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Simple Method of Inducing Sporulation by *Apophysomyces elegans* and *Saksenaea vasiformis*

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Apophysomyces elegans and Saksenaea vasiformis are notorious for their failure to sporulate on routine media. Agar blocks, permeated with the mycelia of A. elegans and S. vasiformis, were cut aseptically from 7-day-old colonies grown on Sabouraud dextrose agar and transferred to plates containing 20 ml of sterile distilled water supplemented with 0.2 ml of 10% filter-sterilized yeast extract solution. When the plates were incubated at 37°C, all 5 isolates of A. elegans and all 10 isolates of S. vasiformis produced abundant, characteristic sporangia within 7 to 10 days. The method is simple to use and yields consistent results.

The zygomycetous pathogens *Apophysomyces elegans* Misra, Srivastava, and Lata and *Saksenaea vasiformis* Saksena are well known for their failure to sporulate on routine mycological media. When Saksena (12) first described *S. vasiformis*, he found that the fungus did not sporulate on potato dextrose agar, and to stimulate sporulation he had to float potato dextrose agar blocks with mycelial growth on sterile distilled water. When Ajello et al. (1) described the first human infection by *S. vasiformis* in 1976, they found that their isolate did not sporulate on either Sabouraud dextrose agar or corn meal agar. Acting on the advice of J. J. Ellis (Northern Regional Research Center, Peoria, Ill.), sporulation was stimulated by growing the isolate on hay infusion agar and then placing blocks of the agar growth in plates of sterile distilled water. The distinctive, vase-shaped sporangia of *S. vasiformis* were observed after 16 days of incubation at 25°C.

While studying the physiological requirements of *S. vasiformis*, Baijal (3) observed that only a few carbohydrates, such as arabinose, rhamnose, sorbose, galactose, lactose, and citric acid, supported good-to-excellent sporulation of *S. vasiformis*. In the last 12 years, various workers have described human infections caused by this fungus and faced the same difficulty with their *S. vasiformis* isolates failing to sporulate on routine media. The only exception was a study by Torell et al. (13) in which *S. vasiformis* was found to form vase-shaped sporangia on the primary isolation medium of Sabouraud dextrose agar when cultures were incubated at

TABLE 1. Isolates of *A. elegans* and *S. vasiformis*

Species and isolate no.	Source	Reference or source
A. elegans		
CDC B-3422	Human; bronchial washing	5
CDC B-3865	Human; abscess on left kidney	8
CDC B-3956	Human; debrided tissue from amputated left leg	14
CDC B-4214	Human; abscess on amputated arm; St. Louis, Mo.	G. Kobayashi, Washington University School of Medicine, St. Louis, Mo.
CDC B-4535	Human; abscess on left knee and pelvic area; Tucson, Ariz.	Mary Fried, Tucson Medical Center, Tucson, Ariz.
S. vasiformis		
CDC B-2189	NRRL[a] 2443	J. J. Ellis, Northern Regional Research Center, Peoria, Ill.
CDC B-2190	NRRL 5251[b]; from soil	J. J. Ellis
CDC B-3164	Human; breast tissue	13
CDC B-3353	Human; tibial fracture	10
CDC B-3883	Human; skin lesions from burn patient; New Orleans, La.	D. L. Greer, LSU Medical Center, New Orleans, La.
CDC B-4078	Human; sphenoid sinus; Reno, Nev.	Steven Praker, University of Nevada, Reno
CDC B-4257	Human; scalp lesion	7
CDC B-4455	Human; lesion on left foot; Vellore, India	Grace Koshi, Christian Medical College, Vellore, India
CDC B-4505	Human; subcutaneous lesion	4
CDC B-4506	Human; subcutaneous lesion	4

[a] NRRL, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Ill.
[b] Subculture derived from the isolate used to prepare the type specimen (12).

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TABLE 2. Sporangial formation by *A. elegans* and *S. vasiformis* isolates in water-yeast extract medium after 10 days at different temperatures

Species and isolate no.	Sporangial formation[a] at:		
	25°C	30°C	37°C
A. elegans			
CDC B-3422	−	+	+++
CDC B-3865	−	++	+++
CDC B-3956	+	++	+++
CDC B-4214	−	++	+++
CDC B-4535	−	+	+++
S. vasiformis			
CDC B-2189	−	−	++
CDC B-2190	−	+	+++
CDC B-3164	−	−	+++
CDC B-3353	−	−	++
CDC B-3883	−	+	+++
CDC B-4078	−	−	+++
CDC B-4257	−	+	+++
CDC B-4455	−	+++	+++
CDC B-4505	−	+	+++
CDC B-4506	−	+	+++

[a] +, <5 sporangia; ++, <20 sporangia; +++, >20 sporangia; −, no sporangia.

37°C. In other cases, nutritionally deficient media, such as corn meal agar (10), Czapek Dox or Czapek solution agar (6, 9, 11), and hay infusion agar (1), have been successfully used to stimulate sporulation in *S. vasiformis*.

A. elegans has also failed to sporulate on routine mycological media (5). To reliably and consistently obtain sporulation, Ellis and Ajello (5) described a method that involved growing the two fungi, *A. elegans* and *S. vasiformis*, on corn meal-glucose-sucrose-yeast extract agar for 7 days at 30°C, cutting 3-mm^3 agar blocks permeated with hyphal growth, and placing them on the surface of sterilized and solidified 1% water-agar petri plates. After incubating the water-agar plate cultures for an additional 7 days at 30°C, both fungi were found to sporulate. The success of their method was confirmed by Ellis and Kaminski (4) for *S. vasiformis* and Lawrence et al. (8) and Wieden et al. (14) for *A. elegans*.

All of the investigators mentioned above recommended a nutritionally deficient medium and an incubation period of 14 to 21 days at 25 to 30°C to stimulate sporulation in *A. elegans* and *S. vasiformis*. Since both fungi are well known for their tolerance of high temperatures (the maximum growth temperatures being 43°C for *S. vasiformis* and 45°C for *A. elegans*), we decided to investigate the effect of incubation temperature variations on sporulation of these two fungi.

Five isolates of *A. elegans* and ten isolates of *S. vasiformis* were selected for study. The isolation data on the 15 isolates are summarized in Table 1. All cultures were maintained on Sabouraud dextrose agar (Difco Laboratories, Detroit, Mich.). Microscopic examination of the 15 cultures after 2 weeks at 25°C showed that their growth consisted of broad, nonseptate to sparsely septate, branched, hyaline hyphae without sporangial formation.

To induce sporulation, we selected a nutritionally deficient medium consisting of 20 ml of sterile distilled water fortified with 3 drops (0.2 ml) of 10% yeast extract solution that was filter sterilized and refrigerated at 4°C before use. This medium was simple to prepare, and its success over the last 30 years in the in vitro hair perforation test for differentiating *Trichophyton mentagrophytes* (Robin) Blanchard and *T. rubrum* (Castellani) Sabouraud (2) was consistent. Six 1-cm^2 agar blocks permeated with the hyphal growth of each isolate grown on Sabouraud dextrose agar were cut aseptically. Two blocks of each isolate were transferred to a plate containing 20 ml of sterile distilled water. Three drops (0.2 ml) of the filter-sterilized 10% yeast extract solution then were added to each plate. For each isolate, six plates were inoculated. Duplicate plates of each isolate were incubated in the dark at 25, 30, and 37°C. After 5 days of incubation, growth (seen as a thin film over the surface of the water) appeared. Portions of the film from each plate were examined microscopically for sporangia on days 5, 10, and 15 of incubation.

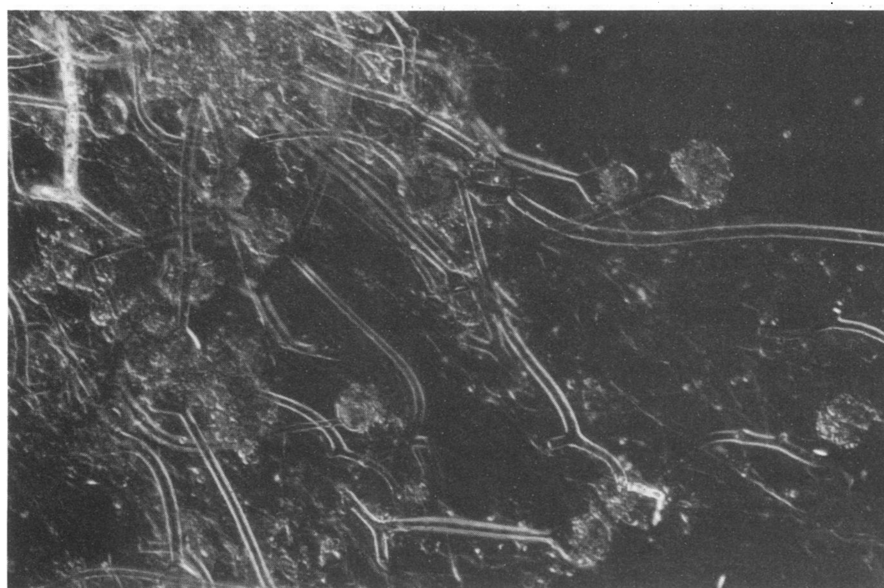

FIG. 1. *A. elegans* (CDC B-4535): numerous apophysate sporangia produced after 10 days at 37°C in distilled water-yeast extract medium. Magnification, ×250.

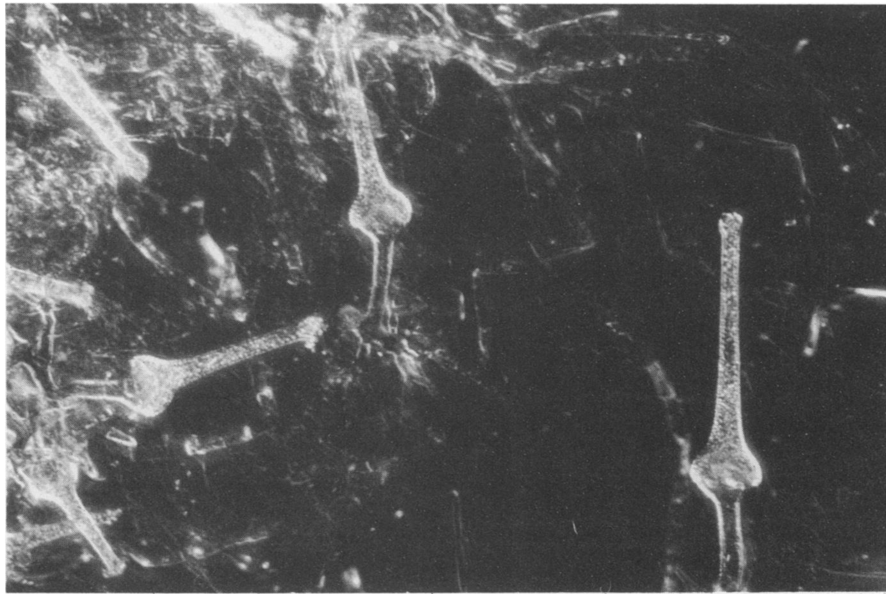

FIG. 2. *S. vasiformis* (CDC B-2190): numerous vase-shaped sporangia produced after 10 days at 37°C in distilled water-yeast extract medium. Magnification, ×250.

The results regarding sporangial formation at the three temperatures are summarized in Table 2. All 15 isolates produced moderate-to-large numbers of sporangia at 37°C (Fig. 1 and 2). Generally, sporangia were produced as early as after 5 days of incubation at 37°C in 2 of the 5 isolates of *A. elegans* and 2 of the 10 isolates of *S. vasiformis*. However, the numbers of sporangia produced were small. The optimum numbers of sporangia were generally observed after 10 to 12 days at 37°C. Significantly fewer sporangia or no sporangia were produced at 25 and 30°C in 10 days. The optimum temperature to stimulate sporulation in *A. elegans* and *S. vasiformis* was 37°C.

The specific and rapid identification of *A. elegans* and *S. vasiformis* isolated from clinical specimens is important because of the rapidity with which they invade the vascular system. This makes it imperative that they be identified rapidly so that treatment with antifungal preparations can be started early. The new procedure is recommended for use in identifying all nonsporulating zygomycetes isolated from clinical specimens.

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