JOURNAL OF BACTERIOLOGY, June 1988, p. 2870–2872
0021-9193/88/062870-03$02.00/0

Vol. 170, No. 6

Saccharomyces cerevisiae Acquires Resistance to 2-Deoxyglucose at a Very High Frequency†

MARÍA F. HEREDIA AND CLAUDIO F. HEREDIA*

Instituto de Investigaciones Biomédicas, Consejo Superior de Investigaciones Científicas, Facultad de Medicina, Universidad Autónoma, 28029 Madrid, Spain

Received 2 November 1987/Accepted 26 February 1988

We have found that *Saccharomyces cerevisiae* acquires spontaneously increasing resistance to 2-deoxyglucose at a very high frequency. This finding allows the easy isolation of different types of resistant strains of interest for metabolic studies with 2-deoxyhexoses. On the other hand, it sounds a note of caution in the widespread use of 2-deoxyglucose as a selective agent for the isolation of yeast mutants with impaired hexose transport or phosphorylation systems.

Yeast growth and fermentation are strongly inhibited by 2-deoxyglucose (deGlc) (2, 3, 8). The degree of inhibition depends on the intracellular concentration of 2-deoxyglucose-6-phosphate (deGlc-6-P), the product of phosphorylation of deGlc (15). We previously isolated a mutant of *Saccharomyces cerevisiae* resistant to the toxic effect of deGlc and established the biochemical basis of this resistance (9). The mutant has high levels of a constitutive phosphatase which prevents intracellular accumulation of deGlc-6-P. Accumulation of this and other deoxysugar phosphates has lethal effects not only in yeasts (13) but also in other eucaryotic organisms (5).

Early studies on the metabolic effects of deGlc suggested that *S. cerevisiae* could easily acquire resistance to this 2-deoxysugar. This prompted us to examine the problem more closely to gain insight into the characteristics of the appearance of this resistance. In this work, we have isolated several *S. cerevisiae* strains that show increasing resistance to deGlc and have found that the spontaneous transition from sensitivity to resistance takes place at a rate much higher than the reported spontaneous mutation rates for other characters. The consequences of these findings are discussed in relation to the widespread use of this deoxysugar in biochemical studies (4, 7, 11, 17).

S. cerevisiae PM-1 was grown as described previously (8) in a basal medium containing 0.3% yeast extract (Difco Laboratories), 2% fructose (Fru), and deGlc (Sigma Chemical Co.) as indicated. The number of viable and resistant cells were estimated by spreading appropriate samples on basal medium with or without deGlc. The number of colonies which appeared after 48 h of incubation at 30°C was taken as the number of viable or resistant cells. Phosphatase activity was determined as previously described (12) except that 100 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; pH 6.5) was used. Either liberated deGlc (14) or P_i (1) was determined. Extracts were prepared essentially as previously described (6) by using 50 mM Tris hydrochloride (pH 7) as the extracting buffer. The method of Lowry et al. (10) was used for protein determination.

When *S. cerevisiae* PM-1 was cultured in medium containing Fru (2%) and deGlc (0.1%), a long lag period (ca. 48 h) preceded the onset of growth. During this lag period, the proportion of cells resistant to 0.1% deGlc increased from about 0.02% in the initial inoculum to around 20% after about 70 h of growth (results not shown). When these resistant cells were isolated and cultured in medium containing a much higher concentration of deGlc (0.5%) (Fig. 1), most of the initial population died owing to the lethal effect of the 2-deoxysugar (9). The proportion of cells resistant to the new concentration of deGlc again increased with time, so that under the conditions illustrated in Fig. 1, after 48 h practically all viable cells were resistant to 0.5% deGlc. The increase in the number of resistant cells was paralleled by an increase in the specific activity of the phosphatase (12), which seems responsible for the resistance to deGlc (9) (Fig. 1). These results indicate that during the lag periods preceding the onset of growth, the selection of preexisting cells resistant to deGlc took place. By successive subculturing in medium with increasing concentrations of deGlc, cells with higher resistance to this deoxysugar (up to 2%) could be isolated. Four of these resistant strains were designated PHR01, PHR05, PHR1, and PHR2 in accordance with the ability to grow in basal medium with 2% Fru and, respectively, 0.1, 0.5, 1, and 2% deGlc. The resistant phenotypes were maintained after 10 serial subcultures in medium without deGlc. In each case, whether the selection of cells resistant to a particular concentration of deGlc is accompanied by killing of the sensitive cells depends, among other factors (8), on the levels of intracellular accumulated deGlc-6-P (8, 9), which in turn is a balance between the rates of phosphorylation and dephosphorylation of deGlc. In the presence of sugars sharing the same transport and phosphorylation systems, the phosphorylation of deGlc will depend on its relative proportion to the sugar being used as the carbon source. On the other hand, the rate of dephosphorylation depends on the levels of the deGlc-6-phosphatase (12).

The fact that the resistant strains consistently emerged in different experiments starting with different yeast populations suggests that the acquisition of resistance takes place at a very high frequency. To verify this assumption, we analyzed in detail the characteristics of the appearance of one of the resistant strains (PHR05). Colonies of strain PHR01, resistant to 0.1% deGlc but sensitive to 0.5% deGlc (Fig. 2), were isolated in agar medium without deGlc, and in each of these colonies, we searched for cells that showed higher resistance to deGlc (0.5%) than the parental cells did. Growth appeared in practically all replicas of the original colonies in medium containing 0.5% deGlc, although with

* Corresponding author.

† This work is dedicated to the memory of Marisol, our mother (M.F.H.) and wife (C.F.H.).

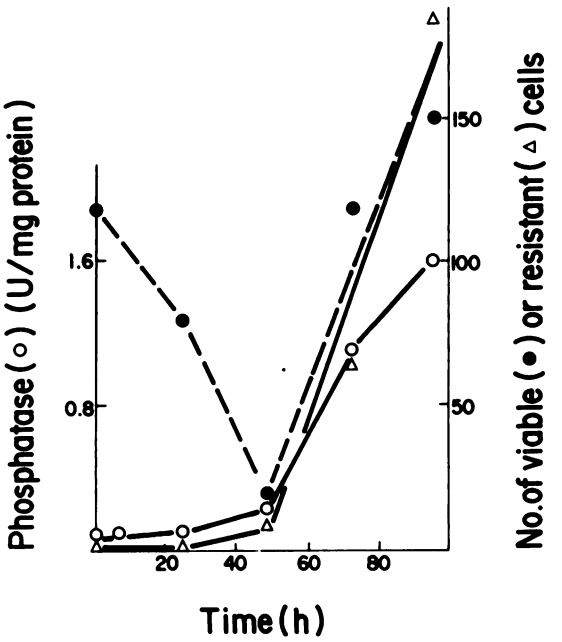

FIG. 1. Selection of yeast cells resistant to 0.5% deGlc. A suspension of strain PHR01 (about 600 mg [wt/wt] in 2 ml of water) was transferred to a flask containing 100 ml of medium with 2% Fru and 0.5% deGlc, and the culture was incubated at 30°C with shaking. Appropriate samples were taken at the times indicated and diluted, and the numbers of viable cells and cells resistant to 0.5% deGlc were estimated by plating in basal medium without or with deGlc, respectively. The number of colonies which appeared after 48 h of incubation at 30°C was taken as the number of viable or resistant cells, which is expressed as the number of cells ($\times 10^7$) per ml of culture.

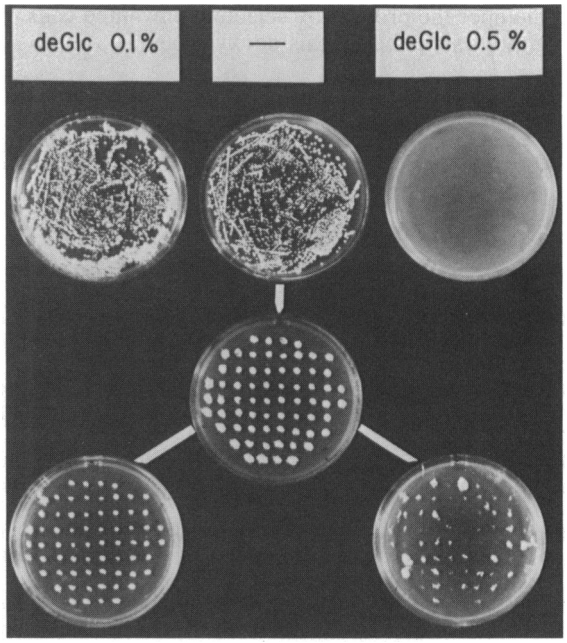

FIG. 2. Analysis of the transition from sensitivity to resistance to deGlc (0.5%) of mutant PHR01. Equal volumes of a suspension of PHR01 were plated in the basal medium with 2% Fru and the indicated concentrations of deGlc. Cells from the colonies grown in medium with Fru were transferred with a toothpick to plates containing the same medium. After incubation at 30°C, the colonies were replicated to medium containing the indicated concentrations of deGlc and incubated at 30°C for 48 h.

different intensities, indicating the widespread occurrence of resistant cells (Fig. 2). Under the conditions illustrated in Fig. 2, no growth of the wild-type cells was apparent in medium with 0.5% deGlc. In a further effort to confirm these observations and to determine the frequency of appearance of the character of resistance, single cells of the same strain (PHR01) were isolated with the aid of a micromanipulator and grown separately in agar medium without deGlc. In each of these single-cell-derived colonies, we looked for cells resistant to 0.5% deGlc as described in the legend to Fig. 1. In 18 of 23 colonies examined, we found the resistant cells. We estimated that there were between 1 and 14 resistant cells for every 10^4 cells (Table 1).

Resistance to deGlc was associated with high levels of a phosphatase active on deGlc-6-P (12) (Table 2 and Fig. 1), confirming previous results (9). In addition, it can be seen (Table 2) that the presence of deGlc in the cultures stimulated (two- to fourfold) the synthesis of this phosphatase. The substrate specificity of this phosphatase is analogous to that found for the 2-deoxyglucose-6-phosphatase characterized previously (12); the phosphatase hydrolyzes deGlc-6-P and fructose-1-phosphate with higher efficiency than other hexose phosphates, although with very low apparent affinity (K_m, $\simeq$15 mM) (results not shown).

It can therefore be concluded that *S. cerevisiae* can acquire spontaneously a progressively increasing resistance to the toxic effect of deGlc at a much higher rate than the standard rate of spontaneous mutations and at a rate similar to those reported for processes in which gene amplification is involved (16). In addition to the potential use of the resistant strains isolated in this work for metabolic studies in which deGlc is used as a glucose analog (4, 7, 11, 17), our findings have practical consequences. deGlc is widely used in the isolation of yeast mutants defective in the hexose transport or phosphorylation system (7). Its use as a selective agent

TABLE 1. Frequency of the transition from sensitivity to resistance to deGlc in strain PHR01

Colony no.	No. of cells/sample		Frequency of resistant cells
	Viable	Resistant to 0.5% deGlc	
1	110,000	160	1.4×10^{-3}
2	170,000	28	1.6×10^{-4}
3	850,000	86	1×10^{-4}
4	240,000	112	4.6×10^{-4}

TABLE 2. Effect of deGlc on the synthesis of 2-deoxyglucose-6-phosphatase by resistant *S. cerevisiae* strains

Yeast strain	% deGlc in culture medium	Phosphatase activity (mU/mg of protein) without (−) and with (+) deGlc	
		−	+
PM-1		22	
PHR01	0.1	50	92
PHR05	0.5	350	900
PHR1	1	533	810
PHR2	2	192	840

might hamper the process by selecting unwanted cells resistant to the toxic effect of this deoxysugar.

This work was supported by a grant from the Comision Asesora de Investigacion Cientifica y Tecnica.

We wish to express our deepest gratitude to Asunción Navarro for technical assistance and to J. M. and C. Gancedo, R. Lagunas, A. Coloma, and C. Mark for their criticism.

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