NOTES

Scanning Electron Microscopic Studies of Candida albicans

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A scanning electron microscopic study of selected morphological stages of Candida albicans is presented. Stages represented are budding yeast cells, mycelial-like forms, chlamydospores, germ tube formation, and an unusual rough cell type.

Candida albicans is a dimorphic organism, capable of growing as a budding yeast or as a pseudomycelial form. In addition, diagnostic chlamydospores may be produced. C. albicans will also produce structures known as germ tubes within 3 hr when incubated at 37 C in the presence of certain human or animal sera (4). Germ tube formation was considered an additional diagnostic procedure for the rapid identification of Candida sp. (3). There were numerous reports concerned with ordinary light and transmission electron microscopy of these characteristic growth stages, but these reports were primarily concerned with detailed studies of particular inner cell organelles (1, 2, 5). A survey of the literature reveals only one scanning electron microscopic (SEM) study concerned with micrographs of C. albicans (6). The authors of that particular report presented micrographs of colonial growth of the organism, which suggested that growth occurred mainly at the base of the colony rather than throughout the colony. In addition, the authors noted that, due to budding, the size of the organisms was variable and that the surfaces of the colonies were covered with a surface film. It is the purpose of this study to present SEM observations of selected morphological stages of the organism.

Cultures of a known strain of C. albicans were grown in Sabouraud-dextrose broth (Difco) at 37 C. Chlamydospore production was carried out by inoculation of corn meal agar (Difco) at 25 to 27 C for 3 days. Germ tube formation was induced by inoculating yeast stage organisms into 0.5 ml of human serum for 3 hr at 36 C. The organisms were then fixed in 0.1% glutaraldehyde in Krebs buffer for 1 hr at 25 to 27 C. After fixation, the samples were washed and centrifuged four times in deionized water, air-dried, coated with a gold palladium alloy (60:40), rotated in a vacuum of 10^-4 Torr, and observed at an angle of 45° (unless otherwise stated). The preparations were examined with a Cambridge Stereoscan electron microscope (model Mark II) at 20 kv.

Figure 1 illustrates an unusual cell type that was observed with SEM in 48-hr cultures. To the knowledge of the authors, this cell type was not previously described. This might be accounted for by the limitations of either ordinary light or transmission EM. A “charge” line can be seen in the upper left portion of the micrograph. In contrast to this “rough” or convoluted surface, typical yeast cells show a uniformly smooth surface. Increased magnification (Fig. 2) reveals the convoluted nature of the surface of the rough cell types. At the present time, the significance of this particular rough cell type cannot be determined. Typical yeast cells appear to be budding from the larger, rough cell (Fig. 1). Since only a few of the total number of cells observed showed this peculiar surface morphology and all of the rough cells presented similar convoluted surface patterns, it was presumed to be a real surface image and not one due to the attachment of extraneous material.

Terminal chlamydospore formation is shown in Fig. 3. With the exception of a shrunken lateral surface, presumably a fixation-processing artifact, the chlamydospore surface appears smooth and homogeneous.

The filamentous stage reveals typical branching mycelial-like elements (Fig. 4). The surface appears relatively smooth. Blastospores are also seen in the micrograph. The “cracked” blasto-
FIG. 1. Appearance of budding yeast cells of C. albicans and a large "rough" cell which exhibits convolutions on its surface. The smooth surface of the budding cells is evident.

FIG. 2. Increased magnification of two adjoining cells of the "rough" cell type showing the surface convolutions. All of the cells observed of this type revealed a uniformly convoluted surface.
**FIG. 3.** Terminal chlamydospore formation of *C. albicans* after 3 days of growth on corn meal agar. The wrinkled surface is considered a fixation-processing artifact. The filamentous processes appear flattened. The surface of all chlamydospores observed appeared smooth.

**FIG. 4.** Filamentous stage of *C. albicans* after 3 days of growth on corn meal agar, showing mycelial-like processes and accompanying blastospore formation.
FIG. 5. Morphology of a chlamydospore, blastospores, and mycelial-like processes of C. albicans when the specimen is tilted to an angle of 60°.

FIG. 6. Germ tube formation after 3 hr of exposure to human serum. Multiple bud scars are also seen in the micrograph. Various degrees of bud scar and scar plug prominence are shown.
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spores, seen in the upper left and lower right portions, are considered processing artifacts.

Figure 5 is a micrograph taken at an angle of 60° showing a chlamydospore, blastospores, and mycelial-like processes. The three-dimensional aspects of these stages were enhanced by tilting the sample to 60°.

Germ tube formation is seen in Fig. 6. The bud scar and scar plug are evident on the surface of the cell undergoing germ tube development and on other cells in the micrograph. Both the bud scars and scar plugs appear more prominent in SEM surface observations than when observed in transmission EM studies of the organism (5).

LITERATURE CITED