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Ethanol Enhancement of Cytochrome P-450 Content in Yeast, *Saccharomyces cerevisiae* D7

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When *Saccharomyces cerevisiae* D7 was cultured in yeast extract-proteose peptone-2% glucose (YPG) liquid medium supplemented with 1.5 and 3% ethanol, the maximum cytochrome P-450 contents in the whole cell suspension were 1.5 and 1.3 times higher, respectively, than that in YPG medium without ethanol. The addition of 6% ethanol to YPG medium decreased the growth rate and final cell population of the yeast markedly, while the cellular content of cytochrome P-450 was similar to that of cells grown in YPG medium. Though the logarithmic phase was extended under glucose-repressed conditions in YPG medium containing 10% glucose, there was no increase in the cytochrome P-450 content compared with the cells grown on 2% glucose. In the medium in which 2% ethanol was the sole carbon and energy source (YPE medium), the growth rate and final cell population of the yeast showed a marked decrease compared with those of cells grown on glucose. Cytochrome P-450 was not detectable in the cells grown on ethanol.

Keywords——cytochrome P-450 content; ethanol enhancement; reduced CO-difference spectrum; yeast; *Saccharomyces cerevisiae*

Saccharomyces cerevisiae has a microsomal cytochrome P-450[1] which is in many respects similar to those found in mammalian microsomes.[2] Though little is known about the metabolic role of yeast cytochrome P-450, yeast has been used as a screening organism for promutagens and procarcinogens without mammalian activation systems such as S-9 fraction and host mediated assay.[3]

Yeast cytochrome P-450 is formed in the cells grown under various conditions, such as in respiratory-repressed cells grown on high glucose concentration (up to 20%),[2a,4] in semi-anaerobically grown cells[2a,5] and in aerobically grown cells on relatively low concentration of glucose.[3a,6,7] In every case, the cytochrome P-450 content in batch-cultured yeast cells was growth-related and reached maximum at the end of the logarithmic phase of growth. Therefore, it was suggested that the formation of cytochrome P-450 in yeast cells is affected by a common metabolite of glucose or an effector generated by the metabolite.[6a] Ethanol is a common metabolite in cultures of yeast grown on glucose.[8] It is also used as a solvent and/or a disinfectant of the compounds tested in mutagenicity assays. The aim of the present paper was therefore to study the effect of exogeneously added ethanol on cytochrome P-450 formation in *S. cerevisiae* D7, which was constructed for mutagenicity assay.[9]

Experimental

Yeast Strain and Culture Conditions——*Saccharomyces cerevisiae* D7 (*a*/α, *ade*2-40/*ade*2-119, *trp*5-12/*trp*5-27, *ilv*1-92/*ilv*1-92)[9] was obtained from Dr. F. K. Zimmermann. Preincubated yeast cells were inoculated into 4 l of each liquid medium at 5×10^4 cells/ml and incubated with vigorous aeration (1.2 l/min) at 30 °C. After various time intervals, samples were withdrawn and cell population and cytochrome P-450 contents were determined. Cell population was counted with a hemocytometer after supersonication (40 W, 29 kHz, 8 s) to remove cell clumps and unseparated daughter cells.

Media——Standard YPG medium contained 1% Difco yeast extract, 2% Difco proteose peptone No. 3 and 2% glucose. YPE medium contained 2% ethanol as a carbon and energy source instead of glucose in YPG medium. YPG medium was also supplemented with 0.5, 1.5, 3 and 6% ethanol. Glucose was sterilized separately and ethanol was added after cooling the autoclaved medium.

Measurement of Cytochrome P-450 Content in Whole Cell Suspensions of Yeast——Cytochrome P-450 was measured principally as described by Omura and Sato.[10] Harvested yeast cells were washed twice in cold distilled water and suspended in 0.1 M phosphate buffer (pH 7.2) at 1.0×10^9 cells/ml. About 0.3 mg/ml of sodium dithionite ($Na_2S_2O_4$) was added and CO gas was bubbled through the sample cuvette for 30 s. The reduced CO-difference spectra were recorded with a Hitachi EPS-3T spectrophotometer. The approximate contents of cytochrome P-450 were calculated as described by Kowal *et al.*[11] by multiplying the absorption differences between 460 and 490 nm by a factor of 2.3 (see Results) and expressed as nmol of cytochrome P-450/10^9 cells. An extinction coefficient of 92/mM/cm[12] was used.

Results

Measurement of Cytochrome P-450 Content and Contribution of Cytochrome Oxidase

In the quantitation of cytochrome P-450 in the whole cell suspensions of *S. cerevisiae* D7, the absorption peak of the cytochrome shifted from 450 to 460 nm after the end of the logarithmic phase. This shift mainly resulted from a deep trough around 443 nm due to the reduced-CO spectrum of cytochrome oxidase (cytochrome a_3). Thus, it was not possible to quantitate the cytochrome P-450 content in the whole cell suspensions on the basis of the absorbancy at 450 nm. We eliminated the contribution of cytochrome oxidase by the method of Kowal *et al.*[11] in which the absorption change between 460 and 490 nm ($A_{460-490}$) was used for calculating the cytochrome P-450 content in bovine liver microsomes.

Table I shows the ratio of $A_{460-490}$ to $A_{450-490}$ in the reduced CO-difference spectra of whole cell suspension and microsome fraction of *S. cerevisiae* D7 harvested before the late logarithmic phase, when little cytochrome oxidase had been formed. The ratio was essentially constant at 43.3 ± 1.9 ($n = 13$). The whole cell suspension from stationary phase culture, which contained a high level of cytochrome oxidase, did not show any absorption at 460 nm (data were not shown). Thus, the approximate contents of cytochrome P-450 in whole cell suspensions were calculated by multiplying $A_{460-490}$ by the factor of 2.3 throughout the

TABLE I. The Ratio of $A_{460-490}$ to $A_{450-490}$ in the Reduced CO-Difference Spectra of Whole Cell Suspension and Microsome Fraction of *S. cerevisiae* D7

Medium	Incubation time (h)	Concentration	$\frac{A_{460-490}}{A_{450-490}}$ (%)
YPG	14	0.5×10^9 cells	45.5
	14	1.0×10^9 cells	44.8
	14	1.0×10^9 cells	44.8
	14	1.7×10^9 cells	42.7
	16	1.0×10^9 cells	45.2
	16	1.0×10^9 cells	44.9
	14	33 mg protein	42.2
	14	55 mg protein	45.1
YPG with 1.5% ethanol	12	1.0×10^9 cells	40.0
	14	1.0×10^9 cells	42.2
	16	1.0×10^9 cells	41.1
YPG with 3% ethanol	14	1.0×10^9 cells	44.3
	16	1.0×10^9 cells	40.0
Average ± SD			43.3 ± 1.9

The percent ratios of $A_{460-490}$ to $A_{450-490}$ in the reduced CO-difference spectra are shown. Concentrations are given as cells/ml for whole cell suspension and as mg protein/ml for microsome fraction. Other conditions were the same as in Figs. 1 and 2.

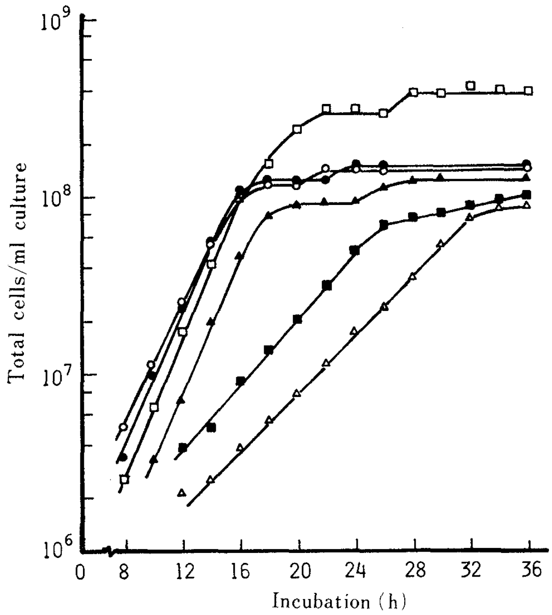

Fig. 1. Growth Curve of *S. cerevisiae* D7 in Each Medium

Media used: yeast extract-peptone–2% glucose (YPG, ●), YPG with 1.5% ethanol (○); with 3% ethanol (▲) and with 6% ethanol (△); YPG with 10% glucose (□); yeast extract-peptone–2% ethanol (YPE, ■). Yeast cells were inoculated into each medium at 5×10^4 cells/ml and incubated at 30 °C aerobically.

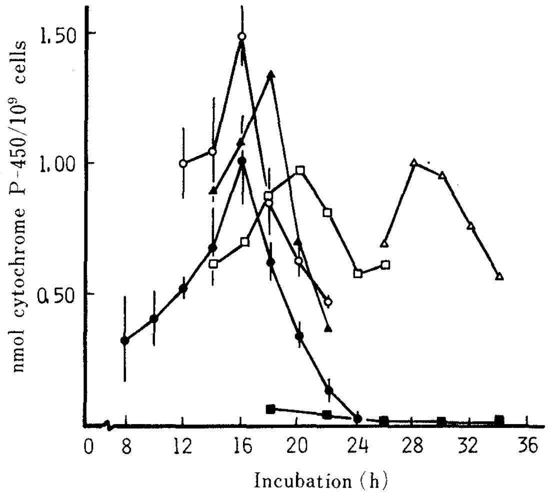

Fig. 2. Time Course of Change of Cellular Cytochrome P-450 Content in *S. cerevisiae* D7 in Each Medium

Media used: Same as in Fig. 1. Samples were withdrawn from each medium, washed and suspended in phosphate buffer at 1.0×10^9 cells/ml. Cytochrome P-450 content was calculated from the reduced CO-difference spectrum as described in the text. Results for YPG and YPG with 1.5% ethanol are averages ± S.D. of 4 and 2 independent experiments, respectively.

experiment.

The Effect of Ethanol on Growth and Cellular Cytochrome P-450 Content of Yeast

Figures 1 and 2 show the time course of growth and cytochrome P-450 content of *S. cerevisiae* D7 in each medium. The addition of 0.5% ethanol to YPG medium did not affect the growth or cytochrome P-450 formation of the yeast (data were not shown). Though the growth and the final cell population were almost the same in YPG medium (control) and that containing 1.5% ethanol, the maximum cellular content of cytochrome P-450 was about 50% higher in the latter. In YPG medium containing 3% ethanol, the growth was somewhat repressed but the maximum cytochrome P-450 content was still 33% higher than the control. Though the growth and final cell population decreased markedly in the presence of 6% ethanol, the cytochrome P-450 content was still equal to that of the control.

In YPG medium containing 10% glucose, the logarithmic phase was extended and the final cell population was almost 2.6 times higher than that in 2% glucose-YPG medium. Though the time course of change of cytochrome P-450 content was rather gradual in 10% glucose-YPG medium, there was no decrease in the maximum content of cellular cytochrome P-450 (Fig. 2). When the yeast was growth on 2% ethanol as a sole carbon and energy source, the growth rate and the final cell population decreased markedly. There was no detectable cytochrome P-450 in the whole cell suspension from the culture grown on ethanol.

Discussion

It has been reported that in aerobically grown yeast cells, cytochrome P-450 is formed only in a medium with high glucose concentration,[2a,4] but in the present experiment, the cytochrome P-450 contents were the same in *S. cerevisiae* D7 grown on 2 and 10% glucose. Callen *et al.*[3a)] and Wiseman *et al.*[7)] have also shown that almost the same amount of cytochrome P-450 is formed in yeast cells grown on 2 or 10% glucose and on 1 or 20% glucose, respectively.

No detectable cytochrome P-450 was formed in *S. cerevisiae* D7 grown on ethanol as a sole carbon and energy source. Cytochrome P-450 was also not formed in other yeast genera, *Candida* and *Torulopsis*, during their growth on ethanol.[13)] In contrast, ethanol increased the cytochrome P-450 contents in higher plant tissues,[14)] cultured hepatocytes,[15)] rats[16)] and hamsters.[17)] The present experiment showed that the addition of 1.5 or 3% ethanol to the medium could increase the cytochrome P-450 content in yeast cells grown on glucose. Though the mechanism of this increase in cytochrome P-450 content is not clear from the present data, it is suggested that ethanol may not be able to induce cytochrome P-450 but may enhance the formation or inhibit the degradation of cytochrome P-450 in the yeast cells grown on glucose. Detailed studies on the formation of cytochrome P-450 and on the activation of promutagens and/or procarcinogens should facilitate the application of the yeast as a screening organism.

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