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Influence of Ethanol on the Lipid Content and Fatty Acid Composition of *Saccharomyces cerevisiæ*

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ABSTRACT. The content of total lipid as well as of ergosterol, squalene, and major fatty acids were compared in the cells of a distillery strain of *Saccharomyces cerevisiæ* incubated for 3, 48 and 120 h in the presence of 5, 10 and 15 % ethanol. Ethanol induced lipid accumulation with preferential ergosterol biosynthesis. The relative contents of palmitic and stearic acid decreased whereas the amount of palmitoleic and oleic acid increased. The total content of all fatty acids rose as a consequence of the ethanol treatment.

Ethanol is known to affect biological membranes already at very low concentrations mostly by fluidizing the lipid matrix (Wood *et al.* 1991). The presence of ethanol in medium during cell growth causes changes in the cell membrane structures which are reflected in membrane reconstitution, redistribution of fatty acids in structural lipids and other processes of homeoviscous adaptation (Alexandre *et al.* 1994). Šajbidor and Grego (1992) studied the influence of ethanol on fatty acid alterations in *Saccharomyces cerevisiæ* and observed an elevation of palmitic and oleic acid level in phosphatidylcholine and phosphatidylethanolamine and also the palmitoleic acid content in esterified sterols and triacylglycerols. Agudo (1992) analyzed the fatty acid and sterol compositions of various *S. cerevisiæ* strains with ethanol tolerance varying from 4 to 12 % (*V/V*) and confirmed a relationship between membrane fluidity and ethanol tolerance, but failed to corroborate a direct role of unsaturated fatty acids in this tolerance. The importance of ergosterol in this phenomenon was discussed. Koukkou *et al.* (1993) studied the effect of ethanol on the sterols of the fission yeast *Schizosaccharomyces pombe*. The presence of ethanol resulted in a decrease in the sterol content under aerobic conditions, but a three-fold increase of total sterols under anaerobic conditions. Novotný *et al.* (1992) reported that the sensitivity of *S. cerevisiæ* cultivated in anaerobic conditions to inhibition by ethanol was higher in the *erg2* mutant in comparison with $\Delta^{5,7}$-synthesizing strains. A high level of these sterols increased the vulnerability of anaerobic growth to ethanol inhibition. Recent findings in the yeast *S. cerevisiæ* and *S. pombe* showed that plasma membrane fluidization took place during the mere aeration of the cell culture in the absence of substrates, and the presence of 200 mmol/L ethanol only slightly increased the extent of this aeration-induced fluidization. Somewhat surprisingly, 200 mmol/L glucose fluidized the cell membrane more strongly than ethanol (Gille *et al.* 1993). The structural modification of the plasma membrane, induced by ethanol insertion into the hydrophobic interior, might be involved in the potency of the inhibition by high concentrations of ethanol of the plasma membrane ATPase, as well as other critical components in the plasma membrane such as nutrient transport systems (Monteiro *et al.* 1994).

In this paper we discuss the influence of ethanol on lipid production, fatty acid profile and the content of ergosterol and squalene in stressed *S. cerevisiæ*.

MATERIAL AND METHODS

The distillery strain *Saccharomyces cerevisiæ* LH 02/2 obtained from B. Janderová (*Department of Genetics, Microbiology and Biophysics, Charles University*, Prague) was used. The basic medium used for cultivation contained (g/L): glucose 150, yeast extract 3, $(NH_4)_2SO_4$ 5, KH_2PO_4 2, $MgSO_4 \cdot 7H_2O$ 1, $Na_2HPO_4 \cdot 12H_2O$ 1, $CaCl_2$ 0.1, NaCl 0.1; pH was adjusted to 5.8. The first stage of the experiment was performed aerobically at 28 °C. The medium for aerobic cultivation was inoculated by 10 % (*V/V*) of inoculum. Cell suspension (220 mL) after 1-d cultivation was added to the fresh medium (280 mL) with 0, 5, 10 or 15 % (*V/V*) of ethanol. Consequently, fermentations were carried out in 600 mL bottles with one-way valves at 28 °C under static conditions. After 3-, 48- or 120-h incubation, the biomass was extracted by the method of Bligh and Dyer (1959), fatty acid profile was analyzed by gas chromatography (Šajbidor *et al.* 1994) and ergosterol and squalene were quantified by HPLC (Rodriguez and Parks 1985).

RESULTS

The influence of ethanol on the lipid content, ergosterol and squalene amounts as well as major fatty acid composition is summarized in Tables I and II. The presence of ethanol in the environment caused preferential lipid accumulation. The process of yeast adaptation to ethanol stress was accompanied by ergosterol and squalene overproduction. For example, the content of ergosterol after a 3-h incubation in 15 % ethanol was approximately 15 times higher than in biomass incubated without ethanol. The amount of ergosterol rose dramatically mainly during the first phase of adaptation. A decrease of both squalene and ergosterol content after was observed 120 h of incubation in 10 or 15 % ethanol.

Table I. Influence of ethanol concentration and time of adaptation on the content (mg per 100 g dry matter) of lipid, ergosterol, squalene and major fatty acids in a distillery strain of *S. cerevisiæ*

Component	Concentration of ethanol[a], % (*V/V*)											
	0			5			10			15		
	Time of incubation with ethanol, h											
	3	48	120	3	48	120	3	48	120	3	48	120
Total lipid	830	420	2130	1060	1140	2460	1100	2240	2670	2670	3720	3920
Ergosterol	21	39	245	42	205	296	214	385	384	389	437	230
Squalene	4	4	62	12	23	85	12	27	39	33	42	20
Palmitic acid	101	51	275	123	93	237	82	163	217	171	274	282
Palmitoleic acid	226	116	652	332	321	778	311	698	906	839	1364	1614
Stearic acid	48	19	91	51	51	101	33	68	77	63	107	93
Oleic acid	276	127	627	400	363	806	356	754	884	745	1246	1412

[a]Added at the start of incubation.

Table II. Influence of ethanol concentration and time of adaptation on the relative content of major fatty acids in a distillery strain of *S. cerevisiæ*

Fatty acid %	Concentration of ethanol[a], % (*V/V*)											
	0			5			10			15		
	Time of incubation with ethanol, h											
	3	48	120	3	48	120	3	48	120	3	48	120
16:0	14.3	14.3	15.9	12.9	10.8	12.0	9.9	9.4	10.3	8.0	8.9	8.1
16:1	31.2	32.6	38.5	34.7	37.5	39.8	37.1	39.8	42.0	38.9	44.3	46.3
18:0	6.8	5.4	5.3	5.4	5.9	5.1	4.0	3.9	3.6	3.0	3.5	2.7
18:1	39.0	36.2	36.2	41.8	42	40.4	42.9	43.4	41.4	34.9	40.9	40.5

[a]Added at the start of incubation.

The major fatty acids were found to be palmitic (16:0), palmitoleic (16:1), stearic (18:0) and oleic (18:1) acids. Ethanol decreased the relative percentage of 16:0 and 18:0. On the other hand, the content of 16:1 and 18:1 after ethanol treatment increased. Quantification of the individual fatty acid expressed as mg per 100 g of dry biomass showed that the absolute level of 16:0, 16:1, 18:0 and 18:1 increased, though differences were observed among individual fatty acids. The highest content – 1614 mg of 16:1 was found after 120-h incubation of *S. cerevisiæ* in 15 % ethanol.

DISCUSSION

Ethanol brings about an adaptation response of the cell which includes both universal adaptation mechanisms and specific reactions peculiar to the particular microorganism. When the integrity of the intracellular environment is threatened, *e.g.* by undesirable membrane fluidization, the universal mechanism of homeoviscous adaptation is switched on, bringing about a stabilization of the cell membrane properties (Alexandre *et al.* 1994).

We may discuss our results in terms of the influence of ethanol on individual parts of lipogenesis. Lipid biosynthesis is generally conditioned by the activity of two inter dependent processes: the biosynthesis of saturated fatty acids *via* condensation reactions catalyzed by the enzyme complex – fatty acid synthase, and a partial dehydrogenation of the fatty acids by specific desaturase enzymes. If the content of lipid increases, condensation reactions can be assumed to be activated. A simultaneous decrease in the relative amounts of 16:0 or 18:0 and their rise in absolute values observed in our experiments was probably linked with preferential ethanol-induced desaturation. Buttke and Ingram (1980) reported inhibition of saturated fatty acid biosynthesis by ethanol. On the other hand, Koukkou *et al.* (1990) described an ethanol-induced elevation of 18:0 in *S. pombe* cultivated in aerobic conditions. It is interesting that anaerobic growth of the same culture in the presence of ethanol caused an increase in 18:1 at the expense of 18:0. A high content of mono-unsaturated fatty acids in cytoplasmic membrane is known to fluidize the membrane and damage cell integrity. Regulation of sterol biosynthesis induced by ethanol can be understood as an individual cell's response to threatening membrane fluidization. Thomas *et al.* (1978) reported that populations of *S. cerevisiæ* with membranes enriched in ergosterol and cetoleic acid lost viability at about the same rate as those enriched in oleyl residues, whereas populations grown in the presence of this sterol and palmitoleic acid were more resistant to ethanol. In contrast to our observations, Walker-Caprioglio *et al.* (1990) detected a reduction of total sterol content in ethanol stressed yeast. It is noteworthy that cells incubated with ethanol take up more exogenous sterol under aerobic conditions than do control cells.

In general, our observations showed that the tested distillery strain of *S. cerevisiæ* accumulated lipid with a high proportion of ergosterol and mono-unsaturated fatty acids in the presence of ethanol. These results are consistent with the previously published results of Koukkou *et al.* (1993) and Šajbidor and Grego (1992).

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