Some Aspects of Cell Division in *Saccharomyces cerevisiae*

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SUMMARY: Two types of scar are formed on yeast cells as a result of the division process: a single 'birth scar' marking the point at which the cell was joined to the parent, and a variable number of 'bud scars' marking the points at which buds have been formed. A regular sequence of bud formations occurs. Cultures grown from the first and last buds appear to be identical.

Interest in the process of budding in yeast cells has centred round the behaviour of cell inclusions. Nevertheless, reproduction by the formation of buds is a cytokinetic process of some interest to cytologists. With a view to extending knowledge of this subject the cell division of a strain of *Saccharomyces cerevisiae* was investigated.

METHODS

The strain of yeast used was D.C.L. baking yeast, a single-cell strain identified as *Saccharomyces cerevisiae* Hansen var. *ellipsoideus*. This was maintained at 30° in a 15 % (w/v) aqueous solution of malt extract (final sp.gr. 1050) and transfers made every 48 hr. Observations were made on living and non-living material. Stained material was mounted in Canada balsam after careful dehydration through a series ethanol-water mixtures of increasing ethanol concentration.

The optical system consisted of Zeiss 2 and 4 mm. apochromatic objectives together with $\times 10$ and $\times 20$ Zeiss Compens eyepieces. Illumination was provided by a 80 c.p. Ediswan Pointolite lamp or a high-pressure mercury-vapour lamp in conjunction with a Watson-Conrady condensing lens and a Wratten 38A or an Ilford 601 filter.

Scars on yeast cells

A study of the process of cell division reveals that the disconnexion of a cell from its bud produces characteristic scars on the wall of both cells. In a nutrient medium the scars on a yeast cell (see 'bud scars' below), are angular projections from the cell wall (Pl. 1, fig. 1), but the similarity in refractive index of cell wall and surrounding fluid precludes further study. The necessary difference in refractive index can be obtained by the transfer of single cells on to a somewhat dry film of agar by a micromanipulator. The cell wall then lies in contact with moist air on the side away from the agar and this, possibly combined with a slight drying of the cell surface, reveals the scars in greater detail (Pl. 1, fig. 2). Each scar is seen to consist of a slightly raised circular rim, approximately 2μ in diameter, enclosing a centrally thickened plug. This marks the last point of cytoplasmic connexion between the mother and daughter

cells. The size and shape of each scar corresponds to that of the neck, or channel, which originally joined the two cells.

In mature yeast cells stained by the Newton crystal violet method, after fixation in osmium tetroxide vapour (Baker, 1945), two types of scar can be demonstrated (Pl. 1, fig. 3): a single scar, termed the 'birth scar', marking the point at which the cell, as a bud, was connected with the parent cell; and a varying number of bud scars, which mark the positions at which buds have been formed. One of these is shown in Pl. 1, fig. 3. Another method for the study of scars is plasmolysis of the cells by heating to *c.* 60°. This is a particularly useful technique when microscopic resolution is increased by photographing the cells under monochromatic mercury-violet illumination (4359 Å.) (Pl. 1, fig. 4).

The 'birth scar' is invariably situated at a point central to the long axis of the cell (Pl. 1, figs. 3 and 4), and can be distinguished from bud scars, which it resembles in the unplasmolysed condition, by a resistance to collapse when the cell is fixed and stained (Pl. 1, fig. 3).

Before separation the rims of the two scars of both cells are believed to lie in contact, strengthening the bond between the cells. When, however, the cytoplasmic connexion is lost, the independent increase in volume of the daughter cell results in a stretching of its birth scar; the shearing action between the two scars then causes the mechanical connexion to break. One consequence is that the birth scar has a greater diameter (3 μ) than the other scars. Any bud scar produced before the maximum size of the cell has been attained will also be stretched, though not to the same extent as the birth scar (Pl. 1, fig. 4).

Site of bud formation

By means of a micromanipulator, the successive buds formed from single cells growing on malt-extract agar were removed as soon as they became free of the parent cell. By using the position of undetached buds as topographical markers the sites of origin of buds one to eight was noted. The points of origin, reduced to two dimensions, are shown in Pl. 1, fig. 5. This positioning was found to be constant in the fifteen cells observed. A bud was never seen to form at the site of a scar.

Effects of age on the cell

A yeast cell was observed to bud twenty-three times. Microscopically, cultures grown from the first and last buds were identical. In the two cases examined, the yield of pressed yeast from a fixed amount of molasses and the dough-raising capacity of the yeast produced were the same for cultures grown from the first and the fifteenth buds of the same cell.

Any tendency to degeneration comparable with that described by Schouten (1935) in yeast was not observed.

The author thanks the Directors of the Distillers Company Ltd. for permission to publish this paper.

REFERENCES

- BAKER, J. R. (1945). *Cytological Technique*, 2nd ed. London: Methuen and Co. Ltd.
SCHOUTEN, S. L. (1985). Altersdegeneration an Hefezellen. *Arch. exp. Zellforsch.* 17, 451.

EXPLANATION OF PLATE

- Fig. 1. Scars (marked *X*) on the cell wall of a yeast cell growing in malt-wort. Wratten 38 A filter; $\times 3600$.
Fig. 2. Scars (marked *X*) on the cell wall of a cell growing on the surface of malt-agar. Wratten 38 A filter; $\times 1600$.
Fig. 3. Birth scar (*A*) and bud scar (*B*) on the cell wall of a yeast cell. The bud scar has collapsed whilst the birth scar has remained intact. Newton crystal-violet method. Wratten 38 A filter; $\times 3600$.
Fig. 4. Cells plasmolysed by heat. The birth scar (*A*) and first bud scar (*C*) are of a greater diameter than the other scars (*B*). Photographed using mercury violet illumination. Ilford 601 filter; $\times 3600$.
Fig. 5. The positions of origin of buds one to eight. The birth scar (*A'*) is at the pole of the long axis of the cell; $\times 8500$.

(Received 28 May 1949)

Figs. 1-4

Fig. 5