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Original Article

Rapid Production of *Candida albicans* Chlamydospores in Liquid Media under Various Incubation Conditions

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Abstract

The production of chlamydospores is a diagnostic tool used to identify *Candida albicans*; these structures also represent a model for morphogenetic research. The time required to produce them with standard methods is 48-72 hours in rice meal agar and tensoactive agents. This time can be shorted using liquid media such as cornmeal broth (CMB) and dairy supplements.

Five media were tested: CMB plus 1% Tween-80, CMB plus 5% milk, CMB plus 5% milk serum, milk serum, and milk serum plus 1% Tween-80, under different incubation conditions: at 28°C and 37°C in a metabolic bath stirring at 150rpm, and at 28°C in a culture stove. The reading time points were established at 8 and 16 hours. The best results were obtained at 16 hours with CMB plus 5% milk under incubation at 28°C and stirring at 150 rpm. The next most efficient methods were CMB plus 5% milk serum and CMB plus 1% Tween-80, under the same incubation conditions. The other media were ineffective in producing chlamydospores. The absence of stirring at 28°C prevented the formation of chlamydospores within the set time points, and incubation at 37°C decreased their production.

This paper reports that the time to form *C. albicans* chlamydospores can be reduced.

Key words: *Candida albicans*, chlamydospores, liquid media, cornmeal

Introduction

The formation of chlamydospores is a morphologic feature of *Candida albicans* widely used in the laboratory for identification purposes. Together with the serum filamentation test, it constitutes a straightforward and low-cost etiologic diagnostic tool[1, 2]. Another important aspect of the production and purification of *Candida albicans* chlamydospores is the recent interest raised by the isolation of these structures in morphogenetic studies[3]. Traditionally, chlamydospores have been obtained using a standard medium, i.e., cornmeal agar (CMA) with added Tween-80, in a minimum period of 48-72 hours[2]. Some authors have described changes in this medium aimed at more efficient production; adding milk has resulted in an increased proportion of chlamydospores from 21.4% to 95.5% in 48 hours[4]. Another variant reported is the use of skim milk and the addition of solid media from processed cheeses, which led to an abundant and quicker development of chlamydospores than with the standard method[5]. With cornmeal broth (CMB), in the same medium, with 5% milk or 1% veal serum, it has been possible to produce chlamydospores in periods ranging from 20 to 72 hours, at an optimal temperature within a range of 24°C to 30°C[6]. This study intends to define conditions providing an abundant production of chlamydospores in a shorter period than the times reported in the literature.

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Materials and methods

Five liquid media were used: CMB plus 1% Tween-80, CMB plus 5% milk, CMB plus 5% milk serum, milk serum, and milk serum plus 1% Tween-80. The CMB was prepared at a 3% concentration in distilled water. Milk serum was

obtained from non-pasteurized cow's milk, with enzymes for coagulation of casein and separation by means of filtration. It was preserved frozen at −20°C. Pasteurized milk was used in the medium consisting of CMB with milk.

Candida species, isolated from different patient specimens and preserved in the strain collection at the Mycology Laboratory, were used in this study; negative controls were one species of *C. guilliermondii* and one of *C. parapsilosis*. Yeasts of the study were isolated and classified by traditional routine: isolation in three media: Biggy-Nickerson, CHROMagar-*Candida* and Sabouraud agar. Each of the strains has a precise typing scheme consisting of morphologic, physiologic as serum-filamentation; pseudohyphae and chlamydospore production in cornmeal + Tween 80 1% (agar-plate) and biochemical tests of carbohydrate assimilation with API 20C. Pre-culture used was potato dextrose agar (PDA), with two days of yeast growth. Broth media were inoculated with 100 μl of turbidity-adjusted suspensions at an approximate concentration of 1×10^6.

The tests were performed in a tube and incubated in a metabolic bath at 28°C and 37°C, stirred at 150 rpm, and in a tube with stove incubation at 28°C (without stirring). Results were read at 8 and 16 hours.

Chlamydospores were observed by a direct examination. A drop of inoculated broth media was placed (after vortex-agitation) between a microscope-slide and a cover-slide; chlamydospore production was quantitated by a semi-quantitative method: we counted a number of them by microscopic fields and quantification was made by two observers.

Results

Results are reported in Tables 1 and 2. Table 1 indicates a percent of *C. albicans* strains with formation of chlamydospores in different liquid media under incubation and metabolic bath at 28°C and stirring at 150 rpm. Table 2 reports a semi-quantitative assessment of *C. albicans* chlamydospore production at 16 hours in different liquid media, with metabolic bath incubation at 28°C and 37°C and stirring at 150 rpm. (Fig. 1 and Fig. 2)

Discussion

Chlamydospores of *Candida albicans* depends on a regulatory protein which influences the transition from yeast to hyphae; 6 genes are needed that depend on certain conditions for their activation such as oxygen, pH and several substrates [7, 8].

In our study, we obtained good results, particularly in three of the culture media: CMB plus 5% milk, CMB plus 5% milk serum and CMB plus Tween-80. In the other two media, milk serum and milk serum plus Tween 80, no chlamydospores were formed under the established conditions. The production of chlamydospores was most effective and was achieved at 16 hours in the medium of CMB plus 5% milk. (Table 1)

Table 1. Percent of formation of chlamydospores in different liquid media

		Culture media					
		CMB + 1% of Tween 80		CMB + 5% of milk		CMB + 5% of milk serum	
Strain	No. strains	8 h (%)	16 h (%)	8 h (%)	16 h (%)	8 h (%)	16 h (%)
C. albicans	25	0	66	16.6	100	16.6	100
C. guilliermondii	1	0	0	0	0	0	0
C. parapsilopsis	1	0	0	0	0	0	0

CMB= Cornmeal broth. Incubation: Metabolic bath at 28°C and stirring 150 rpm.

Table 2. Production of chlamydospores in semi-quantitative assessment of *C. albicans* at 16 hours in different liquid media

		Culture media					
		CMB + 1% of Tween 80		CMB + 5% of milk		CMB + 5% of milk serum	
Strain	No. strains	28°C	37°C	28°C	37°C	28°C	37°C
C. albicans	25	++	+	+++	+	++	+
C. guilliermondii	1	0	0	0	0	0	0
C. parapsilopsis	1	0	0	0	0	0	0

CMB= Cornmeal broth. Incubation: Metabolic bath at 28°C and stirring 150 rpm.
Chlamydospore production: + = limited, ++ = moderate, +++ = abundant.

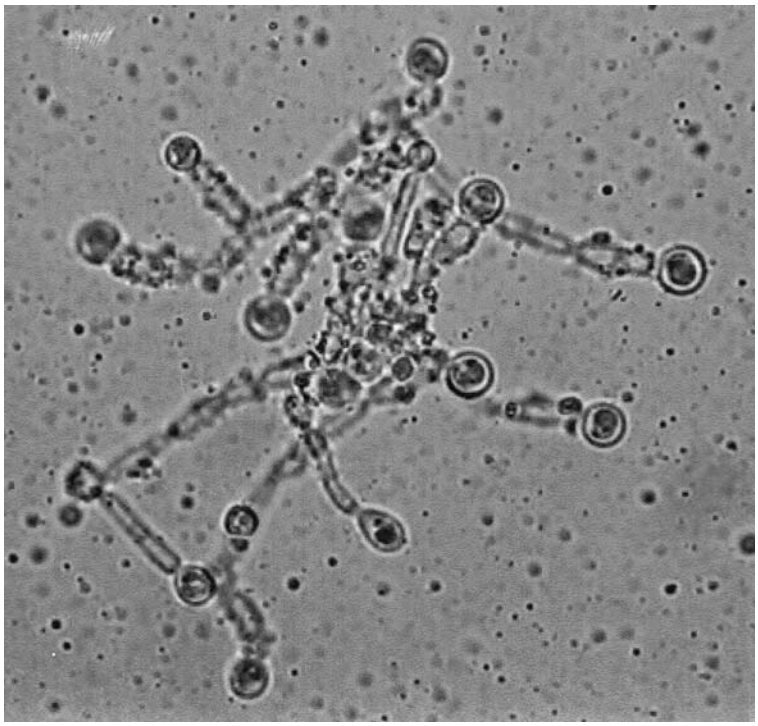

Fig. 1. Chlamydospores of *C. albicans* from CMB plus 5% milk media. (Direct exam, 10×)

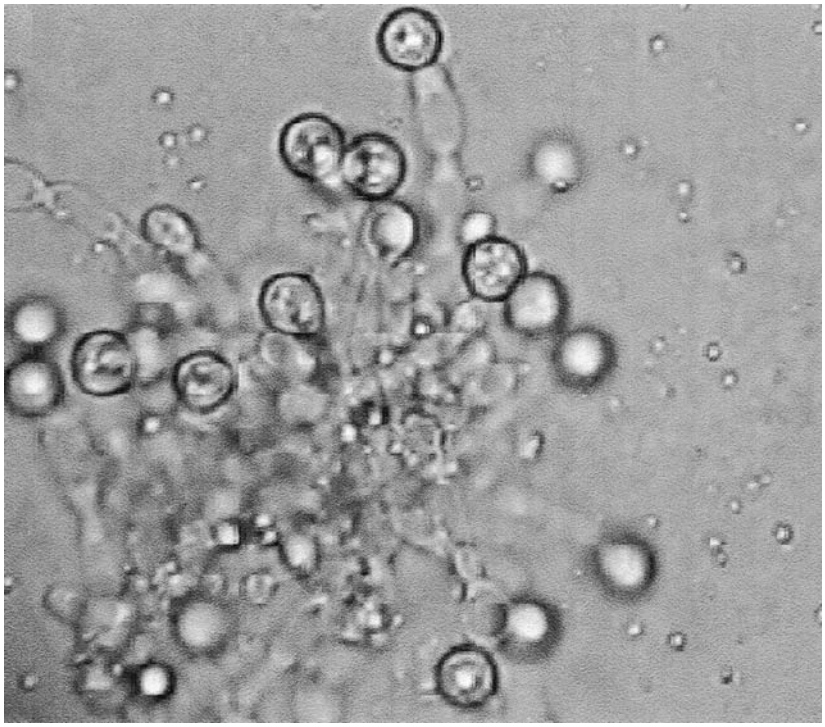

Fig. 2. Chlamydospores of *C. albicans* from CMB plus 5% milk media. (Direct exam, 40×)

The comparison of these results with the literature reports confirmed the effectiveness of milk as a factor that helps in the formation of *C. albicans* chlamydospores, in combination with CMB. To a great extent, milk was also found to favor the production of mycelia from the early stages (8 hours). As regards the time necessary for the production of chlamydospores, stirring in a metabolic bath resulted in a 20% reduction in the time reported by Nakamoto[6], which was 20 hours under similar conditions (without stirring). A shorter time to produce these structures represents an advantage in the daily laboratory diagnostic work. Likewise,

morphogenetic research would benefit from a more effective separation of chlamydospores due to the possibility of producing large amounts under the above established conditions, besides the reduced time.

Casein-free milk (milk serum) was also found to favor the production of chlamydospores, with greater effectiveness than Tween 80 but less than whole milk, and with the added advantage that it can be preserved frozen for long periods of time.

In conclusion, it is important to reduce the time for chlamydospore formation. We also emphasize that the production took place in media without casein, proving that this protein is not necessary for chlamydospore-production

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