Hexokinase activity is affected in mutants of *Kluyveromyces lactis* resistant to glucose repression

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The effect of glucose on a number of mitochondrial and cytoplasmic enzymes involved in carbon metabolism (L-lactate:ferricytochrome-c 2-oxidoreductase, malate dehydrogenase, β -galactosidase, invertase, maltase and NAD-glutamate dehydrogenase) has been analysed in two different strains of *Kluyveromyces lactis* (PM4-4B and JA6 strains). All the above mentioned enzymes were catabolite-repressible in a strain-dependent way. From this study differences in the regulation of some enzymes have been observed between *K. lactis* and *Saccharomyces cerevisiae*. To identify genes involved in glucose metabolism that may also control carbon catabolite repression, 2-deoxyglucose-resistant mutants (Dgr^+) of *K. lactis* that were unable to grow on glucose in the presence of antimycin A (Rag^-) were selected. In this way we identified four classes of mutants. Two of them define genes previously identified: *RAG1* and *RAG5*, encoding the low affinity glucose transport system and hexokinase, respectively. The two remaining classes of mutants define two new genes, *DGR148* and *DGR239*, that control the level of hexokinase, indicating that in *K. lactis* this enzyme is positively regulated by at least two genes. All the mutants devoid of hexokinase showed relief from carbon catabolite repression of several enzymes.

Keywords: *Kluyveromyces lactis*, glucose repression, hexokinase, regulatory mutants

INTRODUCTION

In the yeast *Saccharomyces cerevisiae*, as in other micro-organisms (Magasanik, 1961), the level of activity of several enzymes is subjected to repression by glucose or other rapidly fermentable carbon sources. This phenomenon, called catabolite repression, is a complex regulatory mechanism that intervenes in the control of the expression of several genes involved both in fermentative (Polakis & Bartley, 1965; Gorts, 1967) and oxidative (Slonimski, 1953; Polakis *et al.*, 1965) metabolism in yeast. In order to identify the different elements controlling this regulatory system, many carbon catabolite repression mutants have been isolated and characterized (reviewed by Gancedo & Gancedo, 1986). They suggested that carbon catabolite repression is under the control of interacting genes, and Gancedo (1992) has developed a model to explain the interactions.

Abbreviations: EMS, ethylmethanesulfonate; L-LCR, L-lactate:ferricytochrome-c 2-oxidoreductase (EC 1.1.1.2.3); MDH, malate dehydrogenase (EC 1.1.1.37); NAD-GDH, NAD-dependent glutamate dehydrogenase (EC 1.4.1.3).

In *S. cerevisiae*, one of the glucose-repressed functions is respiration. Thus, carbon metabolism is either oxidative or fermentative. Contrary to what is observed in *S. cerevisiae*, glucose catabolite repression of respiration does not occur in the obligately aerobic *Kluyveromyces lactis* (De Deken, 1966a, b; Ferrero *et al.*, 1978). Therefore, fermentative and oxidative utilization of carbon sources co-exist in *K. lactis*. However, carbon catabolite repression of some cytoplasmic enzymes has been described in *K. lactis* (Ferrero *et al.*, 1978; Dickson & Markin, 1980). In particular, much is known about glucose repression of lactose/galactose metabolism mediated by the *LAC9* gene (Wray *et al.*, 1987; Breunig, 1989; Zachariae *et al.*, 1993), the *K. lactis* homologue of the *S. cerevisiae* regulatory gene *GAL4* (Salmeron & Johnston, 1986; Wray *et al.*, 1987; Breunig & Kuger, 1987). Recently it was found that the two mitochondrial enzymes L- and D-lactate:ferricytochrome-c 2-oxidoreductase are repressible by glucose (Lodi *et al.*, 1994). However, in *K. lactis* mutations affecting the general response to carbon catabolite repression have not hitherto been described.

For this reason we first identified enzymes responding to glucose repression in *K. lactis*. We then isolated mutants

in which the repressing effects of glucose were pleiotropically relieved in order to identify regulatory genes involved in the control of glucose repression. These mutants defined four complementation groups, three of which are affected in hexokinase activity, thus demonstrating that hexokinase has a central role in the regulation of glucose repression in *K. lactis*.

METHODS

Strains and general genetic methods. Strains used in this study are listed in Table 1. Genetic procedures for crossing and sporulation have been previously described (Wésolowski *et al.*, 1982; Goffrini *et al.*, 1989). Rich medium (YP) contained 5 g Difco Bacto yeast extract l⁻¹ and 10 g Difco Bacto peptone l⁻¹. The synthetic minimal medium contained 0.7 g Difco yeast nitrogen base (without amino acids) l⁻¹ supplemented with amino acids and bases, as required.

The different carbon sources utilized were added at 2% (w/v). 2-Deoxy-D-glucose (Fluka) was added at the concentration indicated in Results, according to the minimal inhibitory concentration determined previously (data not shown). Antimycin A (Sigma) was used at a final concentration of 5 µM. For mating and sporulation the medium was 50 g Difco Bacto malt-extract l⁻¹. Media were solidified with 20 g Difco Bacto agar l⁻¹.

Isolation of mutants. Mutants were isolated using ethyl-methanesulfonate (EMS) as a mutagen. The JA6 strain was grown to stationary phase, collected, washed with 0.1 M potassium phosphate buffer, pH 8, and resuspended in the same buffer at 1 × 10⁸ cells ml⁻¹. Cell suspension (3 ml) was divided

into three portions of 1 ml and treated with EMS (20 µl ml⁻¹, 10 µl ml⁻¹, 5 µl ml⁻¹) for 45 min. The cells were then washed three times with water, suspended in complete medium and incubated for 90 min at 28 °C. The cells were harvested, washed with water and plated to determine cell survival. Those samples of EMS-treated cells which exhibited survival rates from 10% to 20% were used to screen for mutants. To select for nonrepressible mutants, cells were plated onto minimal medium containing 20 g raffinose l⁻¹ and 5 g 2-deoxyglucose l⁻¹ and maintained at 28 °C. After a week, approximately 1500 colonies appeared.

Dominance tests and complementation analysis. To test for dominance, each of the mutants was crossed to the wild-type strain PG9-11D. For complementation analysis with *rag1*, mutants were crossed with the PG13-20C strain harbouring a null *rag1* allele. To test the identified mutations for allelism, segregants (PG139-7B, PG148-3A, PG151-7C and PG239-5B) derived from back-crosses of the mutants with the wild-type strain were crossed to the original mutants. In most cases diploids were isolated by prototrophic selection.

For allelism with previously identified mutations conferring the same phenotype (from *rag2* to *rag13* and *pfk2*) heterozygous diploids were constructed by crossing either the original mutant or a segregant to each of the previously identified mutants. When the selection against both haploid parents was not possible, diploids were identified by testing for the ability of single colony derived from the cross to sporulate. Complementation tests were performed by replica-plating of the diploids onto antimycin-A-containing medium and the Rag phenotype was scored after 48 h.

Table 1. List of *K. lactis* strains

Strain	Genotype	Source
HK5-4D	<i>a ade his3-35 leu2-137 ura3-12 pfk2::URA3</i>	Obtained from Dr J. Heinisch
JA6	<i>α ade1 ade2 trp1-11 uraA</i>	Obtained from Dr K. D. Breunig
JA6/138	<i>α ade1 ade2 trp1-11 uraA dgr138</i>	This study
JA6/139	<i>α ade1 ade2 trp1-11 uraA dgr139</i>	This study
JA6/148	<i>α ade1 ade2 trp1-11 uraA dgr148</i>	This study
JA6/151	<i>α ade1 ade2 trp1-11 uraA dgr151</i>	This study
JA6/239	<i>α ade1 ade2 trp1-11 uraA dgr239</i>	This study
MW109-8C/FA39	<i>α lysA1-1 trpA1 rag9</i>	Obtained from Dr M. Wésolowski-Louvel
MW109-8C/FA42	<i>α lysA1-1 rag8</i>	Obtained from Dr M. Wésolowski-Louvel
MW125-21A	<i>α uraA rag4</i>	Obtained from Dr M. Wésolowski-Louvel
MW161-3B	<i>α lysA1-1 rag10</i>	Obtained from Dr M. Wésolowski-Louvel
MW188-1C	<i>a lysA1-1 trpA1 rag12</i>	Obtained from Dr M. Wésolowski-Louvel
PG9-11D	<i>a trp1 metA1 uraA</i>	Parma collection
PG13-20C	<i>a trp1 metA1 uraA rag1::URA3</i>	Parma collection
PG139-7B	<i>a metA1 trp1 uraA dgr139</i>	This study
PG148-3A	<i>a metA1 trp1 uraA dgr148</i>	This study
PG151-7C	<i>a metA1 trp1 uraA dgr151</i>	This study
PG239-5B	<i>a metA1 trp1 uraA dgr239</i>	This study
PM4-4B	<i>α ade1 ade2 uraA</i>	Parma collection
PM4-4B/RAG2	<i>α ade1 ade2 uraA rag2::URA3</i>	Parma collection
PM6-7A/VV10	<i>a ade2 uraA rag11</i>	Obtained from Dr M. Wésolowski-Louvel
PM6-7A/VV13	<i>a ade1 ade2 uraA rag3</i>	Obtained from Dr M. Wésolowski-Louvel
PM6-7A/VV18	<i>a ade2 uraA rag7</i>	Obtained from Dr M. Wésolowski-Louvel
PM6-7A/VV36	<i>a ade2 uraA rag13</i>	Obtained from Dr M. Wésolowski-Louvel
PM6-7A/VV41	<i>a ade2 uraA rag5</i>	Obtained from Dr M. Wésolowski-Louvel

Preparation of cell-free extract and enzyme assays. Cells grown at a density of approximately 5×10^7 cells ml⁻¹ were harvested by centrifugation and washed once with distilled water. The cell pellet was resuspended in extraction buffer (0.1 M Tris/HCl, pH 8.5, 1 mM phenylmethylsulfonyl fluoride) and the cells were disrupted by vortexing at 4 °C in the presence of an equal volume of glass beads (0.5 mm diameter). The suspension was then centrifuged at 5000 *g* for 5 min at 4 °C. The pellet was discarded and the supernatant utilized for measuring the enzyme activity. The Lowry method was used for protein quantification with bovine serum albumin as standard.

β -Galactosidase (EC 3.2.1.23) activity was assayed as described by Cohn & Monod (1951). L-Lactate:ferricytochrome-*c* 2-oxidoreductase (L-LCR) (EC 1.1.2.3) was assayed according to Lodi *et al.* (1994). Total malate dehydrogenase (MDH) (EC 1.1.1.37) was assayed according to Polakis & Bartley (1965). NAD-dependent glutamate dehydrogenase (NAD-GDH) (EC 1.4.1.3) was assayed as described by Grisolia *et al.* (1964). Total α -glucosidase (maltase, EC 3.2.1.20 and isomaltase, EC 3.2.1.10) activity was tested with *p*-nitrophenyl-*O*- α -glucopyranoside as a substrate as described by Zimmermann *et al.* (1977); hexokinase (EC 2.7.1.1) was assayed with glucose as substrate, as described by Clifton *et al.* (1978); invertase (EC 3.2.1.26) was assayed according to Goldstein & Lampen (1975) and to Celenza & Carlson (1984).

RESULTS

K. lactis enzymes repressed by glucose

In order to define the spectrum of enzyme activities sensitive to glucose catabolite repression in *K. lactis*, the cytoplasmic enzymes invertase, maltase, NAD-GDH and β -galactosidase, the total MDH (cytoplasmic and mitochondrial) and the mitochondrial enzyme L-LCR were analysed in cultures grown in the presence or in the absence of glucose. The effect of glucose on the levels of

Table 2. Effect of glucose on the level of maltase, invertase, NAD-GDH, β -galactosidase, MDH and L-LCR in PM4-4B and JA6 strains of *K. lactis*

Strain genotypes are listed in Table 1. Cultures were grown in YP medium supplemented with 2% (w/v) lactate with or without the addition of 2% (w/v) glucose. Enzyme activities were measured as described in Methods. Enzyme units are expressed as nmol substrate min⁻¹ (mg protein)⁻¹. All values are means of three independent experiments. In no case was the variation higher than 15%.

Enzyme	Strain			
	PM4-4B		JA6	
	Lactate	Lactate + glucose	Lactate	Lactate + glucose
Maltase	< 3	< 3	109	< 3
Invertase	83	54	97	< 3
NAD-GDH	380	150	2090	550
β -Galactosidase	411	200	64	22
MDH	2820	1715	1475	323
L-LCR	490	89	98	17

Table 3. Effect of glucose on the level of maltase, invertase and β -galactosidase in PM4-4B and JA6 strains of *K. lactis* grown in the presence of the specific substrates

Strain genotypes are listed in Table 1. Cultures were grown in YP medium supplemented with 2% (w/v) maltose, raffinose or galactose for the determination of maltase, invertase and of β -galactosidase, respectively, with or without the addition of 2% (w/v) glucose. Enzyme activities were measured as described in Methods. Enzyme units are expressed as nmol substrate min⁻¹ (mg protein)⁻¹. All values are means of three independent experiments. In no case was the variation higher than 15%.

Enzyme	Strain			
	PM4-4B		JA6	
	No glucose	Glucose	No glucose	Glucose
Maltase	80	5	55	< 3
Invertase	146	76	189	< 3
β -Galactosidase	2070	1790	909	176

these enzymes was carried out in two different strains: PM4-4B and JA6. Cultures of these strains were prepared on media containing either lactate as a 'nonrepressing' carbon source or lactate plus glucose. The data obtained are reported in Table 2. All the enzyme activities measured were sensitive to catabolite repression, though to different extents. The JA6 strain was more sensitive to glucose than the PM4-4B strain for all the enzymes tested, except for maltase, which in PM4-4B was undetectable in the presence of lactate. In addition, a strain-dependent level of the enzymes was observed in cultures grown in the absence of glucose (nonrepressed level).

We then tested maltase, invertase and β -galactosidase in cells grown in the presence of the specific substrate and possible inducers: maltose, raffinose and galactose, respectively. The analysis of the results reported in Table 3 indicates that, in both strains analysed, there was a high degree of induction of β -galactosidase and a twofold induction only for invertase. For maltase the results were very different depending on the strain used: in PM4-4B maltase was detectable only after growth on maltose while in JA6 maltase had a small repressing effect. Based on these results we decided to use the JA6 strain to develop the study of catabolite repression.

Isolation of nonrepressible mutants

The use of 2-deoxyglucose as a nonmetabolizable glucose analogue that acts also as a gratuitous repressor is one of the most general procedures utilized to obtain non-repressible mutants (Zimmermann & Scheel, 1977). For this reason the JA6 haploid strain was mutagenized with EMS and then plated onto medium containing 5 g 2-deoxyglucose l⁻¹ and 20 g raffinose l⁻¹. The frequency of appearance of resistant colonies was 4×10^{-4} . Among

Table 4. Effect of glucose on the level of maltase, invertase, NAD-GDH, β -galactosidase and MDH in *dgr139*, *dgr148*, *dgr151* and *dgr239* mutants of *K. lactis*

Strain genotypes are listed in Table 1. Cultures were grown in YP medium supplemented with 2% (w/v) raffinose or galactose for the determination of invertase and β -galactosidase, respectively, or 2% (w/v) lactate for the determination of maltase, MDH and NAD-GDH, with or without the addition of 2% (w/v) glucose. Enzyme activities were measured as described in Methods. Enzyme units are expressed as nmol substrate min⁻¹ (mg protein)⁻¹. All values are means of three independent experiments. In no case was the variation higher than 15%.

Enzyme	Strain							
	<i>dgr139</i>		<i>dgr148</i>		<i>dgr151</i>		<i>dgr239</i>	
	No glucose	Glucose	No glucose	Glucose	No glucose	Glucose	No glucose	Glucose
Maltase	140	101	90	43	135	102	63	44
Invertase	200	120	145	179	210	132	215	220
NAD-GDH	1985	863	2100	715	2025	890	1223	530
β -Galactosidase	818	810	675	641	856	838	1158	1050
MDH	1712	1854	1850	1703	1685	1670	1192	1050

600 of these 2-deoxyglucose-resistant (*Dgr*⁺) colonies, retested for their resistance to the analogue, 296 clones that stably maintained the resistant phenotype were analysed further.

In *K. lactis*, *Rag*⁻ mutants are unable to grow on glucose when respiration is blocked by specific inhibitors (Goffrini *et al.*, 1989). It was shown previously that mutants in *K. lactis* which have a block in glycolysis are all *Rag*⁻ (Goffrini *et al.*, 1990, 1991; Wésolowski-Louvel *et al.*, 1988, 1992a, b; Prior *et al.*, 1993; Heinisch *et al.*, 1993). In order to identify genes involved in glucose metabolism that also controlled carbon catabolite repression, we analysed all the *Dgr*⁺ mutants for the *Rag*⁻ phenotype. Among 296 *Dgr*⁺ mutants 184 were *Rag*⁺ whereas 112 were *Rag*⁻.

Since *rag1* mutants, affected in the low affinity glucose transport (Goffrini *et al.*, 1990; Wésolowski-Louvel *et al.*, 1992a), display both the *Rag*⁻ and the *Dgr*⁺ phenotype (P. Goffrini, unpublished data), all the *Dgr*⁺ *Rag*⁻ mutants isolated were tested for complementation to the *rag1* mutant. Among 112 *Dgr*⁺ *Rag*⁻ mutants analysed, 107 were found to be allelic to *rag1*. These mutants will be the subject of another report. The remaining five mutants were analysed further.

Genetic analysis

Crosses of the five mutants (JA6/138, JA6/139, JA6/148, JA6/151, JA6/239) with a wild-type (PG9-11D) indicate dominance for the *Dgr*⁺ phenotype and recessiveness for *Rag*⁻. The above diploids were subjected to tetrad analysis. In all cases a 2:2 segregation for both phenotypes was observed showing that the mutation is monogenic.

Complementation tests between the different mutants indicate that JA6/138 and JA6/139 are allelic and that JA6/151 could also belong to the same complementation

group. JA6/148 and JA6/239 define different genes. The wild-type genes identified by these mutations will be called *DGR139*, *DGR148*, *DGR151* and *DGR239*. Complementation tests with other *Rag*⁻ mutants described (Wésolowski-Louvel *et al.*, 1992b) show that *dgr151* is allelic to the *rag5* mutant (mutant in the unique hexokinase structural gene of *K. lactis*; Prior *et al.*, 1993). The possibility of nonlinked noncomplementors has been discarded because a 4⁻:0⁺ segregation was observed in 20 tetrads. The *dgr139*, *dgr148*, *dgr239* mutants were not allelic to any of the previously described *Rag*⁻ mutants. However, further analysis (see Table 5) showed that *dgr139* was allelic to *rag5*.

Biochemical characterization

To establish whether the mutations that conferred the *Dgr*⁺ phenotype on raffinose also conferred resistance to the analogue on other carbon sources, the ability of the JA6/139, JA6/148, JA6/151 and JA6/239 mutants to grow on lactose, galactose, maltose and sucrose in the presence of 2-deoxyglucose was compared to that of the wild-type. The resistant phenotype was observed also in the presence of the sugars mentioned above, indicating that the mutations had a pleiotropic effect. The wild-type strain was unable to grow under the same culture conditions. Therefore, we expected that these mutations released the enzymes involved in their utilization from glucose repression. We then assayed the levels of invertase, maltase and β -galactosidase activities in the newly isolated mutants under repressing and nonrepressing conditions. As shown in Table 4, these enzyme activities were derepressed in all the four mutants. With respect to other catabolite repressible enzymes, not directly related to the utilization of the mentioned sugars, MDH was nonrepressible in the four mutants analysed, whereas NAD-GDH activity was still repressed. L-LCR was not

Table 5. Hexokinase activity in different strains of *K. lactis*

Cultures were grown in YP medium supplemented with 2% (w/v) glucose. Enzyme activity was measured as described in Methods. Enzyme units are expressed as nmol substrate min⁻¹ (mg protein)⁻¹. All values are means of three independent experiments. In no case was the variation higher than 15%.

Strain	Relevant genotype	Hexokinase activity
JA6	<i>RAG5</i>	436
JA6/151 × PM6-7A/VV41	<i>dgr151 RAG5</i> <i>DGR151 rag5</i>	< 10
JA6/151 × PG139-7B	<i>dgr151 DGR139</i> <i>DGR151 dgr139</i>	23
JA6/148 × PM6-7A/VV41	<i>dgr148 RAG5</i> <i>DGR148 rag5</i>	205
JA6/148 × PG239-5B	<i>dgr148 DGR239</i> <i>DGR148 dgr239</i>	220
JA6/139 × PG239-5B	<i>dgr139 DGR239</i> <i>DGR139 dgr239</i>	330

analysed in the isolated mutants because this enzyme was detectable at a very low level in the parental strain.

All the mutants analysed were able to utilize glucose, fructose, galactose, lactose and lactate both in complete and in minimal medium. However, all of them displayed an increase of the division time only in media containing glucose and fructose (doubling time of approximately 4 h instead of approximately 3 h in minimal medium). Therefore we analysed the hexokinase activity of the mutants. Hexokinase activity was less than 10 enzyme units both in JA6/151 and in JA6/139, JA6/148 and JA6/239 (data not shown). This result was unexpected since the last three mutants complemented each other and they all complemented the hexokinase mutant *rag5*.

The results obtained prompted us to measure the hexokinase level in the diploids. The data, reported in Table 5, confirm that *dgr148* and *dgr239* were not mutations in the structural gene encoding hexokinase since the level of this enzyme was restored in the diploids according to the growth phenotype observed previously. In addition, the hexokinase level in the diploid obtained by crossing *dgr148* with *dgr239* confirmed that *dgr148* and *dgr239* were mutations in different genes. The results indicate that *dgr139* is a mutation of the hexokinase structural gene because the enzyme level in the diploid, obtained by crossing *dgr139* with *dgr151*, was greatly lowered with respect to a wild-type strain. However this low level could be responsible for the partial growth of the diploids in media containing antimycin A.

DISCUSSION

The data reported here demonstrate that several enzymes in *K. lactis* are regulated by catabolite repression. In order to identify genes of glucose metabolism that also controlled catabolite repression, we selected 2-deoxyglucose-

resistant mutants that were also Rag⁻ (in which the glucose fermentation becomes dependent on respiration). In this way we identified mutants that fell into four complementation groups. Two of these define genes previously described: *RAG1* encoding the low affinity glucose transport system, and *RAG5* the unique structural gene encoding hexokinase (Prior *et al.*, 1993). The remaining two genes (*DGR148* and *DGR239*) are required for glucose repression of the enzymes β -galactosidase, maltase, invertase and MDH but not NAD-GDH. Both *dgr148* and *dgr239* mutants lack hexokinase activity suggesting that these genes are either positive regulators of expression of hexokinase or negative regulators of some proteins which inactivate hexokinase.

The isolation of mutants deficient in hexokinase activity but not of other glycolysis mutants by the applied screen supports the important role of the first enzyme in glucose metabolism during glucose repression in *K. lactis*. Hexokinase could intervene in glucose repression: first, by controlling the rate of glucose metabolism either by glucose phosphorylation or glucose transport or both, and second, by being itself a regulatory protein that acts directly or indirectly on genes controlled by glucose repression. In other words, the role of hexokinase in glucose repression could rest either on its catalytic activity or on its molecular structure. The fact that the absence of hexokinase does not affect the repression of NAD-GDH indicates that this enzyme is regulated by a catabolic control distinct from the one identified here.

The growth on glucose and fructose in the absence of detectable hexokinase activity of all the mutants here described is difficult to explain. However, the growth of mutants on glucose is abolished when the respiration is inhibited, and the division time of mutants is longer than that of the parental strain in glucose- and in fructose- but not in galactose, lactose- or lactate-containing media. All together, these observations can be explained by a low level of hexokinase (less than 10 enzyme units) that could represent a bottle-neck for the growth on glucose and fructose only.

The mutations here described are recessive for the Rag⁻ phenotype and dominant for the Dgr⁺ phenotype. This apparent paradox presumably was due to the fact that in the diploid strains the hexokinase activity attains a level sufficient to sustain the cell growth in the absence of respiration (Rag⁺ phenotype), but not sufficient to render the cells insensitive to 2-deoxyglucose (Dgr⁺ phenotype). This suggests that the threshold of hexokinase activity necessary for glucose repression is higher than the threshold for the ability to grow on glucose in the absence of respiration.

From this study an important difference between *S. cerevisiae* and *K. lactis* emerges: in *S. cerevisiae* there are multiple hexokinase-encoding genes with only one of the genes specifically responsible for glucose repression (Entian & Fröhlich, 1984; Ma & Botstein, 1986; Walsh *et al.*, 1991), whereas in *K. lactis* there is a single hexokinase (Prior *et al.*, 1993), and that appears to be regulated by at least two genetic determinants (*DGR148* and *DGR239*).

Results obtained in this study demonstrate that, in *K. lactis*, invertase is induced by the substrate raffinose, in contrast to what occurs in *S. cerevisiae*, in which invertase is regulated only by glucose repression (Carlson & Botstein, 1982). However, recent results have shown that in *S. cerevisiae* maximal expression of invertase actually requires a low concentration of glucose (Vallier & Carlson, 1991; Flick & Johnston, 1991). A similar phenomenon could explain the result in *K. lactis*, where a small amount of glucose resulting from hydrolysis of raffinose results in higher levels of invertase than in cells growing on lactate. Maltase, which is regulated in the PM4-4B strain as in *S. cerevisiae* (Vanoni *et al.*, 1989), i.e. induced by maltose and repressed by glucose, is constitutively present in the JA6 strain and is repressed twofold by its own substrate, maltose. This peculiar situation could be due to rapid catabolism of the inducer that causes glucose repression. The repressing effect of the substrate could be observed in this strain only, due to the constitutivity of the enzyme. The genetic basis of this control is under investigation.

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