Comparison of the Morphology, Fermentation, Assimilation, Lipid Content and Mannans of Rough and Smooth Strains of *Saccharomyces cerevisiae*

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SUMMARY

Tetrad analysis has shown that haploid forms arising from spores of a single ascus of *Saccharomyces cerevisiae* gave smooth and rough strains in the ratio 1:1. These rough and smooth strains fermented the same sugars but differed in their morphology, mode of L-lysine assimilation and lipid content. Immunochemical studies, periodate oxidation and methylation analyses showed that both strains formed the same kind of mannan.

INTRODUCTION

In some yeast species a spontaneous transformation of smooth strains into rough strains has been observed (Kreger-van Rij, 1969). This transformation is associated with biochemical and morphological changes. Differences between smooth and rough forms occur in the adsorption of surface active cationic substances (Šilhánková, 1957). Furthermore, rough forms are hydrophobic and more resistant to lysis by snail juice enzymes (Millbank & Macrae, 1964). Wickerham & Barton (1962) suggested that these hydrophobic properties might be related to the presence of sphingolipids on the cell surface. Kauppinen, Kiviniemi & Erkama (1965) have found that walls of rough strains of *Candida guilliermondii* contain 50 % more lipid than smooth strains. In the genus *Salmonella* changes in antigenic properties of the cell surface result from rough mutants being unable to synthesize complete O-specific polysaccharides (Lüderitz & Westphal, 1966). It would be of interest to determine if there are similar changes in surface layers of walls of rough strains of yeasts.

The purpose of our present work was to elucidate differences in morphology, lipid content, antigenic properties, assimilation and fermentative abilities of rough and smooth strains of *Saccharomyces cerevisiae*.

METHODS

Organisms and media. The monosporogenous cultures used in our study were isolated in 1964 from *Saccharomyces cerevisiae* IXL-A₃ (a commercial yeast strain from England) by micromanipulation. In the present study four strains (64/1–4) arising from the same ascus were used. Their fermentation and assimilation properties were

studied using methods described previously (Kocková-Kratochvilová, 1954). The strains were obtained from the Yeast Division of the Czechoslovak Collection of Micro-organisms (CCY), Institute of Chemistry of the Slovak Academy of Sciences. Further, a non-sporogenous mutant (S 313/1) was obtained from the smooth strain 64/2 by culturing the organism at 28° in media (2 % (w/v) glucose, 1 % (w/v) $\text{NH}_4\cdot_2\text{SO}_4$; 0.1 % (w/v) KH_2PO_4 and 0.05 % (w/v) $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$ in distilled water) containing 1 to 3 mg./l. acriflavin. Either 2 $\mu\text{g.}$ of biotin/ml. or 0.2 % (w/v) DL-serine was added (Zakharov & Inge-Vechtomov, 1966). The *S. cerevisiae* strain CCY 21-4-13 was used to prepare antisera.

The culture medium and the conditions of growth of the studied strains except non-sporogenous mutants have been described previously (Masler, Šikl, Bauer & Šandula, 1966).

Lipid extraction. Lipids were extracted by the methods described by Letters (1966) and Folch, Lees & Sloane-Stanley (1957).

Isolation of polysaccharides. (a) *Extracellular mannans.* The supernatant of centrifuged cultures was concentrated to 2.5 % of its original volume at reduced pressure and extracellular mannans were precipitated by adding 8 volumes of ethanol. The centrifuged precipitate was dissolved in 30-fold amounts of 5 N-NaOH and heated for 15 min. at 100°. After cooling and neutralization with conc. HCl the solution was dialysed against water, concentrated at reduced pressure and precipitated with 4 volumes of ethanol. After centrifugation the precipitate was dissolved in a small amount of water and lyophilized. The mannans precipitated from an aqueous solution of the polysaccharides (1 %, w/v) using Fehling's reagent (Haworth, Hirst & Isherwood, 1937) were washed with ice water and suspended in water. One N-HCl was added with stirring until the precipitate was dissolved. The solution was then passed successively through Zerolit 225 (H^+ form) and Amberlite 402 (OH^- form) columns. The filtrate was concentrated *in vacuo* and lyophilized. The mannans obtained were free of inorganic ash and nitrogen. After hydrolysis for 6 h. at 100° in 1 N-HCl, mannose was identified as the only monosaccharide in solvent A (see below) after paper chromatography.

(b) *Cellular mannans.* After lipid extraction the cells were suspended in a 0.2 M-NaCl solution (cells + solution, 1 + 8 by vol.) and autoclaved for 2 h. at 150°. The extraction was repeated and the pooled supernatants were concentrated *in vacuo* and precipitated with ethanol. They were subsequently treated as described above.

Methylation analysis of the mannans. Methylation analysis was carried out according to Haworth (1915) in sodium hydroxide using dimethylsulphate and according to Kuhn, Trischmann & Löw (1955) in dimethylformamide using methyl iodide in the presence of silver oxide. The methylated mannans were hydrolysed in sulphuric acid as described by Croon, Herrström, Kull & Lindberg (1960). The *O*-methylethers of D-mannose formed by hydrolysis were refluxed for 6 h. in 5 % (w/w) methanolic HCl and one part of the resulting methyl-*O*-methyl-mannosides was analysed using gas chromatography. The remaining part of methyl-*O*-methyl-D-mannosides was trimethylsilylated (Sweeley, Bentley, Makita & Wels, 1963) and then analysed using gas chromatography.

Periodate oxidation. Mannan samples (50 mg.) were dissolved in 30 ml. of water, to which 30 ml. of 0.06 M- NaIO_4 was added. The material was stored in darkness at 4°. The consumption of periodate was determined using the arsenite method described

by Fleury & Lange (1933). The amount of formic acid produced was determined amperometrically as described by Babor, Kaláč & Tihlárík (1964).

Chromatography. A Chrom III (Laboratory Equipments, Prague) fitted with a 115 × 0.6 cm. column packed with 10 % (w/w) Carbowax 6000 on Celite 545 was used for gas chromatography. Methyl-*O*-methyl-mannosides were run at 150° and trimethylsilyl derivatives at 135°.

The descending techniques of paper chromatography were followed with Whatman no. 1 paper. The following solvent systems were used: (a) butanol-1-ol + pyridine + benzene + water (7:3:1:2, by vol., for aldoses) and (b) butanol + ethanol + water (5:1:4, by vol., for methylethers of aldoses). Aniline phthalate was used as detection reagent (Patridge, 1949).

Phosphorus determination. Phosphorus was determined by the method described by Fiske & Subbarow (1925).

Serology. Rabbit antisera were prepared by using intravenous injections of 1 % (w/v) suspensions of heat-killed cells of *Saccharomyces cerevisiae* CCY 21-4-13. Chinchilla rabbits were used. Immunization was carried out three times a week for 5 weeks. The rabbits were bled 1 week after the last immunization, when the level of antibody was found to be sufficient. Antisera were preserved by addition of sodium merthiolate to achieve a concentration of 1:10,000 and stored at -15 to -20°.

Quantitative precipitation reactions between antisera and the corresponding mannans were carried out in 0.9 % aqueous NaCl solution with 0.2 ml. of serum and 0.8 ml. of mannan solution. Different mannan concentrations (50, 100, 150, 200, 500, 1000 µg. in ml.) were used. The polysaccharide-antiserum mixture was incubated for 2 h. at 37° followed by 1 week at 4°. The precipitate was removed by centrifugation at 4°, washed with ice-cold salt solution, dissolved in 0.1 N-NaOH (1 ml.) and assayed for protein spectrophotometrically at 285 nm.

Table 1. *Sporulation activity of Saccharomyces cerevisiae IXL-A₃*

Culture	Percentage of cells producing ascospores*		Presence of diploidization gene D†
	June 1969 test	December 1969 test	
64/1	0.0	0.0	—
64/2	18.5	17.5	+
64/3	4.5	4.0	+
64/4	20.5	22.5	+

* Calculated on 400 vegetative cells.

† —, Diploidization gene D absent; +, diploidization gene D present.

RESULTS AND DISCUSSION

Tetrad analysis has shown that haploid cultures arising from the individual spores gave both smooth and rough strains in the ratio 1:1 (Pl. 1, fig. 1). The original monosporic cultures 64/1, 64/2, 64/3 and 64/4 were retested in 1969. Two retained their original morphology (the rough strain 64/1 and smooth strain 64/2), while two (64/3 and 64/4) gave mixtures of rough and smooth strains (Pl. 1, fig. 2 to 5). The ability of cultures to sporulate was therefore tested (Table 1).

Diploid cultures of strain no. 64 form smooth colonies, but haploid strains can form rough or smooth colonies. This explains the appearance of monosporic cultures:

strain 64/1 has retained its rough colonial character by maintaining a haploid; strain 64/2 has become a diploid and retained its smooth character; while both strains 64/3 and 64/4 appeared as predominantly diploid mutants showing rough sections in smooth colonies. Some morphological features of monosporic cultures 64/1-4 are summarized in Table 2. Morphological features indicate the haploid character of strain 64/1 (high S/V ratio) and the predominantly diploid character of the other cultures which is more precisely explained by Kocková-Kratochvílová & Pokorný, 1964.

Table 2. *Cell morphology of strains 64/1-4*

Culture	Average cell length		Average cell width		Length/ width ratio of cells	Correl. coeff. between length and width of cells	Surface volume (S/V) ratio of cells
	$\mu\text{m.}$	S.E.	$\mu\text{m.}$	S.E.			
64/1	5.62	0.1262	3.78	0.1037	1.49	0.416	1.45
64/2	5.91	0.1165	5.55	0.1142	1.06	0.864	1.04
64/3	6.13	0.1347	5.61	0.1269	1.09	0.773	1.04
64/4	5.48	0.1531	5.03	0.1496	1.09	0.920	1.17

Table 3. *Fermentation and assimilation of cultures 64/1-4*

Culture	Fermentation				Assimilation	
	Saccharose	Maltose	Galactose	Raffinose	KNO ₃	L-Lysine
64/1	+	+	+	1/3	—	(+)
64/2	+	+	+	1/3	—	—
64/3	+	+	+	1/3	—	—
64/4	+	+	+	1/3	—	(+)

+, Fermented; 1/3, culture ferments fructose unit from raffinose after splitting it into melibiose and fructose; (+), weakly assimilated; —, not assimilated.

Table 4. *Biomass, lipid and mannan content of smooth (S) and rough (R) strains of Saccharomyces cerevisiae after 5 days incubation*

Culture	Dry weight of cells*	Lipids†	Mannans†	
			Extracellular	Cellular
R 64/1	9.0	15.8	4.8	2.8
S 64/2	14.0	5.0	3.8	3.5
S 313/1	2.8	4.8	1.7	3.3

* Dry wt of organism/10 l. of medium.

† Expressed as a % of culture dry weight.

In addition we have tested the fermentation and assimilation ability of all four cultures (Table 3).

The results indicate that cultures containing diploidization factor were unable to retain their constant smooth or rough character. Acriflavine was therefore added to cultures to obtain non-sporulating mutants. By this means a culture (313/1) which did not sporulate was obtained from strain 64/2 (Pl. 1, fig. 6). Certain properties of this strain were compared with rough (64/1) and smooth 64/2 strains (Table 4).

Strain 313/1 gave only about 20 % of the biomass of the parent culture 64/2, while

strains 64/1 gave about 66 % of the biomass of strain 64/2. Rough strains 64/1 contained threefold higher lipid content than the smooth strains 64/2 and 313/1.

The results of periodate oxidation (Table 5) and methylation analysis (Table 6) of extracellular and cellular water-soluble mannans from rough and smooth strains showed that they synthesized very similar branched mannans with α -(1 $\rightarrow$ 6), α -(1 $\rightarrow$ 2) and α -(1 $\rightarrow$ 3) glycosidic linkages and with equal chain length. These findings were verified by quantitative precipitation cross-reactions between rough and smooth strains of *Saccharomyces cerevisiae* IXL-A₃ and antisera against the smooth strain of *S. cerevisiae* CCY 21-4-13 (see Table 7).

Table 5. *Physicochemical features and phosphorus content of extracellular and cellular mannans of rough and smooth strains of Saccharomyces cerevisiae*

Culture		[α] _D ²⁴	P	Periodate oxidation moles/1 mannose unit	
				NaIO ₄	HCOOH
R 64/1	EM	+74°	0.40	1.23	0.29
	CM	+71°	0.14	1.20	0.23
S 64/2	EM	+68°	0.09	1.13	0.19
	CM	+75°	0.10	1.17	0.23
S 313/1	EM	+75°	0.12	1.17	0.16
	CM	+63°	0.10	1.12	0.23

EM, extracellular mannan; CM, cellular mannan.

Table 6. *Methylation analysis of mannans from rough and smooth strains of Saccharomyces cerevisiae (mole % composition)*

O-Methyl-D-mannose	R 64/1		S 64/2	
	Cellular mannan	Extracellular mannan	Cellular mannan	Extracellular mannan
2,3,4,6-tetra-	33.8	33.3	33.9	33.5
3,4,6-tri-	15.8	16.4	16.4	17.1
2,3,4-tri-	2.2	2.4	2.3	2.4
2,4,6-tri-	16.7	17.1	15.9	15.9
3,4-di-	31.5	30.8	31.5	31.1

Table 7. *Consumption of extracellular and cellular mannans giving maximum precipitated antibody nitrogen of rabbit antiserum obtained by whole cells of Saccharomyces cerevisiae 21-4-13*

Culture	Extracellular mannan (μ g.)	Precipitated antibody nitrogen (μ g.)	Cellular mannan (μ g.)	Precipitated antibody nitrogen (μ g.)
21-4-13	150	730	150	730
R 64/1	150	810	150	660
S 64/2	100	690	100	690
S 313/1	150	690	150	850

Zakharov & Inge-Vechtomov (1966) studied the genetic background of rough and smooth phenotypes of *Saccharomyces cerevisiae*, while Vezinhet, Galzy & Durand (1968) examined the metabolic differences between the two types. The roughness of

colonies is recessive and the smoothness dominant or semidominant. Šilhánková (1969) supposes the existence of metabolic suppressors of the rough phenotype responsible for the instability of rough mutants. Suppression of the rough phenotype in mutants requiring tryptophan or lysine for the growth falls into this category. Recently Thieme and Ballou (1970) have found that mannans produced by diploid heterothallic strains of *Saccharomyces cerevisiae* contain longer chains than the mannans from haploid strains. On the other hand, we have found that mannans from our monosporic cultures of *S. cerevisiae* IXL-A₃ possess the same average chain length. We explain this fact by the homothallic character of our strains. The indication of this homothally is the presence of diploidization gene D.

Rough strains (Table 4) contained higher amounts of lipids than smooth strains. During aerobic conditions carbohydrates are metabolized into lipids by yeasts but anaerobically grown culture require the incorporation of an unsaturated fatty acid in the medium, i.e. oleic or linoleic acid (Babij, Moss & Ralph, 1969).

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EXPLANATION OF PLATE

- Fig. 1. Cultures arising from the individual spores of one ascus of *Saccharomyces cerevisiae*.
- Fig. 2. Rough strain obtained from original monosporic culture 64/1.
- Fig. 3. Smooth strain obtained from original monosporic culture 64/2.
- Fig. 4. Mixture of rough and smooth strains obtained from original monosporic culture 64/3.
- Fig. 5. Mixture of rough and smooth strains obtained from original monosporic culture 64/4.
- Fig. 6. Smooth strain which did not sporulate obtained by means of acriflavine from smooth strain 64/2.