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## Effect of Light and Temperature On Growth and Conidial Discharge in *Basidiobolus*

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### Abstract

Seventeen isolates of *Basidiobolus* (Entomophthorales) were obtained from 22 specimens of a salamander (*Desmognathus fuscus*) dissected under red light, and formation and discharge of conidia at wavelengths greater than 600 nm was proven. Growth of 177 isolates of *Basidiobolus* (including the six named species) at 25 C and 37 C was compared in the dark and with occasional exposure to light. Growth differences were measured and conidial discharge was noted after 72 hrs. Light apparently has greater effect on growth than on conidial discharge at higher temperatures. Effect of light on conidial discharge is reduced at higher temperatures. Isolates of the fungus differ significantly in response to light at different temperatures, possibly representing four physiologically different strains. *Trans. Kans. Acad. Sci.*, 75 (1), 1972.

### Introduction

The fungus *Basidiobolus* (Entomophthorales) is a saprophytic phycomycete commonly found in the fecal contents of the guts of amphibians and reptiles. Asexual reproduction and dispersal are primarily by means of conidia borne singly at the tips of swollen conidiophores. The conidia are forcibly discharged to a distance of 1–2 cm (Ingold, 1971) and virtually pure isolates can be obtained by placing an appropriate agar medium within range of the conidial discharge.

This two-part investigation was undertaken to determine the effect of light and temperature on the growth and spore discharge of *Basidiobolus*. Callaghan (1969 a, b) found that at 20 C in one isolate of *Basidiobolus ranarum* Eidam light of wavelengths greater than 630 nm did not stimulate spore discharge, and also (1969 b) light did not affect growth rate.

The first part of this study dealt with the isolation of the fungus from fecal material when isolation procedures were conducted under red light only. According to Callaghan (1969 b), success in obtaining isolates under these conditions should have been dubious. The second part of the study tested Callaghan's results on growth and conidia produc-

tion at higher temperatures. One hundred seventy-seven cultures of *Basidiobolus* were grown at 25 and 37 C in the absence of light and the results regarding conidial discharge and colony diameter were compared with those of Hutchison *et al.* (1972), who grew the same 177 cultures under the same conditions used in this study, except that the cultures were periodically exposed to light.

### Methods and Materials

*Isolation Under Red Light.*—Individuals of the salamander *Desmognathus fuscus* (Collected in Montgomery Co., Arkansas) were used as the source of isolation due to the high incidence of *B. ranarum* in this organism (Nickerson and Hutchison, 1971). The method used for obtaining isolates (Hutchison and Nickerson, 1970) depends on discharge of conidia. This consisted of placing fecal material dissected from the large intestine of the specimens in the lids of inverted petri plates, the bottoms of which contained YpSs agar (yeast extract, 4 g ;  $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$ , 0.5 g ; soluble starch, 15 g ;  $\text{K}_2\text{HPO}_4$ , 1 g ; agar, 20 g ;  $\text{H}_2\text{O}$ , 1 liter). Dissection of specimens and inoculation of the inverted petri plates were performed under red light (wavelengths greater than 600 nm) and the plates were placed in a light-proof container and incubated at 25 C. At the end of 72 hrs plates were examined to detect the presence of viable isolates.

*Growth At Two Temperatures Without Light.*—Eight American Type Culture Collection cultures (14449, *Basidiobolus ranarum*; 14448, *B. haptosporus*; 14450, *B. meristosporus*; 15379, *B. magnus*; 14708, *B. microsporus*; 16580, *B. heterosporus*; and 14052 and 16109, isolated from phycomycoses in humans) and 169 Arkansas State University isolates of *Basidiobolus* were grown in the dark at 25 C and 37 C. Established criteria for separating species of *Basidiobolus* are not available (Hutchison *et al.*, 1972) and the ASU isolates have been tentatively identified as *B. ranarum* (Nickerson and Hutchison, 1971).

Inocula were first transferred from YpSs stock agar slants to SDA (Sabouraud's Dextrose Agar) plates (dextrose, 40 g ; peptone, 10 g ; agar, 15 g ;  $\text{H}_2\text{O}$ , 1 liter) and grown for seven days at 25 C. Triplicate SDA plates, to be used for incubation at 25 and 37 C were inoculated with 2 mm<sup>2</sup> agar blocks from the original SDA plates, then sealed in light-proof containers. After 72 hrs, colony diameters were measured and the presence of viable, discharged conidia was confirmed by noting small colonies around the periphery of the main colony. Due to size of the inocula, colony diameters less than 5 mm were considered negative.

This method is a modification of that used by Hutchison *et al.* (1972). However, they did not place the triplicate SDA plates in light-proof containers, but in closed incubators. Measurements and conidial observations were made at 24, 48, and 72 hrs. Colonies were exposed to Westinghouse Cool White Fluorescent Lamps for about 30 min during each measurement, and for occasional, brief periods when incubators were opened for other purposes.

### Results and Discussion

*Isolation Under Red Light.*—Two separate experiments established that conidial discharge in isolating *Basidiobolus* is not necessarily dependent on light of wavelengths less than 630 nm as Callaghan (1969 b) suggested. In the first experiment, eight isolates were recovered from nine individuals of *D. fuscus* under a light source emitting at 600 to 675 nm (red light). For better comparison with Callaghan's (1969 a, b) results at 20 C regarding absence of conidiophore differentiation at longer wavelengths (630 nm or greater), a second isolation experiment was performed using a light source emitting at 678 nm. In this instance, thirteen specimens yielded nine isolates.

These results show that at 25 C (the incubation temperature used), a number of strains of *Basidiobolus* are capable of forming and discharging conidia without being exposed to light of wavelengths shorter than 630 nm. The difference between Callaghan's results and ours could be due to either a different temperature of incubation or the use of different strains, or both.

*Growth At Two Temperatures Without Light.*—The data in Tables 1 and 2 present a comparison of the results of this study with those of Hutchison *et al.* (1972). Comparison of the two tables indicates that at the higher temperatures used in these studies, we observed a reversal of Callaghan's results regarding the influence of light wavelengths less than 510 nm (green to blue light). Callaghan (1969 b) found that at 20 C, shorter wavelengths of light affected conidial discharge more than growth. Comparison of the results of this study with those of Hutchison *et al.* (1972) indicates that at 25 C and 37 C, light had a more pronounced affect on growth than on conidial discharge. It should be noted that Callaghan (1969 a, b) utilized elaborate microscopic techniques for analyzing a single isolate, whereas Hutchison *et al.* (1972) and this study utilized macroscopic techniques with a large number of isolates.

Callaghan (1969 a, b) observed great reduction of conidial discharge in the dark. Comparison of our results and those of Hutchison *et al.* (Table 1) shows a reduction of 10% at 25 C (on the basis of all 177

cultures) in the number of cultures shooting conidia in the dark. A similar reduction of only 2% occurred at 37 C.

Comparison of the data of Callaghan (1969 a, b), Hutchison *et al.* (1972) and from the present study indicates that as temperature increases, the effect of light in promoting discharge of conidia apparently decreases.

Light definitely affected growth in the majority of isolates tested, and the effect varied considerably depending on the temperature (Table 2). It was not possible to show a difference in growth of some cultures (right column, Table 2), due to similar colony diameters or obscuring of the periphery of one or both colonies by secondary colonies produced by discharged conidia.

Ignoring the right column, light stimulated growth at 25 C and inhibited growth at 37 C in most isolates. However, 28% of the isolates grew best in the absence of light at 25 C and 4% grew best in the presence of light at 37 C. Differences in response to light stimuli can be explained by postulating four physiologically different strains.

- 1) Growth stimulated by light
- 2) Growth inhibited by light
- 3) Growth stimulated by light at 25 C but inhibited by light at 37 C
- 4) Growth neither inhibited nor stimulated by light.

**Table 1. Percent of 177 cultures of *Basidiobolus* discharging conidia at two temperatures after 72 hrs.**

Temperature	Light present (Hutchison <i>et al.</i> , 1972)	Light absent (Present study)
25 C	25	15
37 C	27	25

**Table 2. Comparison of 177 cultures of *Basidiobolus* grown for 72 hrs in presence and absence of light.**

Temperature	Best growth:		Growth not measurably different in light and dark (%) <sup>1</sup>
	In light (%)	In dark (%)	
25 C	54	16	30
37 C <sup>2</sup>	4	44	52

<sup>1</sup> At either temperature, growth in light and darkness was considered equal when (a) a difference of 2 mm or less in colony diameter was measured, (b) when the periphery of the main colony of either culture was obscured by daughter colonies produced from conidial discharge or (c) when neither culture grew (37 C).

<sup>2</sup> At 37 C 39 isolates failed to grow in both light and darkness; also, at 37 C 24 isolates failed to grow in light but did grow in darkness.

The inhibitive effects of light at 37 C on some isolates is further indicated by the failure of 63 isolates to grow with exposure to light compared with the failure of only 39 to grow in the dark.

The failure of the isolate used by Callaghan (1969 b) to exhibit growth rate differences in the presence versus absence of light could be due to either the lower temperature at which his studies were conducted, or his isolate belonging to postulated strain 4.

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