CONSTANCY OF THE CELL WALL MANNAN STRUCTURE OF *SACCHAROMYCES CEREVISIAE*

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SUMMARY: The oligosaccharides, obtained by acetolysis of the cell wall mannans of a diploid strain of *Saccharomyces cerevisiae* and its two haploid mating types, have been separated by gel filtration and the ratios compared. Although the haploid forms gave less of the longer fragments than the diploid form, no dramatic difference was observed that might be related to a process of recognition between the two mating types.

A method for fingerprinting yeast cell wall mannan, based on gel filtration of the oligosaccharides produced by controlled acetolysis of the polysaccharide, has recently been described by Kocourek and Ballou (1). From a comparison of the fingerprints of many different mannans, which reflect the nature of the sidechains of the polysaccharides, it appears that there is considerable species specificity in the structure of this wall component.

We have considered the possibility that the mannan, which is at least partly located on the cell surface (2), may have a role in cell-cell interaction or "cell recognition". As a test of this hypothesis, we have compared the mannan fingerprints of two haploid *Saccharomyces cerevisiae* mating types with each other and with the diploid form. The two haploid mating types gave identical fingerprints, and only minor quantitative differences were noted between these fingerprints and that of the diploid form. This constancy of structure reemphasizes the previous suggestion (3) that yeast mannans must be formed by processes subject to a carefully defined genetic control.

EXPERIMENTAL PROCEDURE

Two haploid strains of *Saccharomyces cerevisiae*, X-2180-1A and X-2180-1B, and the diploid product of their mating, were obtained from Dr. R. K. Mortimer Cells were grown with shaking at 30° for 36 hours in a medium containing 1%

yeast extract, 2% peptone and 2% glucose. About 2.1 g of lyophilized cells were obtained per liter.

Mannan was isolated by a modification of the method of Kocourek and Ballou (1). Polysaccharide was precipitated from the citrate extract of autoclaved cells by adding two volumes of ethanol. After redissolving the precipitate in water, protein was precipitated by making the solution 1 M in acetic acid and the precipitate was removed by centrifugation. Mannan was then precipitated with Fehlings solution, the precipitate was redissolved in 0.1 N HCl, the mannan was reprecipitated with methanol-acetic acid (8:1) and then redissolved in water. The last four steps were repeated and the final mannan solution was dialyzed against EDTA and water to remove the last traces of copper. The yield of mannan from each of the *S. cerevisiae* strains was about 45 mg per g of dry cell weight.

After hydrolysis of the mannan with 2 N HCl at 100° for 4 hours, paper chromatography (Whatman No. 1, n-butanol-pyridine-water, 3:1:1, by volume) revealed only mannose as detected by an alkaline $AgNO_3$ dip. The protein (Lowry) and phosphate contents of the mannans are shown in Table 1.

TABLE I

Composition of mannan preparations

Yeast strain	Mannose to phosphate ratio	Protein (%)
X-2180-1A haploid	63	6.7
X-2180-1B haploid	54	7.3
Diploid	53	7.0

Acetolysis of mannan was performed according to Kocourek and Ballou (1). The deacetylated oligosaccharides were applied to a 2 by 200 cm column of Sephadex G-25 and eluted with water (10 ml/hour) at room temperature in 2.5 ml frac-

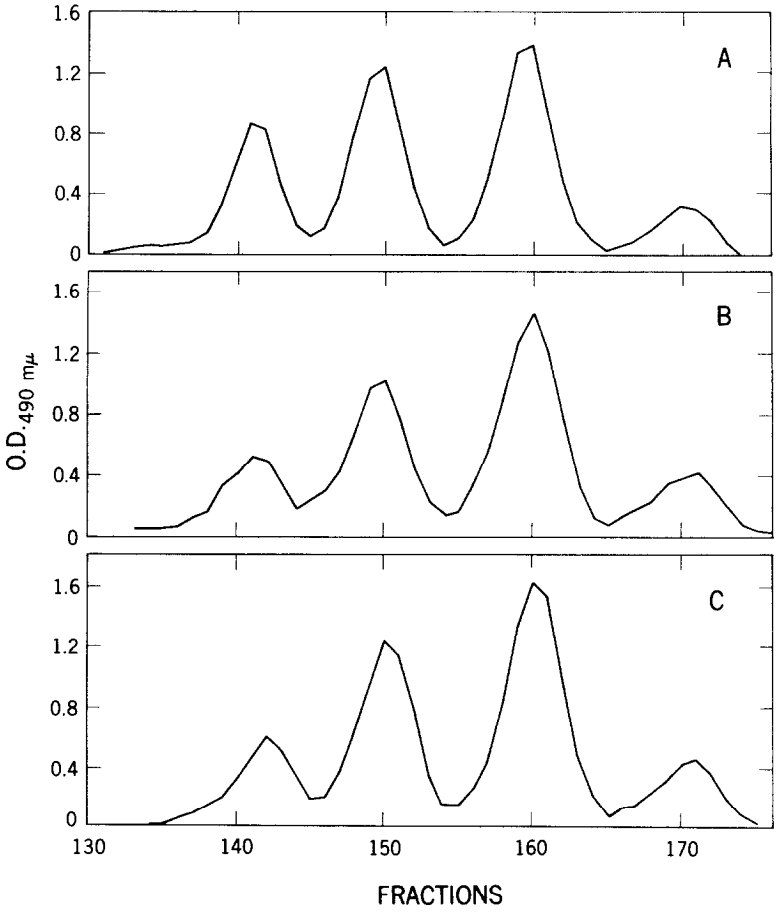

Fig. 1. Gel filtration patterns of oligosaccharides obtained by acetolysis of yeast mannans. A, diploid strain; B, X-2180-1B haploid; C, X-2180-1A haploid. Mannotetraose, M_4, elutes at fraction 142; mannotriose, M_3, at 150; mannobiose, M_2, at 160; and mannose, M_1, at 171.

TABLE II

Areas of the peaks in Fig. 1 relative to the M_2 peak

Yeast strain	M_4	M_3	M_2	M_1
A (Diploid)	0.575	0.875	1	0.258
B (X-2180-1B)	0.320	0.695	1	0.301
C (X-2180-1A)	0.334	0.720	1	0.273

tions. Carbohydrate was assayed by the phenol sulfuric acid method and the area of each peak was determined relative to the disaccharide (M_2) peak with a compensating polar planimeter. As shown in Fig. 1, both haploid strains have very similar acetolysis patterns. The diploid mannan is richer in the longer sidechains (M_3 and M_4) (Table II).

DISCUSSION

In the presence of cells of the opposite mating type, haploid yeast cells swell, elongate and form specialized preconjugation cells. It appears that this transformation may be induced by steroid molecules (4) or small peptides (5) secreted by the opposite mating type. The preconjugation cells then fuse and form a diploid cell. Mating between cells of the same mating type is a rare event. Some heterothallic yeasts agglutinate when mixed with cells of the opposite mating type, a process which increases the chance of zygote formation. In the sexually agglutinating yeast *Hansenula wingei*, it appears that agglutination may be due to association of complementary mannan-protein molecules on the surface of the cells (6).

In the present experiment with nonagglutinating yeasts, we have tested the possibility that haploid cells of opposite mating type might possess structurally different mannans. Such differences could reflect a cell recognition system similar to that in *Hansenula wingei*. Since the two haploid mannans gave nearly identical proportions of the mannose oligosaccharides, we conclude that there is no obvious structural difference in their mannans. Structural differences still might reside in the relative amounts of 1→2 and 1→3 linkages in the oligosaccharides, the order of sidechains on the 1→6-linked mannan backbone, or in the phosphate or protein components of the mannan. We have no explanation for the increased proportion of the longer sidechains in the diploid strain.

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