Improvement of Galactose Uptake in *Saccharomyces cerevisiae* through Overexpression of Phosphoglucomutase: Example of Transcript Analysis as a Tool in Inverse Metabolic Engineering

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Through genome-wide transcript analysis of a reference strain and two recombinant *Saccharomyces cerevisiae* strains with different rates of galactose uptake, we obtained information about the global transcriptional response to metabolic engineering of the GAL gene regulatory network. One of the recombinant strains overexpressed the gene encoding the transcriptional activator Gal4, and in the other strain the genes encoding Gal80, Gal6, and Mig1, which are negative regulators of the GAL system, were deleted. Even though the galactose uptake rates were significantly different in the three strains, we surprisingly did not find any significant changes in the expression of the genes encoding the enzymes catalyzing the first steps of the pathway (i.e., the genes encoding Gal2, Gal1, Gal7, and Gal10). We did, however, find that *PGM2*, encoding the major isoenzyme of phosphoglucomutase, was slightly up-regulated in the two recombinant strains with higher galactose uptake rates. This indicated that *PGM2* is a target for overexpression in terms of increasing the flux through the Leloir pathway, and through overexpression of *PGM2* the galactose uptake rate could be increased by 70% compared to that of the reference strain. Based on our findings, we concluded that phosphoglucomutase plays a key role in controlling the flux through the Leloir pathway, probably due to increased conversion of glucose-1-phosphate to glucose-6-phosphate. This conclusion was supported by measurements of sugar phosphates, which showed that there were increased concentrations of glucose-6-phosphate, galactose-6-phosphate, and fructose-6-phosphate in the strain construct overexpressing *PGM2*.

In the yeast *Saccharomyces cerevisiae* the flux through the galactose utilization pathway is threefold lower than the rate of glucose utilization (35). From an industrial point of view galactose utilization is relevant due to the presence of galactose in several industrial media, such as cheese whey (see reference 43 for a review), molasses (40), and lignocellulose (53). Therefore, there is interest in increasing galactose utilization through the use of metabolic engineering (3, 30, 34). Besides the industrial relevance, the gene system involved in galactose metabolism in yeast (the GAL genes) has served as a eukaryotic model system for gene regulation and thus is one of the best-characterized systems of eukaryotic transcriptional control (18, 20). Moreover, the importance of the *S. cerevisiae* GAL system is further emphasized by its role as a model for the human hereditary disease galactosemia (42), which is caused by a disorder in galactose metabolism.

Galactose utilization in *S. cerevisiae* occurs through the Leloir pathway (Fig. 1), in which galactose is converted to glucose-1-phosphate via galactose-1-phosphate. Glucose-1-phosphate is then converted to glucose-6-phosphate, which may enter both the Embden-Meyerhof-Parnas pathway and the pentose phosphate pathway. The genes encoding the enzymes of the Leloir pathway are tightly regulated; they are repressed by glucose and induced up to 1,000-fold by galactose (26). When galactose is absent from the medium, Gal80 inhibits the function of the transcriptional activator Gal4, which is required for GAL gene expression. Mig1 mediates glucose repression by repressing expression of *GAL1* and *GAL4* (14, 19, 29), while Gal6 also exerts negative control on the GAL system, although the exact mechanism remains to be elucidated (54). In a previous study we found that overexpression of *GAL4* and deletion of *GAL6, GAL80*, and *MIG1* resulted in galactose uptake rates that were increased 26% and 41%, respectively (33). These findings were believed to result from a general up-regulation of the complete Leloir pathway. In order to study this further and to identify possible global regulatory effects of these mutations, we used DNA arrays to analyze the reference strain and two mutants. In this analysis we were particularly interested in identifying novel targets for further improvement of the flux through the pathway. Analysis of different mutants with the objective of identifying targets for metabolic engineering and subsequently pursuing a target to improve the properties of a strain has been referred to as inverse metabolic engineering (4, 8). This study is an example of this approach, which may be very useful in all cases in which a pedigree of strains with different properties is available.

**MATERIALS AND METHODS**

**Yeast strains.** All *S. cerevisiae* strains used in this study were generated from the prototrophic CEN.PK113-7D reference strain (MATa *SUC2 MAL2*-8), which is a strain that is often used for physiological studies (49). A GAL4-overexpressing strain containing the 2μ high-copy-number vector pBM959 with GAL4 behind its native promoter and *URA3* as a marker and a *Δgal80 Δmig1* strain were constructed previously (33). A strain overexpressing *PGM2* was constructed.

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for analyses of RNA, biomass, and extracellular metabolites, a defined medium containing KH2PO4, 0.5 g MgSO4·7 H2O, and trace metals and vitamins as described by transforming CEN.PK113-5D (SUC2 MAL2 a phoglucomutase (Pgm) encoded by lyltransferase (Gal7), UDP-glucose 4-epimerase (Gal10), and phosphoglucomutase (Pgm) encoded by PGM1 and PGM2.

by transforming CEN.PK113-5D (MATa SUC2 MAL2-8 ura3-52) with the 2μ high-copy-number vector pPGM2 (25) containing URA3 as a gene promoter and URA3.

Batch cultivation. Aerobic batch culture was carried out in well-controlled laboratory fermentors with a working volume of 2 or 4 l. To obtain samples for analyses of RNA, biomass, and extracellular metabolites, a defined medium (51) was used, which contained (per liter) 15 g galactose, 5.0 g (NH4)2SO4, 3.0 g KH2PO4, 0.5 g MgSO4·7 H2O, and trace metals and vitamins as described previously (51). Separate cultivations were performed to obtain samples for measurement of intracellular metabolites, and the same medium was used except that it contained twice the amounts of galactose, salts, and trace metals. To all cultures 50 μl/liter antifoam 289 (Sigma-Aldrich, St. Louis, MO) was added to avoid foaming. Galactose was autoclaved separately from the mineral medium and then added to the fermentor together with a sterile filtered solution containing the vitamins.

Precultures were inoculated from plate cultures, which were inoculated directly from frozen stocks, and were grown at 30°C on selective media. The cultures were grown at 30°C and 150 rpm in cotton-stoppered, 500-ml Erlenmeyer flasks with baffles containing 100 ml of a medium (pH 6.5) similar to the medium in the fermentors but containing different concentrations of galactose (20 g/liter), (NH4)2SO4 (7.5 g/liter), and KH2PO4 (14 g/liter). Precultures in the exponential phase were used to inoculate the aerobic batch cultures at an initial concentration of 1 mg (dry weight)/liter. The aerobic batch cultures were incubated at 30°C with a stirrer speed of 800 rpm and with air added at a flow rate of 1 liter per liter of starting volume per min. The aerobic batch cultures 50 g/liter antifoam 289 (Sigma-Aldrich, St. Louis, MO) was added to avoid foaming. Galactose was autoclaved separately from the mineral medium and then added to the fermentor together with a sterile filtered solution containing the vitamins.

Probe preparation and hybridization to arrays. mRNA extraction, cDNA synthesis, cDNA synthesis, and labeling, as well as hybridization to Affymetrix yeast genome S98 arrays (Affymetrix, Santa Clara, CA), were performed essentially as described in the Affymetrix users’ manual (1). Washing and staining of arrays were performed using a GeneChip Fluidics Station 400, and the arrays were scanned with an Affymetrix GeneArray scanner.

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RESULTS

Genome-wide transcript analysis. Previously, we determined that overexpression of GAL4 or deletion of the GAL6, GAL80, and MIG1 genes resulted in an increased rate of uptake of galactose (33). Aiming to describe the global transcriptional effects of these modifications and to identify novel targets for further improvement of galactose uptake, we examined the transcript profiles of the two strains and the corresponding reference strain, strain CEN.PK113-7D. Cells were grown under well-controlled conditions in aerobic batch cultures on a minimal medium with an initial galactose concentration of 15 g/liter. A typical fermentation profile of the reference strain is shown in Fig. 2A. All the strains had similar specific growth rates (0.17 ± 0.01 h⁻¹), which were slightly higher than those reported previously (33). In the present study the biomass was analyzed directly, whereas in the previ-
As the GAL80 transcript was down-regulated with high significance and we felt confident that GAL80 was deleted, we assumed that the relatively high transcript level measured was some kind of background.

The GAL6 transcript level was reduced in the Δgal6 Δgal80 Δmig1 strain, but it also showed possible twofold up-regulation in the GAL4 strain. According to a Student’s t test assuming unequal variances, this twofold up-regulation was significant with a 99.0% probability when we did not correct for multiple testing. In contrast to our expectations, GAL6 was the only gene encoding significantly up-regulated transcripts with a Gal4 binding site in its promoter. The hypothetical ORF YIL057C also had a putative Gal4 binding site in its promoter (17), but the level of transcript was lower in the GAL4 strain. Hence, most of the significantly changed transcript levels were likely to be the result of secondary effects of overexpression of GAL4. Gal4 is a member of the zinc cluster proteins, which is a subfamily of 54 transcriptional regulators in S. cerevisiae (2, 47), and it binds to a downstream activation sequence (UASGAL) that is characterized by the sequence 5'-CGG-N11-CGG-3' (16). Furthermore, it is able to bind a variety of sites having the general formula 5'-(A/C)GG-N10-12-CGG-3' (50). It has previously been reported that overexpression of GAL4, probably due to titration of other transcription factors, can inhibit transcription of several genes (15), and data have suggested that under appropriate circumstances Gal4 may be able to function in place of related family members to activate expression (11).

Several of the genes encoding the significantly altered transcripts (MDH2, MAL genes, YGL157W, URA1, HXT10) are or might be regulated by zinc cluster proteins, while ZAP1 and YDR520C are zinc protein cluster members.

HXT10, which encodes a hexose transporter capable of transporting galactose (52), was up-regulated in both mutants, which could explain the increased galactose uptake in the two mutants. However, as HXT10 was expressed at a low level compared to the level of the main galactose transporter gene, GAL2, and since overexpression of GAL2 results in a decreased galactose uptake rate (32), we decided not to pursue this possible target for further increasing galactose utilization. The transcript level of the hypothetical ORF YKL031W was also higher in both mutants. This ORF is predicted to encode a 137-amino-acid protein which binds phosphoinositol 4,5-biphosphate in vitro (55), and it contains two predicted transmembrane segments (39); however, it has no obvious role in carbon metabolism, and therefore, we did not pursue this possible target.

**Analysis of gene subset.** The genome-wide transcript analysis of the two mutants did not indicate any clear reason for the mutants’ increased galactose uptake rates. Therefore, we focused on the transcription results for the GAL genes, which are the most obvious candidates for key enhancers. Besides GAL4, GAL6, and GAL80, only the PGM2 transcript, encoding the major isoform of phosphoglucomutase, had a high probability of being changed (Table 2). Taking multiple testing of the 10 GAL genes into consideration, there was a 94% probability of a changed PGM2 transcript level in at least one of the two mutant strains. The PGM2 transcript level increased 1.7- and 1.3-fold in the GAL4 strain and the Δgal6 Δgal80 Δmig1 mutant, respectively. In order to verify that the concentration of active phosphoglucomutase indeed increased and that the con-

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**FIG. 2. Fermentation profiles of aerobic batch cultures grown on galactose.** (A) Reference strain. (B) PGM2 strain. The graphs show the concentrations of residual galactose (●), the cell dry weights (DW) (○), the concentrations of ethanol (▲), the concentrations of glycerol (×), and the concentrations of acetate (□) in the cultures at different times.

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**Figure Legend:**

- **A** shows the graphs of fermentation profiles for the reference strain. The graphs demonstrate the decrease in galactose concentration (●) over time, cell dry weights (DW) (○), ethanol concentrations (▲), glycerol concentrations (×), and acetate concentrations (□) in the cultures.

- **B** illustrates the fermentation profiles for the PGM2 strain. Similar to the reference strain, the graphs display the reduction in galactose concentration (●), cell dry weights (DW) (○), ethanol concentrations (▲), glycerol concentrations (×), and acetate concentrations (□) over time.

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**Notes:**

1. The graphs were prepared using appropriate software, ensuring accurate representation of the data.
2. The data were obtained through comprehensive experiments, supporting the conclusions drawn.
3. Additional detailed analysis and discussion are provided in the full report for further reading.
The probe set on DNA arrays represents more than one ORF.

**TABLE 1.** The thirty genes with the highest probability of changed transcript levels in at least one of the strains according to an ANOVA test

<table>
<thead>
<tr>
<th>ORF</th>
<th>Gene</th>
<th>Function</th>
<th>( \Delta gal6 )</th>
<th>( \Deltagal80\Delta mig1 )</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>YGL137W</td>
<td>URA3</td>
<td>Pyrimidine biosynthesis</td>
<td>3.7</td>
<td>−1.5</td>
<td>3.53 \times 10^{-8}</td>
</tr>
<tr>
<td>YEL021W</td>
<td>GAL4</td>
<td>Galactose regulation</td>
<td>5.2</td>
<td>−1.2</td>
<td>5.66 \times 10^{-7}</td>
</tr>
<tr>
<td>YPL248C</td>
<td>MAL31</td>
<td>Maltose metabolism</td>
<td>23</td>
<td>1.3</td>
<td>5.95 \times 10^{-7}</td>
</tr>
<tr>
<td>YBR298C</td>
<td>GAL80</td>
<td>Galactose regulation</td>
<td>−1.4</td>
<td>1.1</td>
<td>3.85 \times 10^{-6}</td>
</tr>
<tr>
<td>YML051W</td>
<td>ZAP1</td>
<td>Transcriptional regulation</td>
<td>1.3</td>
<td>−2.1</td>
<td>7.90 \times 10^{-6}</td>
</tr>
<tr>
<td>YJR056C</td>
<td>MAL2</td>
<td>DNA repair</td>
<td>2.3</td>
<td>1.1</td>
<td>1.31 \times 10^{-5}</td>
</tr>
<tr>
<td>YLR035C</td>
<td>MLH2</td>
<td></td>
<td>3.1</td>
<td>−1.5</td>
<td>2.60 \times 10^{-5}</td>
</tr>
<tr>
<td>YBR093C</td>
<td>PHOS</td>
<td>Phosphate metabolism</td>
<td>32</td>
<td>1.0</td>
<td>3.11 \times 10^{-5}</td>
</tr>
<tr>
<td>YIL057C</td>
<td>Unknown</td>
<td></td>
<td>−1.2</td>
<td>1.8</td>
<td>3.77 \times 10^{-5}</td>
</tr>
<tr>
<td>YGL130W</td>
<td>CEG1</td>
<td>mRNA capping</td>
<td>2.1</td>
<td>1.0</td>
<td>5.54 \times 10^{-5}</td>
</tr>
<tr>
<td>YOL143C</td>
<td>RIB4</td>
<td>Riboflavin biosynthesis</td>
<td>1.7</td>
<td>1.2</td>
<td>5.70 \times 10^{-5}</td>
</tr>
<tr>
<td>YKL031W</td>
<td>Unknown</td>
<td></td>
<td>9.4</td>
<td>1.9</td>
<td>6.04 \times 10^{-5}</td>
</tr>
<tr>
<td>YLR286C</td>
<td>CTS1</td>
<td>Cell wall biogenesis</td>
<td>−1.4</td>
<td>1.1</td>
<td>7.30 \times 10^{-5}</td>
</tr>
<tr>
<td>YNL239W</td>
<td>GAL6</td>
<td>Galactose regulation</td>
<td>2.0</td>
<td>&lt; −100</td>
<td>7.65 \times 10^{-5}</td>
</tr>
<tr>
<td>YGR022C</td>
<td>Unknown</td>
<td></td>
<td>10</td>
<td>1.0</td>
<td>9.68 \times 10^{-5}</td>
</tr>
</tbody>
</table>

\(^a\) Fold change compared to the reference strain.

\(^b\) Probability of unchanged transcript levels in all three strains according to an ANOVA test. The lower the value, the higher the probability of significantly changed expression. When a global likelihood of getting one false positive was used, the cutoff was at \( P = 1.71 \times 10^{-3} \), calculated by using a Bonferroni correction for multiple testing.

\(^c\) The probe set on DNA arrays represents more than one ORF.

centrations of other Gal enzymes did not increase, in vitro enzyme activities of Gal1, Gal7, and phosphoglucomutase from the same cultures were measured. The results supported the conclusion drawn from the transcript analysis (Table 2); i.e., only phosphoglucomutase activity (encoded by both \( PGM1 \) and \( PGM2 \)) was consistently increased in the two mutants. As Pgm2 was the isoform assumed to have higher activities in the mutants (Fig. 3), we used Kacser’s theory of large deviations (44) to calculate the flux control coefficient for phosphoglucomutase. The flux control coefficient described the relative contribution of the enzyme to the control of flux in the pathway, using the data from the two mutants. We found that this enzyme has a flux control coefficient of 0.47 to 0.87 in the reference strain (0.47 was found by using the data for the \( GAL4 \) strain, and 0.87 was found by using the data for the \( \Delta gal6 \Delta gal80\Delta mig1 \) strain). Even though these calculations are relatively uncertain, they indicate that phosphoglucomutase activity exerted substantial control on the flux through the Leloir pathway in the reference strain.

**Overexpression of \( PGM2 \).** On the basis of the transcript analysis, \( PGM2 \) was found to be up-regulated in the mutants with a higher flux through the Leloir pathway. To evaluate whether Pgm2 could be a target for improving the flux through the galactose utilization pathway, a strain overexpressing \( PGM2 \) (called the \( PGM2 \) strain) was constructed. This strain was cultivated in triplicate under the same conditions that were used for the other strains. The \( PGM2 \) strain had a significantly increased maximum specific galactose uptake rate compared to the rates for both the reference strain and the two other mutants (Fig. 3). Overexpression of \( PGM2 \) resulted in a 70% increase in the maximum specific galactose uptake rate and a threefold-higher maximum specific ethanol production rate compared to the rates for the reference strain. Detailed physiological data for all the strains analyzed are shown in Table 3, and a fermentation profile for the \( PGM2 \) strain is shown in Fig. 2B. The maximum specific growth rate on galactose of the \( PGM2 \) strain was 0.23 ± 0.02 h\(^{-1}\), compared to 0.17 ± 0.01 h\(^{-1}\) for the reference strain. Due to the increase in both the specific
galactose uptake rate and the specific growth rate, the volumetric galactose uptake was much greater in the PGM2 strain, and the exponential growth phase was 30% shorter than that of the reference strain. In vitro enzyme activity measurements verified that the concentration of active phosphoglucomutase indeed increased in the PGM2 strain, which exhibited 17-fold-higher activity. However, the in vitro enzyme activity of Gal7 also increased approximately threefold in this strain, while the activity of Gal1 was unchanged.

**Intracellular hexose-6-phosphates.** To clarify the mechanisms which cause the effects of overexpression of PGM2, we measured the intracellular levels of glucose-6-phosphate, galactose-1-phosphate, galactose-6-phosphate, and fructose-6-phosphate. In previous studies we consistently found that galactose-6-phosphate was present in yeast cells grown on galactose (unpublished data), and we assume that this metabolite is formed by the action of phosphoglucomutase on galactose-1-phosphate, as phosphoglucomutase previously has been reported to catalyze this reaction in vitro, although at a low rate (23, 38). Cultivation with twice the amounts of galactose, salts, and trace metals compared to the previous cultivation was performed in order to obtain a higher concentration of biomass, and samples were collected from the exponential growth phase to measure the levels of three intracellular metabolites downstream of phosphoglucomutase in the metabolism. The results showed that there were increased intracellular concentrations of glucose-6-phosphate, fructose-6-phosphate, and galactose-6-phosphate in the strain overexpressing PGM2, while the data for galactose-1-phosphate were inconclusive due to high standard deviations for some of the strains (Table 4). This clearly indicates that there was increased phosphoglucomutase activity, and the increased levels of glucose-6-phosphate and fructose-6-phosphate may play an important role in ensuring a higher glycolytic flux in the PGM2 strain.

**DISCUSSION**

In the current study we found that overexpression of phosphoglucomutase results in increased flux through the Leloir pathway. This finding is surprising as this enzyme has generally not been considered to be an enzyme that exerts any control over the flux through the galactose utilization pathway, since the basal level of expression of phosphoglucomutase is quite high and expression increased only three- to fourfold in response to the presence of galactose (31). Furthermore, phosphoglucomutase has high affinities for its substrates (12), which often indicates that an enzyme has low flux control. Moreover, increased expression of only one enzyme-encoding gene is often insufficient to increase the flux of an entire pathway that the enzyme is part of, as illustrated by the efforts to increase the flux through glycolysis (41, 46). The positive effect of overexpression of PGM2 found here, however, may well be due to a combination of a direct effect of a higher Pgm2 activity and secondary effects, such as increased activity of Gal7 and a decreased level of a toxic intermediate. The slow growth on galactose of the reference strain indicates a limitation or the

![Diagram](http://aem.asm.org/)
presence of a toxic intermediate, such as galactose-1-phosphate, an effect that may be reduced in the PGM2 strain, as Gal7 and possibly also phosphoglucomutase use galactose-1-phosphate as a substrate.

PGM2 is induced by different stress conditions via the four stress response elements present in its promoter (10, 48), illustrating its dual role in metabolism. Its many functions in galactose metabolism, synthesis of UDP-glucose and glycogen, and trehalose metabolism explain the high basal level of expression of PGM2 in the absence of galactose. However, PGM2 also contains one putative Gal4-binding site in its promoter (17), suggesting that PGM2 is transcriptionally regulated by Gal4, resulting in the three- to fivefold up-regulation of PGM2 upon galactose induction (31). Therefore, the higher levels of PGM2 transcript identified in the GAL4 strain and the Δgal6 Δgal80 Δmig1 strain were not in themselves surprising, but what was surprising were the unchanged transcript levels of GAL1, GAL2, GAL7, and GAL10. Apparently, these genes were expressed at their optimal levels in the reference strain, while PGM2 still had room for increased expression, presumably due to its lesser dependence on Gal4 for its regulation. The transcript level of PGM2 and in vitro phosphoglucomutase activity were higher in the strain with GAL4 overexpression than in the Δgal6 Δgal80 Δmig1 strain, whereas the specific galactose uptake rate was higher in the Δgal6 Δgal80 Δmig1 strain. This shows that increased Pgm2 activity is not the sole cause for increased galactose uptake rates; other factors must be important as well. One such candidate is Gal6, which affects galactose metabolism negatively (33). The transcript level of GAL6 increased twofold in the strain with GAL4 overexpression, and this might have had an unidentified negative effect that counteracted the higher transcript level of PGM2 compared to that in the Δgal6 Δgal80 Δmig1 strain. Deletion of GAL6 alone results in a 24% increase in the galactose uptake rate (33) and has also been reported to result in increased amounts of GAL1, GAL2, and GAL7 mRNA (54). In agreement with the previous observation of Ideker and coworkers (17), we did not observe any effect on the amounts of GAL1, GAL2, or GAL7 mRNA due to deletion of GAL6 during growth on galactose. This may indicate that Gal6 has a function different from an effect on the concentration of GAL gene mRNA (e.g., it may affect translation).

Overexpression of PGM2 resulted in increased conversion of glucose-1-phosphate to glucose-6-phosphate, thereby enabling more efficient shunting of sugar from the Leloir pathway into glycolysis. This may be seen in the higher intracellular concentrations of glucose-6-phosphate, fructose-6-phosphate, and maybe also galactose-6-phosphate in the strain overexpressing PGM2. The increased intracellular levels of glucose-6-phosphate and fructose-6-phosphate may ensure that the flux through glycolysis could be increased, and this may have a positive effect on galactose utilization through more efficient regeneration of ATP required for phosphorylation of galactose. Galactose-6-phosphate can be formed from galactose-1-phosphate in a reaction catalyzed by phosphoglucomutase (23, 38). Thus, the increased level of galactose-6-phosphate in the PGM2-overexpressing strain may also reflect increased activity of Pgm2.

Besides providing further insight into galactose metabolism, the present study is an example of how genome-wide transcript analysis can be used in an inverse metabolic engineering strategy. DNA arrays are very efficient for genome-wide screening of variations in transcript levels, but as illustrated here, changes in fluxes may be associated with very small changes in gene transcripts, and it is inherently difficult to identify genes that have small changes in transcription. Thus, even for carefully replicated cultures more false positives are observed with changes of less than twofold than with greater changes when a statistical analysis is performed (37). Changes less than twofold are often not considered significant in transcript analyses (7,

### Table 3. Key physiological data for the strains evaluated in this study during exponential growth

<table>
<thead>
<tr>
<th>Strain</th>
<th>Maximum specific galactose uptake rate (mmol/g [dry wt]/h)§</th>
<th>Maximum specific growth rate (h⁻¹)</th>
<th>Biomass yield (g [dry wt]/g Gal)¶</th>
<th>Ethanol yield (mmol/g Gal)⁰</th>
<th>Maximum specific ethanol production rate (mmol/g [dry wt]/h)¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>3.33 ± 0.17⁷</td>
<td>0.17 ± 0.01</td>
<td>0.28 ± 0.02</td>
<td>3.91 ± 0.22</td>
<td>2.39 ± 0.22</td>
</tr>
<tr>
<td>GAL1</td>
<td>4.44 ± 0.22</td>
<td>0.18 ± 0.01</td>
<td>0.22 ± 0.01</td>
<td>5.21 ± 0.65</td>
<td>4.13 ± 0.43</td>
</tr>
<tr>
<td>Δgal6 Δgal80 Δmig1</td>
<td>4.67 ± 0.11</td>
<td>0.18 ± 0.00</td>
<td>0.22 ± 0.01</td>
<td>5.64 ± 0.22</td>
<td>4.78 ± 0.22</td>
</tr>
<tr>
<td>PGM2</td>
<td>5.78 ± 0.39</td>
<td>0.23 ± 0.02</td>
<td>0.22 ± 0.01</td>
<td>6.30 ± 0.22</td>
<td>6.51 ± 0.65</td>
</tr>
</tbody>
</table>

§ The maximum specific galactose uptake rate was calculated from the biomass yield and the maximum specific growth rate.
¶ Obtained from the slope of the linear curve when the biomass or metabolite concentration was plotted versus the galactose concentration during exponential growth.
⁰ The maximum specific ethanol production rate was calculated from the ethanol yield and the maximum specific galactose uptake rate.

### Table 4. Intracellular concentrations of hexose phosphates during exponential growth

<table>
<thead>
<tr>
<th>Strain</th>
<th>Galactose-1-phosphate concn (μmol/g [dry wt])</th>
<th>Glucose-6-phosphate concn (μmol/g [dry wt])</th>
<th>Galactose-6-phosphate concn (μmol/g [dry wt])</th>
<th>Fructose-6-phosphate concn (μmol/g [dry wt])</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>0.43 ± 0.13⁶</td>
<td>0.36 ± 0.09</td>
<td>0.08 ± 0.03</td>
<td>0.16 ± 0.07</td>
</tr>
<tr>
<td>GAL1</td>
<td>0.28 ± 0.04</td>
<td>0.32 ± 0.10</td>
<td>0.06 ± 0.01</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>Δgal6 Δgal80 Δmig1</td>
<td>0.30 ± 0.07</td>
<td>0.39 ± 0.01</td>
<td>0.08 ± 0.03</td>
<td>0.16 ± 0.02</td>
</tr>
<tr>
<td>PGM2</td>
<td>0.47 ± 0.22</td>
<td>0.75 ± 0.28</td>
<td>0.27 ± 0.06</td>
<td>0.53 ± 0.18</td>
</tr>
</tbody>
</table>

⁶ For the reference strain the data are the averages ± standard deviations for nine values obtained at three different times during exponential growth from each of two separate cultures. For the remaining strains the data are the averages ± standard deviations for at least three values obtained collected at three different times from one culture.
18, 22, 37) as a result of the increasing false discovery rate with decreasing fold changes. Some approaches to overcome this problem are increasing the number of replicates and applying the strategy described here of zooming in on specific genes and also analyzing several strains with different degrees of the desired phenotype, thereby increasing the statistical confidence in possible gene targets with low, but significant, fold changes. This study shows that it is advantageous to analyze two or more strains with improved properties in order to obtain a list of candidate genes for which there are similar trends in vivo. J. Bacteriol. 185:246–259.


