Wall Structure and Bud Formation
in *Rhodotorula glutinis*

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Summary. The first stage in the formation of a bud in *Rhodotorula glutinis* is the production of a tapered plate of new wall material between the existing wall and the plasmalemma. The parent cell wall is lysed, allowing the bud to emerge enveloped in this new wall. Muclage is synthesised to surround the developing bud. As the bud grows a septum forms centripetally dividing the two cells. When the daughter cell reaches maximum size the septum cleaves along its axis, producing the bud scar on the parent cell and the birth scar on the daughter cell. The birth scar is obliterated later as the wall of the young cell grows. A system of endoplasmic reticulum and vesicles is found in young buds and is thought to be responsible for the transport of wall material precursors.

Although yeasts were among the first fungi to be examined using the electron microscope no accurate description of the formation of the new walls during budding has appeared. AGAR and DOUGLAS (1955) examined budding cells of *Saccharomyces cerevisiae*, but thin sectioning techniques were not, at that time, refined enough to reveal the layering of the walls. Other studies of budding yeasts (HASHIMOTO et al., 1959; THYAGARAJAN et al., 1962) have been more concerned with nuclear division and general cytology than with wall structure. Light microscope studies of bud formation in yeasts (THYAGARAJAN and NAYLOR, 1962; McClary et al., 1962) have of course been unable to resolve the problem of how the bud wall is produced and the electron microscope offers the only hope of answering this question. The present study was undertaken to elucidate the stages in bud formation in *Rhodotorula glutinis*, in the light of what is known about spore germination (MARCHANT, 1966a; MARCHANT, 1966b) and wall growth (MARCHANT et al., 1967) in other fungi.

Methods

*Rhodotorula glutinis* (Fres.) Harrison (National Collection of Yeast Cultures, strain 59) was the organism used for this study. It was grown at 30°C in shake cultures containing glucose (15 g/l) as sole carbon source plus NaNO₃ (2 g/l), KH₂PO₄ (1.5 g/l); MgSO₄ (0.5 g/l); KCl (0.5 g/l); FeSO₄ (trace); (pH 5.0). The cells were grown until they reached the early logarithmic phase of growth; they were then harvested and washed with water before being fixed for electron microscopy by one of the following methods.
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1. **1%** unbuffered potassium permanganate for 4 hours at room temperature.
2. **3%** acrolein + **3%** glutaraldehyde in sodium cacodylate buffer (pH 7.2 to 7.4) for 1 hour at room temperature; followed by postfixation for 1 hour at 0°C in **1%** osmium tetroxide in sodium cacodylate buffer (pH 7.2–7.4). The material was soaked for 16–18 hours in 0.5% aqueous uranyl acetate before dehydration (Hess, 1966).

After either of these treatments the material was retained as a pellet and dehydrated in tertiary butyl alcohol mixtures (Johansen, 1940), then embedded in the No. 1 Araldite-Epikote mixture of Molienhauer (1964). Sections were cut with glass knives on an LKB ultratome and viewed in a Siemens Elmiskop 1 electron microscope. Some sections were stained with lead citrate (Reynolds, 1963) before viewing.

The outer surfaces of the cells were examined using a shadowing technique. The cells were extracted with diethyl ether and then treated with 4.5% (w/v) KOH at 100°C for 1 hour. After washing with 20% (v/v) H₂SO₄ and water the material was spread on formvar/carbon coated grids and shadowed at an angle of 22° with gold/palladium (60:40).

**Observations**

The wall of the mature cell of *Rhodotorula glutinis* shows a multi-layered structure, overlaid with mucilage (Fig.1). This mucilage tends to decrease in quantity as the cell ages. The initial stage in the budding process is the formation of a tapered plate of new wall material between the existing wall and the plasmalemma at the point where the bud is to be produced (Fig.2). This situation is analogous to that described by Marchant (1966a) for the production of the germ tubes by *Fusarium culmorum* conidia. Hydrolysis of the existing yeast cell wall now takes place to allow the emergence of the bud; there is very little evidence for mechanical force being utilised to rupture the wall (Fig. 3). The young bud then emerges enveloped by the new wall layer (Fig. 4). Examination of the walls of very young buds shows that they are composed of microfibrils 150–200 Å in width, although the parent cell shows a much less obvious microfibrillar structure by the same hydrolysis treatment (Fig. 5). At the stage when the young bud emerges synthesis of mucilage occurs so that the bud is protected by a thick layer of mucilage. At this time when active wall synthesis is taking place in the bud large quantities of endoplasmic reticulum and vesicles can be seen (Fig.4) and the plasmalemma has the crenulate appearance which Marchant *et al.* (1967) ascribed to fusion of vesicles, carrying wall precursor material, with the plasmalemma. It seems likely that in *Rhodotorula glutinis* the endoplasmic reticulum and vesicles observed in the regions of bud formation are also implicated in cell wall synthesis.

The bud continues to grow in size and to accumulate various organelles while the wall of the parent cell forms a collar round the junction between the two cells (Fig.6). Before the bud cell reaches its maximum size a septum begins to form centripetally in the neck between the cells.