EFFECTS OF ETHANOL ON THERMAL DEATH AND ON THE MAXIMUM TEMPERATURE FOR GROWTH OF THE YEAST KLUYVEROMYCES FRAGILIS

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SUMMARY

The maximum temperature for growth of a strain of Kluyveromyces fragilis was depressed by increasing ethanol concentration from its normal value around 45°C to 26.5°C at 6.4% (w/v) of ethanol. Ethanol enhanced thermal death by increasing $\Delta S^\#$, the entropy of activation of thermal death. Entropy coefficients of 7.6 entropy units per mol of ethanol per liter of medium for unadapted cells and of 5.7 for adapted cells were obtained.

INTRODUCTION

In Saccharomyces cerevisiae ethanol depressed $T_{\text{max}}$, the maximum temperature for growth (van Uden and Duarte, 1981; Loureiro and van Uden, 1982) and enhanced thermal death (Leão and van Uden, 1982).

The recent interest in inulin-fermenting yeasts for the production of ethanol from tubers of the Jerusalem Artichoke (Guiraud et al., 1979, 1981a, 1981b, 1982; Duvnjak et al., 1981; Margaritis et al., 1981; Margaritis and Bajpai, 1981) led us to study the effects of ethanol on the $T_{\text{max}}$ and on thermal death of the inulin-fermenting yeast Kluyveromyces fragilis.

MATERIAL AND METHODS

Microorganism

Kluyveromyces fragilis IGC 2671 was originally isolated from the contents of a sheep's caecum (van Uden et al., 1958) and proved to be able to ferment inulin very efficiently.

The effects of ethanol on $T_{\text{max}}$ were studied by using a Temperature Gradient Incubator, Model TN-3, Toyo Kasaku Sangyo, Tokyo, Japan and culture media as described (van Uden and Duarte, 1981).

The activation parameters of thermal death and the effects of ethanol thereon were determined as described by Leão and van Uden (1982).

RESULTS AND DISCUSSION

Fig. 1. shows the depression of the $T_{\text{max}}$ of K. fragilis by ethanol. $T_{\text{max}}$ decreased with increasing concentrations of ethanol from around 45°C, its value in the absence of ethanol to as low as
26.5°C at an ethanol concentration of 6.4% (w/v). Though the $T_{\text{max}}$ of $K. \text{fragilis}$ in the absence of ethanol is several degrees higher than the $T_{\text{max}}$ of a strain of $S. \text{cerevisiae}$ studied earlier (van Uden and Duarte, 1981), at concentrations higher than about 2% (w/v) of ethanol the $T_{\text{max}}$ values of the former were lower than those of the latter. This is explained by the fact that the $T_{\text{max}}$ of $S. \text{cerevisiae}$ is not significantly changed by ethanol concentrations up to about 3% (w/v) (Fig. 1). $K. \text{fragilis}$ was unable to grow at temperatures above 30°C in the presence of concentrations of ethanol higher than 5.6% (w/v), while this temperature allowed growth of $S. \text{cerevisiae}$ at concentrations around 9% (w/v). Thus $K. \text{fragilis}$ appeared to be less alcohol-tolerant than $S. \text{cerevisiae}$ with respect to $T_{\text{max}}$.

Arrhenius plots of thermal death in the presence and in the absence of ethanol (Fig. 2) were prepared from the thermal death data of cells that had been grown either at 25°C without ethanol (solid lines) or at 32°C in the presence of 4.8% (w/v) of ethanol (broken lines). The plots constituted a family of parallel straight lines with an average value of $\Delta H^\circ$, the enthalpy of activation of thermal death, of $97.4 \times 10^3$ cal mol$^{-1}$.

Plots of the values of $\Delta S^\circ$, the entropy of activation of thermal death, calculated as described by Leão and van Uden (1982) against the concentration of ethanol, were linear (Fig. 3) according to the equation

$$\Delta S^\circ_x = \Delta S_0^\circ + C^A X$$

where $\Delta S^\circ_x$ and $\Delta S_0^\circ$ represent the activation entropy of thermal death at concentrations $x$ and zero of ethanol and $C^A$ is the entropy coefficient i.e. the increase in entropy of activation of thermal death per mol of ethanol per liter of medium.

Its value for the cells pregrown at 25°C without ethanol was 7.6 entropy units per mol of ethanol per liter of medium (Fig. 3) which was higher than the value of 5.1 obtained earlier for $S. \text{cerevisiae}$ in comparable experiments (Leão and van Uden, 1982). Thus, also with respect to the enhancement of thermal death $K. \text{fragilis}$ appeared to be more ethanol-sensitive than $S. \text{cerevisiae}$.

Cells of $K. \text{fragilis}$ that had been grown at 32°C in a medium with 4.8% (w/v) of ethanol, conditions which led to simultaneous growth and death, were more ethanol-resistant with respect to the enhancement of thermal death: the respective Arrhenius plots (broken lines) were shifted to higher temperatures (Fig. 2) while the value of $C^A$ (Fig. 3) fell to 5.7. Comparable data for $S. \text{cerevisiae}$ are not available.

Though it cannot be decided whether the increased ethanol resistance was due to the growth temperature, the specific growth rate or the ethanol concentration at which the cells had grown, the results suggest that during an alcoholic batch fermentation, the ethanol resistance of the population may change with time.

The effects of ethanol on the $T_{\text{max}}$ and on thermal death of $K. \text{fragilis}$ should be taken into account when selecting the process temperature, particularly if one wishes to reuse the yeast in successive batch fermentations.