

A SIMPLE TECHNIQUE FOR TIME-LAPSE PHOTOMICROGRAPHY OF MICROFUNGI IN PLATE CULTURE

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SUMMARY

Modifications of the standard agar petri dish culture to facilitate time-lapse photomicrography of hyphomycetes are described and illustrated. Photomicrographic sequences of anellospore and chlamyospore formation exemplify the potential of the technique.

Developmental characters have recently gained the ascendancy in hyphomycete taxonomy, and this laboratory has undertaken detailed analyses of the diverse methods of conidiophore and conidium ontogeny exhibited by these fungi. Cole and Kendrick (1968a) described an ultra-thin culture chamber which has subsequently been used with considerable success in time-lapse photomicrographic studies (Kendrick, Cole and Bhatt, 1968; Kendrick and Cole, 1968, 1969; Cole and Kendrick, 1968b, 1969).

Unfortunately, some hyphomycetes do not lend themselves to this technique. Three problems have been encountered.

(i) Sporogenous cells which normally produced long chains of conidia in plate culture would form only one or a few spores in the culture chamber before succumbing [e.g., *Scopulariopsis brevicaulis* (Sacc.) Bainier, and *Deightonella torulosa* (Syd.) M. B. Ellis].

(ii) Profuse vegetative mycelium developed before sporulation began, thus obscuring the subsequent events of conidium ontogeny [e.g., *Arthrinium phaeospermum* (Corda) M. B. Ellis].

(iii) In some fungi which normally produced two kinds of spores sequentially, only the first developed; or, if the second did form, previously differentiated elements hid its ontogeny from the camera [e.g., chlamyospores of *Thielaviopsis paradoxa* (de Seynes) Höhnelt were obscured by earlier formed phialides and phialospores, while those of *T. basicola* (Berk. and Br.) Ferr. failed to develop].

These problems necessitated an exploration of alternative techniques. Since those fungi which did not adapt well to the culture

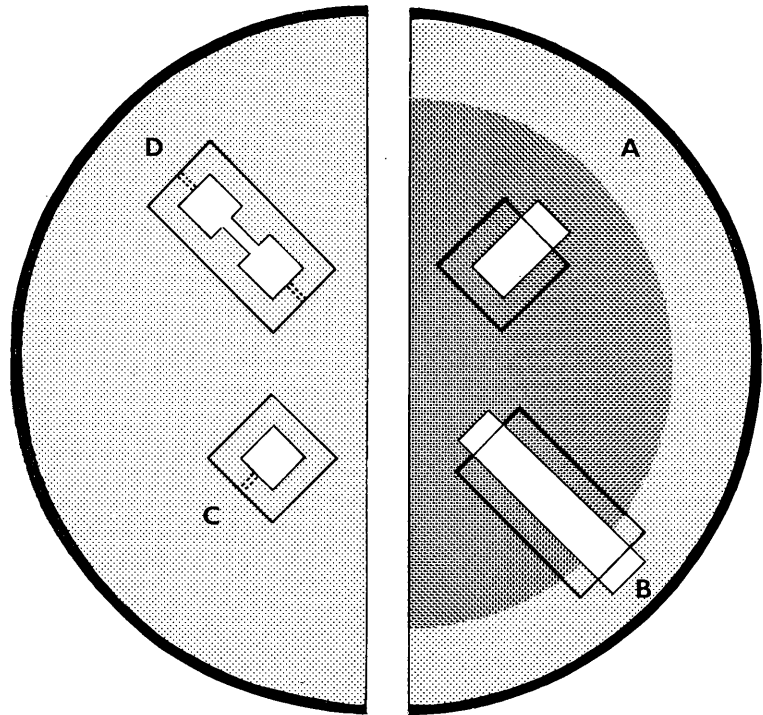


FIG. 1 A-D. Composite diagram of modified plate cultures as seen from above, showing agar (light stippling), fungal colony (dark stippling), channels (blank), coverslips (outlined), and pierced air passages (dotted lines).

chamber all sporulated in plate culture, it seemed reasonable to carry out developmental studies in the petri dish, making only such modifications as were necessary to facilitate time-lapse photomicrography.

Several approaches which have yielded useful results are described and illustrated here (FIG. 1 A-D). As a prelude to each procedure, an approximately 2 mm-thick layer of malt extract agar (2% malt extract and 2% glucose) or potato dextrose agar (2% dextrose) is poured into a standard plastic petri dish. In some cases, it is advantageous to modify the culture only when a sporulating colony has developed (FIG. 1 A, B). At the first signs of sporulation, a rectangular piece of agar and mycelium measuring $24 \times 5-10$ mm is removed with a sterile scalpel, exposing the base of the petri dish (FIG. 1A). The radial channel thus formed extends from the margin toward the center of the colony. The optimum channel width for each organism must be determined by experiment, some fungi needing a large area to accom-

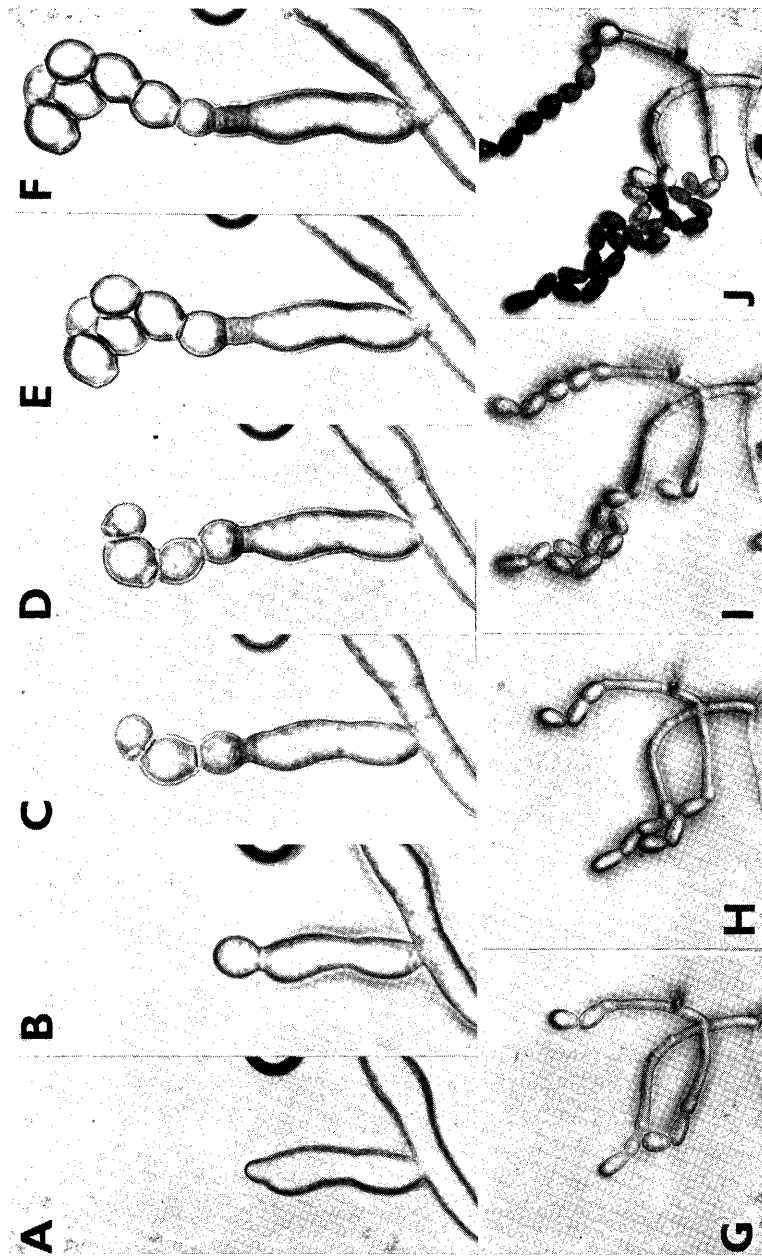


FIG. 2. A-J. 35 mm time-lapse sequences of microfungi in plate culture. A-F. Annelospore formation in *Scopulariopsis brevicaulis* taken under the 100 X oil-immersion objective ($\times 1,100$). G-J. Chlampospore formation in *Thielaviopsis paradoxa* taken under the 40 X objective ($\times 400$).

moderate their profuse vegetative growth. A 22 mm square No. 1 coverslip is then placed over the open channel leaving about 2 mm uncovered for aeration. The coverslip is gently pressed down on the agar surface to make a seal. We have successfully used this approach to record the development of *Deightoniella torulosa*, *Sporendonema purpurascens* (Bon.) Mason & Hughes, and *Scopulariopsis brevicaulis*; annellospore formation in this last species is illustrated in FIG. 2 A-F.

In a second variation (FIG. 1B) a channel 64 × 5-10 mm is excised from the culture, beginning near the point of inoculation and extending radially or tangentially beyond the growing margin of the colony. The channel is covered with a 60 × 24 mm No. 1 coverslip, an opening being left at both ends for greater aeration. The longer coverslip both presents a larger surface area and allows simultaneous observations of sporulation from mycelium of all ages. This approach was particularly useful for examining *Arthrinium phaeospermum* and *Thielaviopsis paradoxa*. Formation of *T. paradoxa* chlamydospores is illustrated in FIG. 2 G-J.

Alternative procedures, diagrammatically shown in FIG. 1C and 1D, involve cutting a shorter piece of agar out of the freshly poured plate, inoculating the walls of the channel thus formed, and then immediately covering it with a glass coverslip, limiting gaseous exchange to that occurring by diffusion through the agar. Aeration may be sharply increased at a later stage of development by simply forming an air passage (dotted lines) under the coverslip with a sterile mounted needle. The chlamydospores of *Thielaviopsis basicola* usually develop from the aerial hyphae as divergent outgrowths and are thus difficult to photograph. Restricted aeration retards the formation of these secondary spores and allows a mycelial mat to grow onto the lower surface of the coverslip. An increase in gaseous exchange then stimulates chlamydospores to develop on the mycelia adhering to the lower surface of the glass, and permits clear observation and photomicrography. A special channel configuration (FIG. 1D) allows for simultaneous production of phialospores and chlamydospores of *T. basicola* after the air passages are formed. Conditions in the narrow channel between the two squares apparently inhibit production of chlamydospores while allowing continued formation of phialospores.

Once a time-lapse sequence has been initiated, the petri dish is left on the microscope stage. In order to prevent movement during or between exposures, the dish is fixed to the stage with adhesive tape, or by a film of water between the stage and the base of the dish. Some hyphomycetes have a relatively low tolerance to bright illumina-

tion, and the light is, therefore, reduced as much as possible for scanning purposes, and turned off between exposures. Heat filters are also placed below the stage of the photomicroscope. Objectives of all available magnifications may be used. A 100 × oil-immersion objective and bright field illumination were used for taking the photomicrographs of *S. brevicaulis* (FIG. 2 A-F), illustrating the excellent definition possible. Between exposures the cover of the dish is replaced in order to maintain high humidity and retard dehydration of the thin layer of agar. With repeated opening of the dish, the introduction of some contaminants is almost inevitable, but since the resident fungus is now well established, there are no noticeable effects on the development of sporogenous cells beneath the coverslip during the time required for a sequence.

Our work does not pretend to be an exhaustive exploration of the possible variations or applications of this simple technique. We have merely established its practicability and utility for time-lapse studies.

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