SHORT COMMUNICATION

Production of Contiguously Arranged Chlamydospores in *Candida albicans*

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Contiguous arrangements of two to three chlamydospores occurred in a clinical isolate of *Candida albicans*. Time-lapse photography showed that the terminal cell of a cell chain was first transformed into a chlamydospore and such transformation proceeded centripetally to the next cell in the chain. Ultrathin sections revealed that the outermost layer of the three-layered chlamydospore wall was continuous throughout the interconnected spores, with the other layers surrounding each spore separately. Chlamydospore chains were common in this organism.

INTRODUCTION

The formation of chlamydospores is one of the characters considered to be of diagnostic importance in *Candida albicans*, and thus has attracted the attention of a number of microbiologists. Although various aspects of the properties of chlamydospores have been studied (for examples, see Bakerspigel & Burke 1974; Cassone *et al.*, 1975; Jansons & Nickerson, 1970a, b; Miller *et al.*, 1974), our understanding of their biological characteristics is still far from satisfactory.

Ordinarily, chlamydospores of *C. albicans* are considered to develop at the extremities or the sides of pseudomyelia, as large, spherical, thick-walled single cells. We found that a number of *C. albicans* strains isolated from clinical specimens produced contiguously arranged chlamydospores when cultured on certain media. This communication describes light and electron microscopic observations on this unusual arrangement of chlamydospores.

METHODS

Organism and media. The strain of *C. albicans* mainly used in this study, K1A-1, was obtained from a patient with skin candidiasis at the clinic of K. Eto, Kumamoto, Japan. This strain was identified as *C. albicans* by sugar fermentation study and a germ tube test in addition to chlamydospore production. Sixty other strains of *C. albicans* used in a wider survey were isolated from a variety of clinical specimens at Kyushu Dental College.

Stock cultures were maintained on Sabouraud dextrose agar (SDA) slants at room temperature. The starch/corn meal/liver/Tween 80 (SCLT) medium contained, per litre: 10 g soluble starch (Wako Pure Chemical Industries, Osaka, Japan), 300 ml corn meal extract, 40 ml bovine liver extract, 8 ml Tween 80 (Katayama Chemical Co., Osaka), 20 g agar and NH₄OH to adjust the pH to 7.5. Sterilization was done by autoclaving at 121 °C for 15 min. Corn meal extract was prepared by extracting 40 g corn meal in 1 litre of water for 1 h at 60 °C followed by...
Fig. 1. Light micrographs of contiguously arranged chlamydospores in C. albicans grown for 4 d on SCLT agar at 25°C. Cotton blue stained. (a) Some of the suspensor cells have apparently been transformed into chlamydospores, while others have not. (b) A pseudomycelium (arrow) and a suspensor cell have changed to chlamydospores. The bar markers represent 10 μm.

Fig. 2. Time-lapse photographs of chlamydospore development in C. albicans grown on SCLT agar. (a) 18 h incubation; (b) 2 d incubation; (c) 3 d incubation; (d) 3 d incubation, stained with cotton blue. The arrows indicate a suspensor cell under transformation into a chlamydospore. The bar markers represent 10 μm.
filtration. Bovine liver extract was prepared by boiling 500 g sliced liver in 1 litre of water for 1 h, followed by filtration after chilling. Both extracts were autoclaved at 121 °C for 15 min.

**Chlamydospore production.** Inoculum was prepared by suspending a loopful of a fresh overnight culture on SDA in 5 ml sterile saline. A 0.5 ml portion was spread on a SCLT agar plate. The plates were incubated aerobically at 25 °C for up to 4 d. An excellent yield of chlamydospores was obtained under these conditions.

**Light microscopy.** Some plate cultures were observed directly under a microscope at a magnification of 400 x. Others were stained by placing on them a drop of 1% (w/v) cotton blue (Kanto Chemical Co., Tokyo, Japan) in 3% (v/v) aqueous acetic acid. A cover glass was then placed on the stained area, and the plates were examined at a magnification of 850 x.

**Electron microscopy.** Cells were scraped off SCLT medium with a spatula and washed three times with a sterile saline. After centrifugation, the pellet was fixed with 2% (v/v) glutaraldehyde in 0.05 M-phosphate buffer pH 7.2 for 18 h. The fixed cells were washed with the same buffer and post-fixed with 2% (w/v) potassium permanganate for 40 h. After fixation, the specimens were dehydrated through a graded series of ethanol and propylene oxide followed by embedding in epoxy resin. Ultrathin sections were obtained on a LKB 4800A Ultrotome with a glass knife and stained with uranyl acetate and lead citrate. Sections were examined in a Hitachi 100HU electron microscope.

**RESULTS AND DISCUSSION**

In 4 d cultures on SCLT medium, *C. albicans* strain K1A-1 produced a number of large, spherical, thick-walled cells with granular contents, occurring peripherally or terminally on pseudomycelia as observed by direct microscopy. These structures were considered to represent typical chlamydospores. Vital staining with 1% (w/v) cotton blue preferentially stained these cells, differentiating them from suspensor cells, pseudomycelia and blastospores. This finding agreed with that of Miller & Finnerty (1979), who used trypan blue for selective staining of chlamydospores.

This strain showed an interesting, hitherto unrecognized mode of arrangement of chlamydospores, i.e. contiguously arranged chlamydospores attached terminally or laterally to pseudomycelial cells and which stained with cotton blue (Fig. 1a). An ellipsoid cell, probably pseudomycelial, was also slightly stained (Fig. 1b, arrow). This might represent an early stage of chlamydospore formation.

The development of chlamydospores was studied by time-lapse photography (Fig. 2). After incubation for 18 h, wall thickening was already apparent in some cells at the extremities, probably representing an early stage of chlamydospore formation (Fig. 2a). Thereafter, one of the suspensor cells was seen to be transformed into a thick-walled cell (Fig. 2b,c, arrows). The ultimate identification of chlamydospores was made by cotton blue staining as shown in Fig. 2d).

Ultrathin sections revealed the wall of each contiguous cell to be three-layered (Fig. 3). The innermost electron-dense layer represents the thick wall characteristic of the chlamydospores. The middle layer is electron-translucent and the outer layer is electron-opaque and continuous throughout the whole dumb-bell-shaped structure. It seems that the cell on the right in Fig. 3 is the proximal chlamydospore and that on the left the apical one, judging from the orientation of the asymmetrical junction structure (cf. Fig. 1). If we assume older chlamydospores to have thicker walls than younger ones, the putative apical spore on the left should have been transformed earlier than the one on the right. This is consistent with the order of chlamydospore formation revealed by the time-lapse photography shown in Fig. 2.

To our knowledge, the present paper is the first to report the occurrence of contiguously arranged chlamydospores in *C. albicans*. Jansons & Nickerson (1970a) considered that the pair of a chlamydospore and a suspensor cell represents a unit, and that much of the content of the suspensor cell is transferred to the chlamydospore. In contrast, we were unable to obtain firm evidence that suspensor cells or pseudomycelia lose their contents, but rather showed that they could be transformed into chlamydospores on SCLT medium. However, not all suspensor cells

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Fig. 3. Transverse section of two contiguously arranged mature chlamydospores of *C. albicans*, including thick cell wall (CW), mitochondria (M), nucleus (N) and lipid inclusion (L). The bar marker represents 1 μm.
or pseudomycelia were transformed into chlamydospores: as shown in Figs 1 and 2, unstained (i.e. non transformed) suspensor cells also existed, while others had already been transformed into chlamydospores.

These morphological features seem not to be limited to the strain used here nor to the use of SCLT medium. Indeed, about one-third of 60 clinical isolates of C. albicans showed such an arrangement. Furthermore, we found that corn meal agar containing N-acetyl-D-glucosamine, with which Strippoli & Simonetti (1975) reported the induction of chlamydospore formation, was also capable of causing chain formation of chlamydospores in this organism (unpublished results). These results indicate that formation of chains of chlamydospores in C. albicans is a rather common phenomenon. It is unclear why this mode of arrangement has apparently eluded documentation.

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REFERENCES