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Autoradiographic Study of Mannan Incorporation into the Growing Cell Walls of *Saccharomyces cerevisiae*

V. FARKAŠ, J. KOVAŘÍK, A. KOŠINOVÁ, AND Š. BAUER

Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Czechoslovakia

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The incorporation of mannan labeled by D-[1-^{3}H]mannose into the growing cell walls of *Saccharomyces cerevisiae* has been followed by means of high-resolution autoradiography. The results show that, in the very first stage of budding, when the shape of the bud is nearly spherical, the new mannan is incorporated uniformly over the whole surface of the emerging bud. Later on, as the bud growth proceeds, the polarized tip growth predominates. In the maturation phase of bud growth, when the size of the bud is about two-thirds that of the mother cell, again a considerable incorporation of the labeled substrate over the whole bud surface was observed. The cell wall of the mother cell remained largely unlabeled. These facts indicate that the ellipsoidal shape of the cell walls in *S. cerevisiae* is the result of the combination of polarized tip growth and nonpolarized spherical extension.

The mode of deposition of new cell wall material in budding yeasts has been the subject of several studies where different techniques were applied. Chung et al. (4) used fluorescent antibodies prepared against whole cells of *Saccharomyces cerevisiae* and concluded that the new cell wall material, presumably mannan-protein, is layered as an annular band at the base of the bud. Contrary to these results, Tkacz (Diss. Abstr. B. **32**:3535-B, 1971), by using fluoresceine-labeled concanavaline A, showed that mannan is incorporated mainly at the distal tip of the bud. The same author (Diss. Abstr. B. **32**:3535-B, 1971), by using fluorescent antibodies prepared against the proteinaceous part of the invertase molecule, an enzyme which is located in the yeast cell wall, found that invertase is, during budding, deposited predominantly into the cell wall of the growing bud.

The sites of deposition of new cell wall material in *S. cerevisiae* were also studied autoradiographically by Johnson and Gibson (9), who followed the incorporation of D-[^{3}H]glucose into the alkali-insoluble cell wall material (presumably glucan). Most of the newly synthesized material was found to be located at the poles of the buds, indicating tip growth.

The sensitivity of the autoradiographic method can be effectively exploited when selective radioactive labeling of the particular cell wall components is achieved. This work represents an attempt to determine the sites of deposition of mannan in *S. cerevisiae* by following the incorporation of the polysaccharide labeled by D-[1-^{3}H]mannose into the growing cell walls. For this purpose, the growth conditions permitting the selective labeling of mannan by radioactive mannose were established.

MATERIALS AND METHODS

Yeast. *S. cerevisiae* strain CCY 21-4-13, whose phenotype was described by Kocková-Kratochvílová et al. (11), was grown at 28 C on a shaker in a synthetic medium containing D-galactose, 20 g, and yeast nitrogen base (Difco Laboratories Inc., Detroit, Mich.) 6.7 g/liter. The cells from the logarithmic phase of growth were used throughout.

Radiochemicals. D-[1-^{14}C]mannose (3.7 mCi/mol) was purchased from the Radiochemical Centre, Amersham, England. D-[1-^{3}H]mannose (3.3 Ci/mmol) was the product of Amersham-Searle Co., Arlington Heights, Ill.

Labeling of the cell wall mannan by radioactive mannose. Cells (5.0×10^7) were suspended in 10 ml of the fresh galactose growth medium containing 2 μCi of D-[1-^{14}C]mannose (1.85 μmol) and incubated for 2 h at 30 C. The cells were then collected by centrifugation, washed three times with cold distilled water, and disintegrated by shaking with a thick suspension of glass beads (size 0.2 mm) at 50 cycles/s for 90 s. Practically complete disintegration of the cells was achieved. The cell walls were then isolated, washed six times with cold water, and hydrolyzed in 1.25 M H_2SO_4 in a sealed tube at 100 C for 8 h. The hydrolysate was neutralized with solid $BaCO_3$ and desalted by passing through a column of Dowex 50 WX 2 (H^+ form). The eluate was concentrated under reduced pressure and applied on Whatman no. 1 chromatographic paper. Descending chromatography was carried out in the solvent system, ethyl acetate-

pyridine-water (8:2:1 vol/vol/vol), at room temperature for 48 h. The chromatogram was dried and cut to 1-cm strips, and the radioactivity was measured in a liquid scintillation counter.

Labeling of the cell walls for autoradiography. Cells (2.6×10^7) were suspended in 1 ml of the fresh growth medium containing 120 μCi of D-[1-^{3}H]mannose (specific activity 3.3 Ci/mmol) and incubated at 30 C for 5 min. The cells were than rapidly centrifuged, and in a portion of the cells the growth was arrested by the addition of 0.1 ml of 36% (wt/vol) formaldehyde to 1 ml of cell suspension. The rest of the cells were washed twice with nonradioactive growth medium and grown in the same medium for different time intervals (10 to 45 min). The cell walls were then isolated as described above and prepared for high-resolution autoradiography.

Autoradiographic technique. Washed radioactive cell walls were transferred to Formvar-coated grids and shadowed with carbon. The specimens were then coated with a 5- to 15-nm-thick layer of Ilford-4 fine nuclear emulsion by using the procedure described by Caro et al. (3). The quality of the emulsion monolayer was checked by electron microscope. After a 26-day exposure time, the autoradiographs were developed with Atomal and examined with JEM-7 and Tesla BS 242A electron microscopes.

RESULTS AND DISCUSSION

To follow the deposition of the cell wall mannan by autoradiographic technique, the conditions for the selective labeling of this polysaccharide by radioactive mannose had to be found.

Since the transport of mannose into the yeast cells is greatly inhibited by glucose (12), we used defined galactose growth medium for the cultivation of our yeast. The galactose is transported by an inducible system, different from that for glucose and mannose (12, 16), and therefore it interferes in the mannose transport much less than glucose. For selectivity of the mannan labeling, it was also necessary to avoid the conversion of exogenously added radioactive mannose to glucose via the phosphomannose-isomerase reaction. We have found that, when the concentration of mannose in the medium was very low, less than 10^{-3} M, in comparison to a 0.11 M concentration of galactose, the label from the exogenously added mannose was incorporated exclusively into the cell wall mannan in our yeast strain. By increasing the mannose to galactose ratio in the medium, an increased portion of the label was found also in the cell wall glucan. The selectivity of the mannan labeling under conditions of our experiment is well demonstrated in Fig. 1. This fact suggests that the exogenously added mannose was not diverted extensively into the glycolytic pathway, but rather was directly metabolized via mannose-1-phosphate to guanosine 5'-diphosphate-mannose and was finally incorporated into the mannan.

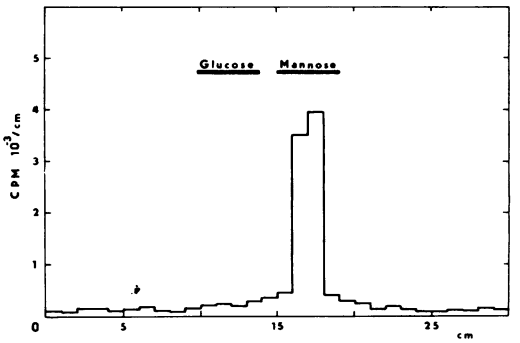


FIG. 1. *Paper chromatographic resolution of hydrolysate of the cell walls of S. cerevisiae incubated with* D-[*1*-^{14}C]*mannose. The details of the experiment are described in the Materials and Methods section.*

The high-resolution autoradiography of the cell walls labeled with D-[1-^{3}H]mannose showed that the mode of growth of *S. cerevisiae* cell walls depends largely on the position of the cell in the cell cycle. In the small, nearly spherical buds, no distinct site of label incorporation could be recognized. The label was uniformly spread over the whole bud surface (Fig. 2, 4). In greater buds the polarization of growth at the bud tip became clearly visible (Fig. 3, 4). When the size of the bud reached about two-thirds that of the mother cell, a more or less uniformly disperse pattern of label incorporation prevailed again (Fig. 5).

The cell walls of the mother cells were in all cases labeled only slightly above the background, indicating that, in these cells, the incorporation of new mannan molecules into the cell walls practically did not occur.

Two phenomena were observed when the cells, after a 5-min radioactive pulse, were submitted to a "cold chase" with nonradioactive medium: (i) dilution of the pre-existing label by nonradioactive material, and (ii) the formation of unlabeled polar caps (Fig. 6 and 7). This label pattern indicates that the incorporation of new mannan molecules into the growing cell walls proceeds predominantly at the tips of the buds. Besides that, as the cell wall extension proceeds, the new mannan molecules are incorporated also over the whole surface of the cell wall in accordance with the rate of growth at the given site. The observed mode of growth in *S. cerevisiae* differs from that observed in hyphae (1, 6) or in the fission yeast, *Schizosaccharomyces pombe* (7, 14, 18), in that, besides the tip growth, the non-polarized or spherical extension also plays an important role. The ellipsoidal shape of the *S. cerevisiae* cells is thus

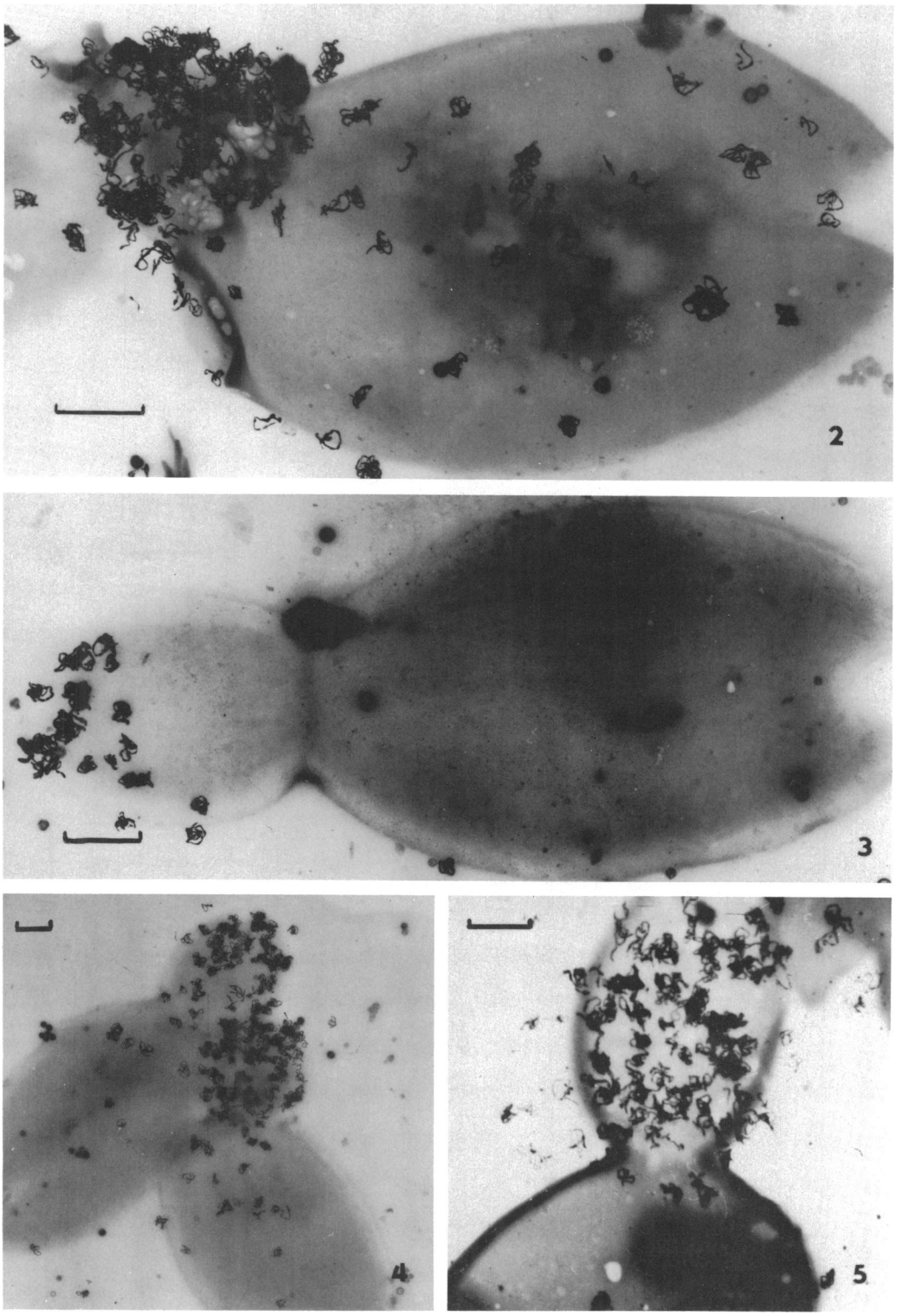
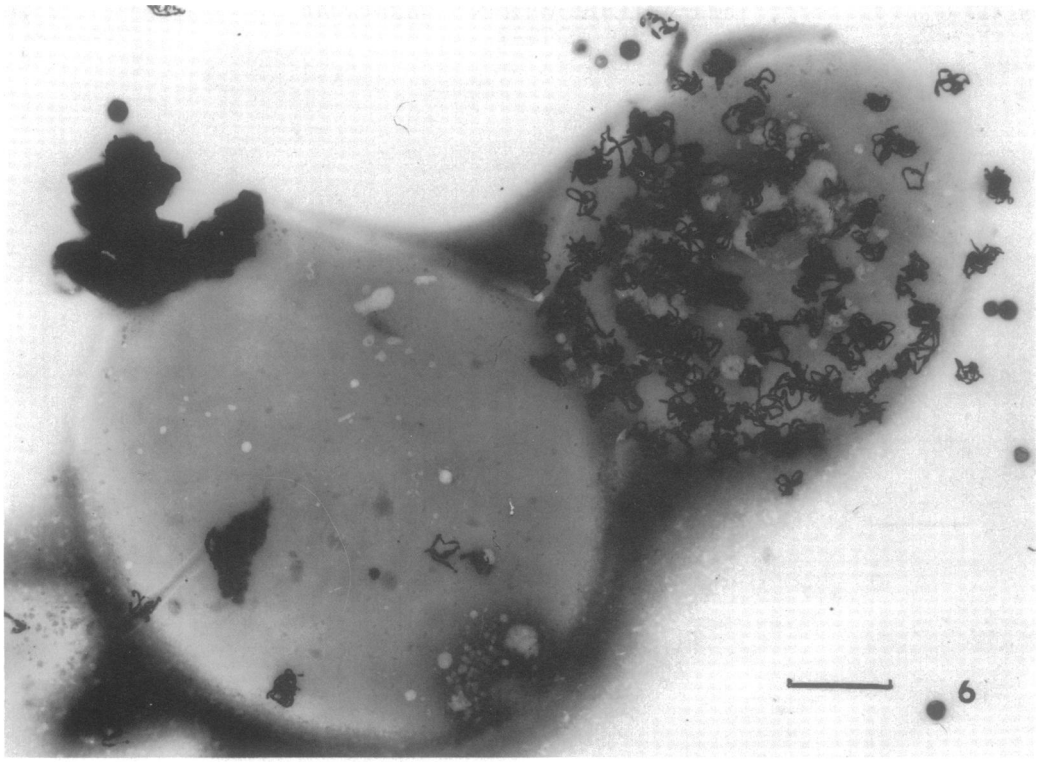


FIG. 2 to 5. *Autoradiographs of the isolated cell walls of S. cerevisiae. The yeast was incubated for 5 min with* D-[1-^{3}H]*mannose before disintegration. The bar represents 1 μm.*



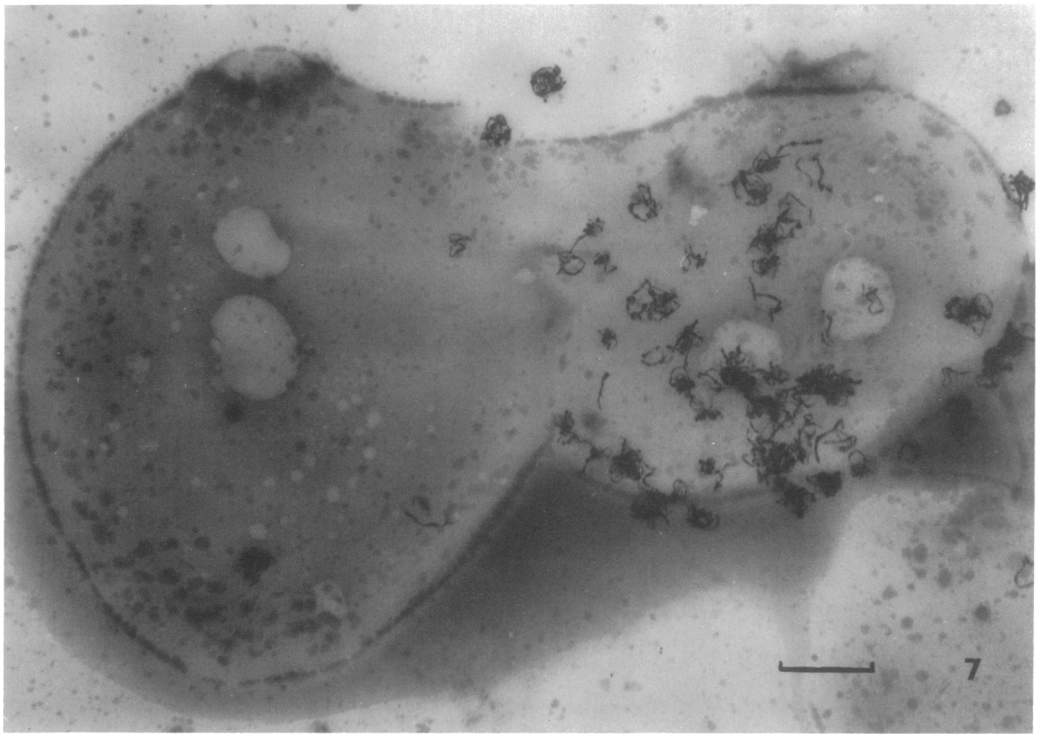


FIG. 6. *Autoradiograph of the cell walls of S. cerevisiae incubated for 5 min with* D-[1-^{3}H]*mannose followed by 30-min incubation in the nonradioactive growth medium. The bar represents 1 μm.*

FIG. 7. *Autoradiograph of the cell walls of S. cerevisiae incubated for 5 min with* D-[1-^{3}H]*mannose followed by 45-min incubation in the nonradioactive medium. The bar represents 1 μm.*

acquired by a combination of these two modes of wall growth. The tip growth apparently predominates in the phase beginning shortly after bud emergence and represents the factor responsible for cell elongation. During maturation of the bud, the cell wall expands by almost uniform insertion of the new building material over the entire surface of the cell.

To sustain the internal turgor pressure, the growth of the yeast cell wall must represent a well-balanced equilibrium between the degrading and building processes. The tip growth requires weakening of the pre-existing cell wall at one site only (2). The enzymes suggested to be involved in this process are the protein disulfide reductase (15) and glucanase (8). The latter enzyme was reported by Matile et al. (13) to be located in the vesicles derived from the endoplasmic reticulum. The vesicles also contained a considerable amount of mannan protein, presumably the building precursors of the cell wall matrix. Indeed, an accumulation of such vesicles at the growth sites in *S. cerevisiae* has been observed by different authors (5, 13, 17). In the very first, as well as in the maturation phases of growth the action of these enzymes may be spread all over the surface of the cell, which consequently leads to a uniform extension of the cell wall (10).

Our results obtained by means of high-resolution autoradiography are compatible with those of Tkacz (Diss. Abstr. B. **32**:3535-B, 1971) obtained by using concanavalin A-fluoresceine labeling of the cell wall mannan. In addition to that, the results of an autoradiographic study made by Johnson and Gibson (9) show that the mode of deposition of another cell wall component, namely glucan, in *S. cerevisiae* is very similar to the mode of deposition of mannan observed in the present study. The apparent contradiction to the results of Chung et al. (4) can hardly be explained on the basis of the different selectivity of the labeling, since in their experiments, as in our case, the mannan was the labeled cell wall constituent.

APPENDIX

As this paper was already mailed for publication, a study of mannan insertion into the growing cell walls of *S. cerevisiae* by using fluorescein-conjugated concanavalin A to label the mannan (J. S. Tkacz and J. O. Lampen, J. Gen. Microbiol. **72**:243-247, 1972) became available to us. The results of Tkacz and Lampen show that the new mannan is deposited almost entirely into the wall which surrounds the bud and that the distal tip of the growing bud is the major site at which the deposition occurs.

Our results presented here, obtained by means of high-resolution autoradiography, confirm in full the findings of these authors and, in addition to that, show that the uniform deposition of the new cell wall material over the whole bud surface is the factor which participates in the formation of the ellipsoidal yeast cell wall.

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LITERATURE CITED

1. Bartnicki-Garcia, S., and E. Lippman. 1969. Fungal morphogenesis: cell wall construction in *Mucor rouxii*. Science **165**:302-304.
2. Burnett, J. H. 1968. Fundamentals of mycology. Edward Arnold Publishers, Ltd., London.
3. Caro, L. G., R. P. van Tubergen, and J. A. Kolb. 1962. High resolution autoradiography. J. Cell Biol. **15**:173-179.
4. Chung, K. L., R. Z. Hawirko, and P. K. Isaac. 1965. Cell wall replication in *Saccharomyces cerevisiae*. Can. J. Microbiol. **11**:953-957.
5. Cortat, M., P. Matile, and A. Wiemken. 1972. Isolation of glucanase-containing vesicles from budding yeast. Arch. Mikrobiol. **82**:189-205.
6. Gooday, G. W. 1971. An autoradiographic study of hyphal growth in some fungi. J. Gen. Microbiol. **67**:125-133.
7. Johnson, B. F. 1965. Autoradiographic analysis of regional cell wall growth of yeasts. *Schizosaccharomyces pombe*. Exp. Cell Res. **39**:613-624.
8. Johnson, B. F. 1968. Lysis of yeast cell walls induced by 2-deoxyglucose at their sites of glucan synthesis. J. Bacteriol. **45**:1169-1172.
9. Johnson, B. F., and E. J. Gibson. 1966. Autoradiographic analysis of regional cell wall growth of yeasts. III. *Saccharomyces cerevisiae*. Exp. Cell Res. **41**:580-591.
10. Kanetsuna, F., L. M. Carbonell, I. Azuma, and Y. Yamamura. 1972. Biochemical studies on the thermal dimorphism of *Paracoccidioides brasiliensis*. J. Bacteriol. **110**:208-218.
11. Kocková-Kratochvílová, A., L. Sedlárová, A. Vojtková-Lepšíková, and J. Šandula. 1970. Taxometric study of the genus Saccharomyces (Meyen) Reess. *Saccharomyces cerevisiae* Hansen and related species, part II. *In* Y. Kolek (ed.), Biologické práce XVI/5. Publishing House of the Slovak Academy of Sciences, Bratislava.
12. Kotyk, A. 1967. Properties of the sugar carrier in baker's yeast. II. Specificity of transport. Folia Microbiol. (Prague) **12**:121-131.
13. Matile, P., M. Cortat, A. Wiemken, and A. Frey-Wyssling. 1971. Isolation of glucanase-containing particles from budding *Saccharomyces cerevisiae*. Proc. Nat. Acad. Sci. U.S.A. **68**:636-640.
14. May, J. W. 1962. Sites of cell wall extension demonstrated by the use of fluorescent antibody. Exp. Cell Res. **21**:170-172.
15. Nickerson, W. J., and G. Falcone. 1956. Identification of protein disulfide reductase as a cellular division enzyme in yeasts. Science **124**:722-723.
16. Robichon-Szulmajster, H. de 1961. Contribution a l'étude génétique et physiologique du métabolisme du galactose chez la levure. Ann. Technol. Agr. **10**:185-256.
17. Sentandreu, R., and D. H. Northcote. 1969. The formation of buds in yeast. J. Gen. Microbiol. **55**:393-398.
18. Streiblová, E., I. Málek, and K. Beran. 1966. Structural changes in the cell wall of *Schizosaccharomyces pombe* during cell division. J. Bacteriol. **91**:428-435.