SHORT COMMUNICATION

Scanning and Transmission Electron Microscopy of *Candida albicans* Chlamydospores

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(Received 22 December 1980)

A simple, convenient method of growing large quantities of *Candida albicans* chlamydospores on a cellulose dialysis membrane has been developed. Long, narrow, cylindrical suspensor cells bearing spherical to ovoid chlamydospores were observed. Ultrastructural observations showed the chlamydospore to have a bilayered cell wall made up of an outer electron-transparent primary layer and an inner electron-dense secondary layer, a large portion of the total cell volume occupied by a single large vacuole and several smaller vacuoles, and cytoplasmic organelles typical of those observed in the yeast-like cell. There are structural similarities between the region of chlamydospore–suspensor cell connection and septa observed in budding yeast-like cells.

INTRODUCTION

Candida albicans typically produces both yeast-like cells and pseudomycelium when grown on laboratory media or in the tissues of man. When the organism is cultured on special media, e.g. corn meal agar, characteristic large chlamydospores are produced. The production of chlamydospores has been of considerable morphological interest for several years (Nickerson & Mankowski, 1953; Liu & Newton, 1955; Miwatani *et al.*, 1956; Tagaki & Nagata, 1962; Bakerspigel, 1964; Jansons & Nickerson, 1970).

The purpose of the work described here was to grow chlamydospores on a cellulose dialysis membrane, so as to examine their morphology *in situ* by scanning electron microscopy, and to compare their ultrastructure with that of the yeast-like cell. A previously reported method of growing chlamydospores on a cellulose dialysis membrane (Jansons & Nickerson, 1970) was found to be time-consuming and laborious as it required special drying of culture plates, adjustment of inoculum concentration, and a 'backwashing' procedure. In the present paper, a modification of this method is described that has been used routinely to produce comparable numbers of chlamydospores.

METHODS

Scanning electron microscopy. Yeast-like cells grown overnight at 37 °C on Trypticase Soy Agar (Difco) were used to inoculate the surface of a pre-sterilized single layer of cellulose dialysis membrane (Carolina Biological Materials, Burlington, N.C., U.S.A.) laid on the surface of freshly prepared corn meal agar (Difco). All plates were incubated lid side up at 27 °C. Every 24 h the lids were replaced with dry sterile lids. At the end of a 72 h incubation period, small samples of membrane were cut from the leading edge of growth and stuck to a glass cover slip with a small drop of adhesive (Tissue Tac Slide Adhesive, Dade Reagents, Miami, Fla, U.S.A.). The cover slip was similarly stuck to a glass microscope slide which was placed in a Petri dish. The cells were fixed by exposure for 1 h at room temperature to the vapour of 1% (w/v) osmium tetroxide or by immersion in a 1% (w/v)

unbuffered solution of potassium permanganate. The samples were washed with distilled water, dehydrated with increasing concentrations of ethyl alcohol, air dried, attached to aluminium stubs with conductive tape, sputter coated with approximately 10 nm of gold/palladium (60/40) using an ISI sputter coater (International Scientific Instruments, Santa Clara, Calif., U.S.A.), and examined in an ISI Mini scanning electron microscope.

Transmission electron microscopy. Chlamydospores used for transmission electron microscopy were grown on a cellulose dialysis membrane as described above. Membranes were lifted from the surface of the growth medium, and the cells were collected by washing the membranes clean with distilled water. After several washings in distilled water, the cells were fixed in 1% (w/v) unbuffered potassium permanganate at ice-bath temperature, washed with distilled water, blocked in warm (50 °C) agar, dehydrated with increasing concentrations of ethyl alcohol, and embedded in ERL 4206 resin (Spurr, 1969). Sections were cut with glass knives, post-stained with lead citrate (Reynolds, 1963) and examined in an RCA EMU-3G electron microscope.

RESULTS AND DISCUSSION

Scanning electron microscope studies of *Candida albicans* have been few in number (Whittaker & Drucker, 1970; Barnes *et al.*, 1971; Joshi *et al.*, 1973). Filamentous forms with accompanying yeast-like cells (blastospores) and chlamydospores were reported by Barnes *et al.* (1971); the chlamydospores were wrinkled in some cases, and none were shown with attached suspensor cells. Rough and convoluted cells were common in the studies of Barnes *et al.* (1971) and Joshi *et al.* (1973). Whittaker & Drucker (1970) removed colonies from growth medium and immediately plunged them into isopentane in liquid nitrogen to minimize shrinkage and morphological changes. Previously observed artefacts (Whittaker & Drucker, 1970; Barnes *et al.*, 1971; Joshi *et al.*, 1973) were not observed in the present study.

The effects of pH, temperature of incubation, carbon source, and other factors involved in the pathway of morphogenesis leading to the production of chlamydospores were studied by Hayes (1966), but the effect of water content in the growth medium was not discussed. It has

Scanning electron micrographs of *C. albicans* chlamydospores. Abbreviations: C, chlamydospore; P, pseudomycelium; SC, suspensor cell; Y, yeast-like cell. The bar markers represent 5 μ m in Fig. 1 and 1 μ m in Fig. 2.

Fig. 1. Chlamydospores grown on a single layer of cellulose dialysis membrane; pseudomycelium and yeast-like cells are also visible. Osmium fixation.

Fig. 2. Chlamydospore attached to a long, narrow, cylindrical suspensor cell. Permanganate fixation.

Transmission electron micrographs of *C. albicans* chlamydospores. Abbreviations: C, chlamydospore; CW, cell wall; N, nucleus; SC, suspensor cell; V, vacuole. The bar markers represent 1 μ m.

Fig. 3. Longitudinal section through a suspensor cell and chlamydospore, showing a vacuole and the absence of an electron-dense secondary cell wall layer.

Fig. 4. Longitudinal section of a chlamydospore showing a vacuole, the outer electron-transparent and inner electron-dense cell wall layers, and a break in the continuity of the chlamydospore cell wall with the wall of the suspensor cell (arrows).

Fig. 5. Section of a chlamydospore showing a vacuole and an abundance of mitochondria and endoplasmic reticulum adjacent to the electron-dense secondary cell wall layer.

Fig. 6. Section of a chlamydospore showing a vacuole, a nucleus, and a thicker than usual electron-dense secondary cell wall layer.

been noted, however, that condensation on cultures inhibits the production of chlamydospores (Jansons & Nickerson, 1970). Incubation of culture plates in the present study with lids up allowed water vapour escaping from the growth medium to condense on the bottom side of the lid. The average water loss from corn meal agar was approximately 5.0% in the first 24 h of incubation, 7.2% in the second 24 h, and 7.8% in the final 24 h. By replacing the wet lids with dry sterile lids at regular intervals, the water content of the growth medium was systematically lowered while still maintaining a constant relative humidity above the medium. The exact effect that removal of water from the growth medium in this fashion may have upon chlamydospore production is not known.

The leading edge of growth on a single layer of cellulose dialysis membrane is shown in Fig. 1. Scattered yeast-like cells, pseudomycelium and chlamydospores can be seen. The chlamydospores are spherical to ovoid, and considerably larger than the yeast-like cells. A chlamydospore attached to a long, narrow, cylindrical suspensor cell is shown in Fig. 2.

Knowledge of chlamydospore ultrastructure is sketchy, reportedly due to fixation difficulties (Tagaki & Nagata, 1962; Bakerspigel, 1964; Jansons & Nickerson, 1970). In the present study, potassium permanganate fixation revealed cytoplasmic organelles comparable to those previously observed in the yeast-like cell (Shannon & Rothman, 1971). The chlamydospore cell wall is about twice as thick as the yeast-like cell wall observed by Shannon & Rothman (1971), and is made up of an outer electron-transparent primary layer and an inner electron-dense secondary layer (Figs 4–6). Mitochondria and endoplasmic reticulum are shown concentrated next to the primary layer in Fig. 3 and next to the secondary layer in Figs 4 and 5. The primary and secondary layers are about equally thick. Occasionally, however, a much thicker secondary layer was observed (compare Fig. 6 with Figs 4 and 5). Continuity of the chlamydospore cell wall with the wall of the suspensor cell appears to be broken in the region of the chlamydospore–suspensor cell connection (Fig. 4, arrows). This region has structural similarities to septa previously observed in budding yeast-like cells (Shannon & Rothman, 1971). Regardless of the presence or absence of the secondary cell wall layer, chlamydospores are characterized by the presence of a large vacuole, occupying a large portion of the total cell volume, and several smaller surrounding vacuoles (Figs 3–6). The plasma membrane is well defined in Fig. 3, but appears to be partially or totally destroyed in Figs 4–6, presumably due to the harsh effect of permanganate fixation. In Fig. 6 the nucleus of a chlamydospore is shown cramped into a limited cytoplasmic space between the large vacuole and the secondary cell wall layer.

The author would like to thank Dr Richard McCullough of the Department of Natural Sciences, Saddleback College, Mission Viejo, California, for the use of the department's scanning electron microscope and sputter coater, and Dr Frank E. Swatek, Chairman, Department of Microbiology, California State University, Long Beach, California, for the strain of *Candida albicans* used in this study.

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