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Blastomyces dermatitidis: Production of the Sexual Stage

Abstract. *Growth of five combinations of strains of* Blastomyces dermatitidis *in paired cultures on yeast-extract agar, plus bone meal, yielded the perfect stage for the first time. These cleistothecia, apparently of a new genus, produced eight spored asci. Monoascospore cultures developed hyphae and conidia typical of* B. dermatitidis. *Mice were successfully infected with inocula from a monoascospore culture.*

The pathogenic fungus *Blastomyces dermatitidis* and the disease it causes, North American blastomycosis, are of medical, mycological, and general interest. The life history of the fungus and its taxonomic position are inadequately known (*1*) and, in spite of reported isolation of the pathogen from nature (*2*), it is generally recognized that the reservoir of infection has yet to be demonstrated (*2, 3*). Our report of the discovery of the sexual stage should contribute to solution of these problems and open up a new field of study of the genetics of this dimorphic, pathogenic organism.

First indications of sexuality were observed during study of the growth characteristics of paired strains of *B. dermatitidis* (*4*). All possible combinations of 34 isolates (595 pairings) were made at 25°C on Sabouraud's dextrose agar in sealed, plastic petri dishes. No sexual structures were seen, but 185 of the plates developed a raised type of growth at the juncture of the two colonies. The ridge-forming pairs were replated on cornmeal agar, a medium that had been employed effectively by Emmons in stimulating cleistothecial formation by *Allescheria boydii* (*5*). Sterile cleistothecia-like structures resulted from six different pairings in separate cultures. These irregularly shaped structures were characterized by being produced near the line of juncture of the two colonies, and by the presence of radiating hyphae in the form of spiral coils reminiscent of the coiled appendages observed in the cleistothecia of some members of the Gymnoascaceae (*6*). Such sterile cleistothecia-like bodies were also obtained in cultures on yeast-extract agar (*7*) with either horse hair or bone meal added.

More recently we have grown fertile cleistothecia at will, using yeast-extract agar (*7*), with or without antibiotics, and bone meal. Yeast-extract agar was used because Smith (*7*) found that it supported slight mycelial growth while stimulating spore production. Bone was used because our own unpublished experiments had demonstrated that it stimulated growth of *B. dermatitidis*. In preparing the medium, steamed bone meal (Armour) is pulverized, sterilized with ethylene oxide, and placed along the midline of a plate of yeast-extract agar before the two inocula for each pairing are placed 2 cm apart near the center of the plate and equidistant from the bone meal.

At 25°C, fruiting bodies become visible by the unaided eye within 2½ to 5 weeks. The most conspicuous structures characterizing these cleistothecia at all ages are thick-walled, tightly coiled spirals radiating from a common source at the base of the young ascocarp (Fig. 1A). In later stages the coils become obscured to some extent by hyphae that arise, at least for the most part, as branches from the coils (Fig. 1, B and D). Dark-field illumination, however, makes it easier to follow the course of the coil in the fruiting body (Fig. 1C).

The offshoots of the spiral hyphae, which become much more abundant in the outer part of the ascocarp as it matures, consist of frequently branched thin-walled hyphae, the cells of which are constricted where they meet at the cross walls. A group of these cells resembles the spongy parenchyma of higher plants.

A mature cleistothecium (Fig. 1D) is 200 to 350 μ in diameter and has a tan color resulting from a pigment located in the coils and in the thin-walled spongy tissue–like cells. Usually young fruiting bodies contain many clusters of ascogenous hypae which are most abundant at the proximal ends of the coils, near the lower-central part of the ascocarp. It appears that the distal ends of the spiral hyphae are forced outward by pressure exerted by growing intercoil cells.

Mature asci contain eight smooth, spherical, hyaline or very light tan, uni-

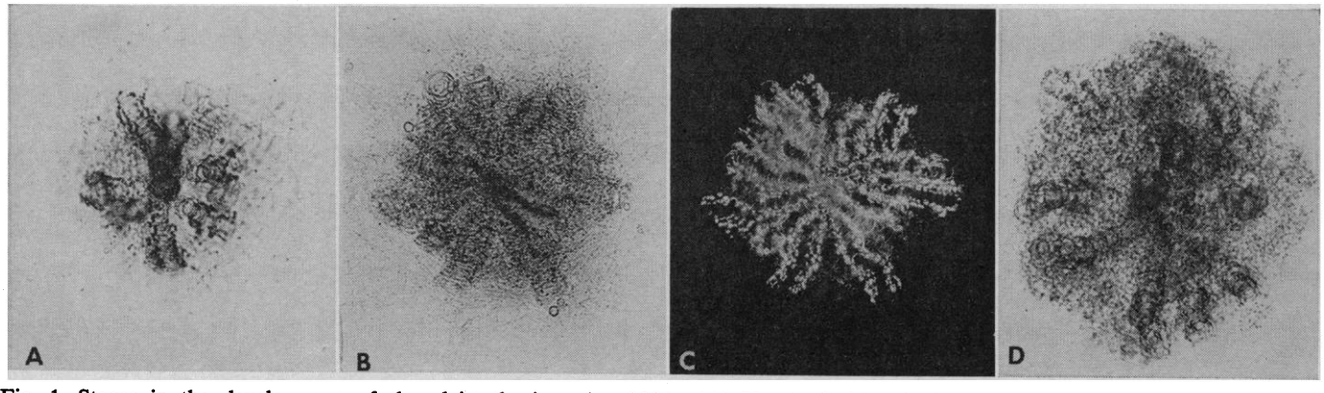

Fig. 1. Stages in the development of the cleistothecium (× 200). (A) Young fruiting body showing characteristic tightly coiled spirals. (B) An intermediate stage of the ascocarp. (C) Same intermediate stage but with dark-field illumination. (D) A young but fully developed cleistothecium containing ascospores. In some instances the coils can still be followed to their place of origin. [Stanislaus Ratajczak, Biology Department, Marquette University]

nucleate ascospores ranging from 1.5 to 2.0 μ in diameter. Such ascospores, when isolated in single-spore cultures (*8*), germinate and give rise to characteristic *B. dermatitidis* colonies that form typical conidia. We have successfully infected mice with inocula from monoascospore cultures and observed the characteristic tissue forms of *B. dermatitidis*.

Since cleistothecia have been produced only after the pairing of cultures, it is assumed that this fungus is heterothallic. Five combinations of strains have produced fertile fruiting bodies. Twenty-six isolates, when paired with at least one other strain, have produced incipient cleistothecia or mature ascocarps. These results are compatible with heterothallism. Since the fungus is multinucleate, we realize that final determination will depend on the results of pairings of monoascospore cultures.

Comparison of this fungus with descriptions (*6*) of members of the Gymnoascaceae indicates that the organism belongs to this family and to a new genus (*9*). A detailed account of the origin and development of the cleistothecium, a Latin diagnosis, and of the results of pairing of monoascospore cultures is in preparation.

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8. The ascospores, isolated with a De Fonbrune micromanipulator, were grown on brain-heart infusion agar containing 5 percent blood and 1 percent yeast extract by Shu-lan Cheng, Mycololgy Section, National Communicable Disease Center, Atlanta, Georgia.
9. R. K. Benjamin has examined prepared slides of the fungus; he agrees with us that it represents a new genus and is a member of the Gymnoascaceae.
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Heritability of Plasma Cholinesterase Activity in Inbred Mouse Strains

Abstract. *The activity of cholinesterase was measured in the plasma of 543 mice of both sexes from 23 inbred mouse strains. Differences between strains were highly significant. The heritability of this characteristic was 0.70 in females and 0.67 in males.*

In a continuing collaborative study designed to characterize experimental animals used in certain biochemical and radiation research, plasma cholinesterase activity has been measured in mice of both sexes from 23 different inbred strains. The data are of particular interest to those working with the genetic control of enzyme activity and to those concerned with correlating enzyme activity and behavior patterns.

All mice were born, raised, and maintained in the same laboratory. At weaning they were transferred from individual breeding rooms to one room where they remained until assay. By this means, stress of shipping was eliminated, and other environmental stresses were minimized and kept uniform. With the exceptions noted, all mice were housed four to six to a cage and were given free access to water and food (Old Guilford laboratory chow). Male mice of strains AKR/J, BALB/cJ, RF/J, and SJL/J fight vigorously; therefore, it was necessary to cage some of them singly. All the mice were 3½ to 4½ months old at the time of assay. Enzyme activity was measured in each of the 543 mice; to minimize possible environmental fluctuations, all measurements were made within a 4-day period. Under light ether anesthesia, the femoral artery and vein were dissected and transected. The freely flowing blood was aspirated into heparinized pipettes. It was then placed in plastic centrifuge cones (0.4 ml) and centrifuged sufficiently to separate the plasma from the red cells. Portions (0.2 ml) of plasma were placed in cups and diluted to 0.5 ml with distilled water. The cups were then placed on automatic analysis equipment which removed a sample of 0.1 ml from each cup for analysis of cholinesterase activity. With the remainder of each sample, assay for other enzymes was performed.

Cholinesterase activity was assayed by the method of Garry and Routh (*1*) as modified by Levine, Scheidt, and Nelson (*2*). This method entails the hydrolysis of the substrate acetylthiocholine by interaction with plasma; the resultant thiocholine is then treated with 5,5′-dithiobis-2-nitrobenzoic acid, a specific thiol indicator. The resultant color is determined colorimetrically at 420 mμ. The released sulfhydryl is determined by comparison to standard solutions of reduced glutathione. The unit of cholinesterase activity is defined as the amount of enzyme per milliliter of plasma that liberates 1 μmole of sulfhydryl groups in 3 minutes of incubation.

Table 1. Plasma cholinesterase activity in 23 inbred mouse strains.

Strain	Activity*			
	Females		Males	
	No.	Mean* ± S.E.	No.	Mean* ± S.E.
A/J	10	37.2 ± 1.20	13	28.7 ± 1.08
AKR/J	16	29.8 ± 0.57	18	16.1 ± 0.71
AU/SsJ	6	67.7 ± 2.97	5	47.2 ± 2.92
BALB/cJ	10	33.1 ± 1.08	12	26.5 ± 0.93
CBA/J	12	41.8 ± 0.87	16	28.1 ± .82
CE/J	9	37.1 ± .94	17	27.9 ± 1.45
C57BL/6J	11	34.5 ± 1.06	10	30.3 ± 0.78
C57BR/cdJ	10	31.9 ± 0.82	18	26.2 ± .49
C57L/J	11	33.8 ± .42	15	26.6 ± .49
C58/J	9	37.8 ± 1.13	16	29.5 ± .65
C3HeB/FeJ	15	33.3 ± 0.67	14	26.1 ± .50
DBA/1J	9	52.9 ± 1.71		
DBA/2J	10	44.7 ± 0.84	10	29.8 ± .29
LP/J			7	20.7 ± 1.41
MA/J	23	45.6 ± .78	21	23.3 ± 0.25
RF/J	12	31.0 ± .44	17	17.8 ± .30
RIII/J	8	42.8 ± 3.07	11	32.7 ± 1.18
PL/J	15	46.0 ± 1.20	16	30.9 ± 0.82
SEC/1ReJ	10	42.6 ± 1.07	10	36.0 ± 1.03
SJL/J	12	34.0 ± 0.60	10	19.4 ± 0.88
SM/J	11	35.5 ± .55	13	25.2 ± .58
ST/bJ	11	35.6 ± .93	13	26.8 ± .48
129/J	11	39.1 ± .83	10	35.8 ± .81

* Enzyme activity is expressed as micromoles of sulfhydryl per milliliter of plasma.