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A Comparative Study of the Cell Wall Structure of Basidiomycetous and Related Yeasts

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SUMMARY

The wall of basidiomycetous and related yeasts showed a lamellar structure in sections of both budding cells and hyphae fixed with potassium permanganate. The yeasts also had a typical way of bud formation and septation. These features differ from those recorded for ascomycetous yeasts. In the hyphae of some species septal pores were observed which were either dolipores or simple pores.

INTRODUCTION

The basidiomycetous yeasts, classified in the Heterobasidiomycetes, are confined to five genera of generally heterothallic species. Most of them have a haploid stage of budding yeast cells preceding a dikaryotic stage of mycelium with clamp connexions. For some of the species in these genera knowledge about the sexual cycle is still incomplete. In three of the five basidiomycetous genera the cultures of the organisms are red, in two of them cream-coloured. Two genera of the first group form ballistospores: *Sporidiobolus* (Nyland, 1949) and *Aessosporon* (van der Walt, 1970). In these and in the third genus, *Rhodosporidium* (Banno, 1967), chlamydospores are produced. In *Aessosporon* conjugation was not observed and dikaryotic mycelium was absent. Of the other two genera with cream-coloured cultures, *Leucosporidium* (Fell, Statzell, Hunter & Phaff, 1969) resembles *Rhodosporidium* in producing chlamydospores but no ballistospores. *Filobasidium* (Olive, 1968), the fifth basidiomycetous genus, differs from all the others by the formation of basidia with sessile basidiospores.

The species considered to be related to the basidiomycetous yeasts show some of the typical features such as: capsulated cells producing starch-like compounds, ballistospores, red cultures and a high GC % of DNA. Sexual reproduction has not yet been observed in them and probably many of their strains are haploids representing one of the mating types.

Electron microscopic studies of *Rhodotorula* (Marchant & Smith, 1967; Ruinen, Deinema & van der Scheer, 1968), *Cryptococcus* (Edwards, Gordon, Lapa & Ghiorse, 1967) and *Sporobolomyces* (Prusso & Wells, 1967), all of them probably related to basidiomycetous yeasts, have shown a lamellar structure of the wall different from that found in ascomycetous yeasts such as *Saccharomyces cerevisiae*. These and our own observations have induced us to a comparative study of the wall of yeast cells and hyphae in a greater number of basidiomycetous and related species to see whether the lamellar structure of the wall was characteristic for all these species and might, therefore, have taxonomic value. Moreover, our observations on bud formation were compared with those by Marchant & Smith (1967) and Prusso & Wells (1967). The structure of the septa was examined in a number of species.

The strains chosen for examination belong to the five basidiomycetous genera mentioned above, and to the probably basidiomycetous genera *Rhodotorula*, *Cryptococcus*, *Sterigmatomyces*, *Sporobolomyces*, *Bullera* and *Itersonilia* of the Fungi Imperfecti. The three latter genera are classified in the Sporobolomycetaceae. *Itersonilia* is not considered to belong to the yeasts. Since, however, Sowell & Korf (1960) obtained budding yeast cells from a culture of *Itersonilia perplexans* we have included this species in our study. From the genera *Candida* and *Trichosporon* a number of species has been chosen.

METHODS

The following strains were examined: *Aessosporon salmonicolor* van der Walt, CBS 5937; *Filobasidium floriforme* Olive, G 469; *Leucosporidium capsuligenum* Fell *et al.*, CBS 4736 and 1906 × 4736; *Leucosporidium frigidum* Fell *et al.*, CBS 5270; *Leucosporidium scottii* Fell *et al.*, CBS 614, 5931 and 5930 × 5931; *Rhodosporidium sphaerocarpum* Newell & Fell, CBS 5939 × 5940; *Rhodosporidium toruloides* Banno, CBS 14 × 349; *Sporidiobolus johnsonii* Nyland, CBS 5470; *Sporidiobolus ruinenii* Phaff, CBS 4999; *Bullera alba* (Hanna) Derx, CBS 501; *Candida curvata* (Diddens & Lodder) Lodder & Kreger-van Rij, CBS 570; *Candida humicola* (Daszewska) Diddens & Lodder, CBS 571; *Candida muscorum* di Menna, G 435; *Cryptococcus diffluens* (Zach) Lodder & Kreger-van Rij, G 188; *Itersonilia perplexans* Derx, CBS 286·50; *Rhodotorula glutinis* (Fres.) Harrison, CBS 20 and 332; *Rhodotorula mucilaginosa* (Jörg.) Harrison, CBS 316; *Sporobolomyces roseus* Kluyver & van Niel, CBS 486; *Sporobolomyces salmonicolor* (Fischer & Brebeck) Kluyver & van Niel, CBS 490; *Sterigmatomyces halophilus* Fell, CBS 4609; *Sterigmatomyces* sp., CBS 5492; *Trichosporon cutaneum* (de Beurm., Gougerot et Vaucher) Ota, CBS 5601, 5597 and 6183; *Trichosporon pullulans* (Lindner) Diddens et Lodder, CBS 2532.

Matings were performed by mixing cells of opposite mating type and inoculating the mixture in a streak on cornmeal agar. Pieces of agar in which dikaryotic mycelium had developed were cut out and placed on fresh cornmeal agar or in potato water. Mycelial growth from these media was used for examination.

Young yeast cells were obtained from shaken malt extract cultures grown for 24 to 48 h. at 15° to 20°. Mycelial hyphae were harvested from malt agar or cornmeal agar cultures grown for 5 to 7 days at room temperature.

Fixation was in 1·5% aqueous $KMnO_4$ for 20 min. at room temperature. The material was dehydrated through a graded ethanol series, stained in a saturated solution of uranyl acetate in ethanol and embedded in Epon 812. By way of exception the cells of *Rhodotorula glutinis* CBS 332 were embedded in Vestopal after dehydration with acetone. The material was cut on an LKB ultramicrotome with a diamond knife. Electron micrographs were taken with a Philips EM 300 and a Philips EM 100. When the contrast was insufficient it was increased by staining the sections with lead citrate (Reynolds, 1963).

RESULTS

Fixation with potassium permanganate, and, if necessary, staining the sections with lead citrate, gave a satisfactory picture of the wall. It did not show the capsule, although a light halo around the cells might indicate its presence.

In the wall of budding yeast cells and hyphae a lamellar structure was observed, which was not equally distinct in all species. The wall of the young bud generally consisted of a single, often vague, greyish layer (Fig. 1). In older cells, showing bud scars or with the bud

still attached, thin dark layers were observed alternating with the greyish layers (Fig. 2). In thick walls the outer layers were often broken. Mostly, the dark layers were equidistant and close together throughout the wall, but occasionally an irregular packing was observed (Fig. 3). The layers stood out most clearly in the collar at the base of the bud where the dark layers were broken up and lay wider apart (Fig. 4). The thickness of the wall and the number of dark layers varied among the cells of a single strain.

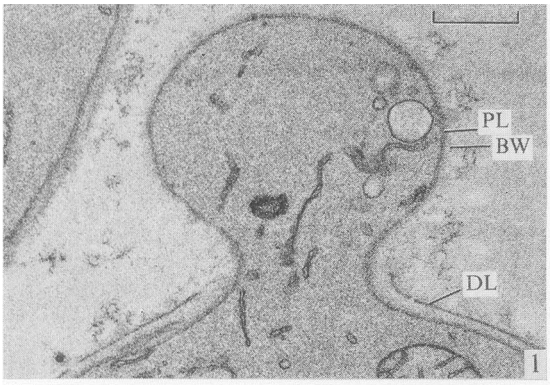
The light lenticular thickenings between the dark layers, as described by Kreger-van Rij & Veenhuis (1971*b*) for *Sporidiobolus ruinenii* (see Fig. 8), *S. johnsonii* and *Rhodosporidium toruloides*, were also observed in the wall of hyphae and chlamydospores of *Leucosporidium scottii*. These thickenings were not found in young cells grown in malt extract. The beginning of bud formation was apparent from the slight bulging of the protoplast and a thickening of the overlying wall with a divergency of the dark layers (Fig. 5). In the protoplast at the site of budding a mitochondrion or strands of endoplasmic reticulum with vesicles were often present. Our observations suggest that subsequently the wall of the mother cell, at least partly, dissolved. A preparation of *Rhodotorula glutinis* showed a light break in the cell wall prior to bud formation (Fig. 6). In a later stage the inner greyish layer of the wall of the mother cell seemed to be continuous with the wall of the bud. In this wall new dark layers arose.

After nuclear division, a cross wall was formed by centripetal growth in the isthmus between mother cell and bud. From the beginning of its development a light inner layer between two grey layers was generally visible in this wall (Fig. 9). The cross wall thickened with greyish material. Separation of mother cell and daughter cell occurred along the light layer. The scar plug of the mother cell consisted of part of the cross wall and the broken lateral wall originally continuous between mother cell and bud (Fig. 10). The dark layer or layers in the latter sometimes formed a distinct second collar on the mother cell. The first collar was formed from the original wall of the mother cell in the place where it was broken up. In some species, after separation of mother cell and bud, a light layer was visible on top of the plug. It was wider than the original light layer of the cross wall and had vague edges. In oval or elongate cells buds were often formed close together at one of the poles of the cell.

In bud formation of strains of *Sterigmatomyces* it deserves special mentioning that the neck of the bud, also indicated as sterigma, is longer than usual. According to Fell (1966), its length may vary from 1·5 to 26 μm. We found short necks in cells grown in malt extract in shaken cultures, and long ones in cells from old malt agar cultures. In *Sterigmatomyces halophilus* the light layers in the wall were very broad. This material clearly showed the continuity of the inner layer of the wall of the mother cell with the wall of the bud (Fig. 11). The dark layers originated from the inner side of the wall. Therefore, the youngest bud had to penetrate all the overlying layers, and buds formed before came from more superficial layers (Fig. 13). In the Sterigmatomyces species studied the cross wall extended over the whole of the sterigma (Fig. 12).

Cross walls in hyphal threads were also formed centripetally and consisted of a light central layer between two greyish layers. They thickened and dark lamellae arose in them (Fig. 7). Arthrospores separated along the light layer.

In the septa of several strains pores were observed. In *Trichosporon cutaneum*, *T. pullulans*, *Leucosporidium capsuligenum*, *Filobasidium floriforme* and *Itersonilia perplexans* (Fig. 17) dolipores occurred, i.e. pores surrounded by a thickened edge of the septum. In all of them a more or less well developed pore cap was present. In *L. capsuligenum* the cap had a peculiar structure and consisted of cone-shaped dilations of the endoplasmic reticulum (Fig. 14, 15).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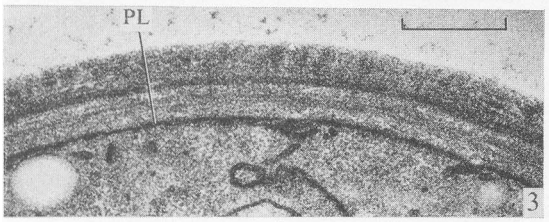
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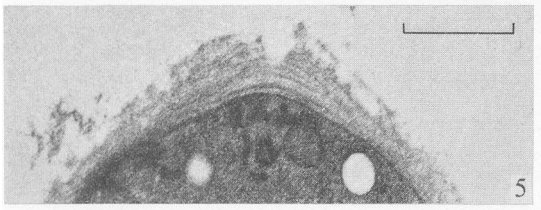
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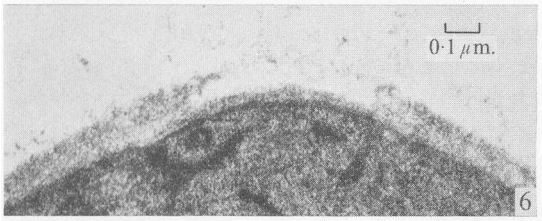
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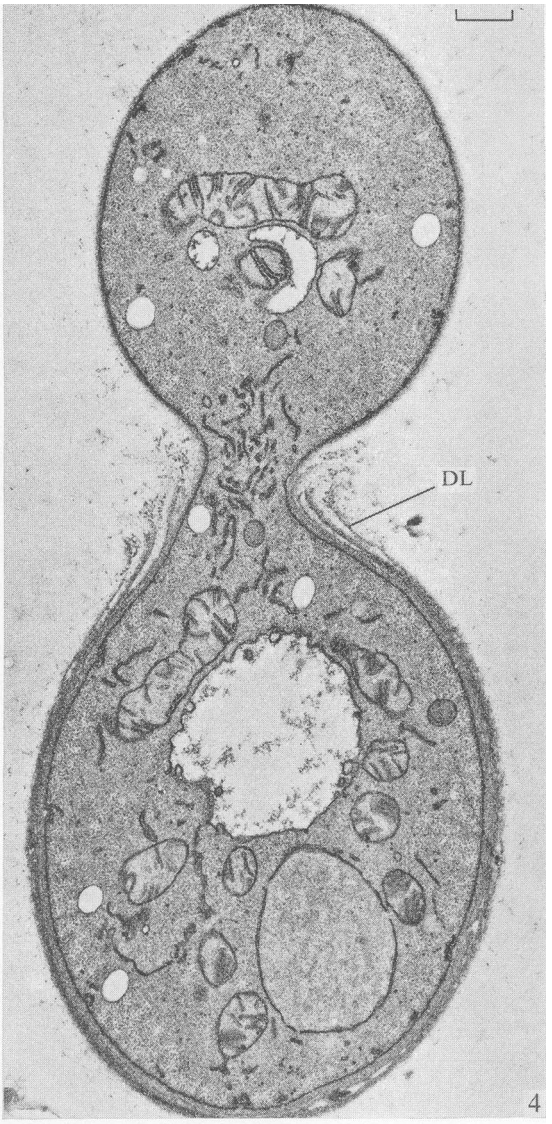
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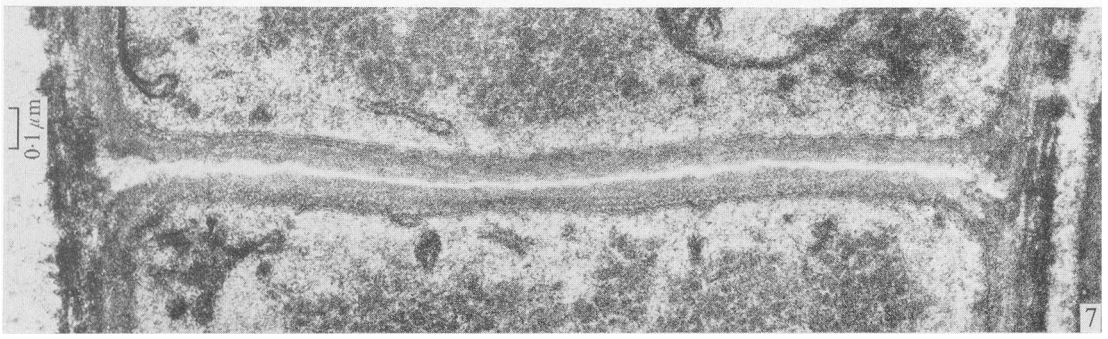
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A simple type of pore was observed in the dikaryotic mycelium of the species *Rhodosporidium sphaerocarpum*, *R. toruloides*, *Sporidiobolus ruinenii* (Fig. 16) and *L. scottii*. The septum decreased in thickness towards the centre where a very narrow pore was present.

DISCUSSION

A lamellar structure of the cell wall, which has been described for *Rhodotorula glutinis*, *Sporobolomyces roseus* and *Cryptococcus neoformans*, was observed in all species presently examined belonging certainly or probably to the Basidiomycetes. Also corresponding among these yeasts are the method of bud formation and separation of daughter cell from mother cell. We did not find a plate of new wall material between the existing wall and the plasmalemma at the site of bud formation described by Marchant & Smith (1967), but we think that the inner layer of the mother cell wall is continuous with the wall of the bud, as mentioned by Prusso & Wells (1967) for *S. roseus*. These latter authors considered the first dark layer in the wall of the mother cell to be also part of the cell wall of the bud. We could confirm this by our observations of continuity of dark layers in the wall of mother cell and bud. However, in the first stage of bud formation only the inner wall grows out to form the bud and the first dark layer is formed later.

The structure of the cell wall and the method of bud formation in basidiomycetous yeasts are distinctly different from these features in most ascomycetous yeasts. The wall of the latter fixed with $KMnO_4$ or OsO_4 does not show dark lamellae, but only one thin, dark outer layer and a broad light inner layer. The wall of the bud arises from under the wall of the mother cell and a light primary wall is initially formed in the isthmus between mother cell and bud.

These differences observed between ascomycetous and basidiomycetous yeasts are worthwhile to consider for taxonomy since the mutual relationship of both groups and their relation to the other Ascomycetes and Basidiomycetes is still uncertain. Taxonomic value has already been attached by several authors to the type of pore in the hyphal septum, the simple pore being regarded as the Ascomycete-type and the dolipore as the Basidiomycete-type (Moore & McAlear, 1962). However, the dolipore observed in the ascomycetous yeast species *Endomycopsis platypodis* (Kreger-van Rij & Veenhuis, 1969) is in contradistinction

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Key to symbols: BS = bud scar, BW = wall of bud, CW = cross wall, DL = dark layer, ER = endoplasmic reticulum, IL = inner layer, L = lenticular thickening, LL = light layer, OL = outer layer, P = pore, PC = pore cap, PL = plasmalemma, S = sterigmen. The markers indicate 0·5 μm. unless indicated otherwise.

Fig. 1. Section through a young bud of *Rhodotorula glutinis*. In the wall of the mother cell one thin dark layer (DL) is visible; the greyish layer underneath is continuous with the wall of the bud (BW).

Fig. 2. Section of the cell wall of *Candida muscorum*. Two dark layers (DL) are visible besides the plasmalemma (PL).

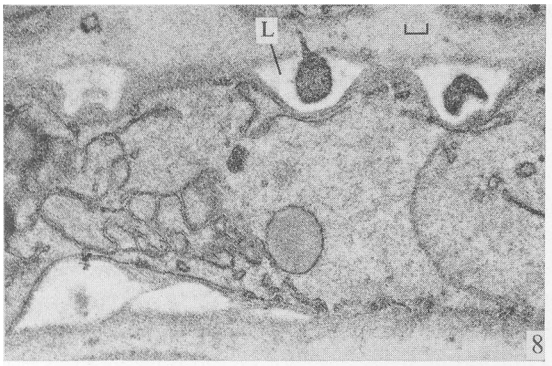
Fig. 3. Section through the wall of an older cell of *Cryptococcus diffluens*. The thin dark layers are unevenly packed.

Fig. 4. Section through a budding cell of *Rhodotorula glutinis*. The broken-up layers (DL) in the wall of the mother cell diverge and form a collar at the base of the bud.

Fig. 5. Initiation of bud formation in *Rhodotorula glutinis*. The wall at the site of budding is thickened and the dark layers are wider apart.

Fig. 6. In an early stage of bud formation in *Rhodotorula glutinis* part of the wall of the mother cell at the site of budding has dissolved and left a break.

Fig. 7. Septum in a hypha of *Rhodosporidium sphaerocarpum*. The lamellar structure of the wall with the light inner layer is visible.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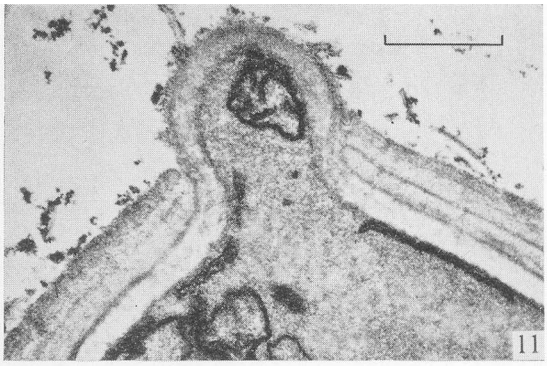
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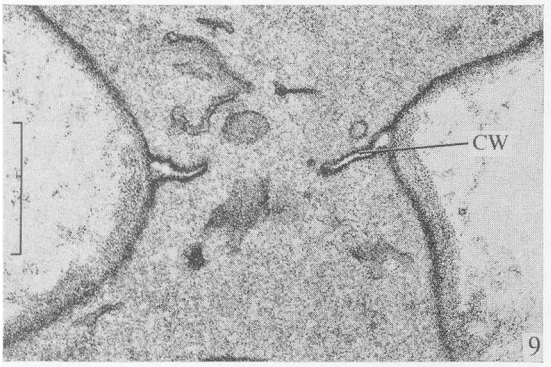
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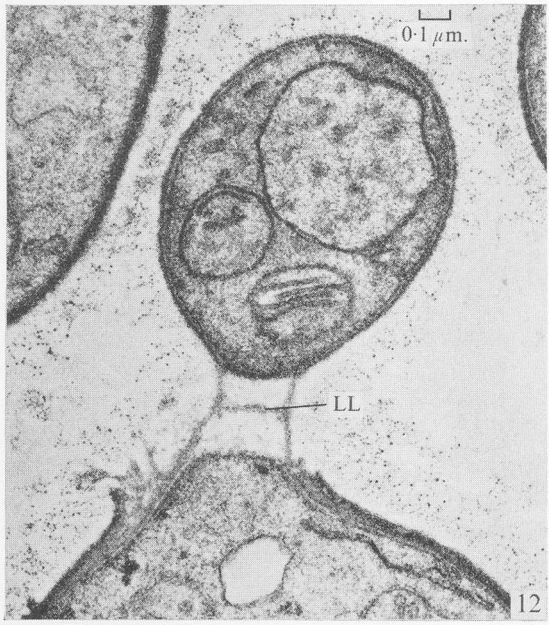
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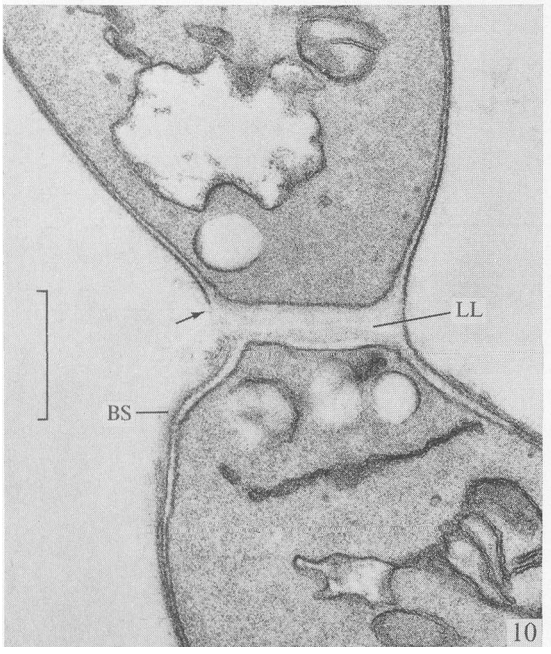
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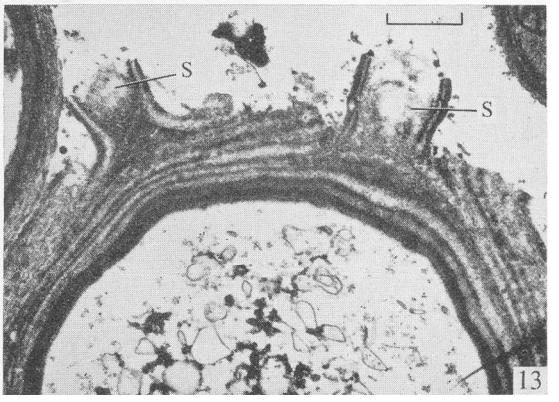
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to this rule. The structure of the wall in this species is of the Ascomycete-type. Further contrasts with this rule are the simple pores observed in some of the basidiomycetous yeast genera as well as the simple pores found in other Heterobasidiomycetes (Bracker, 1967).

From the above data it appears that the ultrastructure of the wall rather than the type of pore may be of assistance in the present classification of the yeasts in the Ascomycetes or Basidiomycetes.

Two types of septal pores were found among the basidiomycetous yeasts studied, and this distinction corroborates other differences. Simple pores occurred in Sporidiobolus and Rhodosporidium strains and in *Leucosporidium scottii*, all forming dikaryotic mycelium, chlamydospores and sporidia. The authors of these genera generally classify them in the Ustilaginaceae–Ustilaginales. In *L. capsuligenum* dolipores were present. In this species no chlamydospores with sporidia, but basidia and basidiospores are formed, and von Arx (personal communication) classified it in the genus *Filobasidium*. Olive (1968) established a new family Filobasidiaceae in the Ustilaginales for this genus.

Van der Walt (1969) classified a sixth yeast genus, *Syringospora*, in the Basidiomycetes. Its single species, *Syringospora albicans*, is considered to be the perfect form of *Candida albicans*. However, the structure of the cell wall and the method of bud formation in this species resemble those of ascomycetous yeasts (Fig. 18). Since other properties such as the GC content of DNA (Stenderup & Leth Bak, 1968) are also more in agreement with the latter, the classification of this species in the Basidiomycetes should be reconsidered.

As mentioned above, the asporogenous yeasts examined exhibited the same ultrastructure of the cell wall and method of bud formation as the basidiomycetous yeasts. Presuming that these yeast strains are heterothallic haploids, mating experiments may be aimed at obtaining dikaryotic mycelium. So far, one type of septal pore has been found among them, the dolipore, in *Trichosporon cutaneum* (Kreger-van Rij & Veenhuis, 1971*a*) and in *Itersonilia perplexans*. This feature may later be of importance for the classification of the perfect forms of these species.

Lodder (1970) placed the genus *Sterigmatomyces* outside the yeasts. The present observations seem to indicate a relation to the basidiomycetous yeasts.

Fig. 8. Section through a hypha of *Sporidiobolus ruinenii*. In the wall between the dark layers light lenticular thickenings (L) are present with a dark central body.

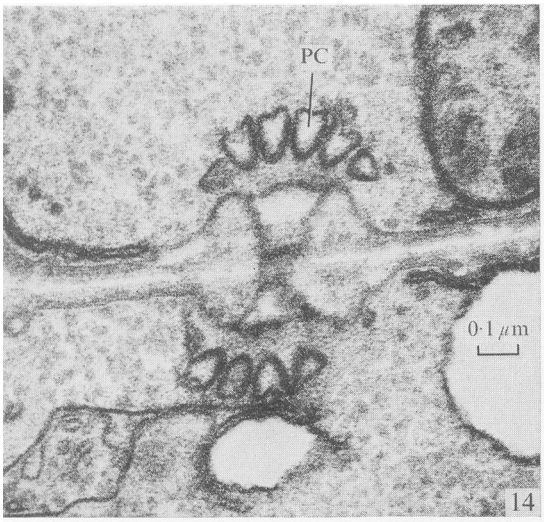
Fig. 9. Partially formed cross wall (CW) in the isthmus between mother cell and bud of *Cryptococcus diffluens*.

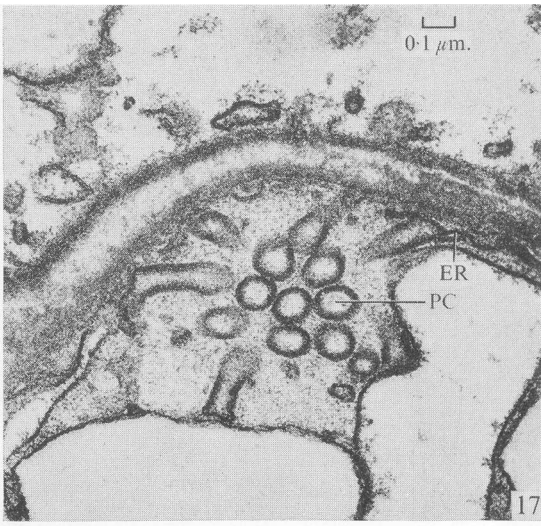
Fig. 10. Beginning of separation of mother cell and bud in *Candida muscorum*. In the middle of the cross wall a light layer is visible (LL). The thin dark layers are partly broken up (arrow). To the left of the bud, a bud scar (BS) is visible.

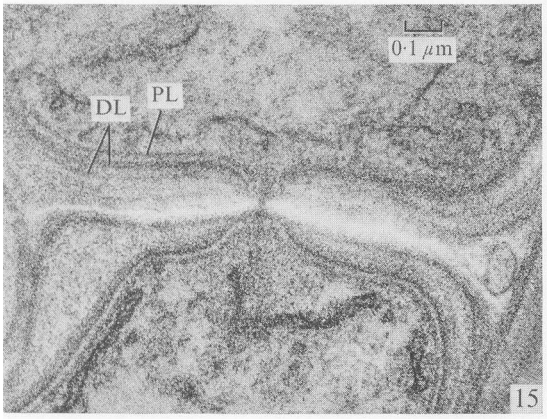
Fig. 11. Section through a young bud of *Sterigmatomyces halophilus*. The light layers in the wall are broad. The inner layer of the wall of the mother cell is continuous with the wall of the bud.

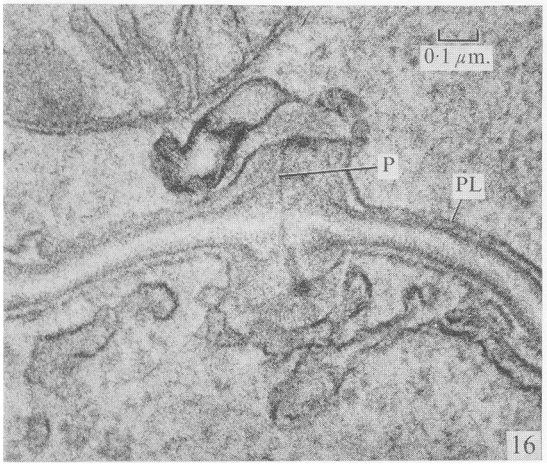
Fig. 12. Bud on sterigmen in Sterigmatomyces species. Dark layers in the wall of the mother cell are continuous in the wall of the bud. Within the sterigmen a thin light layer (LL) between dark material is visible along which the two cells will separate.

Fig. 13. Section through a cell of *Sterigmatomyces halophilus* with two sterigmata (S) in the wall. The right one is a residue of the youngest bud.









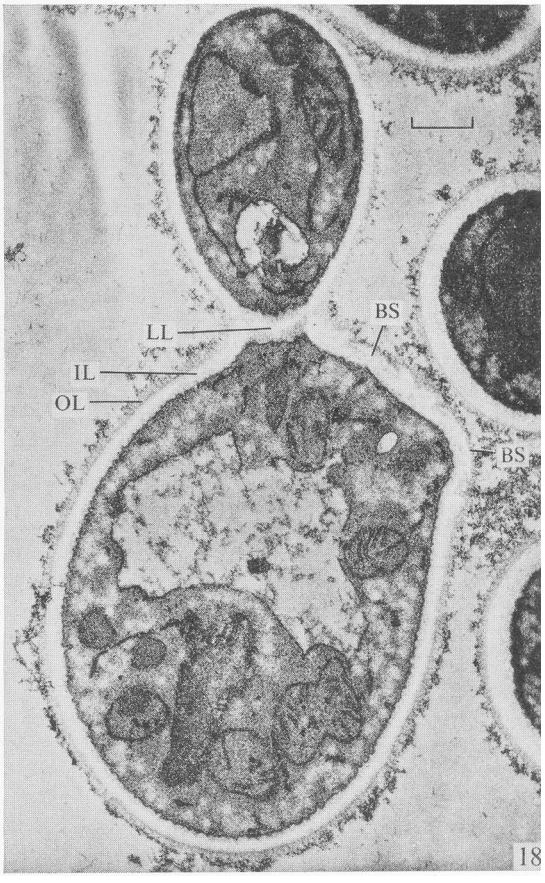


Fig. 14. Longitudinal section through a septum with a dolipore in a hypha of *Leucosporidium capsuligenum*. The pore cap (PC) is constituted of cone-shaped dilations of the endoplasmic reticulum.

Fig. 15. Cross section through a pore cap (PC) of a dolipore in *Leucosporidium capsuligenum*.

Fig. 16. Section through a septum with a simple pore in *Sporidiobolus ruinenii*. In the septum the light inner layer and several dark layers (DL) are visible.

Fig. 17. Section through a septum with a dolipore in *Itersonilia perplexans*. The pore is very narrow. Strands of swollen endoplasmic reticulum lie around the pore.

Fig. 18. Section through a budding cell of *Candida albicans*. The cell wall consists of a broad light inner layer (IL) and a thin dark outer layer (OL). Loose dark material adheres to the cell wall. In the cross wall between mother cell and bud a light inner layer (LL) is visible. In the two bud scars to the right of the bud the light layers are still present on top of the plugs.

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