MICROBIOLOGY AND IMMUNOLOGY

Effect of Ethanol Concentration on the Maximal Specific Growth Rate and Biomass Composition of *Yarrowia Lipolytica* Mutant Strain No. 1

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 121, № 1, pp. 71-73, January, 1996
Original article submitted July 27, 1995

The effect of ethanol concentration on the maximal specific growth rate and biomass composition of *Yarrowia lipolytica* No. 1 was studied during culturing in the pH-auxostat mode. Growth inhibition set in starting from a 2.64 g/liter residual concentration of ethanol. The constant of ethanol inhibition was 11.0 g/liter. Growth inhibition with ethanol was associated with changes of the fatty-acid composition of lipids and a resultant reduction of lipid unsaturation.

Key Words: *Yarrowia lipolytica; citric acid producer; ethanol; pH-auxostat; biomass composition*

Few reports are available on the use of ethanol as a substrate for the growth of citric acid-producing yeast [2,5]. Nonetheless, it has been shown possible in principle to use ethanol for increasing the synthesis of desired products. The use of ethanol as substrate guarantees the formation of a product which may be used in the food and medical industry. However, due to its toxicity, ethanol makes the culturing of microorganisms difficult. High concentrations of ethanol in the medium impede cell growth, inhibit the system of transport of nutrients, and alter the permeability of the cytoplasmic membrane [6].

This study was aimed at investigating the physiological features of growth of a mutant strain of *Yarrowia lipolytica* yeast No. 1 in a medium with ethanol as the sole source of carbon and energy. This mutant strain is remarkable for its ability to produce virtually citric acid alone when cultured on various sources of carbon, including ethanol, with nitrogen limiting cell growth.

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MATERIALS AND METHODS

A specially selected mutant strain of *Yarrowia lipolytica* No. 1 producing citric acids on normal alkanes was used in the study. This strain was obtained in 1972 by N. V. Shishkanova by treating *Y. lipolytica* with nitrosomethylurea [3].

The yeast was cultured in an ANKUM-2M fermenter with a working volume of 1.5 liters in the pH-auxostat regimen. The composition of nutrient medium was as follows (g/liter): KH_2PO_4 0.875, $(NH_4)_2SO_4$ 0.126, $MgSO_4 \times 7H_2O$ 1.5, $CaCl_2 \times 6H_2O$ 0.126; (mg/l): $ZnSO_4 \times 6H_2O$ 81.1, $FeSO_4 \times 7H_2O$ 15.0, $MnSO_4 \times 5H_2O$ 0.2, $CuSO_4 \times 5H_2O$ 5.0; yeast extract (Difco) 0.5 g/liter. Ethanol (rectificate): 1.0-4.0 vol%, 26% ammonium solution 2.5 ml/liter. Silicone antifoam emulsion M-30

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(Serva) was used as foam suppressor. To prevent sedimentation of salts, the components of the medium were delivered in the fermenter through four channels.

The concentration of oxygen dissolved in the medium was maintained at a constant level (Po_2=60% of saturation) by a system of automated oxygen stabilization. The temperature in the fermenter was maintained at 28°C with an accuracy of 0.1°C. The pH of 4.5 was maintained with an accuracy of 0.1.

The duration of fermentation after a stable regimen had been set corresponded to three replacements of the medium in the fermenter.

The composition of the biomass and components of the culture fluid were analyzed as described previously [5].

RESULTS

The effect of ethanol on yeast growth was studied at 28°C and pH 4.5 and concentrations from 2.64 to 17.1 g/liter. Figure 1 demonstrates that the concentration of residual ethanol in the culture fluid higher than 2.64 g/liter caused a reduction of the maximal specific rate of cell growth (μ_{max}). Conversion of the data on the relationship between the μ_{max} value and the concentration of residual ethanol using the reverse values method is presented in Fig. 1, *b*. The line connecting the experimental points intercepts the abscissa at the value of the inhibition constant (K_i) equal to 11.0 g/liter. The K_i value characterizes the residual concentration of ethanol at which the rate of cell growth decreases twofold. The linear dependence between $1/\mu_{max}$ and the residual ethanol concentration indicates that ethanol inhibition of yeast growth rate is described by the equation of noncompetitive inhibition of enzymatic reactions, that is, ethanol evidently inhibits the one enzymatic reaction which is the most sensitive to it. Just which reaction is the most sensitive to high concentrations of ethanol needs to be specially studied. Some authorities report the appearance of acetaldehyde and acetic

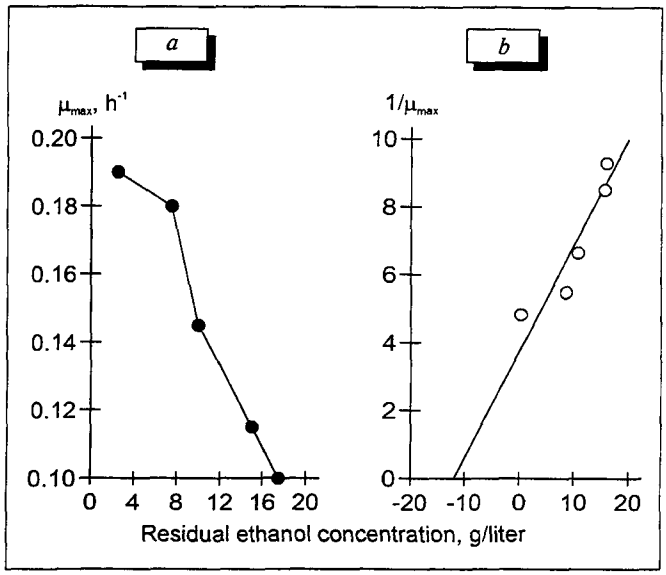

Fig. 1. Relationship between μ_{max} (*a*) and $1/\mu_{max}$ (*b*) and residual ethanol concentration.

acid in the medium during yeast culturing in ethanol [2,5]. These metabolites may be potential inhibitors of many cell functions.

The inhibitory action of ethanol on yeast growth is associated with an increase of membrane microviscosity, a parameter regulating the activity of membrane-bound proteins [7]. Some scientists claim that the inhibitory effect of ethanol on *Saccharomyces cerevisiae* is less expressed in the presence of linoleic acid, which reduces membrane microviscosity. Adaptation of yeast cells to high concentrations of ethanol is known to be associated with an increase of the share of unsaturated fatty acids in the lipid composition [4].

The results of analysis of the fatty-acid composition of *Y. lipolytica* lipids are presented in Table 1. The predominant fatty acids in all the regimens studied were palmitic ($C_{16:0}$), palmitoleic ($C_{16:1}$), oleic ($C_{18:1}$), and linoleic ($C_{18:2}$) acids. As the residual concentration of ethanol increases, the ratio of fatty acids appreciably changes: the content of linoleic acids decreases

TABLE 1. Effect of Ethanol Inhibition of the Growth of *Y. lipolytica* No. 1 on the Fatty Acid Composition of Lipids

Acids, %	Residual ethanol concentration, g/liter			
	2.64	10.3	15.1	17.1
Palmitic ($C_{16:0}$)	14.0	13.6	15.5	17.9
Palmitoleic ($C_{16:1}$)	11.1	9.5	11.3	11.2
Margaric ($C_{17:0}$)	Trace	Trace	Trace	Trace
Heptadecenoic ($C_{17:1}$)	Trace	Trace	Trace	Trace
Stearic ($C_{18:0}$)	0.7	0.8	0.8	0.9
Oleic ($C_{18:1}$)	27.1	36.1	38.1	47.5
Linoleic ($C_{18:2}$)	47.1	40.0	34.3	22.5
CN	132.4	125.6	118.0	103.7

Note. NU: nominal unsaturation coefficient.

from 47.1 to 22.5% and the share of oleic acid increases from 27.1 to 47.5%. Ethanol only slightly affected the content of palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids. The index of nominal unsaturation of lipids, taking account of the number of double bonds in lipids of the studied yeast, fell as the residual ethanol concentration increased.

Hence, we may conclude that under conditions of unlimited cell growth, the fatty-acid composition of *Y. lipolytica* No. 1 lipids is a sensitive indicator of growth inhibition with ethanol.

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