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Effects of Ethanol on the Induction of Respiration-Deficient Mutants in Yeast by Metal Ions

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The effect of ethanol on the induction of respiration-deficient (RD or petite) mutants of *Saccharomyces cerevisiae* by metal compounds was investigated. At concentrations where the cell numbers in 24-h cultures were repressed to from one-third to one-sixth of the control, $PbCl_2$, $CoCl_2$, $NiCl_2$, $MnCl_2$, $CuCl_2$ and $CdCl_2$ induced 2.7—13.5% RD mutants in surviving cells. The induction frequency of RD mutants by these metal compounds, except $CdCl_2$, was increased to 16.0—36.9% by the addition of 2% ethanol to the culture medium. In contrast, the induction frequency of RD mutants by $CdCl_2$ was repressed by ethanol from 13.0% to 4.8% (ethanol alone gave 6.5%). These findings suggest that ethanol may change the uptake of metal ions into the yeast cells or affect some process involved in RD mutation.

Keywords——yeast; *Saccharomyces cerevisiae*; respiration-deficient mutation; metal compound; ethanol-enhancement

In a previous paper,[1] we have reported that the induction rate of cytoplasmic respiration-deficient (RD or petite) mutants of *Saccharomyces cerevisiae* by metal ions such as Zn^{2+}, Hg^{2+}, Ni^{2+}, Cd^{2+}, Pb^{2+} and Cu^{2+} was decreased by the addition of 4% methanol to the culture medium. The same effect of methanol has been observed in the induction of RD mutants of yeast by aromatic alcohols[2] and acriflavine.[3] It was considered that methanol might prevent uptake or accumulation of these inducers of RD mutants.[3]

Though the physiological effects of ethanol on yeast cells have been studied extensively, detailed studies on the mutagenic effects of ethanol have not been carried out. We have reported that ethanol induced RD mutants in yeast.[3,4] Zakharov and Bandas[5] showed that 24% ethanol induced RD mutants in yeast in nearly half of the survivors. Furthermore, ethanol, like methanol, prevented the induction of RD mutants by aromatic alcohols.[2] In this paper, the effects of ethanol on the induction of RD mutants by metal ions in yeast, *Saccharomyces cerevisiae*, are reported.

Experimental

Yeast Strain——*Saccharomyces cerevisiae* ATCC 26422 (*S. sake* Kyokai no. 7) was used.

Chemicals——Ethanol was of specially prepared reagent grade (Nakarai Chemicals Co., Ltd.). Metal compounds were commercial preparations of guaranteed reagent grade.

Cultivation and Detection of RD Mutants——As described previously,[1] yeast cells were incubated with each metal compound in the presence or absence of 2% ethanol, which did not itself show any growth inhibitory effect on the yeast but repressed the induction of RD mutants by aromatic alcohols.[2] After a 24-h incubation at 30 °C, yeast cells were washed and diluted with sterilized water and spread onto Ogur's agar plates[6] to give about 200 colonies per plate. RD mutants were scored by the tetrazolium salt overlay method.[7] Both completely white and sectored colonies were counted as RD mutants. Results were expressed as averages of three independent experiments with 5 plates.

Results and Discussion

The induction of RD mutants in yeast by each metal compound was tested at the highest concentration showing almost no growth-inhibition. If only slight growth inhibition was recognized, metal compounds were also tested at higher concentration by one or two orders of magnitude. As shown in Table I, 2% ethanol did not inhibit the growth of yeast cells but increased the ratio of RD mutants from the spontaneous level (1.4%) to 6.5%.

At concentrations having almost no growth inhibitory effect on the yeast cells, all the metal compounds tested induced relatively small numbers of RD mutants (1.4—3.1%). Addition of 2% ethanol to the culture medium containing each metal compound increased the ratio of RD mutants only to the level induced by ethanol itself.

At a concentration one or two orders of magnitude higher, $HgCl_2$ (1×10^{-4} M) inhibited the growth of yeast cells completely, while $ZnCl_2$, $PbCl_2$, $CoCl_2$, $NiCl_2$, $MnCl_2$, $CuCl_2$ and $CdCl_2$ showed only slight growth inhibition. At the higher concentrations, the induction frequency of RD mutants by metal compounds was slightly higher than the spontaneous level. The addition of 2% ethanol to the culture medium containing $PbCl_2$, $CoCl_2$, $NiCl_2$, $MnCl_2$ or $CuCl_2$ increased the number of RD mutants considerably (up to 36.9%), to a level higher than the sum of the induction frequencies by ethanol and by the metal compound. This result suggests that RD mutant induction by ethanol and metal compounds is synergistic and involves different processes. On the other hand, the induction frequency of RD mutants by 1×10^{-6} M $CdCl_2$ was decreased from 13.0% to 4.8% by the addition of 2% ethanol. The number of colony-forming cells in a 24-h culture with 1×10^{-6} M $CdCl_2$ was increased from 0.7×10^8 to 1.2×10^8 cells per ml by ethanol.

TABLE I. Effects of Ethanol on Cell Growth and the Induction of RD Mutants by Metal Compounds in Yeast

Compounds	Concn. (M)	2% ethanol	Viable cells per ml culture ($\times 10^{-8}$)	Frequency of RD mutants (%)	Compounds	Concn. (M)	2% ethanol	Viable cells per ml culture ($\times 10^{-8}$)	Frequency of RD mutants (%)
None		−	1.8	1.4±0.8	$NiCl_2$	1×10^{-4}	−	1.6	2.3±0.7
		+	1.7	6.5±2.1			+	1.8	7.4±0.9
$HgCl_2$	1×10^{-5}	−	1.7	2.0±1.1		1×10^{-3}	−	0.7	7.2±4.6
		+	1.7	7.1±0.4			+	1.2	16.3±3.0
$ZnCl_2$	1×10^{-4}	−	1.7	1.5±0.4	$MnCl_2$	1×10^{-3}	−	1.6	2.2±1.5
		+	1.7	4.7±1.1			+	1.9	5.8±2.9
	1×10^{-3}	−	1.3	2.1±1.1		1×10^{-2}	−	0.5	13.5±5.0
		+	1.3	4.5±1.8			+	0.3	35.2±16.7
$PbCl_2$	1×10^{-3}	−	1.7	3.1±2.1	$CuCl_2$	1×10^{-5}	−	1.8	2.2±1.5
		+	1.7	15.2±9.4			+	1.7	7.3±2.8
	1×10^{-2}	−	0.6	5.8±2.3		1×10^{-4}	−	0.3	2.7±2.2
		+	0.5	26.1±12.6			+	0.3	18.0±10.1
$CoCl_2$	1×10^{-5}	−	1.6	3.1±2.5	$CdCl_2$	1×10^{-7}	−	1.8	2.0±1.2
		+	1.6	10.1±1.2			+	1.6	6.9±0.4
	1×10^{-4}	−	1.2	5.6±5.2		1×10^{-6}	−	0.7	13.0±5.7
		+	1.2	13.6±4.8			+	1.2	4.8±2.6
	1×10^{-3}	−	0.7	4.6±3.1					
		+	0.2	36.9±0.6					

Yeast cells were inoculated into Ogur's medium at 1×10^5 cells/ml and incubated at 30 °C for 24 h with or without a metal compound and 2% ethanol. Cells were diluted and spread onto Ogur's agar plates and incubated at 30 °C for 2 d. RD mutants were scored by the tetrazolium salt overlay method. Growth is given as colony-forming cells/ml of culture. Results are the average of at least three independent experiments ± S.D.

The growth of normal and RD mutant cells induced by $CoCl_2$, $NiCl_2$ and $CdCl_2$ was compared in the presence of 2% ethanol. All RD mutants were more sensitive to ethanol than the normal strain was (data not shown). Consequently, a selective killing effect of ethanol on RD mutants is not responsible for the increased proportion of RD mutants in the culture.

Bandas and Zakharov[5b] and Cabeca-Silva *et al.*[8] have suggested that the effect of ethanol on RD mutation is indirect and that the primary target site is located in the mitochondrial membrane. However, the mechanism of ethanol-enhanced RD mutation induced by most of the metal ions tested is not clear from the present experiment. Ethanol has also been reported to change the lipid or sterol composition of the plasma membrane of yeast cells.[9] Thus, it is probable that a conformational change of the plasma membrane may allow increased uptake of metal ions into the yeast cells. Another possibility as regards the ethanol-enhancement of RD mutation induced by metal ions is that ethanol may affect some process(es) involved in RD mutation[10] such as repair, replication, excision or amplification after the action of metal ions on mitochondrial deoxyribonucleic acid (DNA), which codes several respiratory enzymes.

The $CdCl_2$-repressed cell growth was restored by the addition of ethanol to the culture medium. This observation supports the speculation that an inhibitory effect of ethanol on the uptake or accumulation of Cd^{2+} ion is responsible for the ethanol repression of RD mutant induction by $CdCl_2$.

The results presented in this paper indicate that RD mutant induction by metal compounds in yeast may be dependent to some extent on the complicated effects of ethanol, which is a product of the metabolic activity of yeast. Detailed studies on the effect of ethanol on RD mutant induction and on the uptake of metal ions by yeast cells are in progress.

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