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Electron Microscopy of Some Special Cell Contacts in Yeasts

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Anastomosis in *Endomycopsis javanensis* and some other filamentous yeasts was brought about by contact of a denticle from one cell with the wall of another cell, resulting in the disappearance of the outer layer and the thickening of the inner layer of the cell wall of the contacted cell. Another form of contact between cells was the penetration of one cell by a denticle on another cell which had grown out to a stalk; this occurred between cells of *E. javanensis* and between cells of this species and other yeast species.

Several species of filamentous, ascomycetous yeasts have anastomosing hyphae. Electron microscopy of these yeasts showed structures of contacting cells. Apart from these, penetration of one cell by another was observed. The latter phenomenon also occurred in mixtures of cells of different species. The observations on *Endomycopsis javanensis* are described in detail.

MATERIALS AND METHODS

Organisms. The following yeast strains were used: *E. javanensis* (Klöcker) Dekker, NRRL-YB-1542 and 2401; *Endomycopsis* (*Guilliermondella*) *selenospora* (Nadson et Krassilnikov) Dekker, CBS 2562; *Saccharomycopsis synnaedendra* Scott et van der Walt, CBS 6161; *Pichia microspora* Batra, no. 1653; and *Pichia crossotarsi* Batra, no. 1658. The yeast strains used in mixtures with *E. javanensis* are listed in Table 1.

Culture conditions. The yeasts were grown in malt extract diluted with water (1:3) in small petri dishes and on corn meal agar, both at room temperature. The cultures in malt extract of cells of different species were mixed after 2 days, and preparations of the mixture were made after 3 days.

Electron microscopy. The cells were fixed with 1.5% $KMnO_4$ for 15 min, dehydrated through an ethanol series, and, during this procedure, post-stained with uranyl acetate. The material was embedded in Epon 812 and cut with a diamond knife. Part of the sections were stained with lead citrate (7). The sections were examined in Philips EM100 and EM300 microscopes.

RESULTS

In *E. javanensis* single cells multiply by the formation of buds on a broad base; i.e., there is a slight constriction between mother cell and bud in which the septum is laid down. These cells may remain attached as pseudomycelium. Besides, hyphae without constriction at the septa occur. Pseudomycelium and true mycelium split up easily at the septa. Single cells are lemon-shaped, long-oval, or elongate. Hyphae and single cells may have denticles or minute short stalks, occasionally with a head. Contact is made via the denticles.

Electron micrographs always showed the presence of a single dark line across the center of the septum connecting the protoplasts of adjacent cells (Fig. 1). This may be a plasmodesma or a closure line of the septum.

As observed in ultrathin sections, the wall of the denticles arose from under the wall of the cell, and the outer layer of the denticle wall was darker than that of other parts of the cell (Fig. 2, 3). In the tip of the denticle a strand of endoplasmic reticulum was usually present. Where a denticle touched the wall of another cell, the dark layer of the latter had disappeared, and the inner layer was lighter and much thicker than in other parts of the wall (Fig. 4, 5). In the light part dark bodies were often found; occasionally membranes could be distinguished around them. Some cells had one or more light spots in the wall, presumably after contact with denticles from other cells (Fig. 6). Several denticles might be present on a single cell, and a cell forming denticles might at the same time have light spots caused by the denticles of other cells (Fig. 7). Most cells contacted by denticles seemed to be in good health at the time of fixation.

Apart from this superficial contact between

cells, we observed penetration of one cell by a denticle from another cell which had grown out to a stalk. In this case, the protoplast of the penetrated cell showed a disorganized condition, and the cell was probably dead at the time of fixation. The head of the stalk had a dark outer wall layer, whereas the wall of the neck of the stalk within the attacked cell was very light (Fig. 8). Endoplasmic reticulum was present in the head and, occasionally, in the narrow neck. In some instances the plasmalemma of the attacked cell was not penetrated, but it surrounded the stalk. Dark bodies were still present around it (Fig. 9).

In a later stage, the wall of the head of the stalk had thickened, the neck was filled with wall material, and only a dark remnant of endoplasmic membranes remained in the upper part of the neck and in the head (Fig. 10). The protoplast of the contacting cell had regained its original shape (Fig. 11).

Outgrowths, described above, frequently occurred in the preparation studied. A single cell might have several stalks, all penetrating different cells (Fig. 12). A cell might form a stalk and be penetrated afterwards (Fig. 13). Cells containing more than one stalk were present. Penetration of asci was observed. Cells bearing outgrown denticles not inside other cells were found (Fig. 14) in samples where penetrating stalks also occurred.

In mixtures of cells of *E. javanensis* with other yeast species, stalks of *E. javanensis* either penetrated cells of the other species (Fig. 15, 16) or no such contacts were observed. The results of these tests are listed in Table 1.

Anastomosing hyphae occurred in the species: *E. selenospora*, *S. synnaedendra*, *P. microspora* and *P. crossotarsi*. In all of these species penetrating stalks were also observed. In *E. selenospora* bridges were generally formed between parallel hyphae, and denticles grown out to stalks entwined crossing hyphae. The denticles arose from under the wall of the cell (Fig. 17). After contacting the other cell, they were broad and had a light wall (Fig. 19). At the site of contact between entwining stalks and wall, the latter had also thickened. Penetration of other cells (Fig. 18) resulted in long stalks with a light wall and a narrow head.

TABLE 1. *Strains of species tested for penetration by Endomycopsis javanensis*

Species	Strain	Penetration
Endomycopsis platypodis	CBS 5560	+
E. capsularis	Y-447	+
E. burtonii	CBS 6141	+
Saccharomyces cerevisiae	G 310	+
Kluyveromyces phaffii	CBS 4417	+
Pichia membranaefaciens	CBS 107	+
Debaryomyces hansenii	CBS 767	+
Candida albicans	G 371	+
C. tropicalis	G 370	+
Schizosaccharomyces pombe	CBS 5682	–
Nadsonia elongata	CBS 2595	–
Hansenula anomala	G 180	–
Sporobolomyces salmonicolor	G 364	–
Rhodotorula glutinis	G 535	–

DISCUSSION

Contacts between hyphae of a single strain of fungi have been described as anastomosis; they may include sexual reactions (2). Contacts of hyphae of different species also may be anastomotic (3, 4) or parasitic (1).

The present study concerns, in the first instance, anastomosis in *E. javanensis*. This species is homothallic. Conjugations preceding ascus formation with a wide, open connection between two cells have been described (8, 9), but frequently no conjugation is found. In the contacts described above, an open connection between the protoplasts of two cells was never observed, and, therefore, the possibility of a sexual reaction may well be discarded.

Contact of the denticles of a cell with the wall of another cell resulted in the disappearance of dark wall material and the thickening of the light material within the contacted cell. The loss of the dark material suggests a direct enzymatic action, whereas the thickening of the wall might be caused either by swelling or by a production of new material. In view of the large areas involved, the latter explanation seems more probable and might be a reaction of the cell to the contact.

Anastomoses are found in several species of the filamentous, ascomycetous yeasts, and this characteristic may have taxonomic value. At present, classification of these species into various genera has not been satisfactorily established and requires further study.

Penetration of cells by denticles grown out to stalks seems to be connected with anastomosis; they originate from a similar contact. The stalks always occurred within dead cells, but we have no evidence that penetration actually killed the cells. It is possible that only dead cells are penetrated, either because the wall is different from that of the living cell or by the absence of extra wall material.

The experiments with mixtures of cells of

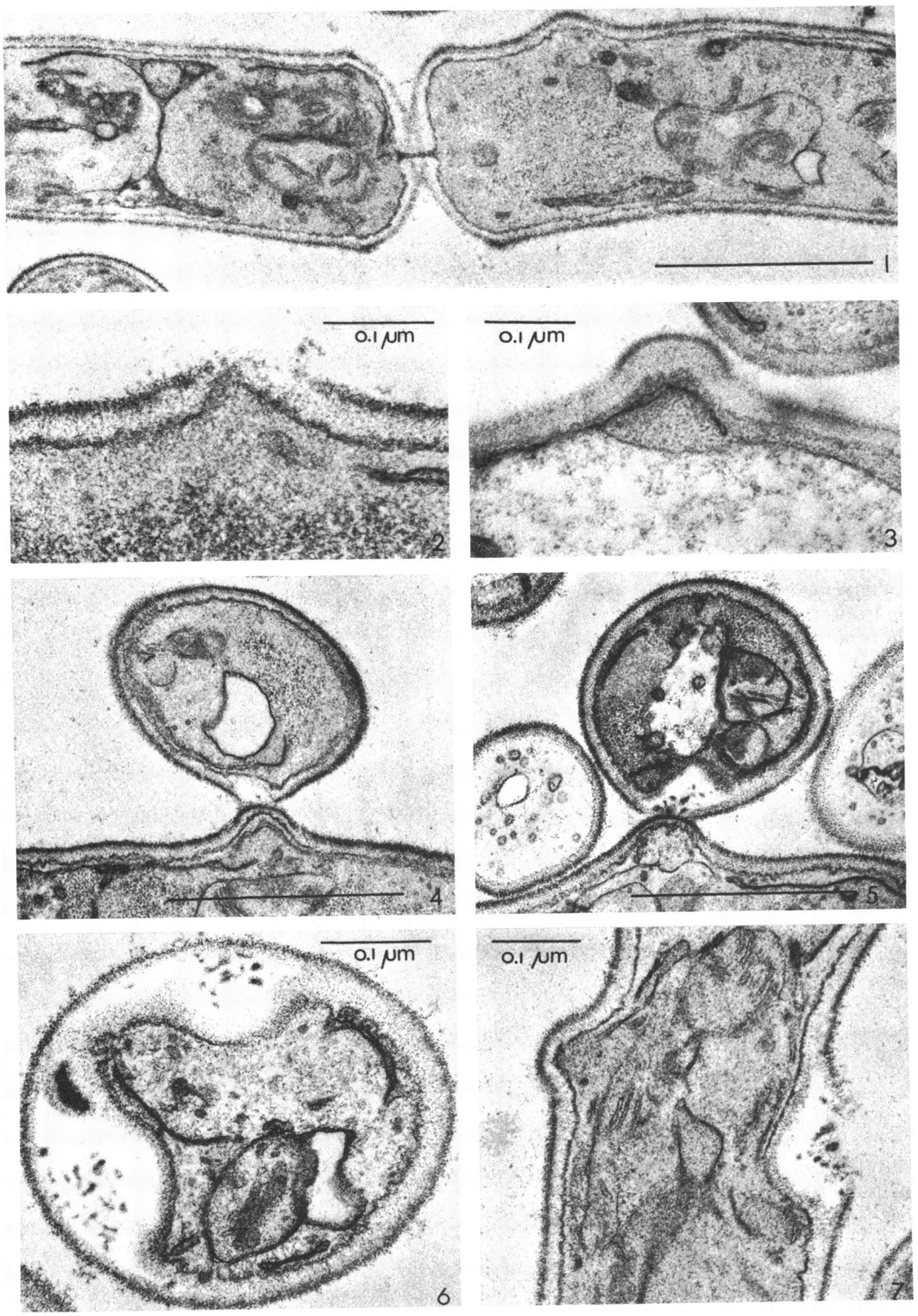
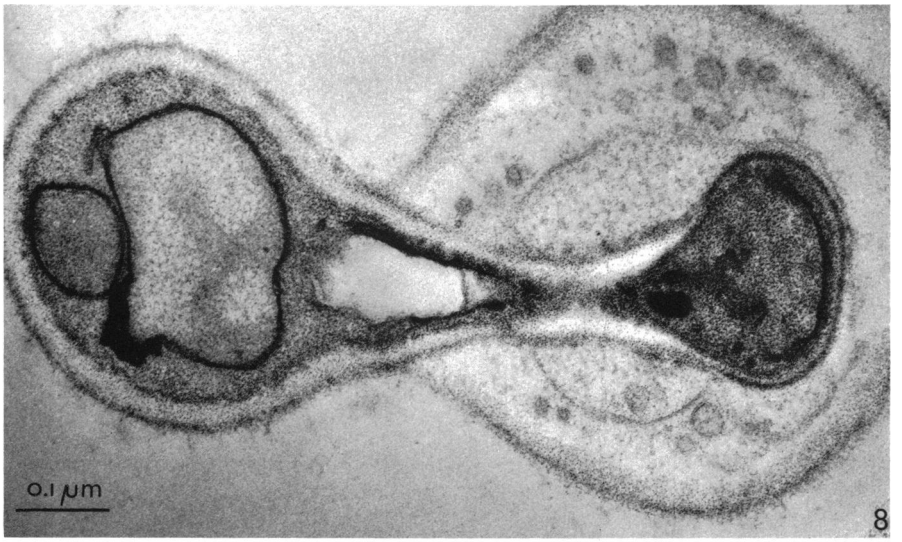
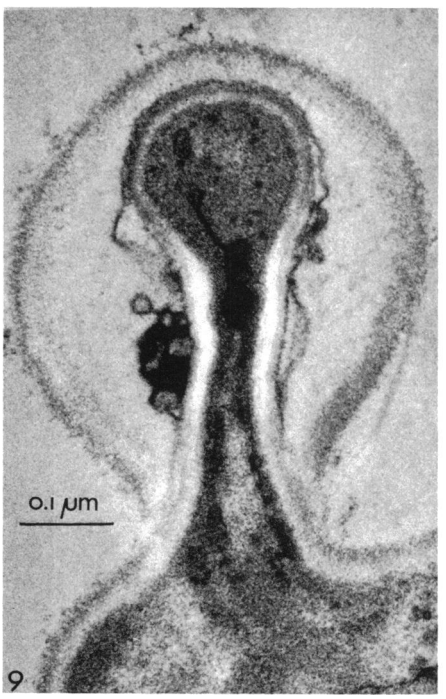


FIG. 1. *Two separating arthrospores with a connection between the protoplasts in the center of the septum. Bar = 0.5 μm.*

FIG. 2. *Beginning of the formation of a denticle.*





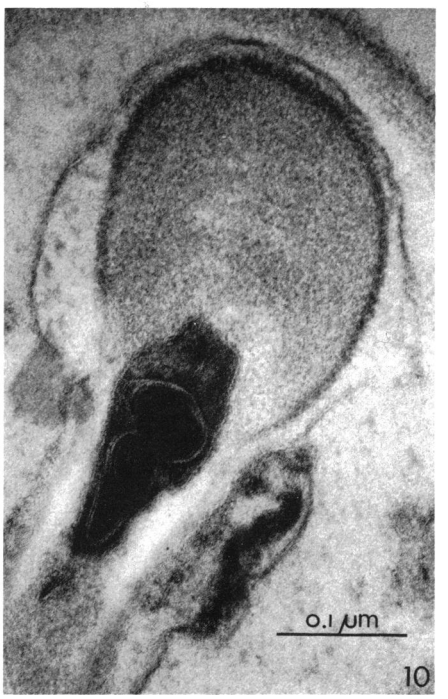


FIG. 8. *Stalk within a cell. The head has a dark outer layer of the wall. The neck has a very light wall. Endoplasmic reticulum is present in the head.*

FIG. 9. *Stalk within a cell surrounded by membranes and dark material.*

FIG. 10. *Head of a stalk filled with wall material. Remnants of the protoplast with membranes are present in the top of the neck.*

FIG. 3. *Denticle with a distinct dark outer wall layer.*

FIG. 4. *At the contact site of a denticle and the wall of another cell, the dark outer layer of the latter has disappeared. Bar = 0.5 μm.*

FIG. 5. *The light part of the wall of the contacted cell has thickened, and dark bodies are present in that part. Bar = 0.5 μm.*

FIG. 6. *Cell with two broad light parts in the wall, presumably after contact with denticles from other cells.*

FIG. 7. *Part of a cell with on one side a denticle and on the other side a light spot caused by a denticle from another cell.*

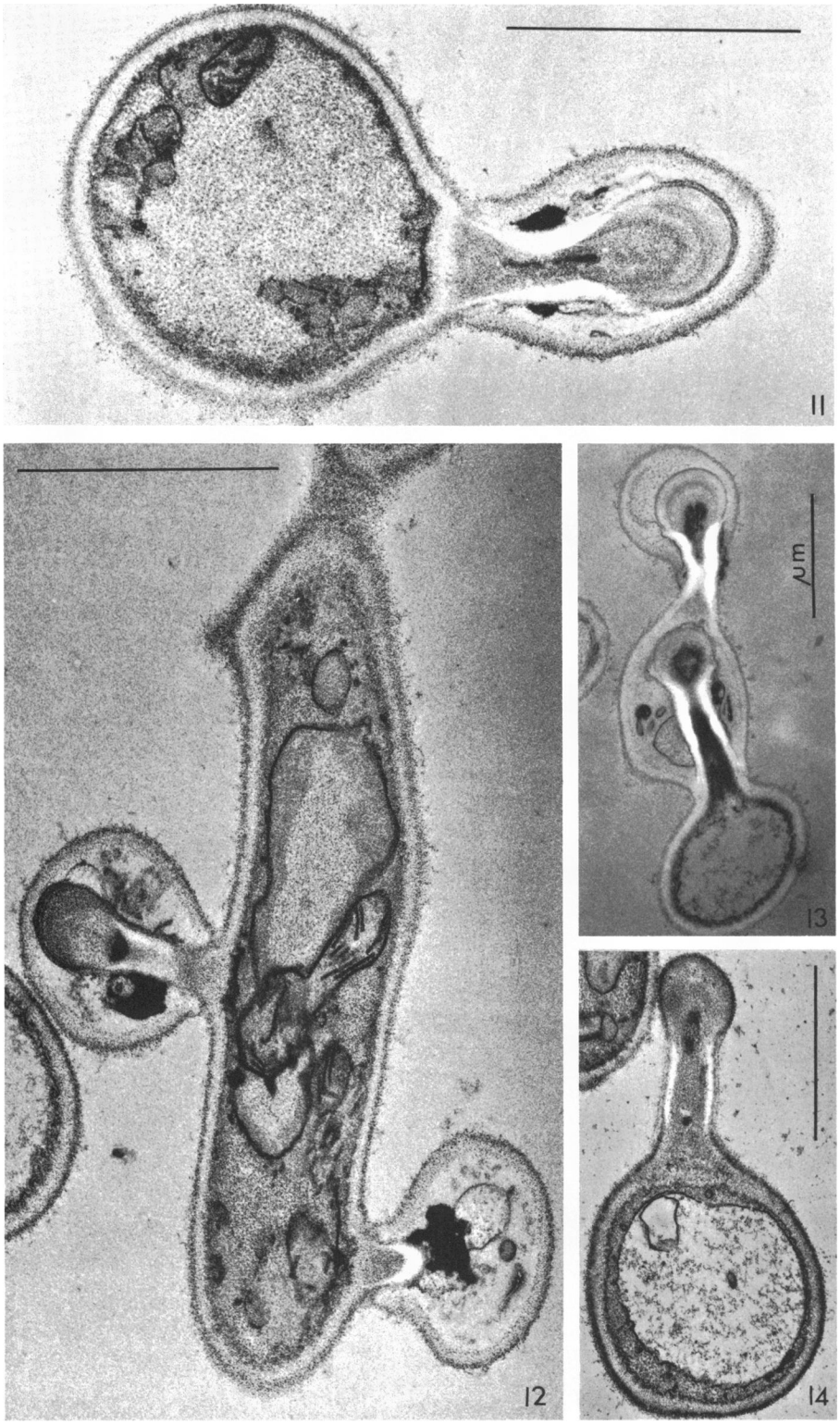


FIG. 11. *Stalk completely filled with wall material. In the head concentric layers are visible. The protoplast of the contacting cell has regained its original shape. Bar = 0.5 μm.*

FIG. 12. *Cell with two stalks within other cells. Bar = 0.5 μm.*

FIG. 13. *One cell, the middle one, has penetrated the top cell and has, in turn, been penetrated by the bottom cell.*

FIG. 14. *Cell with stalk not within another cell. Bar = 0.5 μm.*

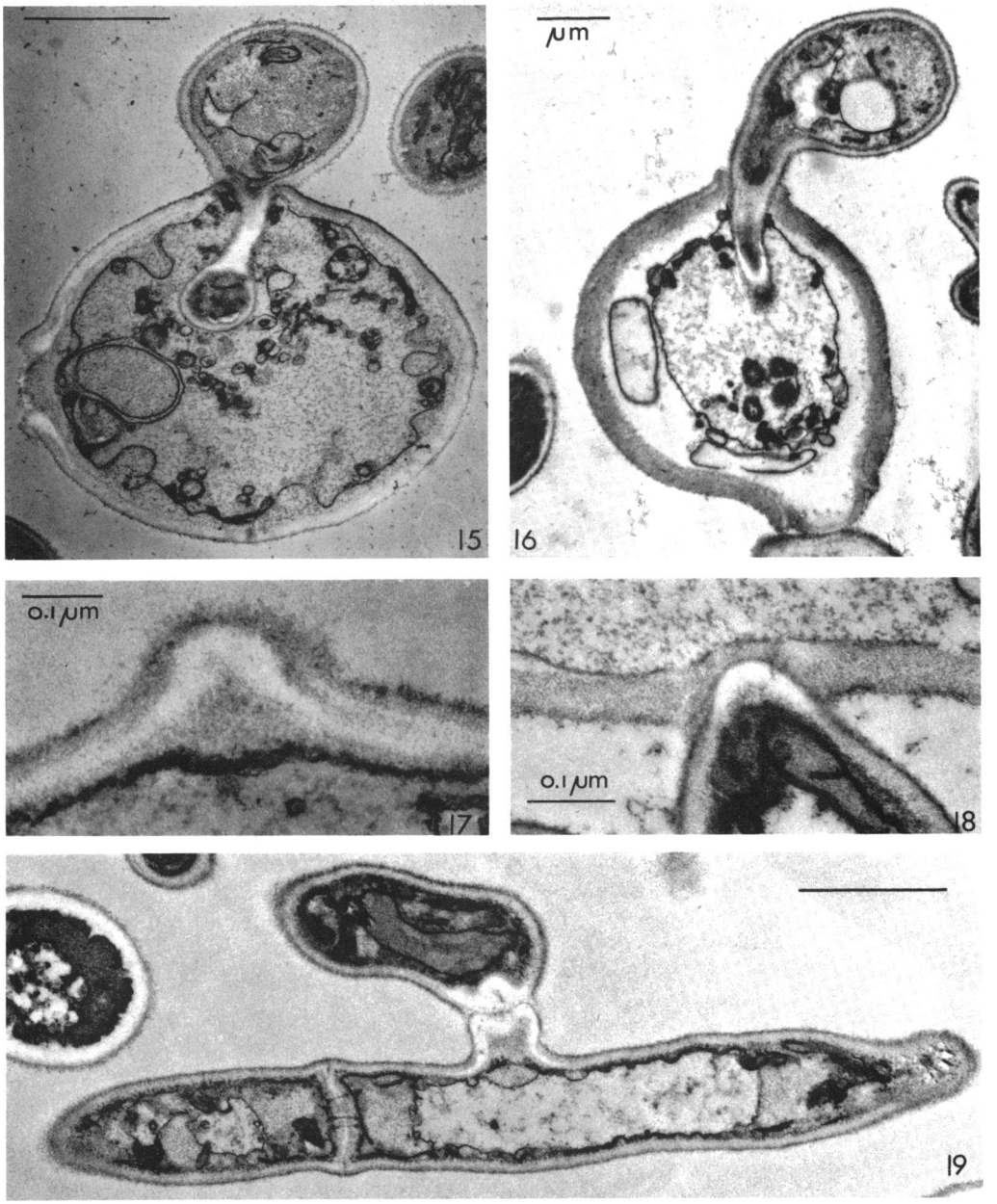


FIG. 15. *Cell of Saccharomyces cerevisiae penetrated by a stalk on a cell of E. javanensis. Bar = 0.5 μm.*
FIG. 16. *Cell of Candida albicans penetrated via a bud scar by a stalk on a cell of E. javanensis.*
FIG. 17. *E. selenospora; denticle arising from under the wall of the cell.*
FIG. 18. *Denticle with a light wall penetrating the wall of another cell.*
FIG. 19. *Hypha with plasmodesmata in the septum and with a denticle contacting another cell. The wall of the latter has thickened with light material. Bar = 0.5 μm.*

different species were preliminary, and they were performed under standard conditions. Therefore, limited value can be attached to negative results. However, it is striking that the cells of species with a cell wall known to differ from that of *S. cerevisiae* were not attacked, namely: *Schizosaccharomyces pombe*, *Nadsonia elongata*, *Sporobolomyces salmonicolor*

and *Rhodotorula glutinis* (5, 6). In the experiments with mixtures we do not know whether only dead cells were penetrated or whether the phenomenon might be considered as parasitism.

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