Transcriptomic Analysis of Extensive Changes in Metabolic Regulation in Kluyveromyces lactis Strains†

Audrey Suleau,1 Pierre Gourdon,2 Joëlle Reitz-Ausseur,2 and Serge Casaregola1*
Laboratoire de Microbiologie et Génétique Moléculaire, INRA/CNRS, INRA-PP, 78850 Thiverval-Grignon, France,1
and SOREDAB, La Tremblaye, 78125 La Boissière-Ecole, France2

Received 24 March 2006/Accepted 17 May 2006

Genome-wide analysis of transcriptional regulation is generally carried out on well-characterized reference laboratory strains; hence, the characteristics of industrial isolates are therefore overlooked. In a previous study on the major cheese yeast Kluyveromyces lactis, we have shown that the reference strain and an industrial strain used in cheese making display a differential gene expression when grown on a single carbon source. Here, we have used more controlled conditions, i.e., growth in a fermentor with pH and oxygen maintained constant, to study how these two isolates grown in glucose reacted to an addition of lactose. The observed differences between sugar consumption and the production of various metabolites, ethanol, acetate, and glycerol, correlated with the response were monitored by the analysis of the expression of 482 genes. Extensive differences in gene expression between the strains were revealed in sugar transport, glucose repression, ethanol metabolism, and amino acid import. These differences were partly due to repression by glucose and another, yet-unknown regulation mechanism. Our results bring to light a new type of K. lactis strain with respect to hexose transport gene content and repression by glucose. We found that a combination of point mutations and variation in gene regulation generates a biodiversity within the K. lactis species that was not anticipated. In contrast to S. cerevisiae, in which there is a massive increase in the number of sugar transporter and fermentation genes, in K. lactis, interstrain diversity in adaptation to a changing environment is based on small changes at the level of key genes and cell growth control.

Kluyveromyces lactis is one of the major yeasts in cheese. It plays a role during the early steps of cheese making; by modifying the pH in the curd, it creates conditions which promote subsequent growth of bacteria. K. lactis is the most studied nonconventional yeast and has become an alternative model to study yeast physiology, as its physiology is very different from that of Saccharomyces cerevisiae. In S. cerevisiae, alcoholic fermentation can occur in addition to respiration in the presence of a high glucose concentration despite aerobic conditions. In contrast to S. cerevisiae, K. lactis is generally described as a strict aerobe and a Crabtree-negative yeast. Sugar transport, a well-described aspect of K. lactis physiology, was shown to be linked to respiration. Until now, two glucose transporters have been described, Rag1, the low-affinity transporter (7, 18, 40), and Hgt1, the high-affinity glucose transporter (2). Some K. lactis isolates do not possess the RAG1 gene but, instead, a pair of genes, KHT1 and KHT2, whose rearrangement corresponds to RAG1 (38). The sensitivity to glucose repression was shown to be linked to that genetic variation, as the strains carrying the KHT1/KHT2 genes are more sensitive to glucose repression than those carrying only RAG1 (38). Many regulators of the genes encoding the hexose transporters have been identified (1–3, 7, 30).

Several studies have suggested a possible effect of the sugar transporters and/or sugar uptake on the general metabolism. Goffrini et al. (15) demonstrated the influence of RAG1 and a glycolytic gene, RAG2, encoding the phosphoglucoisomerase, on respiration during growth in the presence of different sugars, leading to the definition of two groups of strains within the K. lactis species. Those with a Rag+ phenotype are able to grow on glucose even when the respiration is blocked, and those with a Rag− phenotype are not. More recently, Lemaire and Węsolowski-Louvel (24) raised the idea that the glycolysis was probably involved in the regulation of the genes encoding the glucose transporters. A slower glycolytic flux would thus provoke a reduction of the expression of the genes encoding the glucose transporters.

Although intraspecific variability at the level of the genome structure and of the DNA sequence has been shown for a number of species, it has rarely been shown that this variability affects physiological traits. Few studies have addressed the question of the variability of the global regulation of transcription of industrial yeasts. By comparing a laboratory strain and an oenological strain of S. cerevisiae, Brem et al. (5) and Yvert et al. (43) were able to detect and to map the variability of regulatory differences at the level of the DNA sequence between isolates. Recently, growth in copper sulfate was shown to be associated with a finite number of genes when oenological strains of S. cerevisiae were compared (12). With a similar approach, we found differences in gene expression, some of them in response to the carbon sources, between two isolates of K. lactis, CBS2359 which is used as a reference for genetics studies, and B1, an industrial strain used in cheese making (35). Although K. lactis is the most studied and the best known of all the nonconventional yeasts, many aspects of its physiology remain to be understood. In particular, whereas lactose is the most available carbon source in its natural environment, studies using lactose as a carbon source are very rare and

* Corresponding author. Mailing address: Microbiologie et Génétique Moléculaire, INRA UMR1238, CNRS/INRA-PP UMR 2585, 78850 Thiverval-Grignon, France. Phone: 33 1 30 81 52 94. Fax: 33 1 30 81 54 57. E-mail: Serge.Casaregola@grignon.inra.fr.
† Supplemental material for this article may be found at http://ec.asm.org/.
results are contradictory (18). Here, we compare gene expression of these same two strains, in response to lactose in very controlled conditions, combining the global view of gene expression with the production of metabolites and the consumption of carbon sources. We show that the gene expression results, interpreted in relation to the physiological data, can help to understand the way by which \textit{K. lactis} strains adapt to their environment.

**Materials and Methods**

**Yeast strains.** \textit{Kluyveromyces lactis} strains CBS2359 (Centraalbureau voor Schimmelcultures, Delft) and B1, an industrial strain from SORERAB (Bongrain group) isolated from a French soft cheese (Saint Marcelin) were used in this work. Both strains display a Lac\textsuperscript{-} phenotype. For the sake of clarity, we have called CBS2359 the laboratory strain and B1 the industrial strain throughout the text; both strains are wild isolates, although CBS2359 has been used in laboratories for a number of years.

**Batch cultivation in fermentor.** Precultures were grown for 48 h at 28°C in a 1-liter shake-flask containing 100 ml of YPG medium (10 g/liter Bacto peptone, 10 g/liter yeast extract, and 5 g/liter glucose). A preculture of 180 ml was used to inoculate a 20-liter bioreactor (Chemap fermentor) containing 12 liters of culture medium. The cells were cultivated in a medium containing 40 g/liter glucose, 30 g/liter yeast extract, 10 g/liter peptone, 2 g/liter K\textsubscript{2}HPO\textsubscript{4}, 1 g/liter KH\textsubscript{2}PO\textsubscript{4}, 0.05 g/liter CaCl\textsubscript{2}·2H\textsubscript{2}O, and 3 g/liter MgCl\textsubscript{2}·6H\textsubscript{2}O. When the biomass reached the desired concentration, 2 liters of a lactose solution was added to the bioreactor to give an initial concentration of 40 g/liter lactose. The pH was maintained at 6.0 by automatic addition of NaOH (5 N). The fermentation was performed at 20°C. The O\textsubscript{2} concentration was maintained above 80% of the air saturation throughout the culture.

**Analytical methods.** During fermentation, samples were collected every 2 h to determine biomass and the concentration of sugars, organic acids, glycerol, and ethanol. Biomass was estimated by the absorbance at 650 nm. The concentration of the metabolites in the culture supernatant was determined by high-performance liquid chromatography analysis using an HPG-87H column (Bio-Rad) maintained at 48°C with 5 mM H\textsubscript{2}SO\textsubscript{4} as the eluant.

**β-Galactosidase assay.** β-Galactosidase activities were measured as described by Kippert (21). Cells were pelleted and resuspended in 600 μl of Z buffer (60 mM Na\textsubscript{2}HPO\textsubscript{4}, 40 mM NaH\textsubscript{2}PO\textsubscript{4}, 10 mM KCl, 1 mM Mg\textsubscript{2}SO\textsubscript{4}, 5 mM β-mercaptoethanol) containing 0.2% sarcosyl. Samples were incubated at 30°C for 30 min. After this preincubation step, 150 μl of o-nitrophenyl-β-D-galactopyranoside (4 mg/ml) was added and the mixtures were homogenized by gently inverting the tubes several times. When the color turned yellow, the reactions were stopped by adding 400 μl of 1.5 M Na\textsubscript{2}CO\textsubscript{3}. The tubes were then centrifuged at 30,000 × g for 10 min. The activities measured were expressed in arbitrary units.

**Northern blotting.** A total of 10 μg of RNAs was size fractionated by electrophoresis on a 1.2% agarose-formaldehyde gel, transferred to a GeneScreen membrane (Perkin Elmer NEN) by capillarity, and then cross-linked to the membrane using UV. Probes of 409 bp and 423 bp in length were synthesized on CBS2359 genomic DNA by PCR in the genes KLLAC12 and KLA\textit{CT1}, respectively. The primers used were KLA\textit{CT}1 (5′-CCGCTGAAAGAGAAATCGT C-3′) and KLA\textit{CT}1 (5′-AACACCAGTACAGACAGAG-3′) and KLLac12L (5′-TTTTGCTGATCTGTTTTGACA TGATCAGA-3′) and KLLac12R (5′-GCTTTGTGACAGACAGACAGACGACAGAC-3′). PCR amplifications were carried out in a Perkin-Elmer 2400 thermocycler in a total volume of 50 μl with 2.5 units of Ex-\textit{Taq} DNA polymerase (Takara, Madison, WI) in the recommended buffer and about 100 ng of genomic DNA as a template. The following conditions were used: 2 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at the required melting temperature, and 3 min at 72°C, with a final extension step of 3 min at 72°C. The PCR products were purified on a 1% agarose gel. Radiolabeling of each probe, hybridization and radioactive signal detection, and quantification were performed as described in reference 35. Each value was normalized to the corresponding actin signal.

**RNA extraction and fluorescent cDNA preparation.** RNA extraction, reverse transcription, and cDNA labeling were essentially as previously described (35). Briefly, total RNA was extracted from samples using the MIDprep RNeasy kit (QiAGEN). Reverse transcription, from 20 μg of total RNA, and cDNA direct labeling were performed using dCTP-Cy3 and dCTP-Cy5 (CyScribe first-strand cDNA labeling kit; Amersham Bioscience). RNA was then eliminated by a chemical treatment (15 min incubation with 2 M NaOH and then addition of 2 M HEPES free acid), and labeled cDNA was purified on columns and Speedvac concentrated to 5 μl, which was subsequently used for hybridization.

---

**Table 1. Distribution of the 482 \textit{K. lactis} genes represented on the microarray within the diverse cellular functions**

| Functional category | No. of genes represented on microarray |
|---------------------|---------------------------------------|
| **Metabolism of various compounds** |                                   |
| Amino acid metabolism | 67                                   |
| Nitrogen and sulfur metabolism | 11                                   |
| Nucleotide metabolism | 8                                    |
| Phosphate metabolism | 12                                   |
| Lipid and fatty acid, and sterol metabolism | 57                                   |
| Vitamin, cofactor, and prosthetic group metabolism | 20                                   |
| **Energy** |                                   |
| Glucose transport/ regulation/repression | 41                                   |
| Glycolysis/neogluconeogenesis | 17                                   |
| Pentose phosphate pathway | 5                                    |
| Lactose/galactose metabolism | 9                                    |
| Tricarboxylic acid pathway | 16                                   |
| Respiration | 10                                   |
| PDH bypass \textsuperscript{a} | 10                                   |
| Metabolism of energy reserves | 4                                    |
| Glyoxylate cycle | 2                                    |
| **Cell cycle and DNA processing** |                                   |
| Cell cycle and DNA processing | 13                                   |
| Transcription | 5                                    |
| Protein synthesis | 3                                    |
| **Protein metabolism** |                                   |
| Protein folding and stabilization | 11                                   |
| Protein targeting, sorting, and translocation | 25                                   |
| Protein modification | 17                                   |
| Assembly of protein complexes | 10                                   |
| **Proteolysis** |                                   |
| Intracellular organization | 2                                    |
| Extracellular transport | 21                                   |
| Intracellular transport | 28                                   |
| Signal transduction | 4                                    |
| Stress response and cell rescue | 19                                   |
| **Ion homeostasis** |                                   |
| Ion homeostasis | 5                                    |
| Ion transport | 12                                   |
| **Classification not yet clear cut** |                                   |
| Classification not yet clear cut | 9                                    |

\textsuperscript{a} A complete list of the genes may be found in the supplemental material.  
\textsuperscript{b} PDH, pyruvate dehydrogenase.
sion in cultures at different times after lactose addition and gene expression in cultures before (in glucose only) were calculated. The signal/control ratios thus obtained were log2 transformed. For each culture, a control consisted of hybridizing two total cDNAs obtained from the sample in glucose only and differentially labeled (glucose Cy3/glucose Cy5). To correct the incorporation bias of both dyes, the values of the ratios thus obtained were used for normalization per gene in Genespring. The normalized values were thus statistically analyzed using the SAM software (36). For each gene and for each step, a value was assigned to represent the changes in the gene expression compared to the measure in glucose only. The probability that the expression of a given gene varies by chance was represented by a confidence value $P$. We chose the threshold $P$ value of 0.05, considering that, over this value, the observed gene variation was due to chance.

RESULTS

Experimental design. Cells were inoculated into complete medium supplemented with 4% glucose. When the optical density at 650 nm (OD$_{650}$) reached between 10 and 12, lactose was added to a final concentration of 4%. Gene expression was compared before and after lactose addition. In parallel, physiological measurements (sugar consumption and glycerol, ethanol, and acetate production) were performed throughout the cultures. For gene expression measurements, samples were taken from each culture before lactose addition (G) and then 2 h (OD$_{650}$ between 10 and 15), 4 h (OD$_{650}$ between 15 and 20), and 20 h (OD$_{650}$ between 28 and 35) after lactose addition (L$_{2h}$, L$_{4h}$, and L$_{20h}$, respectively). The G sample was used as a control to compare gene expression before and after lactose addition by calculating the ratios L$_{2h}$/G, L$_{4h}$/G, and L$_{20h}$/G.

Sugar consumption during the fermentation varies according to the strain. Figure 1 represents the growth of both strains and sugar consumption. As expected, the industrial strain...
FIG. 2. Schematic representation of the number of preferentially expressed genes in each strain and in both strains 4 h after lactose addition. The number of genes whose variation in expression is significant, as described in Materials and Methods, are presented. Genes preferentially expressed in CBS2359 (black), in B1 (light gray) and in both strains (dark gray) are indicated.

seemed fitter than the laboratory strain, whose growth appeared affected by lactose (Fig. 1A). Whereas they utilize glucose at a similar rate, slightly lower in B1 (Fig. 1B), a striking difference in lactose consumption was seen, since the laboratory strain, CBS2359, did not consume lactose immediately, unlike the industrial strain, B1 (Fig. 1C). CBS2359 lactose consumption was marked by two phases: a first phase about 4 h following lactose addition, during which no lactose was consumed, and a second phase, during which little lactose was consumed, reaching 15% of the total lactose at the end of the fermentation, i.e., 20 h after lactose addition (OD650 between 28 and 35). Measurements carried out after glucose exhaustion showed that the lactose was finally entirely consumed in this strain after about 20 h (data not shown). During the phase of lactose consumption following glucose exhaustion, the growth rate of the strain CBS2359 was divided by six (data not shown). In strain B1, glucose and lactose consumption occurred simultaneously, but we noted variations in the rate of sugar consumption coinciding with variations in the growth rate throughout the culture. In strain CBS2359, the rate of lactose consumption reached its maximum of 0.15 mmol/g/h 4 h after lactose addition, whereas in strain B1, the rates of glucose and lactose consumption reached their maximum of 0.07 mmol/g/h and 0.3 mmol/g/h 4 and 6 h after lactose addition, respectively. Although the two strains are both Lac+, they behaved differently in response to lactose. These results seem to indicate that strain CBS2359 is completely sensitive to repression by glucose (see Discussion).

Ethanol, acetate, and glycerol accumulation during fermentation. Cell growth was accompanied by the production of ethanol, acetate, and glycerol, as presented in (Fig. 1D, E, and F). In both strains, we observed a sudden increase in ethanol concentration at around 4 h after lactose addition (Fig. 1D), concomitant with the moment at which the strains reach the maximal rate of sugar consumption. Interestingly, B1 accumulated 80 mM ethanol in 2 h and then began to slowly reuse it, whereas in CBS2359, the accumulation of ethanol was constant and reached 140 mM. When the glucose concentration reached 20 mM, CBS2359 started to utilize some of the accumulated ethanol (Fig. 1B and D). The amount of ethanol produced was higher in strain CBS2359 than in strain B1, while the CBS2359 biomass was lower than that of B1 (Fig. 1A and D).

Concomitant with the accumulation of ethanol, accumulation of glycerol and acetate was observed in both strains, but to different levels: the maximum amounts of glycerol and acetate were, respectively, three- and sixfold higher in B1 than in CBS2359 (Fig. 1E).

Reduced expression due to glucose repression of the lactose permease gene LAC12 is likely responsible for the nonconsumption of lactose by CBS2359. Two hours after lactose addition, only 19 genes were up-regulated in strain B1, with a low error probability \( P < 0.03 \), and 9 in the strain CBS2359, with a \( P \) value of \( <0.01 \), including the genes LAC4, GAL10, and GAL7, whose transcription levels were increased in both strains (25- to 40-fold, on average), reflecting an expected reaction of the cell to lactose through an induction of the lactose assimilation genes.

Four hours after lactose addition, more genes had their expression changed (Fig. 2): 112 genes in strain B1 \( (P < 0.052) \) and 150 genes in strain CBS2359 \( (P < 0.013) \) had increased levels of expression. Among them, 52 were common to both strains. The expression of the genes of the system GAL/LAC \( (GAL1, GAL10, GAL7, GAL80, and LAC4) \) was increased in both strains CBS2359 and B1 (Table 2). This is in agreement with the well-known induction of expression of the genes of the GAL/LAC regulon in \( K. lactis \) in the presence of the substrate,

### TABLE 2. Relative lactose and galactose metabolism gene expression in CBS2359 and B1 4 and 20 h after lactose addition

| Gene name | S. cerevisiae ORF | KLLA_ID | CBS2359 | B1 | CBS2359 | B1 |
|-----------|------------------|---------|---------|----|---------|----|
| GAL1      | YBR020w          | KLLA0F08393g | 9.3     | 21.1 | >100    | 1  |
| GAL10     | YBR019c          | KLLA0F08415g | 9.7     | 23.1 | >100    | 9.6|
| GAL7      | YBR018c          | KLLA0F08437g | 19.0    | 41.0 | 39.7    | 1  |
| GAL80     | YML051w          | KLLA0A08162g | 5.4     | 17.5 | 1       | 1  |
| LAC4      |                  | KLLA0B14883g | 16.8    | 52.3 | 34.4    | 19.0|
| LAC12     |                  | KLLA0B14861g | 1       | 38.9 | 82.0    | 1  |
| LAC12     |                  | KLLA0B14861g | 1       | 18.7 | >100    | 1  |
| LAC9      | YPL248c          | KLLA0D12672g | 1       | 1    | 1       | 1  |

\( a \) ORF, open reading frame.

\( b \) Ratio of expression level of cells grown in lactose to expression level of cells grown in glucose.

\( c \) Corresponds to two different oligonucleotides within the LAC12 gene.
where the genes GAL1 and GAL10 have a similar induction of expression and GAL7 is the most highly expressed gene (37).

Interestingly, 2 and 4 h after the addition of lactose, the \textit{LAC12} gene encoding the lactose permease was increased 40-fold and over 20-fold, respectively, in strain B1, but not in CBS2359 (Table 2). This was confirmed by Northern blotting, where the \textit{LAC12} RNA level was monitored as shown in Fig. 3. \textit{LAC12} was nevertheless expressed in CBS 2389 at the end of the fermentation, when the glucose concentration was lower and lactose was being utilized (Fig. 3, lane 4), suggesting that glucose affects the expression of the \textit{LAC12} gene. The lack of induction of \textit{LAC12} gene expression by lactose is consistent with the nonconsumption of lactose by CBS2659 (Fig. 1C), but with the nonconsumption of lactose by CBS2659 (Fig. 1C), but

Induction level

\begin{tabular}{|c|c|c|c|c|}
\hline
 & CBS2359 & & B1 & \\
\hline
\textit{LAC12} & 1.3 & 1.5 & 3.7 & 2.7 & 2.5 & 1.0 & \\
\hline
\textit{ACT1} & & & & & & & \\
\hline
\end{tabular}

**FIG. 3.** Northern blot analysis of \textit{LAC12} mRNA. Total RNAs were extracted from both strains before lactose addition (lanes 1) and 2, 4, and 20 h after lactose addition (lanes 2, 3, and 4, respectively). Around 15 µg of total RNA was separated on an agarose gel and blotted onto a nylon membrane. The probes used to detect the \textit{HGT1} and \textit{ACT1} genes were amplified using oligonucleotides described in Materials and Methods. The probes used to detect the \textit{HGT1} and \textit{ACT1} genes were amplified using oligonucleotides described in Materials and Methods. The probes used to detect the \textit{HGT1} gene signal in each lane. The ratios of the normalized signals in lanes 2, 3, and 4 compared to that of lane 1 for each strain are indicated below each corresponding lane.

one single base change at position −224 with respect to the \textit{LAC9} start of transcription between B1 and CBS2359. This contradicts the claim that the 2-bp differences in the URS of \textit{LAC9} between the glucose-sensitive strain JA6 and a glucose-insensitive strain were responsible for a reduced expression of \textit{LAC9} and therefore a noninduction of \textit{LAC12} in the former strain (23) (see discussion). We found six base changes and one insertion when the \textit{LAC12} promoter sequences of CBS2359 and B1 were compared and a single transversion in the \textit{LAC12} coding sequence that resulted in the replacement of a serine by an alanine at position 213. None of the base changes affected the \textit{LAC9} binding sites, but the sequence differences observed in the promoter region may be responsible for the difference with respect to glucose repression, although these sequence changes do not affect the \textit{LAC12} induction and activity in the absence of glucose (35) (data not shown).

Central metabolism is differently regulated in the laboratory strain and the industrial strain and involves genes that are not under glucose regulation. One of the most striking observations in our studies concerns the way the glucose metabolic pathways diverge in the two studied strains. We found three groups of genes whose expression differed between the two strains (Table 3): (i) the genes subjected to glucose repression in \textit{S. cerevisiae} and in \textit{K. lactis}, such as \textit{INV1} (the \textit{S. cerevisiae SUC2} orthologue) and \textit{MAL2} (the \textit{S. cerevisiae MAL32} orthologue), had a higher expression only in strain B1, (ii) the genes involved in glucose repression, such as \textit{GSF2}, involved in glucose-dependent repression in \textit{S. cerevisiae} (34), are highly expressed in CBS2359 only, and (iii) the genes involved in glucose utilization, such as \textit{SWI1} and \textit{GCY1}, which showed a 17-fold increase in B1, and \textit{GCR1}, a positive regulator of the glycolytic genes \textit{RAG2} (\textit{KIPG1}), \textit{RAG17} (\textit{KINO}), and \textit{RAG6} (\textit{KIPDC}) (29), which was induced 5-fold in strain B1 only. Overall, this confirms a differential sensitivity to glucose repression in the two strains. We also observed, in both strains, higher transcription levels for the gene \textit{FOG1}, which encodes a protein with high similarity to the members of the \textit{S. cerevisiae GAL83/SIP1/SIP2} gene family, whose product is involved...
TABLE 3. Expression of genes involved in repression by glucose or sensitive to glucose repression in CBS2359 and B1 4 and 20 h after lactose addition

| Gene name | S. cerevisiae ORF | KLLA_ID | L/G ratio\(^a\) for strain at time (h) post-lactose addition: | Function |
|-----------|-----------------|---------|---------------------------------|-----------|
| CBS2359   | CBS2359         |         |                                 |           |
|          | 4               | 20      |                                 |           |
| CCR4      | YPL075w         | KLLA0E23507g | 5.0 1 1 1 | Transcriptional activator of genes involved in glycolysis |
| GSF2      | YML048w         | KLLA0A08107g | 5.1 1 1 1 | Involved in glucose repression |
| FOG1      | YER027c         | KLLA0B00583g | 3.7 46.3 1 1 | GAL83, Snf1 kinase complex |
| INV1      | YIL162w         | KLLA0A10417g | 1 32.4 \(\gg\) 100 40.0 | Invertase |
| MAL22     | YBR299w         | KLLA0D00231g | 1 27.0 1 23.2 | Maltase |
| SWI1      | YPL016w         | KLLA0D12323g | 1 17.4 1 1 | Component of SWI/SNF global transcription activator complex |
| GCY1      | YOR120w         | KLLA0D10109g | 1 17.9 2.3 1 | Galactose-induced protein of aldo/keto reductase family |

\(^a\) ORF, open reading frame.  
\(^b\) Ratio of expression level of cells grown in lactose to expression level of cells grown in glucose.

TABLE 4. Expression of genes of the pentose phosphate pathway in CBS2359 and B1 4 and 20 h after lactose addition

| Gene name | S. cerevisiae ORF | KLLA_ID | L/G ratio\(^a\) for strain at time (h) post-lactose addition: | Function |
|-----------|-----------------|---------|---------------------------------|-----------|
| CBS2359   | CBS2359         |         |                                 |           |
|          | 4               | 20      |                                 |           |
| XKS1      | YGR194c         | KLLA0E09746g | 3.5 1 1 8.4 | Xylulokinase |
| RPE1      | YJL121c         | KLLA0E15136g | 6.0 1 1 5.9 | \(\delta\)-Ribulose-5-phosphate 3-epimerase |
| GND1      | YHR183w         | KLLA0A09339g | 11.0 1 4.2 1 | 6-Phosphogluconate dehydrogenase |
| TAL1      | YLR354c         | KLLA0A02607g | 1 1 2.7 1 | Transaldolase |
| ZWF1      | YNL241c         | KLLA0D19855g | 1 1 38.1 1 | Glucose-6-phosphate 1-dehydrogenase |

\(^a\) ORF, open reading frame.  
\(^b\) Ratio of expression level of cells grown in lactose to expression level of cells grown in glucose.
used in the study of Denis and Malavar (9), while we used a fermentor.

In addition to an increase in the expression of many genes involved in general biosynthetic processes, such as lipid and amino acid metabolism, we observed, in particular, a higher expression of genes involved in the metabolism of some amino acids, such as glutamate, proline, and arginine, which may reflect a high general activity in the cells at this stage of the cultures (see the supplemental material). This was accompanied by the most striking difference between the strains, with the high expression in the B1 strain of genes encoding specific amino acid transporters: PUT4, DIP5, and the two K. lactis GAP1 orthologues (Table 7). On the other hand, other transporter-encoded genes like CAN1 (amino acid permease) and MUP1 (high-affinity methionine permease) had an increased expression in both strains, indicating that these two groups of amino acid permease-encoding genes are not under the same regulation.

It was proposed that differential gene redundancy between species reflects physiological traits (for a review, see reference 4). We examined the specific expression of two pairs of duplicated genes in K. lactis that are singletons in S. cerevisiae, YKL215c, strongly related to rat and bacterial 5 oxo-prolinase, and YKR097w, encoding the general amino acid permease GAP1. Considering the weaker overall gene redundancy of K. lactis with respect to S. cerevisiae, the role of these genes in the former species might be of importance. As mentioned above, there was a 30-fold difference in the expression levels of the two GAP1 orthologues when strains were compared. The YKL215c orthologue KLLA0C18879g showed a 3.5-fold increase but in CBS2359 only. It has been proposed that duplicated genes could evolve differently. While one of the paralogues keeps its primary function, the other one evolves more rapidly to perform a different role. The two GAP1 paralogues show 91% identity at the protein sequence level, and the two YKL125c-like paralogues show 65% sequence identity. We have here the examples of (i) a pair of duplicated genes that share the same regulation but display a differential expression in the two strains studied and (ii) a pair of duplicated genes whose regulation diverged in the two strains for each copy. It will be of interest to find out, in the second example, whether the function of each duplicated gene has also diverged and if this functional divergence correlates with the observed different expression pattern.

The number of glucose transporter genes and their expression vary between strains. Glucose entry has been proposed as one of the key mechanisms of regulation of the growth and physiology of K. lactis, and the very limited number of glucose transporters support this hypothesis. We have seen that regulation of the lactose permease varied according to the strain. We therefore sought to determine whether the regulation of the two major K. lactis glucose transporters RAG1 and HGT1 (the low- and high-affinity glucose transporters, respectively) also showed an intraspecific variation (Table 8). Whereas CBS2359 behaves as previously observed, with an increased expression of RAG1 by high glucose concentration and no effect of glucose on the expression for HGT1 (2, 40), we observed, in strain B1, that RAG1 was not induced by high glucose concentration and that HGT1 had a large 32-fold increase in expression. Our results show that the latter gene is regulated in a different way in the two strains. We wondered if a variation

| Gene name | S. cerevisiae ORF | KLLA_ID | CBS2359 | B1 | CBS2359 | B1 |
|-----------|----------------|---------|---------|----|---------|----|
| ICL1      | YER065c        | KLLA0C08107g | 1 | >100 | 1 | >100 |
| MLS1      | YNL117w        | KLLA0F23914g | 1 | 52.2 | 1 | >100 |
| PCK1      | YKR097w        | KLLA0A00484g | 1 | >100 | 48.6 | >100 |

a. ORF, open reading frame.

b. Ratio of expression level of cells grown in lactose to expression level of cells grown in glucose.

| Gene name | S. cerevisiae ORF | KLLA_ID | CBS2359 | B1 | CBS2359 | B1 |
|-----------|----------------|---------|---------|----|---------|----|
| ADH1      | YOL086c        | KLLA0F21010g | 1 | 1 | 1 | 7.3 |
| ADH4      | YGL250w        | KLLA0F13530g | 1 | 1 | 1 | 11.9 |
| ADH3      | YMR083w        | KLLA0B09064g | 1 | 15.3 | 1 | >100 |
| ADH2      | YMR303c        | KLLA0F18260g | 7.8 | 1 | 3.4 | 1 |
| ACS1      | YAL034c        | KLLA0A03333g | 3.4 | 1 | 1 | >100 |
| DCPY      | YLR044c        | KLLA0E16357g | 1 | 1 | 2.4 | 0.03 |
| ALD6      | YPL061w        | KLLA0E23144g | 1 | 1 | 2.3 | 36.4 |

a. ORF, open reading frame.
b. Ratio of expression level of cells grown in lactose to expression level of cells grown in glucose.
in the DNA sequence of one or both RAG1 and HGT1 genes, especially in their promoter regions, or in one of their regulators, RAG4, could explain the different expression observed between the strains. Sequencing of the RAG1 and RAG4 genes, including their promoter, did not reveal any sequence variation in B1 with the sequence strain. But we discovered that a point mutation consisting of a transition from C to A at codon into a stop codon in the industrial strain B1. This leads to a short truncated protein of 70 amino acids, rather than of a full-length protein, consistent with our proposal. Importantly, our results confirmed the observation by Gonzalez Siso et al. (19), that a point mutation consisting of a transition from C to A at codon into a stop codon in the industrial strain B1. This leads to a short truncated protein of 70 amino acids, rather than of a full-length protein, consistent with our proposal.

**DISCUSSION**

In our previous work (35), using the same 482-gene microarray, we have shown differences between the K. lactis reference strain and an industrial strain cultivated in flasks in the presence of glucose or lactose. However several parameters may affect growth and response to the environment like limitation of oxygen (20). In the present work, we used strictly controlled oxygenation conditions to avoid such a bias. We were able to identify clear differences in the physiological response of the strains studied, and we correlated them to variations in the regulation of gene expression.

**Ethanol is accumulated in the two strains but is metabolized differently.** In contrast to a number of reports (see reference 20), we confirmed the observation by Gonzalez Siso et al. (19), that the peak of ethanol production coincided with the moment when cells reached the maximal rate of glucose consumption. As a result, lactose consumption was impaired in CBS2359, whereas B1 utilized glucose concomitantly. Expression of the GAL/LAC genes was induced immediately after lactose addition in both strains. Production of ethanol in K. lactis approaches that of S. cerevisiae with a relatively high glucose concentration (1%), by noting a high ethanol production in both strains. Production of ethanol is the result of a lack of oxygen or an overflow of carbon. Considering the high oxygen supply and high glucose concentration used here, the latter explanation may be the cause of ethanol production in our experiment. In our strains, this overflow of carbon was managed differently. Both strains started accumulating ethanol suddenly 4 h after lactose addition, which may reflect an effect of lactose. As this sugar was not utilized by CBS2359, ethanol accumulation was very likely independent of the presence of lactose. Interestingly, we found that the peak of ethanol production coincided with the moment when cells reached the maximal rate of glucose consumption, consistent with our proposal. Importantly, our results have shown that the K. lactis strains clearly display a different regulation of ethanol metabolism; in B1, ethanol was reused a few hours after being produced, whereas it accumulated in cultures of CBS2359. This observation correlated with the differential expression of the genes encoding the alcohol dehydrogenases.

**Sensitivity to glucose repression of CBS2359 partly explains the physiological differences between the two studied strains.** In contrast to previous conclusions (38; see reference 39 for a review), we have shown that the reference strain CBS2359 was very sensitive to glucose repression. As a result, lactose consumption was impaired in CBS2359, whereas B1 utilized glucose and lactose concomitantly. Expression of the GAL/LAC genes was induced immediately after lactose addition in CBS2359, whereas B1 displayed a delay of expression in the presence of lactose. These results highlight the importance of glucose repression in the physiological differences observed in this study.

**TABLE 7. Expression of genes encoding amino acid transporters in CBS2359 and B1 4 and 20 h after lactose addition**

| Gene name | CBS2359 | B1 | CBS2359 | B1 |
|-----------|---------|----|---------|----|
| CAN1      | 4.8     | 3.0| 44.2    | 1  |
| MUP1      | 2.9     | 11.4| 1       | 42.0|
| PUT4      | 1       | 5.8| 1       | 77.4|
| GAP1      | 1       | 33.0| 2.6     | 16.9|
| GAP2      | 1       | 31.5| 1       | 1  |
| DIP5      | 1       | 40.3| 1       | 1  |
| YKL215c   | 3.5     | 1  | 1       | 1  |
| YKL215c   | 1       | 13.0| 1       | 5.5|

*a* ORF, open reading frame.  
*b* Ratio of expression level of cells grown in lactose to expression level of cells grown in glucose.  
*c* Corresponds to two paralogues in K. lactis homologous to a single S. cerevisiae gene.

**TABLE 8. Expression of genes encoding glucose transporters in CBS2359 and B1 4 and 20 h after lactose addition**

| Gene name | CBS2359 | B1 | CBS2359 | B1 |
|-----------|---------|----|---------|----|
| HGT1      | 32.4    | 1  | 50.1    | 1  |
| RAG1      | 3.6     | 1  | 1       | 1  |

*a* ORF, open reading frame.  
*b* Ratio of expression level of cells grown in lactose to expression level of cells grown in glucose.  
*c* Pseudogene.
CBS2359, including LAC4, as shown in the glucose-sensitive strain JA6 (10). Sequence comparison indicated that B1 and CBS2359 shared the same LAC9 promoter region, whereas JA6 carried two base changes in the URS that were proposed to be responsible for the difference in response to glucose between JA6 and CBS2359 by lowering the level of Lac9p in JA6 (23). Our result clearly indicates that glucose repression does not influence LAC9 expression in the same fashion in the two glucose-sensitive strains. Accordingly, we did not observe any difference on the level of LAC9 expression between B1 and CBS2359, which would have been expected if LAC9 control was similar in CBS2359 and JA6. We observed a few base changes in the B1 LAC12 promoter that could play a role in the LAC12 expression, but none of these changes affected the two LAC9 binding sites. An interesting possibility is that glucose repression is effective in CBS2359 in high-oxygenation conditions and that laboratory conditions did not permit detection of this phenotypic trait, which could explain the moderate sensitivity of CBS2359 to glucose (38). Considering the intraspecific variability with respect to glucose repression, it will also be of interest to test whether what we observed for CBS2359 holds true for other “laboratory” strains.

A number of genes known to be sensitive to glucose repression were shown to be repressed in the presence of glucose in CBS2359. This was the case for several glycolytic genes and some of their regulators (Table 3). We also have observed that, independently of glucose repression regulation, genes are subject to different regulation between the two strains. This is the case of the glyoxylate cycle genes ICL1 and MLS1, known to be induced when ethanol is used (8, 33). In contrast to genes like INV1 (Table 3) and LAC4 (Table 2), whose repression in CBS2359 is alleviated when the glucose is exhausted, they are kept repressed until the end of the fermentation. This was also seen with a number of amino acid permeases (Table 7), showing the extent of the variability of gene regulation within the K. lactis species. It is tempting to speculate that the clear difference between the two strains is due to an adaptation to its environment to deal with extreme conditions. Another possibility is that this set of genes entirely repressed in CBS2359 is also sensitive to lactose repression. Further work will be needed to choose between these possibilities.

Our results also confirm that regulation by glucose is different in K. lactis and in S. cerevisiae. This has been shown in a number of cases: ICL1 gene expression with respect to catabolite repression (25), independent regulation of INV1 by MIG1 (13), and uncoupling of the regulation of gluconeogenesis and the glyoxylate cycle by CAT8 (14).

A variable hexose transporter content differentiates K. lactis isolates. Sugar transport plays a key role in regulation of carbon metabolism and in the balance between respiration and fermentation. Our observations on the sugar transporters of the studied strains indicate that the content and regulation of these transporters vary between strains. RAG1 was expressed at a higher level in strain CBS2359 only, in agreement with its being inducible in the presence of several hexoses, such as glucose and galactose, at concentrations above 1% (7, 40). In contrast, HGT1 is regulated at the transcriptional level in strain B1 but not in strain CBS2359, where it had been described as constitutive in a CBS2359 derivative (2). With the discovery that B1 carries a mutation inactivating HGT1, our results demonstrated the existence of a new type of K. lactis strain with respect to glucose transport, different from the two most studied strains, CBS2359, which has RAG1 and HGT1, and JA6, which has KHT1 and KHT2 in the place of RAG1 but no
This difference in glucose transporter gene complement is accompanied by differences at the level of the regulation of their expression as schematized in Fig. 5.

Several studies have shown a correlation between respiration/fermentation and sugar transport (16, 24, 27, 38), suggesting control of assimilation by fermentation due to a limitation of sugar uptake. It is notable that HGTL1 is highly inducible, as the introduction of additional sugar transporter genes was shown to allow K. lactis to grow in the absence of respiration (16), and it is even more notable that the HGTL1 gene is inactivated, suggesting that the mutation in HGTL1 of strain B1 may compensate for the effects of the increased HGTL1 gene expression and limit sugar uptake, thus preventing a too massive carbon uptake, which would be deleterious for the cell. This observation also suggests either that RAG1 will have a higher basal level or that there exist other transporters in the industrial strain. In this respect, Breunig et al. (6) have mentioned that the RAG1 locus was a rearrangement hot spot, and natural isolates with an inactivated RAG1 gene were described previously (15).

Taken together, these observations support the idea that K. lactis is therefore able, by modifying the sequence and the number and the nature of the sugar transporter genes, to adapt its growth to environmental conditions. The interplay between sugar transporters seems to be very subtle. Indeed, a new hexose transporter, called Kht3p, has recently been identified whose overexpression could improve the growth of the snf1 and lac12 mutants on galactose but not on lactose (41).

Our results seem to reflect an adaptation of the two strains, both originating from cheese, to their environments. CBS2359 and K. lactis have shown global metabolic changes and little molecular differentiation combined with specific genome reshuffling rather than by chromosomal amplification. Further work on the analysis of transport/fermentation and sugar transport (16, 24, 27, 38), suggests a regulatory control of assimilation by fermentation due to a limitation of sugar uptake, which would be deleterious for the cell. This observation also suggests either that RAG1 will have a higher basal level or that there exist other transporters in the industrial strain. In this respect, Breunig et al. (6) have mentioned that the RAG1 locus was a rearrangement hot spot, and natural isolates with an inactivated RAG1 gene were described previously (15).

ACKNOWLEDGMENTS

A.S. benefited from an INRA/SCOREDB grant. INRA and CNRS supported this work. This work was partially supported by a grant from the “Bureau des Ressources Genetiques.”

We are grateful to Cécile Neuvéglise for Northern blotting and Renaud Garçonnet for technical assistance with the fermentation cultures. We thank Hiroshi Fukuhara, Josef Deutscher, and Colin Timsley for critical reading of the manuscript.

REFERENCES

1. Betina, S., P. Goiuri, I. Ferrero, and M. Wesolowski-Louveil. 2001. RAG4 gene encodes a glucose sensor in Kluyveromyces lactis. Genetics 158:541–548.
2. Billard, P., S. Menart, J. Blaisonneau, M. Bolotin-Fukuhara, H. Fukuhara, and M. Wesolowski-Louveil. 1996. Glucose uptake in Kluyveromyces lactis: role of the HGT1 gene in glucose metabolism. J. Bacteriol. 178:5860–5866.
3. Blaisonneau, J., H. Fukuhara, and M. Wesolowski-Louveil. 1997. The Kluyveromyces lactis equivalent of eaeA in Escherichia coli is required for the transcription of the gene encoding the low-affinity glucose permease. Mol. Gen. Genet. 253:469–477.
4. Bolotin-Fukuhara, M., S. Casaregola, and M. Aigle. 2006. Genome evolution: lessons from Genolevures. Top. Curr. Genet. 15:165–196.
5. Brem, R. B., G. Yvert, R. Clinton, and L. Kruglyak. 2002. Genetic dissection of transcriptional regulation in budding yeast. Science 296:752–755.
6. Breunig, K. D., M. Bolotin-Fukuhara, M. M. Bianchi, D. Bourgarel, C. Falcone, I. I. Ferrero, L. Frontali, P. Goiuri, J. J. Krijger, C. Mazzoni, C. Milikowski, H. Y. Steensma, M. Wesolowski-Louveil, and A. M. Zeeman. 2000. Regulation of primary carbon metabolism in Kluyveromyces lactis. Microbiology (London). 146:791–798.
7. Chen, X. J., M. Wesolowski-Louveil, and H. Fukuhara. 1992. Glucose transport in the yeast Kluyveromyces lactis. II. Transcriptional regulation of the glucose transporter gene RAG1. Mol. Gen. Genet. 233:97–105.
8. de Jong-Gubbels, P., J. Bauer, P. Niederlegerer, T. Stockrath, P. Kotter, J. P. van Dijken, and J. T. Pront. 1998. Physiological characterization of a pyruvate-carboxylase-negative Saccharomyces cerevisiae mutant in batch and chemostat cultures. Antonie Leeuwenhoek 74:253–263.
9. Denis, C. L., and T. Malvar. 1990. The CCR4 gene from Saccharomyces cerevisiae is required for both nonfermentative and sp-mediated gene expression. Genetics 124:283–291.
10. Dong, J., and R. C. Dickson. 1997. Glucose represses the lactose-galactose regulon in Kluyveromyces lactis through a SNF1-dependent pathway that modulates galactokinase (GAL1) gene expression. Nucleic Acids Res. 25:3657–3664.
11. Dujon, B., D. Sherman, G. Fischer, P. Durrens, S. Casaregola, L. Lafontaine, J. de Montgolfier, C. March, C. Neuvéglise, E. Talla, N. Goffard, L. Frangue, M. V. Anthoniou, V. Barbie, S. Barnay, S. Bianchi, J. M. Beckerich, E. Beyne, C. Bleykasten, A. Boisrame, J. Boyer, L. Cattolico, F. Confanioliere, A. De Daruvar, L. Despuns, E. Fabre, C. Fairhead, H. Ferry Dumazet, A. Groppi, F. Hantryave, C. Hennenquin, N. Janniaux, J. Joyet, R. Kachaouri, A. Kerrest, R. Koszul, M. Lemaire, I. Lesuer, L. Ma, H. Muller, J. M. Nicouad, M. Nikolaki, S. Oztas, O. Oziez-Kalogeropoulos, S. Pellenz, S. Potier, G. F. Richard, M. L. Straub, A. Suleau, D. Swennen, F. Tekia, M. Wesolowski-Louveil, E. Westhof, B. Wirth, M. Zeniou-Meyer, I. Zivanovic, M. Bolotin-Fukuhara, A. Thierry, C. Boucher, A. Cauldron, C. Scarpelli, C. Gailardin, J. Weissenbach, P. Wincker, and J. L. Souciet. 2004. Genome evolution in yeasts. Nature 430:35–44.
12. Fay, J. C., H. L. McCullough, P. D. Sniegowski, and M. B. Eisen. 2004. Population genetic variation in gene expression is associated with phenotypic variation in Saccharomyces cerevisiae. Genome Biol. 5:R26.
13. Georls, I., J. P. Cassart, K. D. Breunig, and J. Vandenhaute. 1999. Glucose repression of the Kluyveromyces lactis invertase gene KLINV1 does not require Mig1p. Mol. Gen. Genet. 261:193–203.
14. Goiuri, P., A. A. Alberg, C. Donnini, M. Wesolowski-Louveil, and I. Ferrero. 1989. RAG1 and RAG2: nuclear genes involved in the dependence/independence on mitochondrial respiratory function for growth on sugars. Yeast 5:111–116.
15. Goiuri, P., I. Ferrero, and C. Donnini. 2002. Respiration-dependent utilization of sugars in yeasts: a determinant role for sugar transporters. J. Bacteriol. 184:427–432.
16. Goiuri, P., A. Picarelli, C. Donnini, T. Lodi, P. P. Puglisi, and I. Ferrero. 1996. FG01 and FG02 genes, required for the transcriptional activation of glucose-repressible genes of Kluyveromyces lactis, are homologous to GAL83 and SNF1 of Saccharomyces cerevisiae. Mol. Biol. Evol. 13:132–140.
17. Goiuri, P., M. Wesolowski-Louveil, I. Ferrero, and H. Fukuhara. 1990. RAG1 gene of the yeast Kluyveromyces lactis codes for a sugar transporter. Nucleic Acids Res. 18:5294.
18. Gonzalez Siso, M. L., E. Ramil, M. E. Cerdán, and M. A. Freire-Picos. 1996. Respiratory metabolite metabolism in Kluyveromyces lactis: ethanol production and the Crabtree effect. Enzyme Microb. Technol. 18:544–546.
19. Kiers, I. A. A. M. Zeeman, M. Luttik, C. Tiele, I. J. Castrillo, H. Y. Steensma, J. P. van Dijken, and J. T. Pront. 1998. Regulation of alcoholic fermentation in batch and chemostat cultures of Kluyveromyces lactis CBS 2359. Yeast 14:459–460.
20. Kippert, F. 1995. A rapid permeabilization procedure for accurate quantitative determination of beta-galactosidase activity in yeast cells. FEMS Microbiol. Lett. 128:201–206.
21. Kogut, P., A. Godcak, and K. D. Breunig. 1990. A mutation in the Zn-finger of the GAL4 homolog LAC9 results in glucose repression of its target genes. Nucleic Acids Res. 18:745–751.
22. Kuzhandaivelu, N., W. K. Jones, A. K. Martin, and R. C. Dickson. 1992. The signal for glucose repression of the lactose-galactose regulon is amplified through subtle modulation of transcription of the Kluyveromyces lactis KI/GAL4 activator gene. Mol. Cell. Biol. 12:1924–1931.
23. Lemaire, M., and M. Wesolowski-Louveil. 2004. Enolase and glycolytic flux...
play a role in the regulation of the glucose permease gene RAG1 of Kluyveromyces lactis. Genetics 168:723–731.

25. Lopez, M. L., B. Redruello, E. Valdes, F. Moreno, J. J. Heinisch, and R. Rodicio. 2004. Isocitrate lyase of the yeast Kluyveromyces lactis is subject to glucose repression but not to catabolite inactivation. Curr. Genet. 44:305–316.

26. Mazzoni, C., M. Saliola, and C. Falcone. 1992. Ethanol-induced and glucose-insensitive alcohol dehydrogenase activity in the yeast Kluyveromyces lactis. Mol. Microbiol. 6:2279–2286.

27. Milkowski, C., S. Krampe, J. Weirich, V. Hasse, E. Boles, and K. D. Breunig. 2001. Feedback regulation of glucose transporter gene transcription in Kluyveromyces lactis by glucose uptake. J. Bacteriol. 183:5223–5229.

28. Naumov, G. I., E. S. Naumova, and E. J. Louis. 1995. Genetic mapping of the alpha-galactosidase MEL gene family on right and left telomeres of Saccharomyces cerevisiae. Yeast 11:481–483.

29. Neil, H., M. Lemaire, and M. Wesołowski-Louvel. 2004. Regulation of glycolysis in Kluyveromyces lactis: role of KIGCR1 and KIGCR2 in glucose uptake and catabolism. Curr. Genet. 45:129–139.

30. Prior, C., L. Tizzani, H. Fukuhara, and M. Wesołowski-Louvel. 1996. RAG3 gene and transcriptional regulation of the pyruvate decarboxylase gene in Kluyveromyces lactis. Mol. Microbiol. 20:765–772.

31. Saliola, M., and C. Falcone. 1995. Two mitochondrial alcohol dehydrogenase activities of Kluyveromyces lactis are differently expressed during respiration and fermentation. Mol. Gen. Genet. 249:665–672.

32. Saliola, M., J. R. Shuster, and C. Falcone. 1990. The alcohol dehydrogenase system in the yeast, Kluyveromyces lactis. Yeast 6:193–204.

33. Schuller, H. J. 2003. Transcriptional control of nonfermentative metabolism in the yeast Saccharomyces cerevisiae. Curr. Genet. 43:139–160.

34. Sherwood, P. W., and M. Carlson. 1997. Mutations in GSF1 and GSF2 alter glucose signaling in Saccharomyces cerevisiae. Genetcs 147:557–566.

35. Suleau, A., N. Jacques, J. Reitz-Ausseur, and S. Casaregola. 2005. Intraspecific gene expression variability in the yeast Kluyveromyces lactis revealed by micro-array analysis. FEMS Yeast Res. 5:595–604.

36. Tusher, V. G., R. Tibshirani, and G. Chu. 2001. Significance analysis of microarrays applied to the ionizing radiation response. Proc. Natl. Acad. Sci. USA 98:5116–5121.

37. Webster, T. D., and R. C. Dickson. 1988. The organization and transcription of the galactose gene cluster of Kluyveromyces lactis. Nucleic Acids Res. 16:8011–8028.

38. Weirich, J., P. Goffrini, P. Kugler, I. Ferrero, and K. D. Breunig. 1997. Influence of mutations in hexose-transporter genes on glucose repression in Kluyveromyces lactis. Eur. J. Biochem. 249:248–257.

39. Wesołowski-Louvel, M., K. D. Breunig, and H. Fukuhara. 1996. Kluyveromyces lactis, p. 139–201. In K. Wolf (ed.), Non conventional yeast in biotechnology. Springer-Verlag, Berlin, Germany.

40. Wesołowski-Louvel, M., P. Goffrini, I. Ferrero, and H. Fukuhara. 1992. Glucose transport in the yeast Kluyveromyces lactis. I. Properties of an inducible low-affinity glucose transporter gene. Mol. Gen. Genet. 233:89–96.

41. Wiedemuth, C., and K. D. Breunig. 2005. Role of Snf1p in regulation of intracellular sorting of the lactose and galactose transporter Lac12p in Kluyveromyces lactis. Eukaryot. Cell 4:716–721.

42. Winzeler, E. A., C. I. Castillo-Davis, G. Oshiro, D. Liang, D. R. Richards, Y. Zhou, and D. L. Hartl. 2003. Genetic diversity in yeast assessed with whole-genome oligonucleotide arrays. Genetics 163:79–89.

43. Zeeman, A. M., M. Kopper, J. T. Pronk, J. P. van Dijken, and H. Y. Steensma. 2000. Regulation of pyruvate metabolism in chemostat cultures of Kluyveromyces lactis CBS 2359. Yeast 16:611–620.