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Sugar uptake in a 2-deoxy-D-glucose resistant mutant of *Saccharomyces cerevisiae*

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SUMMARY

The non-metabolizable and toxic glucose analogue 2-deoxy-D-glucose (2-DOG) has been widely employed to screen for regulatory mutants which lack catabolite repression. A number of yeast mutants resistant to 2-DOG have recently been isolated in this laboratory. One such mutant, derived from a *Saccharomyces cerevisiae* haploid strain, was demonstrated to be derepressed for maltose, galactose and sucrose uptake. Furthermore, kinetic analysis of glucose transport suggested that the high affinity glucose transport system was also derepressed in the mutant strain. In addition, the mutant had an increased intracellular concentration of trehalose relative to the parental strain. These results indicate that the 2-DOG resistant mutant is defective in general glucose repression.

INTRODUCTION

Glucose or catabolite repression is a major regulatory mechanism in yeast. In the presence of glucose, the synthesis of most enzymes, as well as transport proteins involved in the utilization of various sugars, is strongly repressed [8]. Despite the considerable amount of data published on this subject, the mechanism of glucose repression is still poorly understood. One approach taken to study glucose repression in yeast has involved the isolation of repression resistant or derepressed mutants [10,14,18,19]. The non-metabolizable and toxic glucose analogue 2-deoxy-D-glucose (2-DOG) acts as a gratuitous repressor for a number of enzymes and transport proteins in yeasts [2,6,8,9,13,18,19]. Consequently, cells which are resistant and able to grow in the presence of 2-DOG may exhibit the derepression phenomenon.

A number of 2-DOG resistant mutants have previously been isolated and characterized in this laboratory [10,14]. Some of these mutants exhibit derepression of maltose uptake and fermentation in the presence of glucose. In the fermentation of brewer's wort, the uptake of maltose is under direct control of glucose repression. Only when approximately 50% of the wort glucose has been taken up by the yeast will the uptake of maltose and other repressible sugars commence [7,10]. Therefore, the isolation of mutants resistant to glucose repression may have great industrial importance since many commercial substrates contain a mixture of sugars. One particular mutant obtained from a *Saccharomyces cerevisiae* haploid strain was further investigated and the results presented in this manuscript. Fermentation studies indicated that the utilization of commonly repressed sugars such as maltose, sucrose and galactose was derepressed in the mutant strain. Furthermore, the high affinity glucose transport system was also observed to be derepressed in the mutant strain.

MATERIALS AND METHODS

Chemicals. D-[U-^{14}C]Glucose (270 mCi/mmol) was obtained from ICN Biomedicals (Irvine, CA). All other chemicals were obtained from commercial sources and were of the highest available purity.

Yeast strains and growth medium. The yeast strains employed in this study were, with the Labatt Culture Collection numbers, *Saccharomyces cerevisiae* strain 1190 and its 2-DOG resistant mutant strain 1620. The 2-DOG resistant mutant was isolated as described previously [10,14]. The yeast cells were subcultured in PYN medium which consisted of: peptone, 3.5 g; yeast extract, 3.0 g; KH_2PO_4, 2.0 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; $(NH_4)_2SO_4$, 1.0 g; glucose, 100 g; all dissolved in 1 liter of distilled water and adjusted to pH 5.6.

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Fermentation conditions. Fermentations were conducted in PYN medium containing varying concentrations of glucose and other sugars (maltose, sucrose and galactose) as described in the RESULTS AND DISCUSSION section. Fermentations were carried out at 21 °C in 300 ml Erlenmeyer flasks containing 100 ml of medium and shaken at 150 rpm on an orbital shaker. The yeast inoculum employed in all cases was 3.5 g wet weight cells/l.

Sugar analysis. At specified times during fermentation, 10 ml of cell suspension was withdrawn. The samples were centrifuged at 4000 × *g* for 10 min. The supernatant was subjected to HPLC analysis for determining sugar concentration as described elsewhere [7]. A Spectra-Physics model SP8100 high performance liquid chromatograph incorporating a Bio-Rad oligosaccharide column (Aminex HPX-42A) for glucose and maltose analysis and an HPX-87P column for sucrose and galactose analysis were employed. Both were operated in conjunction with a Spectra-Physics model SP6040 XR refractive index detector and a Spectra-Physics SP4270 computing integrator.

Uptake studies. Mid-exponential phase yeast cells grown on 4% glucose were harvested by centrifuging at 4000 × *g* for 10 min at 4 °C. The cells were washed twice with ice-cold 100 mM potassium phosphate buffer pH 6.6 and suspended in the same buffer at room temperature to a cell density of 5 mg dry wt./ml. Uptake studies were initiated by addition of 1 ml of cell suspension to 2 ml of radioactive substrate to the desired final concentration as described previously [7]. At 15 s intervals, 200 μl of cell suspension was withdrawn, filtered through 0.45 μm nitrocellulose filters and washed twice with ice-cold phosphate buffer. The filters were solubilized in scintillation fluid and radioactivity determined on a liquid scintillation counter.

Trehalose determination. Yeast strains were grown overnight in PYN medium containing 10% glucose. The cells (20 ml suspension) were washed twice with cold distilled water and centrifuged at 4000 × *g* for 10 min. The trehalose was extracted from the cells with 4 ml of cold 0.5 M trichloroacetic acid by agitation for 20 min followed by centrifugation at 4000 × *g* for 10 min. This procedure was repeated three times. The supernatants from the extractions were pooled and intracellular trehalose determined by the anthrone method [17].

RESULTS AND DISCUSSION

The yeast strains employed in this study were pregrown over night in a PYN medium containing 10% glucose to ensure highly repressed conditions at the moment of inoculation. When these cells were inoculated into media containing a mixture of glucose and maltose, the parental *S. cerevisiae* strain 1190 clearly demonstrated glucose repression (Fig. 1). That is, maltose uptake did not occur until most of the glucose was taken up from the media. It has previously been determined that only when approximately 50% of the glucose is taken up by the yeast will the uptake of maltose and other repressible sugars commence [7,10]. On the other hand, the uptake of maltose and glucose was observed to occur simultaneously in the 2-DOG resistant mutant. These results were observed in medium containing an equal concentration of glucose and maltose (Fig. 1A) and in medium

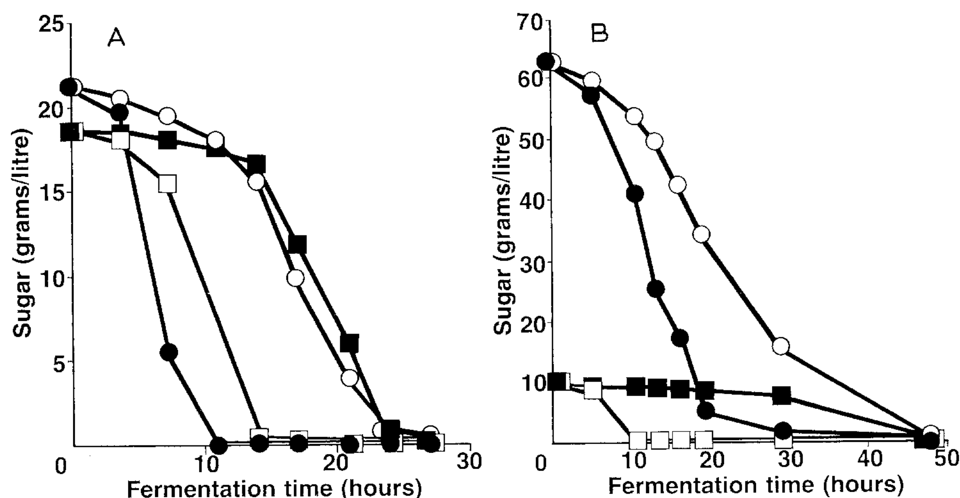

Fig. 1. Sugar uptake during fermentation of glucose-maltose media by *S. cerevisiae* strain 1190 and its 2-DOG resistant mutant. (A) Medium containing 2% glucose and 2% maltose. (B) Medium containing 6% glucose and 1% maltose. Glucose uptake: parent (●) and mutant (○). Maltose uptake: parent (■) and mutant (□).

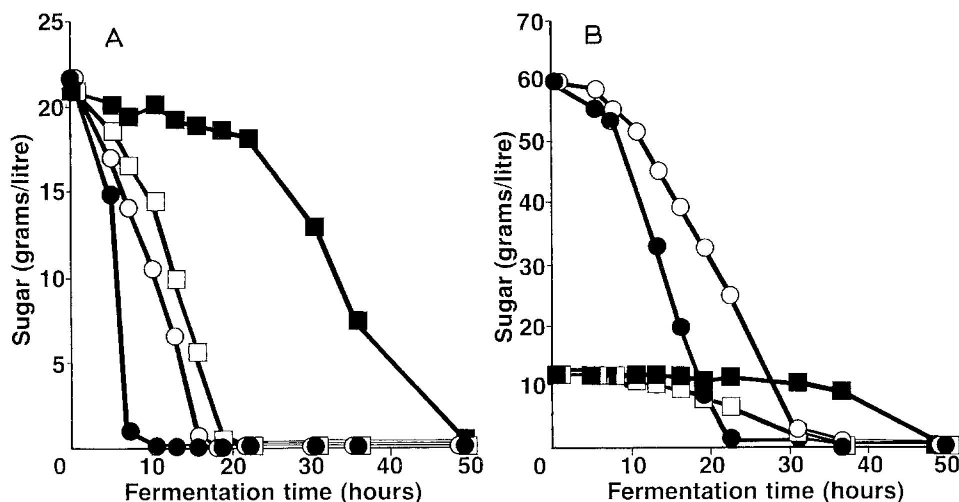

Fig. 2. Sugar uptake during fermentation of glucose-galactose media by *S. cerevisiae* strain 1190 and its 2-DOG resistant mutant. (A) Medium containing 2% glucose and 2% galactose. (B) Medium containing 6% glucose and 1% galactose. Glucose uptake: parent (●) and mutant (○). Galactose uptake: parent (■) and mutant (□).

containing a higher concentration of glucose (Fig. 1B). Furthermore, maltose was observed to be taken up at a faster rate and glucose taken up at a slower rate in the mutant strain compared to the parental strain, suggesting mutations affecting the transport systems for these two sugars in the mutant strain.

The experiments described above were repeated employing media containing mixtures of glucose and galactose or glucose and sucrose (Fig. 2 and 3). As in the case with maltose, galactose uptake was repressed in the parental strain, but was derepressed in the mutant strain (Fig. 2). The uptake of galactose was faster and glucose slower in the mutant strain compared to the parental strain. Similarly, sucrose uptake was repressed in the parental strain but was derepressed in the mutant strain (Fig. 3). Again, sucrose uptake was faster and glucose uptake slower in the mutant strain. These results support the presence of a common regulatory mechanism for several repressible functions in yeast [4,8]. The simultaneous utilization of commonly repressed sugars in the presence of glucose allows for faster fermentations and greater rates of ethanol production [7,10].

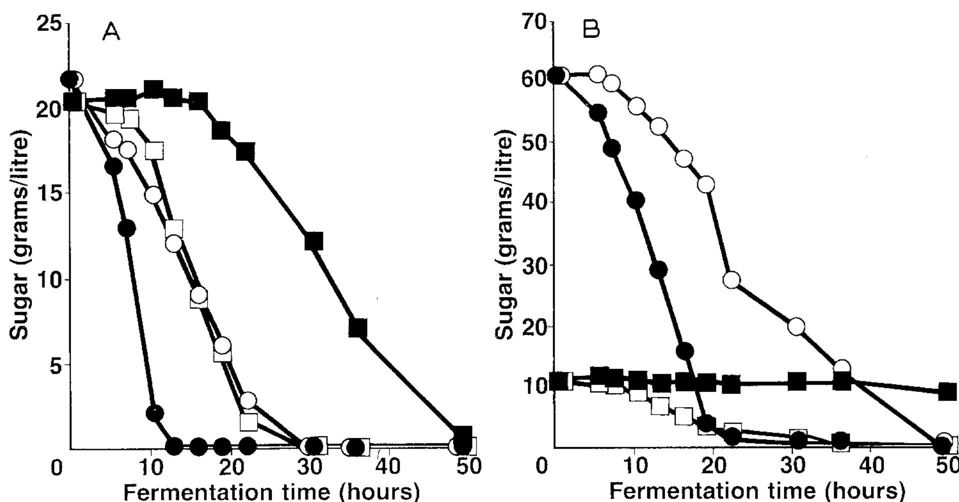

Fig. 3. Sugar uptake during fermentation of glucose-sucrose media by *S. cerevisiae* strain 1190 and its 2-DOG resistant mutant. (A) Medium containing 2% glucose and 2% sucrose. (B) Medium containing 6% glucose and 1% sucrose. Glucose uptake: parent (●) and mutant (○). Sucrose uptake: parent (■) and mutant (□).

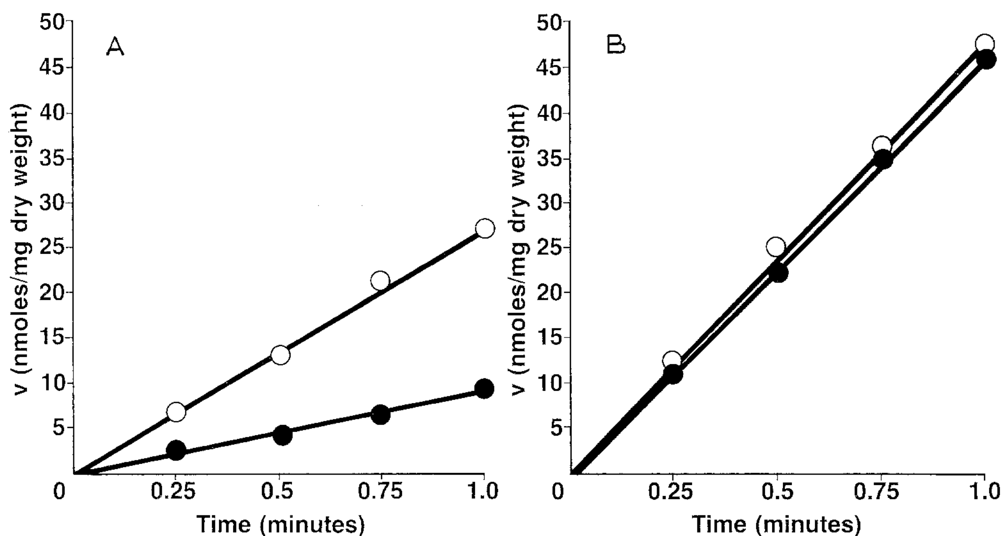

Fig. 4. Transport of glucose into *S. cerevisiae* strain 1190 and its 2-DOG resistant mutant. (A) 2 mM glucose. (B) 50 mM glucose. Parental (●) and mutant (○) strains.

Kinetic analysis of glucose transport in *S. cerevisiae* has demonstrated that glucose is transported by two systems; high and low affinity transport systems with K_m values of approximately 2 mM and 20 mM, respectively [3]. The low affinity transport system is expressed constitutively, whereas the high affinity transport system is under general glucose repression control [4]. Glucose concentrations of 2 mM and 50 mM were employed in this study to reflect the high and low affinity transport systems, respectively. Cells were harvested during the mid-exponential growth phase from media containing 4% glucose to ensure highly repressed conditions. The uptake of 2 mM glucose was observed to be approximately 3-times higher in the mutant strain compared to the parental strain, whereas no significant difference between the two strains was observed when 50 mM glucose was employed (Fig. 4). It should be noted that when higher glucose concentrations were employed, glucose uptake was lower in the mutant strain similar to that observed in the fermentation studies (data not shown). These results suggest that the high affinity glucose transport system is repressed in the parental strain but derepressed in the mutant strain.

Since the high affinity transport system for glucose was determined to be regulated by glucose repression [4,5], it is not surprising that the derepressed mutant strain had higher glucose transport activity. These results suggest that the mutant is not only derepressed for maltose, galactose and sucrose utilization but also for high affinity glucose transport. A recent report on 2-DOG resistant mutants of *Neurospora crassa* describes a class of mutants in which glucoamylase, invertase and the high affinity glucose transport system were derepressed [1]. In addition, yeast mutants defective in glucose repression have recently been isolated by mutagenesis [5]. However, these strains did not exhibit the general derepression of maltose, sucrose and galactose utilization observed in the 2-DOG resistant mutant isolated from *S. cerevisiae* strain 1190. Genetic characterization of the 2-DOG resistant mutant is in progress.

Growth of yeast on glucose has been shown to inhibit trehalose accumulation [15]. Recent studies indicate that trehalose accumulation is closely associated with maltose utilization [15,16]. An essential requirement for trehalose accumulation is that the *MAL4* gene be in the constitutive form. In this form, yeast cells were shown to accumulate trehalose even in the presence of glucose [15,16]. The intracellular accumulation of trehalose has been suggested to make the yeast more resistant to adverse conditions [11]. For example, trehalose has been shown to be an important factor in stabilizing membranes, playing a protective role in osmoregulation and protecting the membrane and cytoplasm under conditions of stress [11,12]. The intracellular trehalose content was determined in *S. cerevisiae* strain 1190 and its 2-DOG resistant mutant (Table 1). It can be seen that the mutant strain contains approximately 2-times more trehalose compared to the parental strain. These results confirm that the mutant strain is defective in general glucose repression, resulting in a higher trehalose content compared to the parental strain which is repressed in the presence of glucose and, therefore, has a lower trehalose

TABLE 1

Trehalose content in glucose grown cells

Yeast strain	Trehalose content (mg/g dry wt.)
Saccharomyces cerevisiae 1190	6.1 ± 0.7
2-DOG resistant mutant 1620	11.7 ± 1.1

The results are the average of three trials. Trehalose was determined as described in MATERIALS AND METHODS.

content. Furthermore, these results suggest that the mutant strain may be more resistant to adverse conditions than the parental strain.

In conclusion, the 2-DOG resistant mutant of *S. cerevisiae* strain 1190 carries a pleiotropic mutation, in which maltose, galactose and sucrose uptake and the high affinity glucose transport system are derepressed. In addition, the mutant has an increased intracellular trehalose content. Further studies on sugar transport with this mutant are in progress.

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