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Characterization of sugar transport in 2-deoxy-D-glucose resistant mutants of yeast

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SUMMARY

A number of 2-deoxy-D-glucose (2-DOG) resistant mutants exhibiting resistance to glucose repression were isolated from various *Saccharomyces* yeast strains. Most of the mutants isolated were observed to have improved maltose uptake ability in the presence of glucose. Fermentation studies indicated that maltose was taken up at a faster rate and glucose taken up at a slower rate in the mutant strains compared to the parental strains, when these sugars were fermented together. When these sugars were fermented separately, only the 2-DOG resistant mutant obtained from *Saccharomyces cerevisiae* strain 1190 exhibited alterations in glucose and maltose uptake compared to the parental strain. Kinetic analysis of sugar transport employing radiolabelled glucose and maltose indicated that both glucose and maltose were transported with higher rates in the mutant strain. These results suggested that the high affinity glucose transport system was regulated by glucose repression in the parental strain but was derepressed in the mutant.

INTRODUCTION

Glucose or catabolite repression is the effect conferred by high concentrations of glucose on many strains of *Saccharomyces* yeast that renders a variety of substrate utilizing systems inactive [3,11,17]. For example, the repressing influence of glucose on maltose uptake and fermentation in yeast has been well documented [3,8,12,18,22,25]. Despite the considerable volume of published data on this subject, its regulatory mechanism is still poorly understood. One approach taken to study the regulation of glucose repression in yeast has been to isolate repression resistant mutants [2,17,29].

The non-metabolizable glucose analogue 2-deoxy-D-glucose (2-DOG) has been employed by a number of investigators to isolate resistant mutants [1,9,10,14,20,22,25,28,29]. This compound is transported by the glucose transport system and is readily phosphorylated by yeast hexokinases into 2-DOG-6-phosphate, without further metabolism. The subsequent accumulation of 2-DOG-6-phosphate in the cell causes several toxic effects such as inhibition of glycolytic enzymes, interference of glucose and mannose incorporation into the cell wall polysaccharides and depletion of cellular ATP pools [4,19,23,24]. In addition, 2-DOG elicits a glucose repression response for a number of enzyme systems in yeast. Consequently, cells which are resistant to 2-DOG may exhibit alterations in the activity of enzymes and/or proteins which are under control of glucose repression (i.e., exhibit the derepression phenomenon).

The isolation of mutants resistant to glucose repression may have great industrial potential since many commercial substrates contain a mixture of sugars. For example, in the fermentation of brewer's wort, the uptake of maltose and other sugars 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 [12,13,22,25]. The simultaneous utilization of commonly repressed sugars in the presence of glucose allows for faster fermentations and greater rates of ethanol production [13,22]. This paper reports on the uptake and fermentation of glucose and maltose in several recently isolated 2-DOG resistant mutants [22,25]. In addition, the trans-

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port of glucose and maltose into these yeast strains was also investigated.

MATERIALS AND METHODS

Chemicals. D-[U-^{14}C]Glucose (270 mCi/mmol) and D-[U-^{14}C]maltose (420 mCi/mmol) were obtained from ICN Biomedical (Irvine, CA). All other chemicals were obtained from commercial sources and were of the highest available purity.

Yeast strains and growth medium. Table 1 lists the genotypes of the strains employed in this study. Mutant strains resistant to 2-DOG were isolated as described previously [22]. 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; $(NH_4)_2SO_4$, 1.0 g; $MgSO_4 \cdot 7H_2O$, 1.0 g; glucose, 100 g; all dissolved in 1 litre of distilled water and adjusted to pH 5.6.

Fermentation conditions. Fermentations were conducted in PYN medium containing varying concentrations of glucose and/or maltose as described in RESULTS. Fermentations were carried out at 21 °C in 300 ml Erlenmeyer shake flasks containing 100 ml of medium, with constant agitation of 150 rpm. The yeast inoculum employed in all cases was 3.5 g (wet weight) cells/l.

Estimation of sugar uptake during fermentation. 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 concentrations. A Spectra-Physics model SP8100 high performance liquid chromatograph incorporating a Bio-Rad oligosaccharide column (Aminex HPX-42A) for glucose and maltose analysis was employed. The column was operated in conjunction with a Spectra-Physics model SP6040 XR refractive index detector and a Spectra-Physics model SP4270 computing integrator.

Uptake studies. Mid-exponential grown yeast cells were harvested by centrifuging at 4000 × *g* for 10 min at 4 °C. For glucose uptake studies, glucose grown 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 30 mg wet weight/ml [12]. For maltose uptake studies, maltose grown cells were washed twice with ice-cold distilled water and suspended in 100 mM tartaric acid-Tris buffer, pH 4.2 [27]. Uptake studies were initiated by addition of 1 ml of cell suspension to 2 ml of radioactive substrate to the desired final concentration. At 15-s intervals, 200 μl of cell suspension was withdrawn and filtered through 0.45 μm nitrocellulose filters and washed twice with 5 ml of ice-cold phosphate buffer. The filters were solubilized in liquid scintillation fluid and radioactivity determined on a liquid scintillation counter.

RESULTS

Sugar uptake during fermentation

Fermentations were initially carried out in media containing equal amounts of glucose and maltose (20 g/l of each sugar). Yeast strains were grown on PYN medium containing 100 g/l of glucose so that the yeasts were highly repressed at the moment of inoculation. Glucose repression can be clearly observed in all four parental strains examined (Fig. 1). In all cases, maltose uptake did not occur until most of the glucose was utilized from the medium. On the other hand, the uptake of maltose and glucose was observed to occur simultaneously in the

TABLE 1

List of strains employed

Labatt Yeast Culture Number	*Saccharomyces* species	Genotype
154	*Saccharomyces cerevisiae*	Ale brewing polyploid
1190	*Saccharomyces cerevisiae*	*a, MAL2, his4, leu2*
1393	*Saccharomyces diastaticus*	*a/α, DEX1/DEX1, DEX2/DEX2, STA3/STA3, MAL6/mal*
1384	*Saccharomyces diastaticus*	*a/α, DEX1/DEX1, DEX2/DEX2, STA3/STA3, mal/mal*
3021	*Saccharomyces uvarum (carlsbergensis)*	Lager brewing polyploid
1400	*Saccharomyces diastaticus*	Fusion product (1384 × 3021)

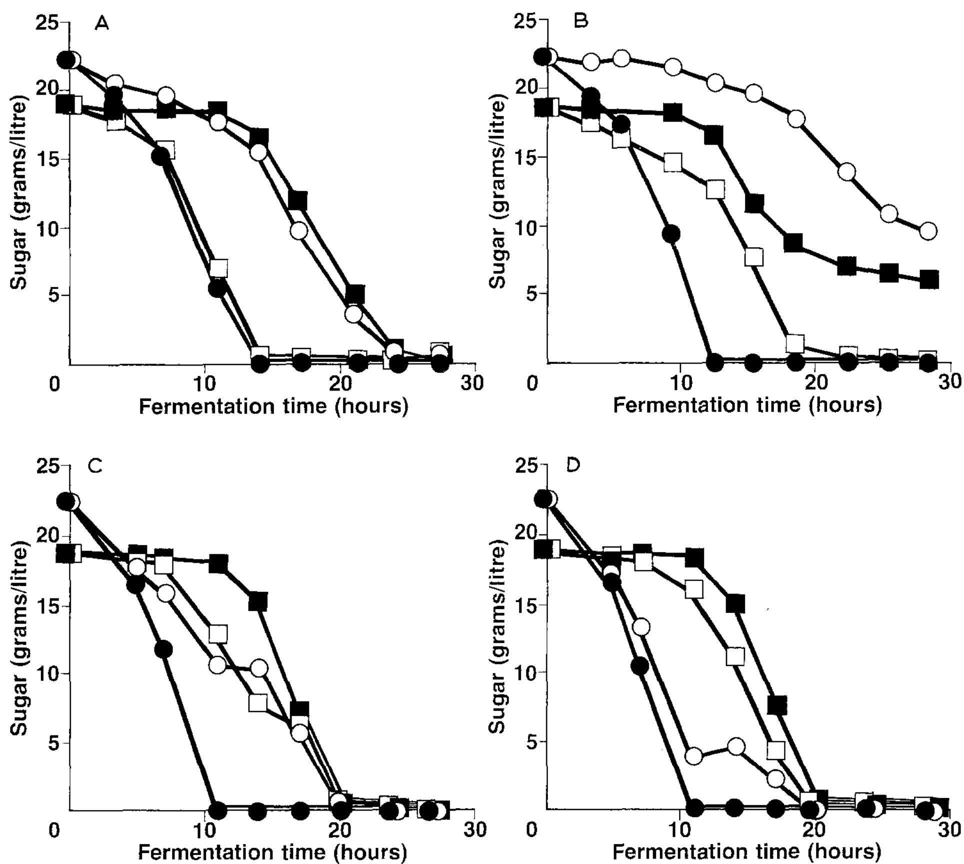

Fig. 1. Sugar uptake during the fermentation of a 2% glucose/2% maltose medium. The strains employed were (A) *Saccharomyces cerevisiae* 1190 (B) *S. cerevisiae* 154, (C) *S. diastaticus* 1393 and (D) *S. diastaticus* fusion product 1400 and their respective 2-DOG resistant mutants. The plots show glucose uptake in the parental (●) and mutant strains (○) and maltose uptake in the parental (■) and mutant strains (□).

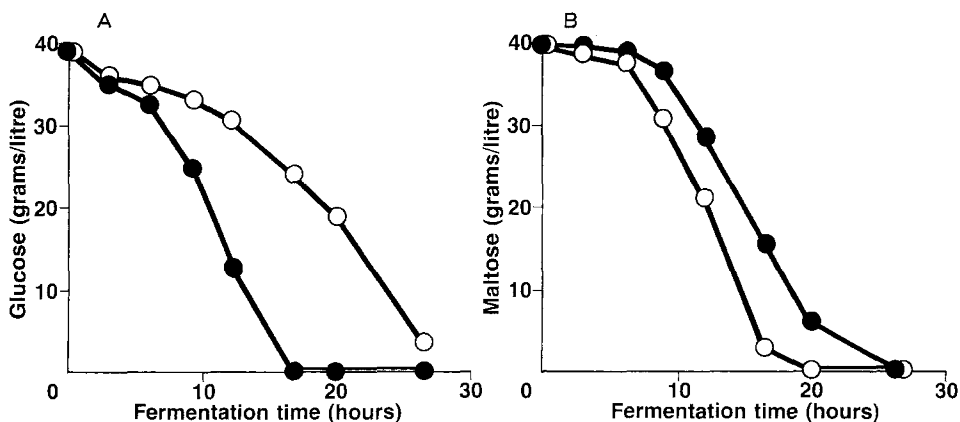

Fig. 2. Glucose and maltose uptake by *Saccharomyces cerevisiae* strain 1190 (●) and its 2-DOG resistant mutant (○). Fermentations were conducted in (A) 4% glucose and (B) 4% maltose media.

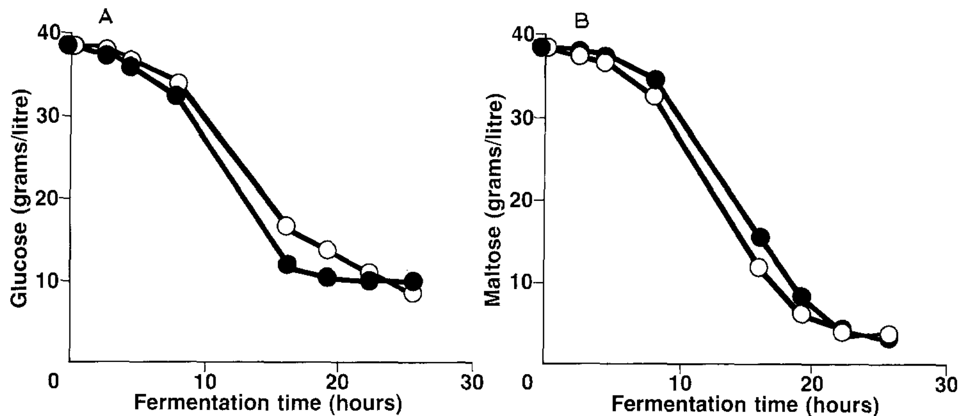

Fig. 3. Glucose and maltose uptake by *Saccharomyces cerevisiae* strain 154 (●) and its 2-DOG resistant mutant (○). Fermentations were conducted in (A) 4% glucose and (B) 4% maltose media.

2-DOG resistant mutants. Maltose was taken up approximately twice as fast and glucose taken up approximately two times slower in the mutant strains compared to the parental strains demonstrating that the mutants are resistant to glucose repression (i.e., derepressed).

Fermentations were also carried out separately on 40 g/l glucose and 40 g/l maltose. The results indicate that *Saccharomyces cerevisiae* strain 1190 and its 2-DOG resistant mutant (Fig. 2) possess uptake profiles similar to Fig. 1A. Maltose was, again, taken up approximately 2-times faster and glucose approximately 2-times slower in the mutant strain compared to the parental strain. On the other hand, *Saccharomyces cerevisiae* strain 154 and its 2-DOG resistant mutant exhibited very little differences in maltose and glucose uptake profiles under these conditions (Fig. 3). Similar results were observed with the other yeast strains and their respective 2-DOG resistant mutants that were examined (data not shown). These results suggest that the 2-DOG resistant mutant obtained from *Saccharomyces cerevisiae* strain 1190 may have alterations in glucose and maltose uptake. Therefore, the transport of glucose and maltose in these two strains was further investigated.

Transport studies

The results of 1.0 mM glucose and 1.0 mM maltose uptake studies in *Saccharomyces cerevisiae* strain 1190 and its 2-DOG resistant mutant are presented in Fig. 4. It can be seen that the rate of maltose uptake was more than 2-times higher in the mutant strain than in the parental

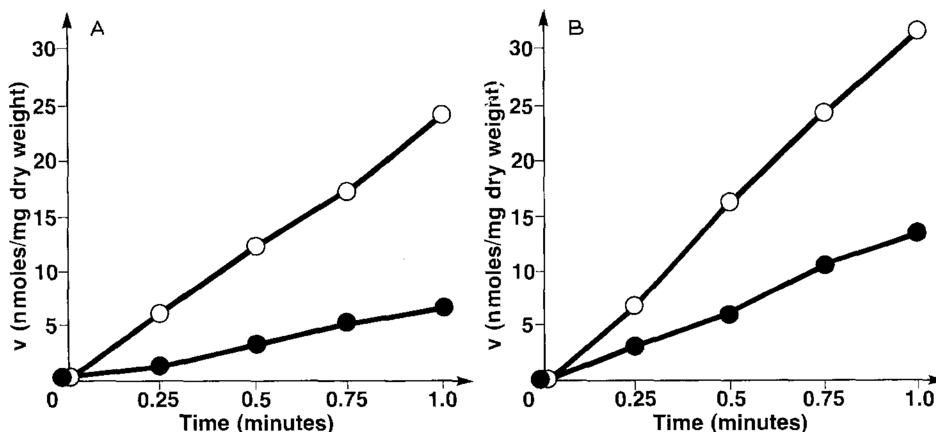

Fig. 4. The transport of glucose and maltose into *Saccharomyces cerevisiae* strain 1190 (●) and its 2-DOG resistant mutant (○). Transport studies were performed with (A) 1 mM glucose and (B) 1 mM maltose.

strain. These results are in agreement with the fermentation profiles presented in Figs. 1A and 2. Surprisingly, the rate of glucose uptake was also higher (approximately 4-times) in the mutant strain, which at first glance is in contradiction to the fermentation profiles. However, a possible explanation for this apparent discrepancy is discussed in the next section.

DISCUSSION

The incubation of parental yeast strains in a medium containing equal concentrations of glucose and maltose resulted in the characteristic pattern of glucose repression (Fig. 1). That is, maltose uptake did not commence until most of the glucose had been depleted from the medium. On the other hand, the 2-DOG resistant mutants utilized both sugars simultaneously, clearly illustrating the derepression phenomenon. Glucose was observed to be taken up at a slower rate and maltose taken up at a faster rate in all of the mutant strains examined compared to their respective parental strains. In addition, the rate of ethanol production was observed to be greater in the mutant strains than in the parental strains (data not shown). The simultaneous utilization of commonly repressed sugars in the presence of glucose allows for faster fermentations and greater rates of ethanol production [12,13,22,25].

The derepression of maltose uptake and fermentation is more pronounced in the mutants obtained from *Saccharomyces cerevisiae* strains (Fig. 1A, B) than from *Saccharomyces diastaticus* strains (Fig. 1C, D). It should be noted that *Saccharomyces diastaticus* produces an extracellular glucoamylase which will hydrolyse maltose resulting in a transient increase in glucose concentration [16]. The production of this enzyme is under control of glucose repression [10,14,16,22,25]. However, the results in Fig. 1C and D suggest that the activity of this enzyme is derepressed in the mutant strains.

The uptake of glucose and maltose during the fermentation of media containing equal concentrations of these sugars indicated that the uptake systems for both sugars were altered in the mutant strains, although to different extents. When fermentations were performed with media containing only glucose or maltose, very little difference in glucose and maltose uptake between some of the mutant strains and their respective parental strains were observed (Fig. 3). This would suggest that the repression regulatory mechanism was altered in the mutant strains such that the mutants do not exhibit repression of maltose uptake in the presence of glucose but were unaffected under derepressed conditions (i.e., the absence of glucose). However, the uptake profiles for glucose and maltose in *Saccharomyces cerevisiae* strain 1190 and its 2-DOG resistant mutant were virtually identical to that observed when these sugars were fermented together (compare Fig. 2 with 1A). In both cases, maltose was taken up approximately 2-times faster and glucose taken up approximately 2-times slower in the mutant compared to the parental strain. These results demonstrate that the 2-DOG resistant mutant obtained from *Saccharomyces cerevisiae* strain 1190 has alterations in glucose and maltose uptake. Therefore, kinetic analysis of sugar transport was further investigated in these two strains.

In *Saccharomyces cerevisiae*, glucose and maltose are transported into the cell by different carriers [3]. Maltose transport is induced by maltose and repressed by glucose [18] and is driven by a proton-symport mechanism [26,27]. Kinetic studies have identified two transport components, a high and low affinity transport system with K_m values of approximately 4 and 70 mM, respectively [8]. Furthermore, both components of the maltose transport systems are rapidly and irreversibly inactivated upon glucose addition into the medium [8,21]. Glucose is transported into the cell by facilitated diffusion and again two transport components have been identified, a high and low affinity transport system, with K_m values of approximately 2 and 20 mM, respectively [6]. Kinetic parameters for both glucose and maltose transport similar to these have also been determined for *Saccharomyces cerevisiae* strain 1190 (in preparation). The low affinity system for glucose seems to be expressed constitutively, whereas the high affinity system is operating under glucose repression [5,7]. Furthermore, at least one of the glucose phosphorylating enzymes is necessary for its function [6]. Glucose repression of the high affinity glucose uptake system has recently been detected in a number of fermentative yeast species [15].

The results of glucose and maltose transport in *Saccharomyces cerevisiae* strain 1190 and its 2-DOG resistant mutant demonstrate that the rate of maltose uptake was approximately 2-times higher in the mutant strain compared to the parental strain. These results are in agreement with the maltose uptake profiles in the fermentation trials. Similarly, the rate of glucose uptake was observed to be approximately 4-times higher in the mutant strain (Fig. 4). This is in contradiction to the fermentation results in which glucose was observed to be taken up at a slower rate in the mutant strain compared to the

parental strain. The differences in these results are based on the sugar concentration employed in the separate studies. For example, in the fermentation trials, the concentration of glucose and maltose employed was approximately 40 g/l (Fig. 2) which is equivalent to approximately 220 mM and 110 mM, respectively. This concentration is high enough for repression of the high affinity transport component [5,7]. On the other hand, the concentration of glucose and maltose employed in the transport studies was only 1 mM, which would reflect predominantly the high affinity transport system. Since the high affinity system for glucose uptake was determined to be under glucose repression, it is not surprising that the mutant strain had higher glucose uptake activity (i.e. derepressed). This would suggest that the mutant is not only derepressed for maltose uptake 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 general glucose repression have also recently been identified [5]. Studies of glucose and maltose uptake, as well as other sugars, in the 2-DOG resistant mutant are continuing in this laboratory.

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