Ultrastructure of the Surface of Multiple Scars in *Saccharomyces ludwigii*

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ABSTRACT

The use of induced primuline fluorescence led to the discovery of a new type of yeast scars (multiple scars) in the genera *Kloeckera, Saccharomyces, Nadsonia* and *Hanseniaspora*. The structure and ultrastructure of their surface was studied by electron microscopy, using carbon replicas and isolated cell walls.

In proliferating yeasts, permanent structures are formed both on mother and daughter cells as a result of the processes accompanying multiplication. The origin of these structures, which are known as scars, has been studied in detail in budding yeasts (Nickerson, 1963). The structure of the cell wall and scars of budding yeasts has been studied mainly by electron microscopy (Barton, 1950; Northcote & Horne, 1952; Houwink & Kreger, 1953; Bartholomew & Mittwer, 1953; Agar & Douglas, 1955; Bartholomew & Levin, 1955; Bradley, 1957).

Streiblová and Beran (1963a, b) described a new fluorescence staining method, by means of which scars on the cell surface can be observed directly in the fluorescence microscope. This technique led to the discovery of a new type of scar in the group of apiculate yeasts, which the authors termed "multiple" scars. The present paper reports the results of study of the ultrastructure of the surface of multiple scars in *Saccharomyces ludwigii*, using carbon replicas and shadow isolated cell walls.

MATERIALS AND METHODS

The test yeast, *Saccharomyces ludwigii* 371 (National Collection of Czechoslovak Academy of Sciences), was cultivated on Olson and Johnson's medium (1949) on a rotary shaker at 30°C. Cell walls were isolated by the technique of Mendoza and Villanueva (1963). When preparing carbon replicas, the washed cell suspension was first applied to a formvar film. The cells were then coated with a layer of carbon 500 Å thick. The formvar film was dissolved with chloroform and the cells were removed in a mixture of concentrated hydrochloric acid and glacial acetic acid (20%) under boiling point for five minutes. After washing in dilute alcohol, the carbon replicas were shadowed with chromium or W₂O at an angle of 30°. Metallic shadow-casting of isolated cell walls were prepared in a similar manner. A cell wall suspension was applied to a formvar film and chromium shadowed at an angle of 30°. The specimens were studied in a BS 413 electron microscope (Institute of Scientific Apparatus, Czechoslovak Academy of Sciences). The fluorescence microscopy technique was described in previous papers (Streiblová & Beran, 1963a, b).

RESULTS AND DISCUSSION

In apiculate yeasts, daughter cells are formed at both poles of the mother cell. As
Fig. 1. Fluorescence photomicrograph of *Saccharomyces ludwigii* cells (× 2,000). Photograph by J. Fiala

Fig. 2. Carbon replica of apiculate process of *Saccharomyces ludwigii* cell. (× 12,000)