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Bipolar budding in yeasts — an electron microscope study

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KREGER-VAN RIJ, N. J. W. and VEENHUIS, M. 1971. Bipolar budding in yeasts — an electron microscope study. *Antonie van Leeuwenhoek* 37: 125–136.

Bud formation in yeasts with bipolar budding was studied by electron microscopy of thin sections.

Budding in yeasts of the species *Saccharomyces ludwigii*, *Hanseniaspora valbyensis* and *Wickerhamia fluorescens* resulted in concentric rings of scar ridges on the wall of the mother cell. The wall between the ridges consisted of the scar plug left by the former budding and opened up in the formation of the next bud. The wall of the bud arose from under the wall of the mother cell.

In the yeasts of the species *Nadsonia elongata* more than one bud might be formed from the same plug.

In *Schizoblastosporion starkeyi-henricii* the scar ridges were close together and apparently not separated by the entire plug.

In all species a cross wall was formed between mother cell and bud which consisted of an electron-light layer between two layers of more electron-dense material. The cells separated along the light layer.

INTRODUCTION

Certain yeasts reproduce vegetatively by budding on a broad base at the two poles of the cell. These yeasts are classified in the ascosporogenous genera *Hanseniaspora*, *Saccharomyces*, *Wickerhamia* and *Nadsonia*, and in the asporogenous genera *Kloeckera* and *Schizoblastosporion*. The genus *Hanseniaspora* comprises sporulating forms of *Kloeckera* species.

Several authors have considered that yeasts with bipolar budding have a common ancestry and have therefore classified the sporogenous genera in a single family, subfamily or tribe. Nevertheless, there are marked differences between these genera, in the mode of ascus formation, in the shape of the spores, and in the composition of the cell wall.

Streiblová and Beran (1963) and Streiblová et al. (1964) were the first to

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draw attention to the peculiar features that distinguish bipolar from multilateral budding. They studied bipolar budding in the genera *Saccharomycodes*, *Hanseniaspora*, *Kloeckera* and *Nadsonia*, and described the concentric ridges left by successive buds at each pole as multiple scars.

The present paper describes a comparative electron microscopic study of bipolar budding in species of the genera *Saccharomycodes*, *Hanseniaspora*, *Wickerhamia*, *Nadsonia* and *Schizoblastosporion*, giving special attention to the cell wall and its formation.

MATERIAL AND METHODS

The following strains were studied:

Saccharomycodes ludwigii Hansen, CBS 5929;

Hanseniaspora uvarum (Niehaus) Shehata, Mrak et Phaff, CBS 5973;

Wickerhamia fluorescens Soneda, CBS 4565;

Nadsonia elongata Konokotina, CBS 2593 and 2595;

Schizoblastosporion starkeyi-henricii Ciferri, CBS 2159.

The yeasts were grown in shaken malt-extract cultures for 16–24 hr at 25 C, with the exception of the strains of *Nadsonia elongata* which were grown at 15 C. After washing with water, the cells were fixed with 1.5% aqueous KMnO_4 for 20 min at room temperature, washed with water, and suspended in agar. During dehydration through an ethanol series, the material was poststained with a saturated solution of uranyl acetate in 100% ethanol. The specimens were embedded in Epon 812. Some of the sections were poststained with lead citrate (Reynolds, 1963).

RESULTS

Bud formation is very similar for each yeast of the genera *Saccharomycodes*, *Hanseniaspora* and *Wickerhamia*, but differs for *Nadsonia elongata* and *Schizoblastosporion starkeyi-henricii* which will be treated separately.

In *S. ludwigii*, *H. uvarum* and *W. fluorescens* the first bud on a new cell is formed at the opposite end from the birth scar with a wide isthmus between mother cell and bud (Figs. 1a and 2). The wall of the bud arises from under the mother-cell wall, the latter lying with a tapered end against the former. In *W. fluorescens* the wall of the first bud within the mother cell is lighter than that of the mother cell, whilst in *S. ludwigii* and *H. uvarum* no corresponding difference in electron-density is detectable. After nuclear division a light primary wall is formed centripetally in the isthmus (Figs. 1b and 3). Later this wall thickens

Fig. 1. Diagram of bud formation in *S. ludwigii* and *W. fluorescens* (for description see text).

Fig. 2. Budding cell of *S. ludwigii*. The wall of the bud (B) arises from under the wall of the mother cell (M), and a dark line (arrow) separates both walls.

Fig. 3. Budding cell of *W. fluorescens*. A light primary layer has been formed in the isthmus between mother cell (M) and bud (B).

Fig. 4. A complete cross wall in a budding cell of *W. fluorescens* (M = mother cell, B = bud).

Fig. 5. Section through a cross wall of *S. ludwigii* which in other sections of the same series was still incomplete. The light primary layer has already thickened with darker material.

Fig. 6. Bud scar of *W. fluorescens* showing the light primary layer (PL) left on the plug of the scar. The scar edge is two-layered with a light inner layer.

with darker material (Figs. 1c and 4), but in *S. ludwigii* darker material is present even before the light layer closes the aperture between bud and mother cell (Fig. 5). The mother and daughter cells separate along the primary wall. In *S. ludwigii* and *W. fluorescens* remnants of this wall remain attached to the plug on the mother cell (Figs. 1d and 6); whilst as the bud in *H. uvarum* separates, the primary wall disappears leaving no trace on the plug. The ridge of the first bud scar is composed of the edges of the walls of mother and daughter cells. In *W. fluorescens* the ridge consists of a darker and a lighter part. The ridge of the birth scar on the daughter cell is made from the daughter cell wall only and is less distinct than the bud scar.

After the first, buds are formed at each pole of the cell from the plugs of birth and bud scars, with an isthmus between mother cell and bud at some distance

Fig. 7. Plug of a cell of *S. ludwigii* about to open up. The plug is cone-shaped; at the top a small crack in the wall is visible.

Fig. 8. Plug of a cell of *S. ludwigii* in which a thickened dark layer is present between plasmalemma and the light layer of the wall at the fracture.

Fig. 9. Budding from the plug in *W. fluorescens*. Between the scar ridge of the first bud (SR) and the wall of the new bud, the light primary layer (PL) of the first cross wall is still present.

from the former scar edge. The wall of each of these buds arises from under the wall of the plug, the latter lying tapered along the wall of the bud and in sections separated from it by a dark line (Fig. 1e), which is, however, not always distinct.

Preparations of *S. ludwigii* show a small crack in the centre of the plug in the dark outer layer of the wall; and this crack is interpreted as an early stage of bud formation (Fig. 7). The dark layer between the plasmalemma and the light layer of the wall is notably thicker at the fracture (Fig. 8).

Cross-wall formation and separation of the cells occur as described for the first bud. The primary cross wall is formed at the transition of the plug and the wall of the bud. The wall between the two scar ridges consists of the plug and, for *S. ludwigii* and *W. fluorescens* is often still covered by the light primary layer (Figs. 1f and 9).

The number of scar ridges at either apex suggests that buds are formed alternately at each pole (Fig. 10).

In sections of *N. elongata* and *Sch. starkeyi-henricii* no separating line is visible between the walls of the first bud formed at the pole opposite to the birth scar and the wall of the mother cell. A cross wall is formed centripetally in the wide isthmus between both cells. This wall is, from the beginning of its formation, of darker material with a thin, light layer in the centre (Fig. 11). The cells separate at the light layer which leaves no visible residue (Fig. 12). The edges of

Fig. 10. Section through a cell of *H. uvarum* with four scar ridges at the pole where a new bud develops, and four at the opposite pole (arrows).

Fig. 11. Partly formed cross wall between mother cell and bud in *N. elongata* (Strain CBS 2593). In the centre of the cross wall a thin light layer is visible.
Fig. 12. Separating cells of *N. elongata*.

Fig. 13. Cell of *N. elongata* (M) with one partly separated daughter cell (D) and one growing bud (B) on the same plug. The ridge of the first scar is visible (arrowed).

the bud and birth scars are narrow, with little difference between them, the edge of the scar on the mother cell protruding more than that on the daughter cell. The outer layer of the plug wall is not as dark as that of the cell wall and appears more fuzzy. Very often the plug bulges and widens considerably.

In *N. elongata* a second bud at a given pole is formed either from the middle of the plug or from its side. In the latter case the new scar ridge is placed asymmetrically on the cell (Fig. 13). Third and fourth buds may be formed from different parts of the original plug, new ones being formed before the older buds are split off. Occasionally, from the free part of a birth-scar plug a new bud arises while the mother cell is still partly attached to the other part of the plug. The formation of more than one bud from a single plug distinguishes this type of bipolar budding from that in *H. uvarum*, *S. ludwigii* and *W. fluorescens*. However, the latter type also occurs in *N. elongata*.

In *Sch. starkeyi-henricii* a slightly narrowed area occurs between the mother cell and the bud formed at the side where a plug is present. In this area, presumably consisting of the original plug, the wall is very fuzzy. The cross wall is

Fig. 14. Cell of *Sch. starkeyi-henricii* with a beginning cross wall (arrowed). The nuclear division is still incomplete.

Fig. 15. Mother cell (M) and bud (B) of *Sch. starkeyi-henricii* with a complete cross wall. The scar ridge (SR) of a former bud lies close to the cross wall.

laid down close to the former scar edge (Figs. 14 and 15) leaving part of the fuzzy wall on the bud. This type of budding differs from that of the first three species discussed in that the distance between two scar ridges is too small to be considered as the entire original plug.

In *N. elongata* and *Sch. starkeyi-henricii* buds are not formed alternately at each pole.

DISCUSSION

Bud formation shows several features which vary among the yeasts, such as the site of budding on the cell, the way in which the bud emerges from the mother cell, the formation of the cross wall and the separation of the cells.

In budding confined to the two poles of the cell the scar plugs of former buds are involved with each new bud, unlike multilateral budding where buds are never formed at the same site.

In the genera *Saccharomyces*, *Hanseniaspora* and *Wickerhamia* a dark line was usually observed separating the wall of the mother cell, including the plug, from the growing bud. *W. fluorescens* was the only yeast examined in which the part of the wall of the first bud within the mother cell was lighter than the wall of the mother cell as has also been observed in the buds of *Saccharomyces cerevisiae*. The emergence of the wall of the bud from under the wall of the mother cell also occurs in *Sacch. cerevisiae*. However, Streiblová and Beran (1963) and Streiblová et al. (1964) observed a difference between *Sacch. cerevisiae* and *S. ludwigii* by fluorescence microscopy. Primulin-stained preparations of *Sacch. cerevisiae* showed a fluorescing collar between mother cell and bud. Such a collar was not seen in *S. ludwigii*, nor did it appear in electron micrographs of carbon replicas. The present observations do not explain this difference.

In *S. ludwigii* a crack was observed in the outer layer of the wall at the pole where a new bud was due. Opening up of the wall coincided with the formation of a thickened dark layer between plasmalemma and the light layer of the cell wall. Vitols et al. (1961) and Streiblová (1968) considered that this layer was part of the cell wall. Nečas and Kopecká (1969) found that this layer consisted of non-etchable material in freeze-etched yeast protoplasts.

In the formation of the light primary layer which later thickens and along which mother cell and bud are separated, the species *W. fluorescens*, *S. ludwigii* and *H. uvarum* conform with *Sacch. cerevisiae* (Sentandreu and Northcote, 1969) and *Endomycopsis platypodis* (Kreger-van Rij and Veenhuis, 1969). As in the two latter species, in *S. ludwigii* and *W. fluorescens* the remains of the primary layer could be observed on the plug of the mother cell.

From the above results it appears that bipolar budding shows different features

among the yeasts of the genera examined. Since these genera differ also in other respects, it is doubtful whether the character of bipolar budding indicates a close relationship, or whether it serves merely as a convenient means of recognizing a group of yeasts.

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