- Manners, D. J. & Marshall, J. J. (1969). *J. Inst. Brew.* **75**, 550.
 Manners, D. J., Marshall, J. J. & Yellowlees, D. (1970). *Biochem. J.* **116**, 539.
 Manners, D. J. & Yellowlees, D. (1970). *Biochem. J.* **117**, 22P.
 Palmer, G. H. (1969). *J. Inst. Brew.* **75**, 536.

Observations on the Purity of some Yeast Glycogen Preparations

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Recent investigations by Bacon and his co-workers (Bacon & Farmer, 1968; Bacon, Farmer, Jones & Taylor, 1969) and by Manners & Masson (1969) have shown that yeast cell-wall preparations contain a soluble β -(1 $\rightarrow$ 6)-glucan in addition to the branched insoluble β -(1 $\rightarrow$ 3)-glucan. A survey of the literature shows a considerable variation in the specific rotation and β -amylolysis limits of various yeast glycogen preparations, and this is rather surprising in view of the generally accepted molecular structure of the polysaccharide (Manners, 1957). It is possible that this variation could be due, in part, to contamination of the glycogen preparations to differing extents with β -(1 $\rightarrow$ 6)-glucan, and we now describe experimental evidence to support this suggestion.

Baker's yeast was fractionated by the method of Trevelyan & Harrison (1956) to give the following fractions: trehalose, mannan and alkali-soluble glycogen, acetic acid-soluble glycogen, perchloric acid-soluble glycogen and residual β -(1 $\rightarrow$ 3)-glucan. The glycogen samples were then deproteinized by treatment with ice-cold 5% trichloroacetic acid solution.

Samples of the acetic acid-soluble glycogen and perchloric acid-soluble glycogen were partially hydrolysed (0.167M-sulphuric acid at 100°C for 30min). Paper chromatography showed the presence of glucose and maltosaccharides (degree of polymerization 2–5), but no other oligosaccharides. However, when the conditions for partial hydrolysis were altered to those that would be suitable for the β -(1 $\rightarrow$ 6)-glucan luteose, i.e. 1M-sulphuric acid at 100°C for 1h, the partial hydrolysates contained glucose and oligosaccharides with the chromatographic mobility of gentiobiose and gentiotriose.

The yeast glycogens were then treated with a purified amyloglucosidase preparation, which had no action on luteose but with rabbit liver glycogen gave 98.4% conversion into glucose. By using both glucose oxidase and Nelson-Somogyi reducing-power methods of analysis, the acetic acid-soluble

glycogen gave 78 and 79% conversion into glucose, and the perchloric acid-soluble glycogen gave 83 and 85% conversion respectively. The enzyme digests were then heated to inactivate the amyloglucosidase, dialysed to remove the glucose, concentrated and then partially hydrolysed with 1M-sulphuric acid at 100°C for 1h. The two hydrolysates both contained glucose and oligosaccharides having the chromatographic properties of gentiobiose, gentiotriose and gentiotetraose. These results show clearly that the glycogen preparations were contaminated with a significant amount of β -(1 $\rightarrow$ 6)-glucan.

Fractionation procedures of the type devised by Trevelyan & Harrison (1956) have been used in studies of the changes in carbohydrate composition that occur during the growth of yeasts (see, e.g., Trevelyan & Harrison, 1956; Suomalainen & Pfäffli, 1961; Chester & Byrne, 1968). The present work shows that these results can now only have a semi-quantitative significance, since they do not take into account the rate of synthesis of the β -(1 $\rightarrow$ 6)-glucan. The existence of the latter was, of course, not known when the fractionation procedures were carried out.

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- Bacon, J. S. D. & Farmer, V. C. (1968). *Biochem. J.* **110**, 34P.
 Bacon, J. S. D., Farmer, V. C., Jones, D. & Taylor, I. F. (1969). *Biochem. J.* **114**, 557.
 Chester, V. E. & Byrne, M. J. (1968). *Archs Biochem. Biophys.* **127**, 556.
 Manners, D. J. (1957). *Adv. Carbohydr. Chem.* **12**, 261.
 Manners, D. J. & Masson, A. J. (1969). *FEBS Lett.* **4**, 122.
 Suomalainen, H. & Pfäffli, S. (1961). *J. Inst. Brew.* **67**, 247.
 Trevelyan, W. E. & Harrison, J. S. (1956). *Biochem. J.* **63**, 23.

The Cell and Exocellular Mannans from *Hansenula holstii* N.C.Y.C. 560

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Mannose-containing polysaccharides have been isolated from the cell walls of most yeasts. These may occur as homoglycans or in combination with other monosaccharides, phosphate and protein. Certain species will also produce exocellular mannans that may be conveniently isolated from the culture media after removal of the cells. In

particular the exocellular phosphomannans from several species of *Hansenula* have been described (Jeanes & Watson, 1962; Slodki, 1962; Slodki & Boundy, 1970). One of these, *H. holstii* N.R.R.L. Y-2448, forms an essentially linear polymer with a mannose/phosphorus molar ratio 5:1. This report describes the results of our studies on the mannans from the related organism *H. holstii* N.C.Y.C. 560.

The yeast was grown in the medium described by Slodki, Wickerham & Cadmus (1961) at 30°C. Exocellular mannan was isolated in high yield from the cell-free medium. Mannan was extracted from the cells by the procedure of Peat, Whelan & Edwards (1961).

The phosphorus content of the exocellular mannan was found to vary, depending on the ages of the culture, the product after 1 day having a mannose/phosphorus molar ratio 3.8:1, which altered to 30:1 after 5 days. This variation was not observed with the cell mannan, which had a molar ratio 100:1 at both stages of growth.

Ion-exchange chromatography on DEAE-cellulose of mannans from the 5-day culture indicated that each contained at least two components with similar charge and, in addition, that there were two more highly charged species in the exocellular mannan.

Partial degradation by the acetolysis technique (Gorin & Perlin, 1956), followed by molecular-sieve chromatography on Sephadex G-15, converted the exocellular mannan into a mixture of mannose (2%), mannobiose (5%), mannotriose (13%), mannotetraose (29%) and a fraction of molecular weight greater than 5000 (51%). Digestion of the original polysaccharide with an α -mannosidase from *Arthrobacter* GJM-1 (Jones & Ballou, 1968) removed 75% of the mannose units. Acetolysis of the residual material produced a chromatographic pattern from which the high-molecular-weight fraction was absent. The product from the cell mannan contained only one fraction, which was eluted near the void volume of Sephadex G-15, suggesting that few (1→6)-mannopyranoside linkages were present. Although prior degradation with α -mannosidase removed 39% of the mannose units, no change in the acetolysis pattern was observed.

There was little difference in the oxidation of the two mannans with periodate, both reducing about 0.75 mol of periodate and forming 0.17 mol of formic acid/mol.prop. of mannose. In contrast, after α -mannosidase hydrolysis, the residue from the exocellular mannan required 1.8 mol of periodate to produce 0.9 mol of formic acid/mol.prop. of mannose, whereas the values for the cell mannan remained almost unchanged.

Consideration of these results suggests that both mannan preparations are structurally distinct, the exocellular mannan resembling the branched

structure normally associated with 'baker's yeast' (Stewart, Mendershausen & Ballou, 1968) rather than the structure proposed for *H. holstii* N.R.R.L. Y-2448 (Jeanes & Watson, 1962). It is also possible that each preparation may contain more than one type of mannan.

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Gorin, P. A. J. & Perlin, A. S. (1956). *Can. J. Chem.* **34**, 1796.

Jeanes, A. & Watson, P. R. (1962). *Can. J. Chem.* **40**, 1318.
Jones, G. H. & Ballou, C. E. (1968). *J. biol. Chem.* **244**, 1043.

Peat, S., Whelan, W. J. & Edwards, T. E. (1961). *J. chem. Soc.* p. 29.

Slodki, M. E. (1962). *Biochim. biophys. Acta*, **57**, 525.

Slodki, M. E. & Boundy, J. A. (1970). *Devs ind. Microbiol.* **11**, 86.

Slodki, M. E., Wickerham, L. J. & Cadmus, M. C. (1961). *J. Bact.* **82**, 269.

Stewart, T. S., Mendershausen, P. B. & Ballou, C. E. (1968). *Biochemistry*, Easton, **7**, 1843.

Formation of Yeast Protoplasts by Using an Enzyme Preparation from *Cytophaga*

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Enzyme systems from the snail *Helix pomatia* (Anderson & Millbank, 1966) and various microbial sources (Villanueva, 1966) will form protoplasts from a number of yeasts. A preparation from *Cytophaga* that contains significant amounts of endo- β -(1→3)-glucanase activity (Manners & Patterson, 1966) attracted our attention as being a suitable source of enzymes for the study of yeast protoplast formation.

Exponential-phase cells of *Saccharomyces cerevisiae* N.C.Y.C. 1006 and N.C.Y.C. 239, and *S. chevalieri* N.C.Y.C. 394 were prepared by using the media as described by Darling, Theilade & Birch-Anderson (1969). Cells were removed by centrifugation and washed with 0.6 M-potassium chloride before suspending them in a suitable volume of 0.04 M-potassium phosphate buffer, pH 6.0, containing 1.2 M-potassium chloride. Generally the cell suspension (1 vol.) was shaken at 37°C with the enzyme preparation (1 vol.; 0.24% in distilled water) containing either 2-mercaptoethanol or dithiothreitol. The extent of formation of osmotically sensitive protoplasts was determined by diluting samples (0.4 ml) with distilled water (2.4 ml) and measuring the change in extinction at 660 nm