A SIMPLIFIED MEDIUM FOR GROWTH AND SPORULATION OF *PILOBOLUS* SPECIES

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Formation of sporangia induced by light in *Pilobolus* species has been reported by several workers (**1**, **4**, **6**, **9**, **12**, **13**, **14**). Since 1952 synthetic media containing specific growth factors: hemin (**8**, **10**, **11**), coprogen (**2**, **3**, **15**), or ferrichrome (**7**, **9**) have been used for culturing members of this genus. Because *Pilobolus* can be grown on synthetic media and sporangium formation is induced with light, this genus has been suggested for use in studying problems dealing with sporangium development (**9**). The present research deals with the study of various synthetic media to determine which would be best suited for sporangium development.

In the initial experiments (**5**) a strain of *Pilobolus kleinii* van Tiegham was separately incubated on various media described in the literature. Best results were obtained using a solid hemin-asparagine medium described by Page (**11**). Although good vegetative growth resulted and numerous trophocysts were formed, sporangia were seldom observed. Changes in the *p*H, light regime, and culturing conditions with Page's medium and modification of the other synthetic media failed to provide cultures which regularly produced sporangia (**5**). After numerous experiments using the constituents of Page's medium, coprogen medium, ferrichrome medium, as well as many other compounds used in various concentrations and combinations, a simplified hemin medium was developed (**5**). In contrast to other media, cultures on this new medium were slightly more vigorous, had more densely pigmented vegetative growth, and produced abundant sporangia.

Page's hemin-asparagine medium (PM) was prepared as described (**11**) except that the concentration of $(NH_4)_2SO_4$ was decreased from 1.32 g/l to 0.66 g/l and the concentration of $MgSO_4 \cdot 7H_2O$ was decreased from 1.0 g/l to 0.5 g/l. These changes improved the rate and density of vegetative growth but did not improve the extremely sparse

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production of sporangia even though trophocysts were abundant (**5**). The coprogen (CM) and ferrichrome (FM) media were prepared as described (**3**, **9**).

The simplified hemin medium (SHM) contained hemin, 10 mg (dissolved in 37.5 ml of 0.1 N NaOH); sodium acetate ($CH_3COONa \cdot 3H_2O$), 10 g; thiamine·HCl, 10 mg; $(NH_4)_2SO_4$, 0.66 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; and deionized distilled water to bring the volume to one liter. For solid medium 15 g of agar were added. Media were inoculated with cylinders of agar permeated with hyphae. Each cylinder was approximately 6 mm diam and was obtained by cutting into the agar with a No. 4 cork borer at the periphery of an actively growing colony. Cultures were incubated in a walk-in growth room at 26 ± 1 C under a 12 h light-dark photoperiod. During the light phase cultures were exposed to an illumination of 25 foot-candles incandescent light. Vegetative growth was evaluated by measuring the linear increase of colony diam of cultures grown on solid media (FIG. 1) and the dry weight of cultures grown in liquid media (**5**). Qualitative observations of mycelial density and pigmentation were also made. Evaluation of asexual reproduction on solid media was based on counts of the number of sporangia which adhered to the lid of the petri dish. Sporangia were counted 2 wk after the medium was inoculated. Beyond this period of incubation, formation of sporangia ceased in the majority of cases.

Since vegetative growth on the coprogen and ferrichrome media (FIG. 1) and modifications of these was poor, they were not used for additional studies. Asexual reproduction on these media was also poor; sporangia never formed on the coprogen medium and rarely formed on the ferrichrome medium. The greatest number ever formed on the latter was 20.

Vegetative growth on Page's medium did not differ from that described in the literature (**8**, **9**, **11**). The mycelium, which was coenocytic and initially unpigmented, grew in the agar medium and never on the surface. The yellow pigment was evident on the 3rd da following inoculation. Starting on the 4th da trophocysts began developing from thickenings of the mycelium. Numerous trophocysts could be found in 5-da-old and older cultures. Subsequent stages in the development of asexual reproductive structures—elongation of the sporangiophore and differentiation of the sporangium and subsporangial swelling were seldom present. The maximum number of sporangia found in a culture on this medium was 75. In liquid culture the fungus grew entirely submerged and produced a fairly dense mycelial mat. No trophocytes and consequently no sporangia ever developed from this submerged mycelium.

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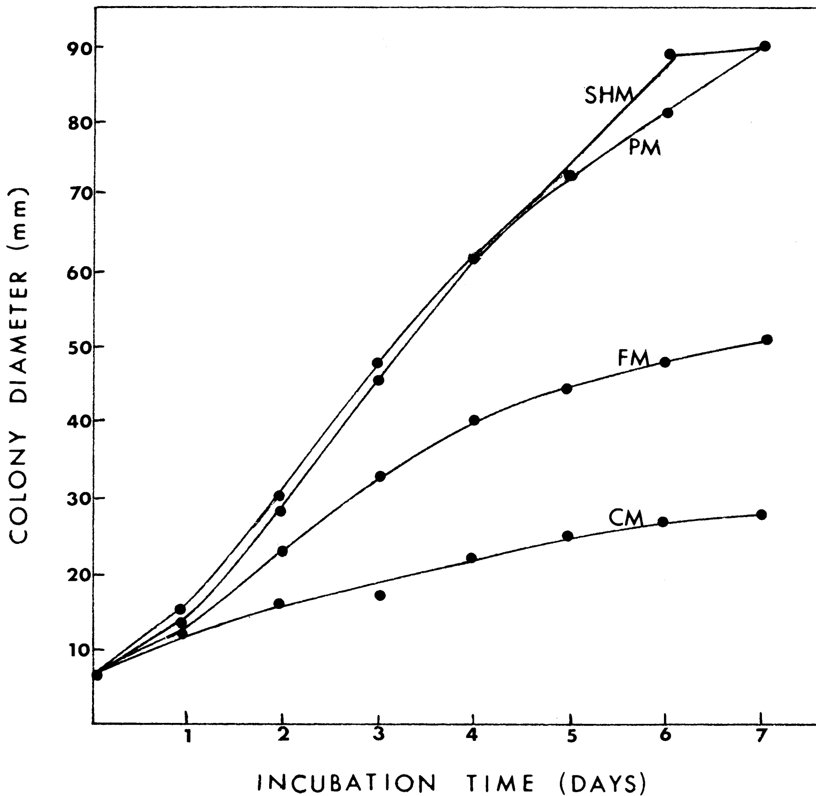

FIG. 1. Colony diameter of *P. kleinii* grown on simplified hemin medium (SHM), Page's hemin-asparagine medium (PM), ferrichrome medium (FM), and coprogen medium (CM).

During vegetative growth, the appearance of the cultures and the rate of linear growth on the simplified hemin medium were essentially the same as on Page's medium (FIG. 1), however, the cultures did appear somewhat denser and more deeply pigmented. Many trophocysts were visible 4 da after inoculation. Starting on the 5th da of incubation cultures began producing sporangia. This daily production and discharge of sporangia continued for at least one wk. The average number of sporangia that adhered to the petri dish lid was approximately 1,500, but the range was from 1,000 to 3,500 (**5**). In liquid culture the average dry weight of the cultures after 9 da incubation was 50.2 mg/25 ml medium compared to 35.3 mg/25 ml of Page's medium. Except for the increase in dry weight these cultures appeared no different than

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those on Page's liquid medium; that is, the fungus grew entirely submerged producing no sporangia.

The medium was also effective for a second isolate of *P. kleinii* and for *P. crystallinus* (Wigg.) Tode. When grown on solid media these fungi showed vigorous vegetative growth and abundant production of sporangia. The second isolate of *P. kleinii* produced an average of 1,600 sporangia/petri dish and *P. crystallinus* produced an average of 1,200.

The simplified hemin medium described here permits good growth and asexual reproduction of at least two species of *Pilobolus*. Under the conditions of this investigation it proved to be the best medium for culturing these fungi for developmental and physiological studies.

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AN ISOLATE OF *SAPROLEGNIA AUSTRALIS* FROM SOUTHEASTERN NORTH CAROLINA [1]

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There are no known reports of the occurrence of *Saprolegnia australis* Elliott since its initial isolation (Elliott, **1968**). *Saprolegnia australis* is prevalent, however, in small streams feeding Bradley Creek, Wilmington, North Carolina. The most striking dissimiliarity of the present isolate (UNC-W No. W10b, ATCC No. 32940) from the type species is sporangium size (FIGS. 1–4). Elliott reported the median primary sporangium length to be 40–80 μm, while those of W-10b are 189–243 μm (median 70%). Secondary sporangia in W-10b, like the type species, usually are short and arise by internal proliferation; but in W-10b secondary sporangia up to 600 μm in length are not uncommon at the tips of robust hyphae (FIG. 5). In 6-da-old cultures several sporangia exhibited dictyoid spore discharge, a characteristic not noted by Elliott.

Although W-10b is clearly an isolate of *S. australis* several aspects of its sexual morphology serve to broaden the species concept. Neither the antheridial branches no antheridia are persistent (FIGS. 6–7) while the reverse was true in Elliott's isolates. Fertilization tubes were found in three cases in W-10b, (e.g., FIG. 8), but none were noted by Seymour (**1970**) in preserved slides of the type culture. Oöspheres are variable in size as in Elliott's isolates, the smaller ones being produced by budding of large oöspheres (FIGS. 9–12). Mature subcentric oöspores of greatly varying sizes thus can be found in the same oögonium (FIG. 7), but

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