

NOTES

Cell Wall Residues in Yeast Protoplast Preparations

J. S. D. BACON, D. JONES, AND P. OTTOLENGHI

*Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen, Scotland, and
Carlsberg Laboratorium, 2500 Copenhagen, Valby, Denmark*

Received for publication 17 April 1969

Protoplast preparations made from *Saccharomyces cerevisiae* by prolonged treatment with snail digestive juice contained fibrils and chitinous bud-scar residues from the original cell wall.

Ottolenghi (4) has asked whether yeast cells are converted to true protoplasts by the action of snail digestive juice. Viewed by phase-contrast, some cells still possessed thin membranes, which were shed under osmotic stress. Remains of bud scars could also be seen, occasionally constricting a "protoplast" to a dumbbell shape. We have extended these observations.

A protoplast preparation was made from a diploid strain of *Saccharomyces* (4) grown at 30 C on a yeast extract-peptone medium containing 2% lactate. Cells were harvested after 36 hr while still growing, washed with 0.1 M mercaptoethanol, and suspended in 40 ml of 0.6 M KCl containing 0.5 ml of snail juice (Industrie Biologique Française). After 20 hr at 33 C, the protoplasts were lysed. The residues were washed five times with water, suspended in 8 M urea, and stirred for 15 hr at 22 C. On centrifuging at $10,000 \times g$, some debris, unrecognizable under phase-contrast, floated. The precipitate was washed repeatedly with 8 M urea and with water; electron microscopy (Fig. 1-3) showed it to consist largely of bud-scar residues. After freeze-drying, it gave the infrared spectrum of a mixture of chitin and β (1 \rightarrow 3) glucan, the former predominating, with traces of mannan and ester carbonyl.

Another preparation was made by using 1.0 M sorbitol instead of 0.6 M KCl. After 20 hr of incubation no cells were left, but the protoplasts were accompanied by some cellular debris, including free bud scars. These contaminants were removed by washing the protoplasts repeatedly in 1.0 M sorbitol. They were then lysed by shaking in chloroform-water (1:1, v/v). On centrifuging, much debris accumulated in the interface; above it was a layer rich in bud-scar

residues. Repetition of chloroform treatment yielded additional material rich in bud scars. This was then centrifuged in cesium chloride gradients, and two fractions were selected. One fraction had an infrared spectrum similar to that of the sample prepared in 0.6 M KCl, except that there was more mannan, perhaps 20%; electron microscopy (Fig. 4) confirmed that this fraction consisted mainly of bud-scar residues. The second fraction showed, under phase contrast, mainly groups of bud scars attached by thin membranes (see Fig. 5 and 6). The infrared spectrum indicated mannan, glucan, and high protein which obscured absorption by chitin.

Thus, even after a period of incubation considerably exceeding that normally used, elements of the original wall can be recovered from the "protoplasts." The presence of chitin is consistent with the previously reported association of this substance with bud scars (1). The fibrils (Fig. 1, 3, and 6) recall those seen in regenerating cell walls (3).

We cannot explain the failure of the snail digestive juice to complete the degradation of the chitin and glucan components. Isolated cell walls were completely lysed in 20 hr by snail juice when suspended in water, but the presence of 0.6 M KCl or 1.0 M sorbitol delayed this noticeably. Eddy (2) found that walls lysed more rapidly at low salt concentrations.

With more enzyme and a longer digestion period, bud-scar residues eventually disappear, but it would seem of importance when wall regeneration is being studied, to decide whether the much shorter periods of incubation usually employed do lead to the production of true protoplasts.

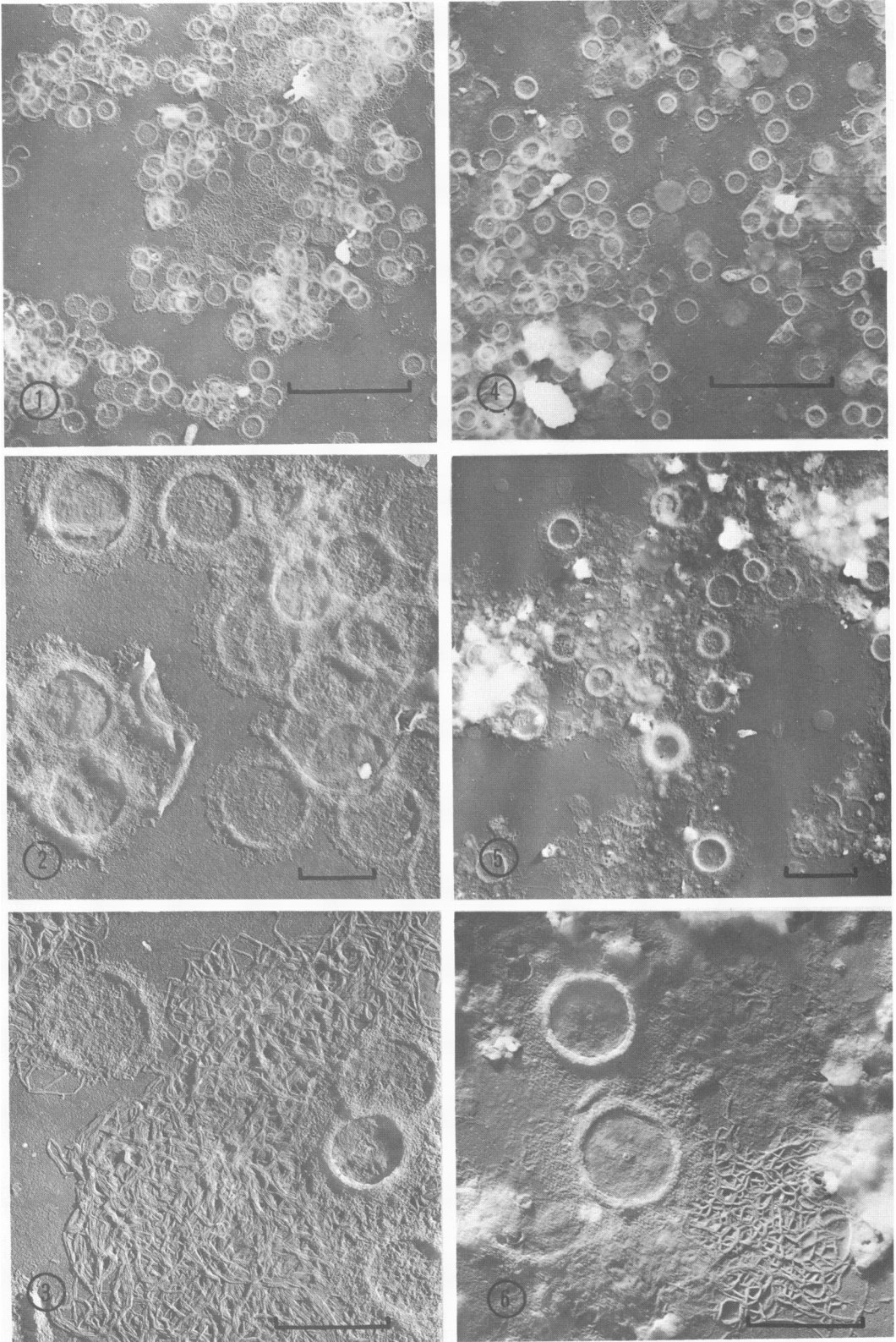


FIG. 1-6. The specimens for electron microscopy were air dried from aqueous suspension on carbon-coated support grids and shadowed with nickel-palladium at an angle of $\arctan \frac{1}{3}$. Markers indicate 5 μm (Fig. 1 and 4), 2 μm (Fig. 5), and 1 μm (Fig. 2, 3, 6). Fig. 1-3. Insoluble residues from yeast protoplasts prepared in the presence of KCl. Fig. 4-6. Insoluble residues from yeast protoplasts prepared in the presence of sorbitol.

This study was supported by U.S. Public Health Service grant GM 12180 from the National Institute of General Medical Sciences.

We are grateful to V. C. Farmer for the interpretation of the infrared spectra.

LITERATURE CITED

1. Bacon, J. S. D., E. D. Davidson, D. Jones, and I. F. Taylor. 1966. The location of chitin in the yeast cell wall. *Biochem. J.* **101**:36C-38C.
2. Eddy, A. A. 1958. The structure of the yeast cell wall. II. Degradative studies with enzymes. *Proc. Roy. Soc. (London) Ser. B.* **149**:425-440.
3. Eddy, A. A., and D. H. Williamson. 1959. Formation of aberrant cell walls and of spores by the growing yeast protoplast. *Nature* **183**:1101-1104.
4. Ottolenghi, P. 1966. Do yeasts form true protoplasts? *C. R. Trav. Lab. Carlsberg* **35**:363-368.