can provide a relatively synchronous population of basidia and spores.

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