JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1984, p. 594–596
0095-1137/84/090594-03$02.00/0

Vol. 20, No. 3

Conversion of *Blastomyces dermatitidis* to the Yeast Form at 37°C and 26°C

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Received 9 April 1984/Accepted 11 June 1984

A partially defined agar medium, KT, has been developed and compared with brain heart infusion agar for the conversion of *Blastomyces dermatitidis* to the yeast form. On the KT medium, the mold form converted to a yeast form within 72 h of incubation at 37°C or after 3 weeks at 26°C. A nutritionally dependent dimorphism in *B. dermatitidis* was observed.

The conversion of the mold form of *Blastomyces dermatitidis* Gilchrist et Stokes 1898 to a yeast form has been shown to occur at 37°C (1, 3, 6, 7, 9, 12, 14, 19, 20, 21, 22, 25) on a wide variety of substrates. I have observed that *B. dermatitidis* will convert to a yeast form on many different types of media, provided that an incubation temperature of 37°C is maintained and that the time of incubation is prolonged. At 37°C, clinical isolates cultured on conventional conversion media occasionally require repeated subculture and often require extended incubation for conversion to the yeast form (5). Several agar media have been recommended for the conversion of *B. dermatitidis* from its mold form to its yeast forms, including brain heart infusion (BHI) agar, blood agar, blood agar supplemented by glutamine, Kelley agar, and cotton seed agar (1, 2, 5, 11, 13, 19, 24). McGinnis has indicated that total colonial conversion is not necessary for the identification of *B. dermatitidis* (17). Most of the *B. dermatitidis* strains used in this study were isolated from clinical specimens which had been plated on blood agar with the addition of 3% egg white (4, 10).

In 1973, several isolates of *B. dermatitidis* failed to convert to the yeast form on BHI media incubated at 37°C. Because of this problem, Tween-albumin-niacin (TAN) medium (23) was inoculated with the mold form of *B. dermatitidis*. Within 72 h at 37°C, conversion to the yeast form occurred. The cultures showed complete conversion with masses of large, thick-walled, budding yeast cells. These cultures were left in the safety cabinet at 26°C, and after 4 weeks it was observed that the isolates did not convert back to the mold form as would be expected (19). Microscopic examination again showed masses of large budding yeast cells and some hyphae.

For the experimental work in this study, 19 strains of *B. dermatitidis* and 3 isolates of *Chrysosporium* species Corda 1833 were randomly selected for study. The isolates of *Chrysosporium* sp. were obtained from human skin scrapings, and the isolates of *B. dermatitidis* were obtained from a variety of clinical specimens. Confirmation of the identity of *B. dermatitidis* was made by conversion of the mold to the yeast form at 37°C on BHI agar (Difco Laboratories, Detroit, Mich.). The isolates were maintained at −20°C on peptone-dextrose agar and in distilled water, in the mold form (18), before use.

Cultures were inoculated onto peptone-dextrose agar and grown for 3 weeks at 26°C. Each culture was then ground in a glass grinder containing 5 ml of sterile distilled water. Three drops of the mycelial suspension, comparable to a no. 3 McFarland nepholometer standard, were inoculated with a Pasteur pipette onto each of the media (Table 1) and incubated at 37 and 26°C.

The TAN medium, a partially-defined formulation, was prepared as recommended by Tarshis (22; Table 1).

Conversion was considered to have occurred when yeast cells bearing blastoconidia attached by a broad base were seen, irrespective of their number. The number of isolates that converted to the yeast form on each of the media tested is shown in Table 2.

After 3 weeks of incubation of the TAN, KT, D, and C media at 37°C, each of the 19 strains of *B. dermatitidis* exhibited typical yeast cells with colonies showing a soft consistency. In contrast, two of the isolates showed poor growth on BHI agar, and the remaining cultures on BHI showed colonies with waxy or dry appearance. These results confirm the observation by Weeks (23) that BHI agar is not an efficient medium for primary conversion of *B. dermatitidis* at 37°C. All 19 isolates showed good growth after 3 weeks of incubation on the various media at 26°C. The colonies on TAN and KT media were yeastlike with a rough and crumbly appearance. Growth on C, D, and BHI media produced the mold form with white to gray aerial mycelium, particularly on the modified D medium. A yeast form was absent. This medium has been used for rapid conversion of primary clinical isolation of *B. dermatitidis* at 37°C. The three isolates of *Chrysosporium*, including *C. parvum* (Emmons et Ashburn) Carmichael 1962, *C. keratinophytum* Frey et Carmichael 1963, and an unidentified species, showed no conversion at either 37 of 26°C.

Microscopic examination of the *B. dermatitidis* strains revealed large budding yeast cells (Fig. 1) of different shapes and sizes, ca. 12.5 by 22.5 μm in diameter. Yeast cells were abundant in some isolates and less so in others and were arranged in groups or found singly mingled with transitional forms and wide hyphae (Fig. 2). On KT medium, early hyphal coversion of *B. dermatitidis* to the yeast form may occur after 10 days of incubation at 26°C. Complete conversion at 26°C did not occur, however, under the specific experimental conditions tested. The hyphae were considered to be those left from the partial conversion of the yeast cells

TABLE 1. Composition of experimental media

Medium	Composition	Amt
TAN	Tween 80	0.2 ml
	Potassium sulfate	0.5 g
	Magnesium citrate	1.5 g
	Asparagine	5 g
	Albumin	5.0 g
	Dextrose	7.0 g
	Sodium chloride	0.85 g
	Agar	15 g
	Glycerol	20.0 ml
	Distilled water	1 liter
Formula C	TAN medium without glycerol or albumin	
Formula D	Formula C supplemented by 0.3% Casamino Acids	
Formula KT	TAN medium supplemented by 0.3% Casamino Acids	

at 26°C. These observations suggested that temperature may not be the only important factor in dimorphism of *B. dermatitidis*.

Classically, it has been considered that the conversion of the mold form of *B. dermatitidis* to a yeast form was entirely dependent on the incubation temperature of 35 to 37°C. The media used in this study show that nutrition is an additionally important factor in the conversion of *B. dermatitidis* at 26°C. All ingredients listed in the TAN formula were required for conversion at 26°C. The addition of Casamino Acids to the TAN medium induced the production of yeast growth. The incomplete C and D media did not support conversion to the yeastlike form at 26°C. Conversion back to the mold form did not occur when the yeast form of *B. dermatitidis* grown at 37°C on the KT medium was further incubated for several months at 26°C. Microscopic examination of the yeast colonies revealed only large budding yeast cells, with no evidence of hyphal formation.

The mold form of *B. dermatitidis*, after conversion to the yeast form on KT medium at 26°C, showed no reconversion to the mold form after several weeks of incubation. Subculturing of the yeast cells to other media resulted in typical mold colonies. When a light suspension of the yeast form of *B. dermatitidis*, grown at 37°C, was inoculated onto KT medium and incubated at 26°C for ca. 3 weeks, a two-step reproduction process occurred (Fig. 3). First, a transitional mycelial form developed, which was followed by the transformation of this intermediate stage into a yeast form, the shape of which would vary with the nature of the isolate.

The addition of chloramphenicol to the experimental conversion media inhibited the growth of the yeast form at 37°C, confirming previously reported findings by McDonough et al (15, 16). Other antimicrobial agents did not interfere with yeast growth at 37°C within 2 weeks. Since chloramphenicol inhibits protein synthesis in bacteria by interfering with the function of mRNA (8), it is tempting to speculate that a similar inhibitory action takes place in the yeast phase of *B. dermatitidis*.

The demonstrated nutritionally dependent mold-to-yeast conversion at 26°C contributes a new insight to our understanding of dimorphism in *B. dermatitidis*.

TABLE 2. Efficacy of five media for converting 19 isolates of *B. dermatitidis* to the yeast form at 37°C within 72 h

Medium	No. of isolates showing the following degree of conversion from mold to yeast form (%)[a]:				% Conversion
	None	1+	2+	3+	
TAN		9 (47)	8 (42)	2 (11)	100
C		10 (53)	7 (37)	2 (10)	100
D		7 (37)	9 (47)	3 (16)	100
KT		6 (32)	9 (47)	4 (21)	100
BHI	8 (42)	11 (58)			58

[a] Conversion was graded as follows: 1+, few budding yeast cells; 2+, moderate budding yeast cells; 3+ abundant budding yeast cells.

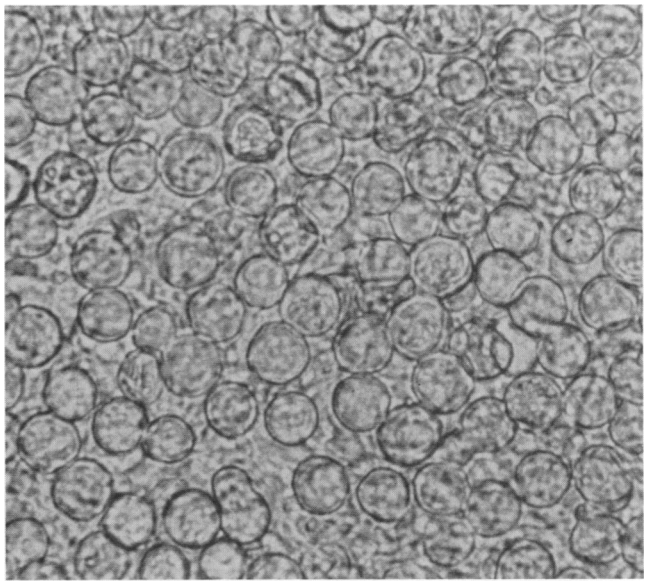

FIG. 1. Conversion of *B. dermatitidis* (OMH 1714) within 72 h at 37°C on KT medium; figure shows masses of yeast cells. Magnification, ×400.

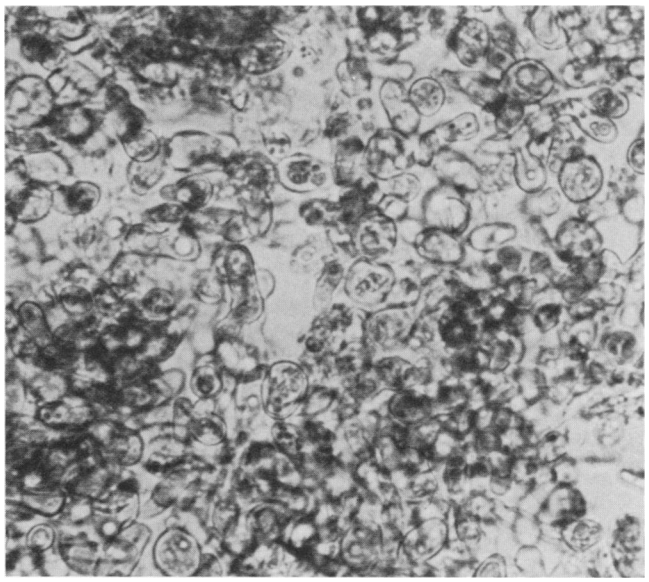

FIG. 2. Partial conversion of *B. dermatitidis* (OMH 889) to the yeast form at 3 weeks of incubation at 26°C on KT medium. Magnification, ×400.

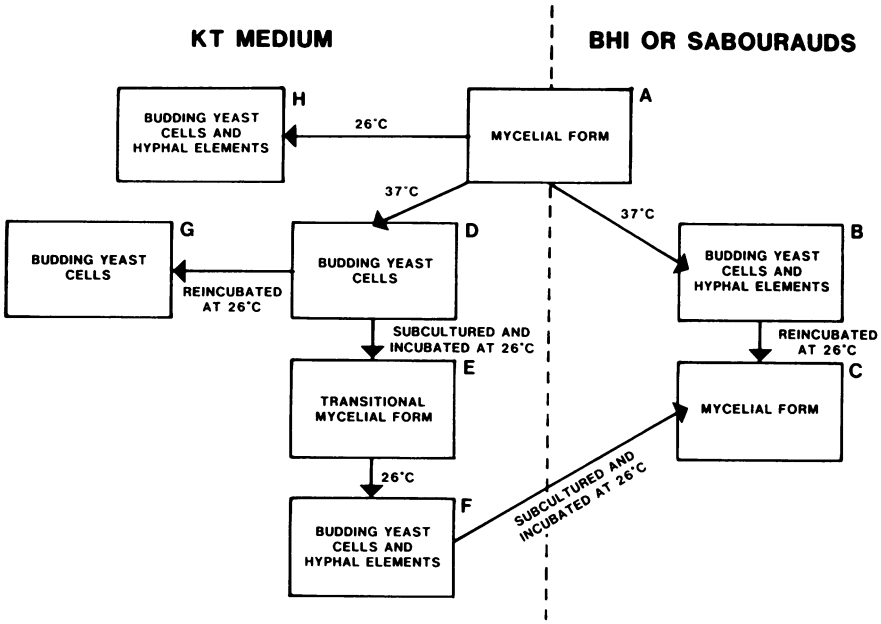

FIG. 3. Cultural conversion cycle of *B. dermatitidis* on conventional media and KT medium at 37 and 26°C. Classic conversion of *B. dermatitidis* is shown in A, B, C, and D on BHI agar, Sabouraud agar, and KT medium. Subculturing the yeastlike growth onto KT medium at 26°C (E) yielded a transitional mycelial phase which, upon continued incubation, reconverted to the yeastlike phase. Conversion of the mycelial to the yeastlike form at 26°C is shown in H.

I thank S. A. Rosenthal for reviewing this manuscript and for helpful suggestions; the Audio-Visual Department of the Ministry of Health for photography; and S. Albreish and G. Riley for technical assistance.

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