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NOTES

Growth of *Paracoccidioides brasiliensis* Yeast Phase in a Chemically Defined Culture Medium

ANGELA RESTREPO* AND BEATRÍZ E. JIMÉNEZ

Corporación de Investigaciones Biológicas, Hospital Pablo Tobón Uribe, Medellín, Colombia

A slight modification of the chemically defined medium of McVeigh and Morton resulted in an excellent substratum for the cultivation of *Paracoccidioides brasiliensis* yeast phase.

Repeated attempts to grow the dimorphic fungal pathogen *Paracoccidioides brasiliensis* in chemically defined media have proven unsuccessful (10, 11, 13, 15). In spite of reports concerning the adequacy of two synthetic formulations (4, 6), the results of such studies have not been reproducible (10, 11). Investigators have thus been forced to cultivate the fungus in conventional complex or basal media, the latter containing one or more undefined ingredients, e.g., yeast extract or casein hydrolysate. Consequently, previous findings concerning physiological processes (10, 11), pattern of growth curves (1, 14), antigen production (3, 9, 12), and drug susceptibility (2, 15) have met with only qualified success. The yeast phase is particularly difficult to grow in liquid media, even if the media are complex (11, 13). Since this phase corresponds to the one in tissues, obtaining yeast-phase antigens and determining the physiological characteristics of that phase would be greatly

TABLE 1. *Composition of MMcM medium*[a]

Component	Amt (per liter)
Glucose	10.0 g
KH_2PO_4	1.5 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$CaCl_2 \cdot 2H_2O$	0.15 g
$(NH_4)_2SO_4$	2.0 g
L-Asparagine	2.0 g
L-Cystine[b]	0.2 g
Vitamin supplement[c]	10.0 ml
Trace element supplement[d]	1.0 ml

[a] All components except the vitamin supplement were mixed, and the pH was adjusted to 7.0 with 1 N NaOH. The vitamin solution was filter sterilized and added after the remainder of the medium had been autoclaved at 121°C for 15 min and cooled.

[b] The cystine was dissolved, before addition to the remainder of the medium, by heating it in a small volume of distilled water to which 1.0 N NaOH was added dropwise until the cystine was completely dissolved.

[c] The stock vitamin solution contained, per 100 ml: thiamine hydrochloride, 6.0 mg; niacin, 6.0 mg; calcium pantothenate, 6.0 mg; inositol, 1.0 mg; biotin, 0.1 mg; riboflavin, 1.0 mg; folic acid, 10 mg; choline chloride, 10 mg; and pyridoxine hydrochloride, 10 mg.

[d] Trace element solution contained, per 100 ml: H_3BO_3, 5.7 mg; $CuSO_4 \cdot 5H_2O$, 15.7 mg; $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$, 140.4 mg; $MnSO_4 \cdot 14H_2O$, 8.1 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 3.6 mg; $ZnSO_4 \cdot 7H_2O$, 79.2 mg.

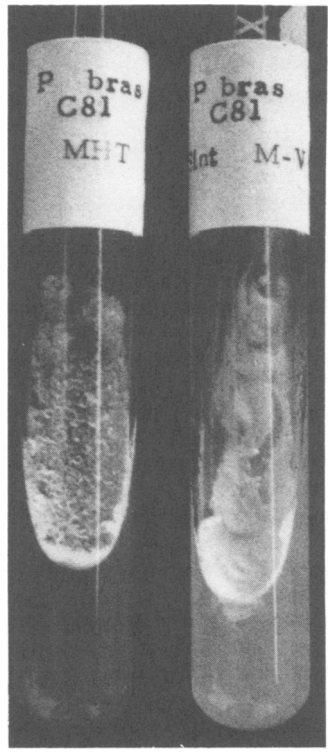

FIG. 1. *P. brasiliensis yeast-phase cultures. Tube at left, growth in complex medium. Tube at right, growth in MMcM medium.*

facilitated if the organism could be grown in a totally synthetic medium. Such studies would, in turn, facilitate other studies involving the host-parasite relationship. We report here a modification of the medium described originally by McVeigh and Morton for *Histoplasma capsulatum* (8), as reported by Levine et al. (7). Addition of four vitamins, namely, riboflavin, folic acid, choline, and pyridoxine, resulted in excellent growth of *P. brasiliensis*. This modified McVeigh-Morton (MMcM) medium can be solidified by addition of 1.3% purified agar. The components of the medium are listed in Table 1.

Studies were conducted with 20 human isolates of *P. brasiliensis* available in our collection. Stock cultures maintained at room temperature were initially reverted to the yeast phase by culturing in Kelly's hemoglobin agar (5) with incubation at 36°C for 10 days. After conversion, adaptation to solid MMcM medium was attempted by repeated transfers to fresh slants every 5 days, followed by incubation at the same temperature. After four to six such passages, all isolates produced abundant growth after 3 days. With the exception of strain B 339, growth was pasty and homogeneous, in comparison with the wrinkled colonies regularly obtained on richer media (Fig. 1). Microscopic observation revealed yeast cells with typical multiple budding. Once

TABLE 2. *Growth of P. brasiliensis yeast phase in MMcM medium*

Strain	Turbidity at h of growth[a]:		
	0	60	120
LA	50.5	9.5	1.0
C 81	52.5	24.0	3.0
MM	25.0	1.0	0.05
MTC	43.5	22.0	1.0
LS	35.5	7.0	3.8
B 339	76.0	76.0	70.0

[a] Turbidity measured by transmittance at 550 nm in a Beckman DU Spectrophotometer after growth at 36°C in liquid MMcM medium.

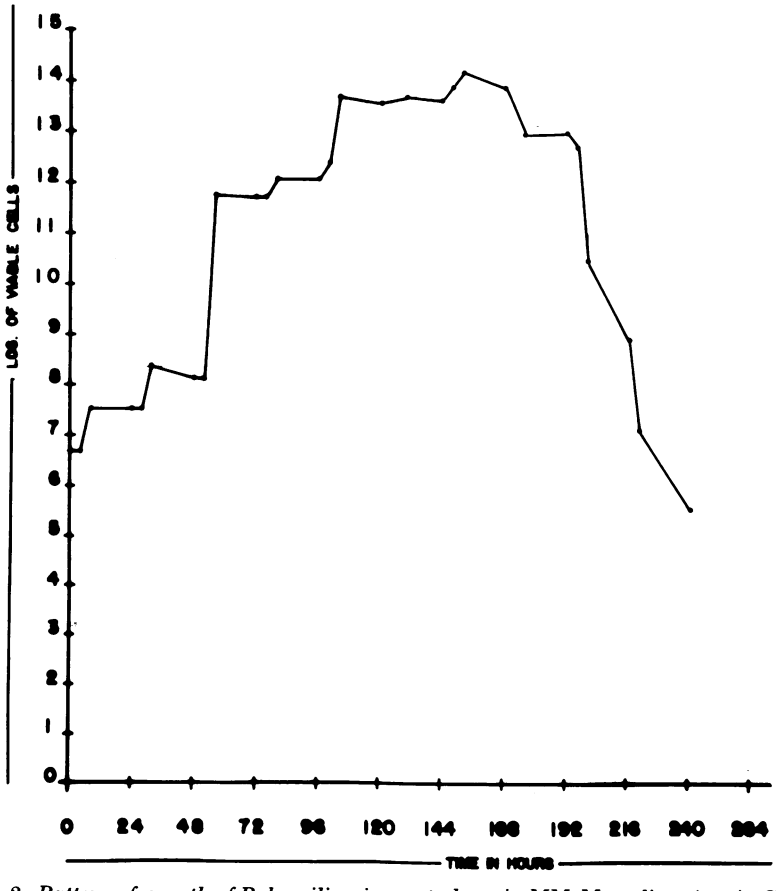

FIG. 2. *Pattern of growth of P. brasiliensis yeast phase in MMcM medium (strain C 81).*

adapted, 3-day-old cultures could be transferred to liquid MMcM medium in Erlenmeyer flasks, and abundant growth occurred if the liquid cultures were incubated at 36°C with constant agitation (120 rotations per min) on a gyratory shaker (New Brunswick Scientific). Of the six isolates studied in liquid MMcM medium, five grew such that an increase in turbidity was obvious after 60 h of incubation, with a heavy growth after 120 h of incubation. Turbidimetric readings taken from the cultures are presented in Table 2. One strain, B 339, did not grow well in the MMcM medium.

The pattern of growth of one of the isolates, C 81, was determined as described previously (1) (Fig. 2). Colony-forming units increased logarithmically during the first 108 h, after which time the stationary phase began. The stationary phase was 84 h long. The decline occurred thereafter and was relatively abrupt. By 240 h, when the experiment was terminated, colony-forming units were below the initial numbers at 0 h. This pattern is similar to the one previously described for *P. brasiliensis* grown in complex media (1, 14).

The results of this study corroborate previous findings (1) concerning the growth of *P. brasiliensis* yeast phase and indicate the points at which young cells can be obtained for physiological and antigenic studies to avoid the risk of using degenerated yeast cells.

Preliminary experiments with *P. brasiliensis* mycelial phase indicate that the mycelial phase grows well in either liquid or agar MMcM medium. This is an added advantage because any work involving comparison between the two phases requires identical substrata.

The availability of an adequate, totally defined medium for cultivation of *P. brasiliensis* will facilitate studies on the metabolic, physiological, and antigenic characteristics of this microorganism.

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