an enzyme that converts some of the phosphorylated mannotetraose chains to unphosphorylated mannoheptaose chains.

The observation that the mnnl mutant of X2180 has the phenotype of strain 4484-24D raises the question as to whether the latter is a true "wild type" strain or one that has been selected fortuitously during the time it has been under laboratory investigation. While there is no clearcut answer to this question since the exact history of the strain is unknown (see Footnote 2 in Reference 6), we feel it probably does represent a wild phenotype because we have found the same kind of mannan in K. brevis (4) and in several S. cerevisiae brewing strains (5).

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APPENDIX

A COMPARISON OF YEAST MANNAN MUTANTS BY ELECTRON MICROSCOPY

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In correlation with investigations on the properties of yeast mutants with altered mannan (1, 2), a study was conducted of cell wall ultrastructure to determine whether the mutations had visible effects. As a primary function, yeast mannan is believed to provide physical protection for the cell, but mannans also appear to be involved in cell flocculation (3), as well as in the attachment of hydrodases such as invertase to the cell wall (4) and mannan-protein complexes which are involved in sexual agglutination (5). In addition, mannan is known to be the principal antigenic determinant on the yeast cell (6), and therefore it must be located in part on the cell surface. Thus, differences in cell wall appearance resulting from altered mannan structure might well be revealed in the thickness, electron density, or surface features of the wall. To investigate these possibilities, the various yeast mannan mutants have been compared by both thin sectioning and carbon replication techniques.

Growth of Bacteria—The following Saccharomyces cerevisiae strains were supplied by Dr. C. E. Ballou, University of California, Berkeley, California (1, 2): X2180-1A (wild type with respect to mannan, characterized by a mannotetraose side chain unit); X2180-1A-4 (an mnnl mutant lacking the mannotetraose side chain); X2180-1A-5 (an mnn2 mutant lacking almost completely the ability to make side chains); X2180-1R-2 (an mnn3 mutant characterized by a decreased amount of mannotriose side chains); X2180-1B-6 (characterized by loss of mating ability with either haploid mating type); 4484-24D-(a) (wild type strain characterized by mannosylphosphorylmannotriose side chain units) phenotypically equivalent to mnnl mutants; and the diploid strain from a cross between X2180-1A and X2180-1B. All strains were grown in shaking flasks for a period of 12 hours at 30°C in a medium containing 1% yeast extract, 2% Bacto-peptone, and 2% glucose. Cells were harvested by centrifugation and washed twice in Veronal acetate (Kellenberger) buffer, pH 6.0 (7).

Thin Sectioning—In preparation for thin sectioning, the cells were suspended in 2% potassium permanganate at 0°C for 15 min (8), rinsed twice in Kellenberger buffer, and post-stained for 1 hour at 4°C with 0.5% uranyl acetate in the same buffer. The yeast cells were then dehydrated by transfer through solutions of increasing alcohol concentration, and then were embedded in Spurr epoxy resin. Sections, cut with a diamond knife on an LKB ultratome, were mounted on copper grids. After post-staining with lead citrate for 15 min (9), the specimens were examined with an RCA EMU-3C electron microscope.

Carbon Replication—Carbon replicas were made of cells which had been washed twice in Kellenberger buffer and resuspended in 3% glutaraldehyde in the same buffer. Cells were placed on parlodion-coated copper grids and coated with approximately 10 nm of evaporated carbon. The parlodion was then removed with amyl acetate vapor, and the specimen was dissolved in a potassium permanganate-potassium dichromate solution. After washing well with distilled water, the replicas were dried and shadowed using a carbon-platinum pellicle.

In the electron micrographs obtained by the above procedures, no difference could be detected between the appearance of the wild type yeast cell (Fig. 1) and the mannan mutants (Fig. 2). In all strains, the electron-dense outer layer of the cell wall appeared to be intact and of approximately the same thickness. Even in mutant X2180-1A-5 (Fig. 2), which was almost devoid

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Fig. 1 (top). Electron micrographs of *Saccharomyces cerevisiae* X2180-1A, in (A) thin section at 31,000 magnification, and (B) carbon replica at 14,000 magnification.

Fig. 2 (bottom). Electron micrographs of *Saccharomyces cerevisiae* X2180-1A-5, a *mnn5* mutant, in (A) thin section at 32,000 magnification, and (B) carbon replica at 13,000 magnification.
of mannan side chains but contained a normal amount of linear polysaccharide, the cell wall retained its usual thickness and electron opacity. Mutant X2180-1B-6 (not shown), which lacked the ability to mate with either haploid mating type, also failed to exhibit any distinguishing surface feature that could be correlated with its behavior. Significant differences in cell surface characteristics were also absent in the carbon replicas. Thus, the kinds of mannan mutants studied do not result in changes in the cell wall thickness or surface characteristics which can be detected by the methods employed in this study.

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