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Intercellular Matrix in Colonies of *Candida*

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The histochemistry and fine structure of typical colonies of six species of *Candida* were studied, using a total of 31 clinical isolates. The colonies consisted of viable and degenerate cells which lay in an intercellular matrix. This matrix was made up of amorphous, granular, and fibrillar components, the relative proportions and total amount of which varied from species to species. The cells of all species were surrounded by a zone of homogeneous amorphous material, which may be a highly cross-linked carbohydrate. This separated intact cells from irregularly distributed granular debris derived from the cytoplasm of degenerate cells. Focal cellular degeneration and associated granular debris were present within the colonies of all species and were most common in the surface layers of cells of colonies of *C. albicans* and *C. tropicalis*. The large amounts of intercellular matrix in this region formed a surface coat on colonies of these two species. Intercellular strands of cell wall material, and to a lesser extent other membranous elements from degenerate cells, formed a prominent fibrillar meshwork in the colonies of *C. albicans* and *C. tropicalis*, but were less common in those of *C. pseudotropicalis* and *C. guilliermondii* and seldom seen in those of *C. parapsilosis* and *C. krusei*.

Although both the microorganisms and the intercellular matrix contribute to the overall form and appearance of microbial colonies, the intercellular component has not been extensively studied. A recent scanning electron microscope study has demonstrated that the intercellular matrices of colonies of six species of *Candida* contain several components which appear to be important in maintaining colonial integrity (2). The present paper describes a histochemical and fine structural investigation of intact colonies of *Candida* undertaken to define this intercellular material.

MATERIALS AND METHODS

Thirty-one clinical isolates of *Candida* from the Microbiology Laboratory, Auckland Public Hospital, identified (1) as *C. albicans* (ten), *C. krusei* (five), *C. tropicalis* (five), *C. parapsilosis* (five), *C. pseudotropicalis* (three), and *C. guilliermondii* (three), were subcultured on fresh Sabouraud dextrose agar for 48 h at 22 C.

Typical colonies from each preparation were coated with a thin layer of molten agar (45 C) and excised with some of the underlying agar. Frozen sections 5 μm thick were prepared from some of these agar-embedded colonies and stained with 0.5% alcoholic ninhydrin for 30 s for light microscopy. Other colonies were placed, either directly or after rapid quenching in liquid nitrogen and subsequent thawing (K. R. Jashi, E. E. Wheeler, and J. B. Gavin, Mycopathol. Mycol. Appl., in press), in 0.15 M phosphate-buffered (pH 7.2) 3% glutaraldehyde for 18 to 24 h and then trimmed so that only a thin layer of agar surrounded each colony. The colonies were next postfixed for 3 h in phosphate-buffered 1% osmium tetroxide, dehydrated in graded ethanol, and embedded in either conventional or low viscosity epoxy resin (6).

An ultramicrotome was used to cut 2- to 3-μm-thick sections through whole colonies. These sections were transferred to glass slides where the resin was removed and the osmium bleached (5) before staining with 0.1% aqueous toluidine blue, periodic acid-Schiff (PAS) (4), alcian blue (7), acrolein-Schiff (8) or methyl green-pyronin Y (3). Thin sections (50 to 100 nm) through selected regions of the resin-embedded colonies were then cut and stained with uranyl acetate and lead citrate and examined in a Philips EM 300 electron microscope.

RESULTS

The majority of the agar-embedded colonies remained intact during processing, and thin sections for electron microscopy could be prepared from regions of interest identified by light microscopy in thicker sections. Although the internal structure of many of the cells fixed directly in glutaraldehyde was indistinct (Fig. 1), membranous and granular organelles and large lipid droplets were clearly evident in the majority of cells from colonies freeze-thawed before fixation (Fig. 2). All colonies consisted of a mixture of apparently viable and degenerate cells lying in an intercellular matrix which

consisted of granular, amorphous, and fibrillar components.

The apparently viable cells were of various sizes (Fig. 1), often showed evidence of budding (Fig. 2) and, when fixation was adequate, contained well-defined cytoplasmic organelles (Fig. 2). On the other hand, degenerate cells tended to be larger and more irregular in shape and had cell walls and cell contents which stained less deeply with PAS and ninhydrin (Fig. 3). Such degenerate cells contained heterogeneous granular material which included poorly defined membranous structures and small lipid droplets. This cytoplasmic debris was continuous through breaches in the cell wall with the granular phase of the intercellular matrix (Fig. 4), and like it, stained lightly with toluidine blue, PAS, acrolein-Schiff and pyronin, and strongly with ninhydrin (Fig. 3).

The majority of cells were separated from this granular material by a clear zone of amorphous material (see Fig. 1, 3, 8) which remained unstained in all preparations.

Fibrillar structures within the intercellular matrix were of two types. In the colonies of some species some cells were interconnected in the vicinity of bud and birth scars (Fig. 5) by strands of homogeneous material which, like the cell walls, stained only with PAS and alcian blue (Fig. 6). These fibrillar structures formed a well-developed meshwork throughout the colonies of *C. albicans* and *C. tropicalis*, were less common in those of *C. pseudotropicalis* and *C. guilliermondii* and were seldom observed in those of *C. parapsilosis* and *C. krusei*. Other sparsely distributed elements observed in all species were associated with the granular phase and had a trilaminar structure similar to that of cytoplasmic membranes (Fig. 7).

The relative proportions of viable and degenerate cells varied with the species. In the basal parts of most colonies of all species focal collections of degenerate cells were observed. In addition, the superficial layers of organisms in colonies of *C. albicans* and *C. tropicalis* were predominantly degenerate and associated with large amounts of granular material. In the colonies of these two species the comparatively large total amount of intercellular matrix formed a thick surface coat over the superficial organisms (Fig. 8).

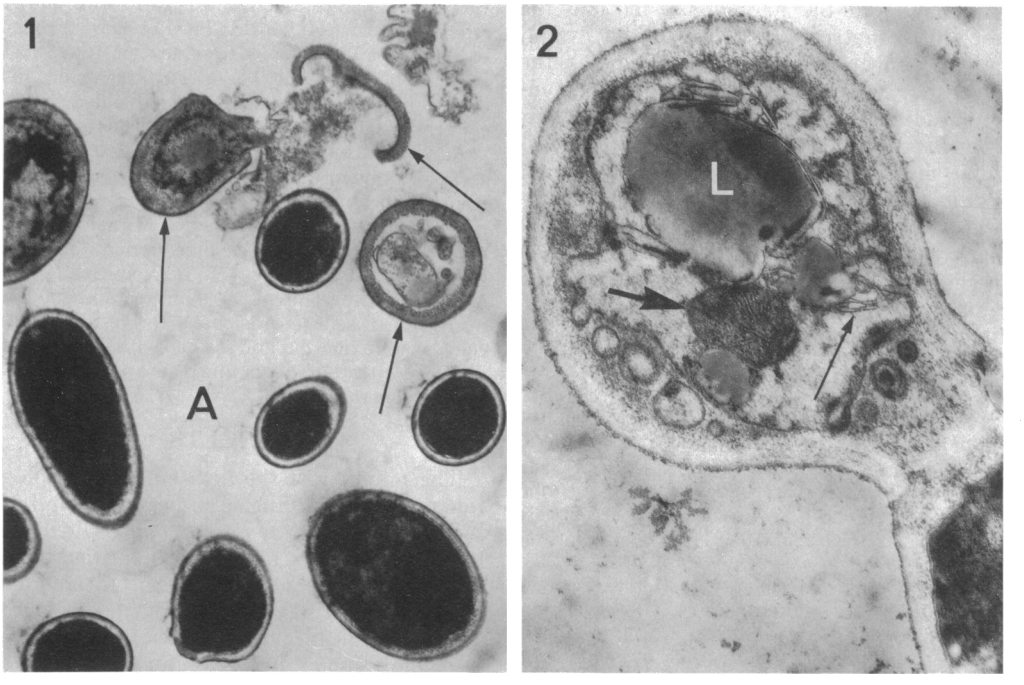
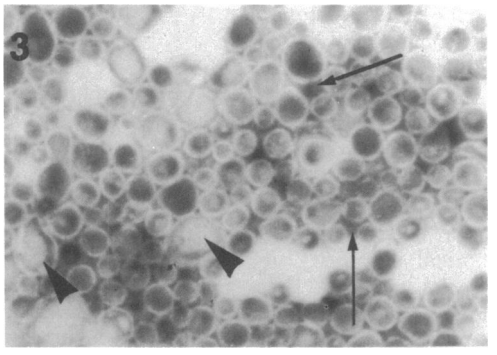


FIG. 1. *Viable and degenerate (arrows) yeast cells separated by amorphous matrix (A) in a colony of C. krusei fixed in glutaraldehyde.* ×*4,500.*

FIG. 2. *A daughter cell containing a large lipid droplet (L), endoplasmic reticulum (small arrow), and a mitochondrion (large arrow) in a freeze-thawed, glutaraldehyde-fixed colony of C. tropicalis.* ×*12,000.*



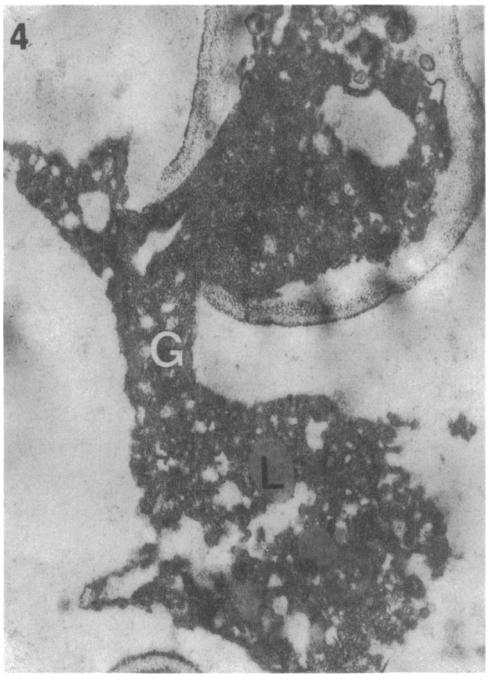


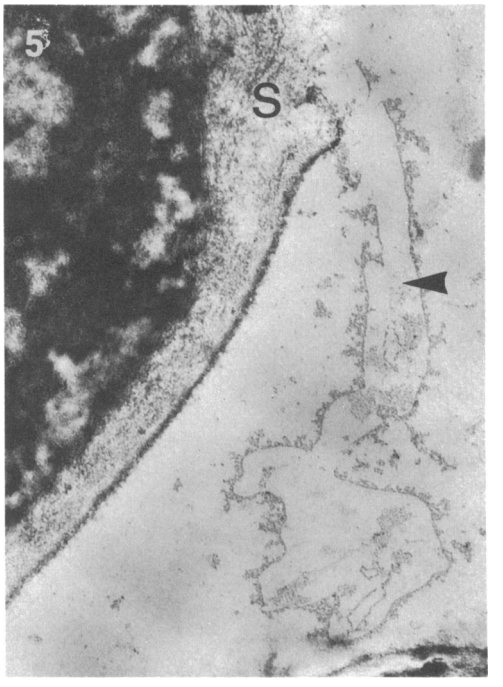
FIG. 3. *Degenerate yeast cells (large arrows) and intercellular matrix (small arrows) are densely stained by ninhydrin in a frozen section through a freeze-dried colony of C. albicans. Amorphous material forms a halo around each cell.* ×*1,000.*

FIG. 4. *Heterogeneous granular material (G) containing small lipid droplets (L) extends from within a degenerate cell (above) into the intercellular matrix (below) of a colony of C. pseudotropicalis.* ×*11,000.*

DISCUSSION

This study has demonstrated that the intercellular matrices of colonies of *Candida* consist of granular, fibrillar, and amorphous components, the relative proportions of which vary in the species studied. The amorphous material, which forms a clear zone surrounding each intact cell, is invariably present in all species. Its pericellular distribution suggests that this material is a cellular secretion and its lack of histochemical reaction for protein or glycosaminoglycans, together with its negative reaction to PAS, suggest that it may be a highly cross-linked carbohydrate.

Differences in the total amount of intercellular matrix, which determine the presence or absence of a surface coat (2), are due almost entirely to variations in the proportion of the granular component in the intercellular matrix. The present investigation has established that this material is cytoplasmic debris and its



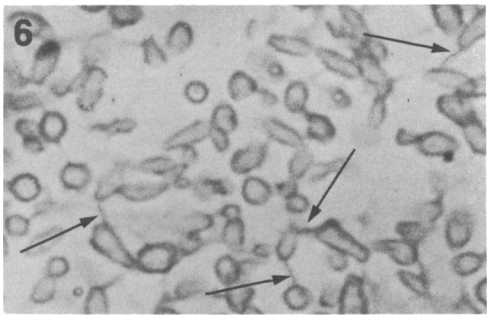


FIG. 5. *An intercellular band of homogeneous material (arrow) extending from a bud scar (S) on a yeast cell in a colony of C. albicans.* ×*17,500.*

FIG. 6. *Alcian blue-stained fibrils (arrows) unite adjacent cells in a colony of C. albicans.* ×*1,000.*

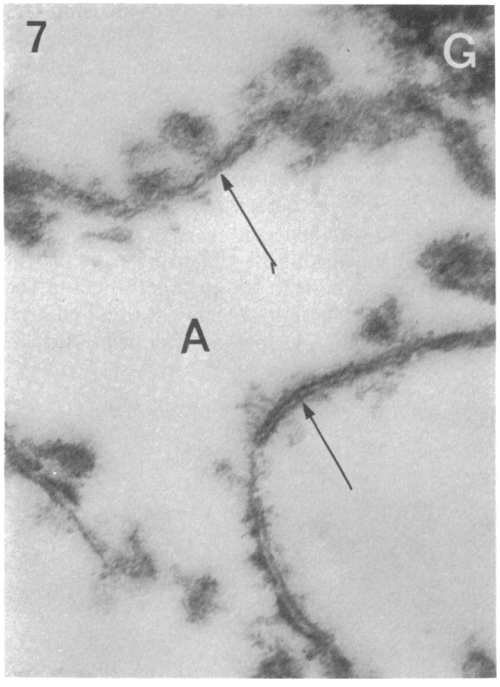


FIG. 7. *Trilaminar strands (arrows) lying in amorphous matrix (A) close to granular material (G) in a colony of C. albicans.* ×*36,000.*

distribution is correlated with the incidence of cell degeneration.

The fibrillar meshwork, previously recognized in scanning electron micrographs of colonies of *Candida* prepared in aqueous solvents (2), has been shown to consist partly of membranous elements from degenerate cells and predominantly of intercellular strands attached to scars on the surfaces of adjacent cells. The histochemical similarity of the latter to cell wall material suggests that they are intercellular connections which persist after cell division in some species. Further study of other species of *Candida* are necessary to determine the biological and taxonomic significance of variations in the three components of the intercellular matrix in this genus.

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LITERATURE CITED

1. Dolan, C. T. 1971. A practical approach to identification of yeast-like organisms. Am. J. Clin. Pathol. **55**:580–590.
2. Joshi, K. R., E. E. Wheeler, and J. B. Gavin. 1973. Scanning electron microscopy of colonies of six species of

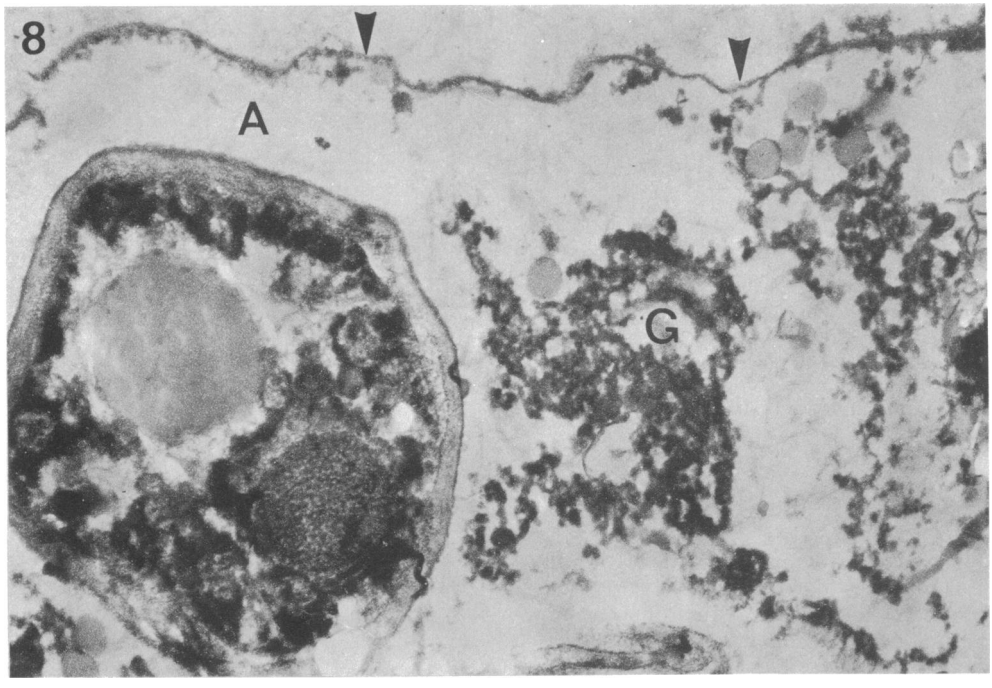

FIG. 8. *Amorphous (A) and granular (G) material in a colony of C. albicans forming a surface coat (arrows) over superficial organisms.* ×*15,000.*

Candida. J. Bacteriol. **115**:341-348.
3. Kurnick, N. B. 1955. Pyronin Y in the methyl green-pyronin histological stain. Stain Technol. **30**:213-230.
4. Pearse, A. G. E. 1968. Histochemistry theoretical and applied, vol. 1, 3rd ed., p. 659. J & A Churchill Ltd, London.
5. Snodgrass, A. B., C. H. Dorsey, W. H. Bailey, and L. G. Dickson. 1972. Conventional histopathologic staining methods compatible with Epon-embedded osmicated tissue. Lab. Invest. **26**:329-335.
6. Spurr, A. R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**:31-43.
7. Steedman, H. F. 1950. Alcian blue 8GS: A new stain for mucin. Q. J. Microsc. Sci. **91**:477-479.
8. Van Duijan, P. 1961. Acrolein-Schiff, a new staining method for proteins. J. Histochem. Cytochem. **9**:234-241.