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 Tieghem 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 pH, 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 (NH₄)₂SO₄ was decreased from 1.32 g/l to 0.66 g/l and the concentration of MgSO₄·7H₂O 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...
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 (dis-
solved in 37.5 ml of 0.1 N NaOH); sodium acetate (CH₃COONa·
3H₂O), 10 g; thiamine·HCl, 10 mg; (NH₄)₂SO₄, 0.66 g; K₂HPO₄,
1.0 g; MgSO₄·7H₂O, 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 coeno-
cytic 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 con-
sequently no sporangia ever developed from this submerged mycelium.
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
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.

**LITERATURE CITED**

AN ISOLATE OF Saprolegnia australis
FROM SOUTHEASTERN NORTH CAROLINA

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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 dissimilarity 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 nor 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. Oöspores are variable in size as in Elliott’s isolates, the smaller ones being produced by budding of large oöspores (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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