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An Electron Microscope Study of the Yeast *Pityrosporum ovale*

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Summary. Cells of *Pityrosporum ovale* were prepared for electron microscopy by different methods of fixation and embedding, all of them causing some degree of damage to the cells. Apart from the usual organelles seen in other yeast cells, a body was found which showed an electron-dense outer layer and an electron-light centre when stained with permanganate. The cell wall showed layers of different electron-density. Buds were formed at one pole only, leaving a collar on the mother cell.

The genus *Pityrosporum* is conspicuous by its morphology and physiology. It is distinguished from other yeasts by monopolar budding. The cells are small, round or oval, and buds are formed on a rather broad base at the same site as the birth scar. From the physiological standpoint, Benham (1939) found that *P. ovale* requires fatty substances for growth. The same holds for the second species, *P. orbiculare*; the third, *P. canis*, can grow on malt agar without the addition of lipids.

Barfatani *et al.* (1964) were the first to study the ultra-structure of the cells of *P. orbiculare.* They observed a relatively thick cell wall consisting of two layers, the inner one showing a regular pattern of indentations.

Swift and Dunbar (1965) examined the fine structure of *P. ovale* and *P. canis* which appeared to be similar to that of *P. orbiculare.* These authors mention the multi-layered cell wall with a corrugated inner surface; the corrugations were concentric with the cylindrical nature of the wall. According to Swift (1966), they spiral along the inner side of the wall.

Keddie (1966) made an electron microscope study of human skin prepared from lesions of tinea versicolor. *P. orbiculare* can be cultured from these lesions, but not the hyphae which are also present and which, together with the yeast cells or conidia were described as the fungus

Malassezia furfur. Sternberg and Keddie (1961) and Keddie and Shadomy (1963) assumed that *Malassezia furfur* was identical with *Pityrosporum orbiculare*. In the preparations of the skin mentioned above, Keddie (1966) observed in sections in both hyphae and conidia a sculptured inner surface of the thick fibrous wall.

The typical structure of the cell wall in *Pityrosporum* with its possible taxonomic implications led us to a closer examination of the ultrastructure of *P. ovale*, both as whole cells and in the separated walls.

Materials and Methods

The strain studied was the type strain of *Pityrosporum ovale* (CBS 1878), obtained from the Yeast Division of the Centraalbureau voor Schimmelcultures at Delft. This strain was grown (1) on Littman oxgall agar containing peptone 1%, Difco oxgall 1.5%, glucose 1%, and agar 2%, for 2 days at 25° C, and (2) in a liquid medium of the same composition but without agar. The liquid culture was shaken for 2 days at 30° C.

The cells were fixed: (a) with 1.5% $KMnO_4$ solution for 20 min, (b) with 1.5% $KMnO_4$ solution for 20 min and, after washing, with 1% osmium tetroxide solution for 1 hr, (c) as for (b), but with osmium tetroxide solution for 16 hrs. The fixed and washed material was suspended in agar and dehydrated through a graded acetone or alcohol series. It was post-stained with a saturated solution of uranyl acetate in either absolute acetone or absolute alcohol for one hr. The material dehydrated via acetone was embedded in Vestopal W, that treated with alcohol in Epon 812.

Cell walls were prepared from two different batches of cells grown in liquid medium (2). A suspension of cells was shaken with glass beads in a "Bühler" shaker. The walls were separated from the protoplasm by centrifugation. They were fixed with 1.5% $KMnO_4$ solution for 20 min at room temperature. After washing, the material was suspended in agar and dehydrated through an alcohol series. During dehydration the material of one batch was post-stained with a saturated solution of uranyl acetate in 70% alcohol. Both batches were embedded in Epon 812.

Ultrathin sections were cut with a diamond knife on an LKB ultramicrotome. Part of the sections was post-stained with lead citrate (Reynolds, 1963). Electron micrographs were taken with a Philips EM 100.

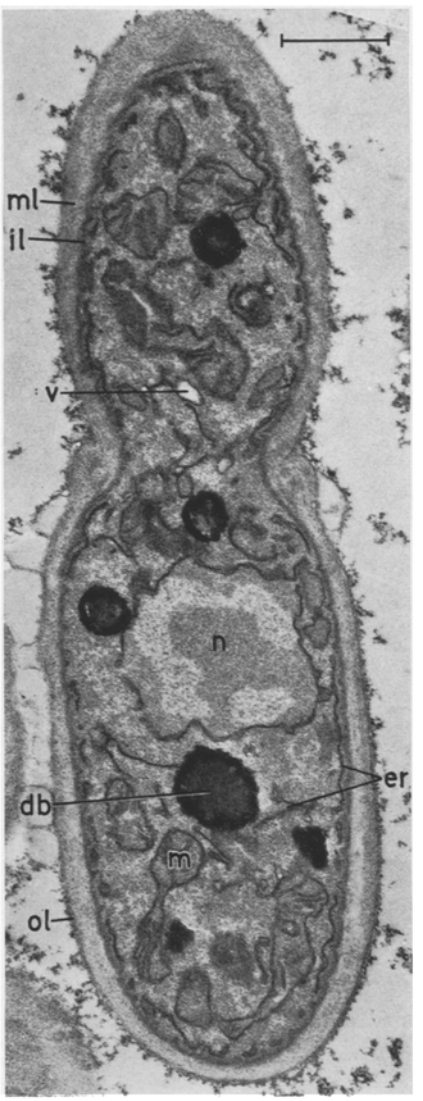
All figures are from cells grown in liquid medium and treated with permanganate, osmium tetroxide and uranyl acetate (method c), unless otherwise indicated. The marker bars denote 0.5 μ.

Fig. 1 shows a section of a cell with a growing bud. It contains a nucleus *n*, mitochondria *m*, endoplasmic reticulum *er*, a vacuole *v*, and electron-dense bodies *db*. The wall has a thin, dark outer layer *ol*, a light middle layer with corrugations at the inside *ml* and a dark undulating inner layer *il*

Fig. 2. Section of a cell grown on solid medium with distinct pits *p*. ($KMnO_4$, lead)

Fig. 3 shows small vesicles *vs* in the top of the growing bud

Fig. 4 shows arranged membranes, resembling a Golgi apparatus *g*



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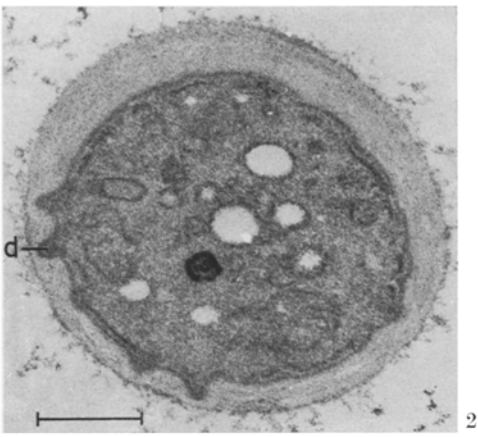

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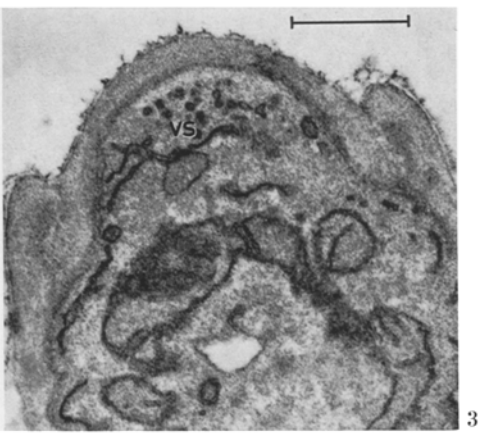

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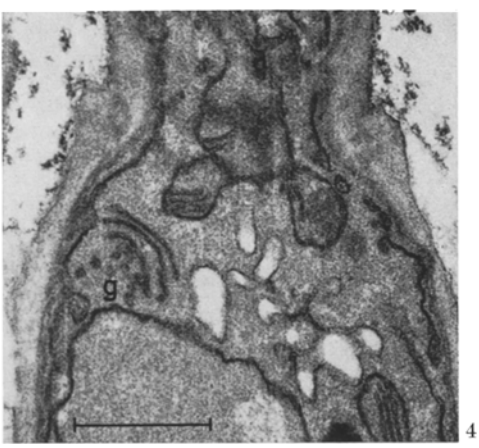

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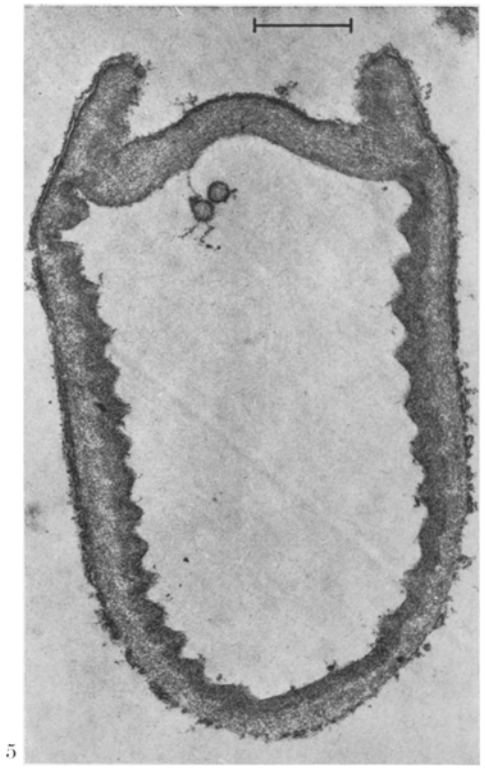
Figs. 1—4



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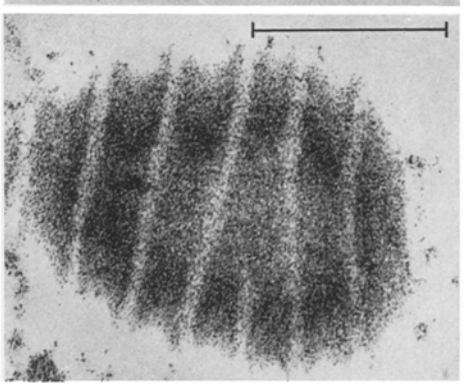

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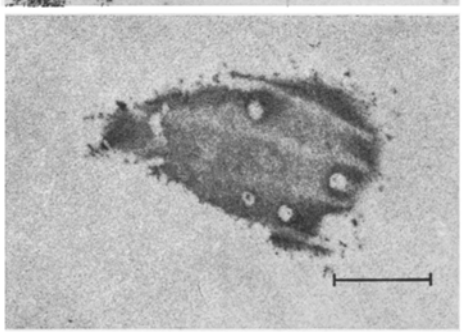

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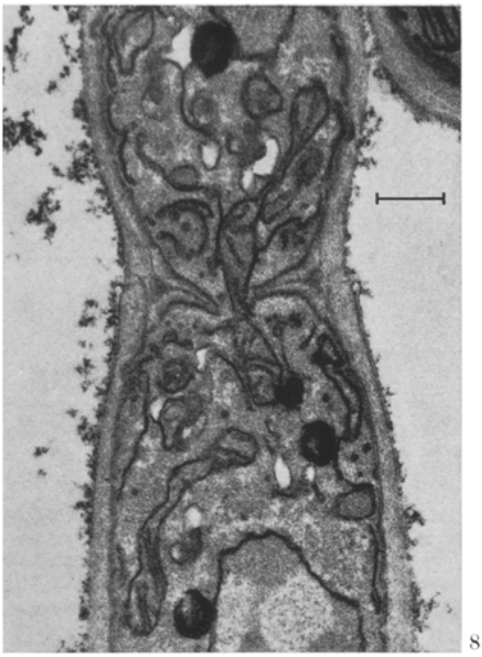

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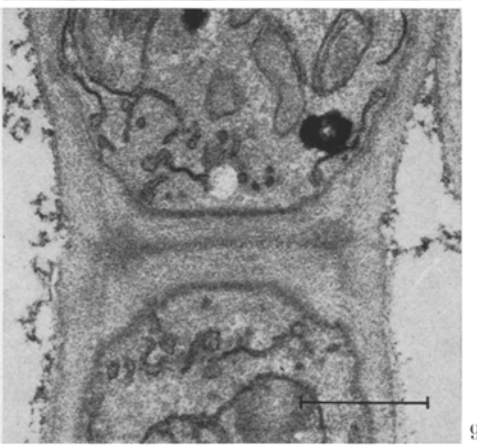

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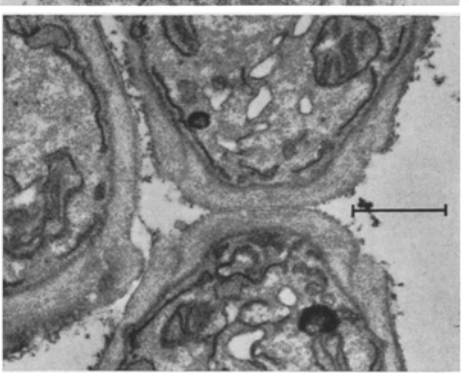

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Figs. 5—10

Results

The different methods of fixation and embedding all yielded material in which damaged cells were present, besides presumably normal cells. The damage was especially apparent in the wall from the broken electron-dense outer layer and the swollen electron-light middle-layer. Sections of separate walls also showed these deviations, and besides, occasionally, a broken dark inner layer. The number of damaged cells and the extent of the damage was much greater in material embedded in Vestopal than that, embedded in Epon. There was no distinctive difference as regards the damage to the cell wall among the three methods of fixation in the same plastic.

Ultrathin sections of the cells (Figs. 1—4) showed the protoplasm enveloped in the plasmalemma, which followed the undulating inner side and the deeper pits (Fig. 2) of the cell wall, but lay at some distance from it. The cortical endoplasmic reticulum was generally lacking at the top of the growing bud and here many small vesicles were found (Fig. 3). These also occurred at the transition between mother cell and bud when a cross wall was formed. In some cells a number of concentric membranes were observed which may be a Golgi apparatus (Fig. 4).

In the cells from agar culture fixed with permanganate several electron-light bodies occurred, which probably had contained lipids. In the same preparation electron-dense bodies were observed of round to short-oval shape and of varying size. These bodies consisted of a thick dark outer layer and a lighter centre. After post-fixation with osmium tetroxide the centre was more electron-dense but not as dark as the outer layer. In a single section up to six bodies might be present at various sites of the cell and also in the growing bud. The electron-dense bodies were also found in cells grown in the liquid medium.

The cell wall in sections of whole cells and separately showed layers of different electron-density (Fig. 1, 5 and 6).

At the outside of the wall two thin, dark, parallel lines were observed with a light space in between. In cells lying close together, the outer dark

Figs. 5—7. Sections through separate cell walls, all fixed with $KMnO_4$ and post-stained with lead. In Fig. 5 the double-lined dark outer layer is partly broken up or lacking. The dark inner layer is thicker than in whole cells. Fig. 6. Section through the inside of the cell wall showing the electron-light corrugations of the middle layer. Fig. 7. Similar section showing pits

Fig. 8. Partly formed cross wall between mother cell and bud. The dark line in the middle of the cross wall is broadened to triangles near the lateral wall

Fig. 9. The cross wall is complete

Fig. 10. Section through two separating cells

line was occasionally lacking between the cells. As mentioned before, this layer might be damaged, i.e. broken up or even entirely lacking, or the outer line might be partly separated from the inner one with irregular spaces in between.

Underneath the dark lines lay a relatively electron-light layer. It was smooth at the outside but it had indentations at the inner side which were only observed in longitudinal or oblique sections and not in cross sections of the cell. The cell was slightly flattened at the end opposite to the site of budding. The indentations were also present in the growing bud. Apart from these regular indentations, deep cone-shaped pits occurred in the wall in varying numbers and at various sites (Fig. 2, 7). In damaged cells the light layer was more or less swollen which was especially distinct where the outer dark layer was interrupted or had disappeared.

At the inside of the light layer a dark layer was observed which followed the indentations of the light layer. In separate walls this dark layer was very distinct and sometimes much thicker than in whole cells (Fig. 5). Occasionally, it was interrupted by the corrugations of the light layer. Both, greater thickness and interruptions of the dark layer in separate walls seem to be artefacts.

From the observation of sections we have deduced the following scheme of bud formation (Fig. 8—11). A young cell, just separated from the mother cell, shows a birth scar consisting of the edge of the lateral cell wall where it has broken from the mother cell and a central plug which is part of the cross wall between mother cell and bud (Fig. 11 a). This plug bulges out and the edge of the lateral wall does not appear as a ridge but is generally more apparent from the different arrangement of the layers within the middle layer of the wall; these are perpendicular to those of the plug.

The bulging plug grows out to a bud (Fig. 11 b), and after nuclear division, a cross wall is formed centripetally between mother cell and bud (Fig. 8, 11 c). The cross wall has a broad connection with the lateral walls of mother cell and bud and during growth it is tapered towards the centre. In sections, a thin dark line is visible in the middle of the cross wall which connects the points of transition of the lateral walls of mother cell and bud. At the place of connection it may be broadened to a triangle, especially in a later phase (Fig. 9). Thus, part of the cross wall is connected with the cell wall of the mother cell, and part of it with that of the bud. The complete wall is of equal thickness in the centre, but much thicker towards the edge. As mentioned before, many small vesicles were visible in the vicinity of the cross wall in the protoplasm of the mother cell and the bud.

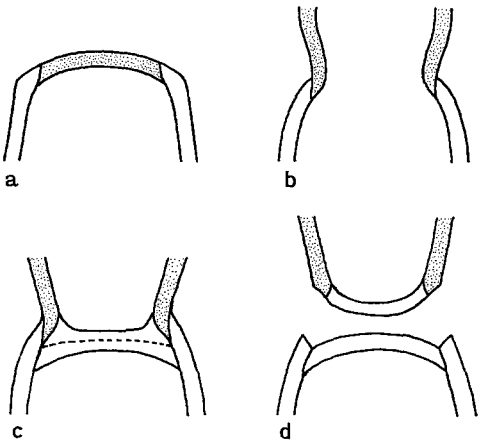

Fig. 11. Diagram of bud formation

Separation of mother cell and bud occurs along the dark line in the cross wall and the division line between the lateral walls of both cells (Fig. 10, 11 d). The lateral walls may remain for a longer time attached to each other at one place. The bulging plugs force the still-attached cells to come into a parallel position with each other. After separation of the bud from the mother cell, the latter shows a ridge consisting of the edges of both birth and bud scars but the birth scar is not distinct.

The formation of a second bud proceeds in the same way. The cross wall lies again at the place of transition between mother cell and bud, partly connected with both of them. Its connection with the mother cell lies underneath that of the former cross wall and the cleft between the plug and the edge of the lateral wall of the cell gets deeper with every bud; occasionally the wall curls inwards.

Discussion

The ultra-structure of *Pityrosporum ovale* shows some remarkable characteristics not recorded for yeasts of other genera.

In the first place, the cell wall consists of several layers, among them an electron-dense layer at the inside of the electron-light layer. The latter is corrugated so that the inside of the wall shows parallel or nearly parallel ribs. According to Swift (1966) who examined defatted, fragmented and shadowed cell walls of the three *Pityrosporum* species, the corrugations spiral along the inner surface of the wall. He considered the corrugations as a typical feature of the genus *Pityrosporum*. Apart from the regular indentations, we found conical pits to be present.

Although the cell wall seems to be relatively thick in many cells, this may be an artefact, since thinner walls do occur and of the thicker walls the outer layer is generally broken. An explanation for the damage could be the presence of a compound which is not fixed by permanganate and osmium tetroxide, and which, during embedding is removed thereby causing disintegration of the wall.

In the protoplast the electron-dense bodies are peculiar. The fact that the contents are stainable with osmium tetroxide and not with permanganate suggests that they contain lipids. The outer layer of these bodies also stains with permanganate alone. In one of the micrographs by Swift and Dunbar (1965) these electron-dense bodies are present, but the authors tentatively identified them as nuclear material. We also observed electron-dense bodies in cells of *P. canis* and *P. orbiculare*.

As mentioned before, *Pityrosporum* is the only yeast genus with monopolar budding. Moreover, bud formation differs from that described for yeasts with bipolar budding where buds are also formed at the same sites of the cell. In the latter cases, the plug of the bud scar grows out to form a bud presumably in the same way as in *Pityrosporum*, but the cross wall is formed at some distance from the former one. In this way every separated bud leaves a new ridge, and the so-called multiple scars arise (Streiblová *et al.*, 1964). In *Pityrosporum*, however, the cross wall is formed partly connected with the wall of the mother cell and partly with that of the bud. This results in a collar on the mother cell which gets larger with every bud, and not in multiple scars. From the beginning of its formation the cross wall in *Pityrosporum ovale* shows a thin, dark centre plate where the bud is split off later on. This differs from the formation of a primary wall which later thickens as described for *Saccharomyces cerevisiae* by Sentandreu and Northcote (1969), and for *Endomycopsis platypodis* by Kreger-van Rij and Veenhuis (1969).

The present study partly corroborates former observations and adds some new ones. It shows the desirability for finding a better way of preserving the material.

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