Galactose transporters discriminate steric anomers at the cell surface in yeast

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GAL10; galactose metabolism; epimerase; mutarotase; sugar transporter; yeast.

Abstract
Aldose-1-epimerase or mutarotase (EC 5.1.3.3) catalyzes interconversion of α/β-anomers of aldoses, such as glucose and galactose, and is distributed in a wide variety of organisms from bacteria to humans. Nevertheless, the physiological role of this enzyme has been elusive in most cases, because the α-form of aldoses in the solid state spontaneously converts to the β-form in an aqueous solution until an equilibrium of α:β = 36.5:63.5 is reached. A gene named GAL10 encodes this enzyme in yeast. Here, we show that the GAL10-encoded mutarotase is necessary for utilization of galactose in the milk yeast Kluyveromyces lactis, and that this condition is presumably created by the presence of the β-specific galactose transporter, which excludes the α-anomer from the α/β-mixture in the medium at the cell surface. Thus, we found that a mutarotase-deficient mutant of K. lactis failed to grow on medium, in which galactose was the sole carbon source, but, surprisingly, that the growth failure is suppressed by concomitant expression of the Saccharomyces cerevisiae-derived galactose transporter Gal2p, but not by that of the K. lactis galactose transporter Hgt1p. We also suggest the existence of another mutarotase in K. lactis, whose physiological role remains unknown, however.

Introduction
Galactose is important for most living organisms including humans, where this sugar is not only an essential carbon source in newborns as a constituent of lactose but also a building block of nerve tissue as galactolipid. Galactose is metabolized through the so-called ‘Leloir pathway’ as shown in Fig. 1, which is conserved among most organisms. The genes encoding the enzymes responsible form an operon in Escherichia coli K12 (Buttin, 1963). In 1994, Adhya and his colleagues identified the galM gene encoding mutarotase as a new member of the galactose operon in E. coli K12 (Bouffard et al., 1994). Introduction of a deletion in galM does not affect utilization of galactose at all, however. Instead, the defect leads to slow growth in the medium containing phenyl-β-D-galactopyranoside (hereafter phenyl-β-D-galactose) as the sole carbon source. These authors suggested that mutarotase is involved in efficient utilization of lactose due to the following reason: phenyl-β-D-galactose, when taken up by the cell through the function of the β-galactoside transporter (Kennedy, 1970), is split into a phenyl group and β-D-galactopyranoside (hereafter β-D-galactose) by the catalytic action of β-galactosidase. The latter compound, but not the former, suitable as a carbon source, has to be converted to α-galactose by the action of mutarotase before entering the Leloir pathway, because the first enzyme of the pathway, galactokinase, uses only α-galactose, but not its β-anomer, as the substrate (Howard & Heinrich, 1965, see Fig. 1). Therefore, the galM mutants normally fail to grow in the medium containing phenyl-β-D-galactose as the sole carbon source. As a corollary, they concluded that autocatalytic water-dependent conversion of β-galactose to its α-form is not efficient enough for the Leloir pathway to support normal cell growth. To determine whether mutarotase is really required for utilization of galactose or lactose, we used Kluyveromyces lactis, which is capable of utilizing both galactose and lactose as the carbon source, exceptionally among yeasts.

In most organisms including bacteria (Poolman et al., 1990; Bouffard et al., 1994) and humans (Timson & Reece, 2009).
mutarotase and UDP-glucose-4-epimerase (hereafter called epimerase), the third enzyme of the Leloir pathway, are encoded by two independent genes, *galM* and *galE*, respectively. In contrast, these enzymes are produced as a single peptide in yeasts, such as *Saccharomyces cerevisiae*, *K. lactis*, and *Kluyveromyces fragilis*. While epimerase was purified from yeasts including *K. fragilis* and *S. cerevisiae* since 1963 through 1980, it was noticed, surprisingly, that the molecular size of yeast enzymes was twice or more larger than that of epimerase from other sources, such as *E. coli*, wheat germ, porcine, or bovine (see Supporting Information, Table S1). This was solved by Poolman *et al.* (1990), who found that the mutarotase gene cloned from *Streptococcus thermophilus* has strong similarity to the 3'-half of *GAL10* from *S. cerevisiae* (Citرون & Donelson, 1984). Majumdar *et al.* (2004), Brahma & Bhattacharyya (2004), and Scott & Timson (2007), respectively, confirmed this fact using purified enzymes from *S. cerevisiae* and *K. fragilis*, showing that the epimerase and mutarotase activities reside separately in N- and C-terminal domains. The putative active sites have been located in the respective domains in a three-dimensional structure visualized by X-ray diffraction analysis (Thoden & Holden, 2005).

**Materials and methods**

**Yeast genetic methods including media**

Yeast genetic methods including media were those described by Sherman (2002). Yeast transformation was performed according to the 'high efficient protocol' described by Gietz & Woods (2002).

**Yeast strains and plasmids**

JA6gal10Δ was constructed in this work by insertion of the 'popping-out' URA3 gene from *S. cerevisiae* between BglIII and SalI sites in *GAL10* from *K. lactis* JA6 (MATα, ade1-600 trp1-11 ura3-12), followed by selecting 5-fluoroorotic acid-resistant clones from Ura<sup>+</sup> transformants (Alani *et al.*, 1987). JA6gal10Δ gal80Δ was also constructed in this work.
by replacing the GAL80 locus of JA6gal10Δ with the XhoI fragment of pD802, a plasmid carrying gal80::URA3 (Zenke et al., 1993). The ORFs of ScGAL10 (P04397), KIGAL10 (P09609), and SpGAL10a (CAC21414) were PCR-cloned from S. cerevisiae W303-1a, K. lactis KAS-6C, and Schizosaccharomyces pombe Y742, respectively. Codes in parentheses indicate the accession numbers of NCBI. The yeast Saccharomyces pombe (P09609), and Spet al., 1993). The ORFs of ScGAL10 possesses two GAL10 genes: a and b; the former encodes epimerase/mutarotase, whereas the latter encodes only epimerase. No evidence has been available to indicate whether these genes are transcribed in situ. The primers used for cloning are as follows:

ScGAL10: 5′-(Met)TCATCGAGATGACAGCTCACGTAC
3′-AGTCAGCTGGCAATCAGAGAAAATC;
KIGAL10: 5′-(Met)AAACCTCGAGATGTCTGAGATAAAT
3′-TGTCAGCTGTATTTCCATCACAAAG;
SpGAL10: 5′-(Met) CTCTTCGAGATGCCGTTCAAGACGA
3′-GCTTAATTCGCTGTTTATTAAGT.

Cloned GAL10 fragments were first inserted into pVT102U, an expression vector for S. cerevisiae (Vernet et al., 1987). The resulting plasmid then received a 70-bp-fragment-containing sequence for the histidine tag (His10) from pET16b (Novagen) at the 5′-end of GAL10. The region encompassing the His10-GAL10-ADH1 terminator was excised and inserted into p4XX, another expression vector of S. cerevisiae, which contains the promoter region of ScGAL1 and the selection marker of GAL1.

Affinity purification of KlGal10p

Affinity purification of KlGal10p was carried out using TALON metal affinity resin (Clontech Laboratories Inc., Palo Alto, CA), essentially according to the supplier’s protocol. In brief, cells of K. lactis strain JA6gal10Δ gal80Δ carrying either pK394 or pK411, expressing the wild-type or mutarotase-domain-deleted Gal10p were grown to the late-log phase in a tryptophan-omitted synthetic medium at 30 °C. When the cell density reached an OD650 nm of 1.2–1.3, cells were harvested and washed with an equal volume of chilled water and then 0.1 M Tris/HCl (pH 8.0) containing 0.25 M NaCl. Cells were disrupted by vigorous agitation on a Vortex mixer in the presence of glass beads (d = 0.5 mm), and the clear lysate obtained after high-speed centrifugation (100 000 g for 20 min) was gently mixed with TALON metal affinity resin for 30 min. The mixture was transferred into a column, and the resin was washed in a gravity-flow manner successively with buffer and buffer containing 5 mM imidazole. Gal10p or its deletion was eluted with buffer containing 50 mM imidazole. All the steps were carried out at a temperature < 5 °C.

Preparation of concentrated crude extracts and ammonium sulfate fractionation

Cells of JA6gal10Δ gal80Δ were grown to the late-log phase in 500 mL of YPD fortified with 20 μg mL⁻¹ of adenine sulfate. When the cell density reached an OD650 nm of 1.2–1.3, cells were collected, washed twice with an equal volume of water, and finally 250 mL of 20 mM Tris/HCl buffer (pH 8.0) containing 0.2 M NaCl and 1 mM each of EDTA and dithiothreitol. Cells were disrupted on a Vortex mixer in the presence of glass beads. Cell debris and unbroken cells were removed by centrifugation at 1100 g for 20 min in a clinical centrifuge, and the supernatant sample was subjected to high-speed (100 000 g) centrifugation for 20 min to yield 100 mL of a clear lysate. Solid ammonium sulfate was added to the resulting clear lysate to obtain precipitates at the indicated concentrations. All steps were carried out at a temperature < 5 °C.

UDP-glucose-4-epimerase

UDP-glucose-4-epimerase was assayed using the two-step method described previously (Fukasawa et al., 1980); UDP-galactose was incubated with a sample in the first reaction, and the UDP-glucose formed was determined by formation of NADH in the second reaction consisting of UDP-glucose-dehydrogenase and NAD⁺ in a spectrophotometer. Mutarotase was determined with a polarimeter based on the change in optical rotation of α-D-glucose according to the method described by Majumdar et al. (2004), whose validity is shown in Fig. S3. Mutarotase is assayed enzymatically in some experiments with β-glucose-specific dehydrogenase (Sigma/Aldrich Co.) according to the method described by Bouffard et al. (1994).

Results

Construction of the gal10 deletion K. lactis strain

First, we introduced deletion covering both domains of GAL10 in K. lactis strain JA6 requiring uracil and tryptophan to yield JA6gal10Δ. Because JA6gal10Δ was unable to grow in the presence of galactose, it is difficult to obtain sufficient amount of induced cell mass for an enzyme assay. We therefore introduced deletion into the GAL80 gene that encodes the specific inhibitor of Gal4p to generate JA6gal10Δ gal80Δ, using pD802, a plasmid containing gal80Δ (Zenke et al., 1993). The loss of Gal80p function
leads to constitutive synthesis of the enzymes in the Leloir pathway, so that these activities are fully expressed, even when cells were grown on glucose. The regulatory proteins Gal4p and Gal80p exert the respective functions in the same manner both in *S. cerevisiae* and in *K. lactis* (Schaffrath & Breunig, 2000). As shown in Table 1, the activity of epimerase and mutarotase became undetectable in JA6-gal10Δ gal80Δ. By contrast, JA6gal80Δ grown on glucose exhibited both activities comparable to those in the original strain JA6 grown on galactose.

### Construction of mutarotase-domain deleted gal10 gene on an expression vector

We PCR-cloned *GAL10* from *K. lactis* in an expression vector as described in Materials and methods. Next, we introduced an internal deletion into the mutarotase domain of KlGAL10 in the plasmid (see Fig. S2). These plasmids pK394 or pK411 carrying either the wild-type *GAL10* (KlGAL10) or the mutarotase-deleted gal10 (Klgal10mutΔ) were transferred into JAgal10Δ gal80Δ. As is shown in Table 1, KlGAL10-carrying cells grown on glucose exhibited normal activities of both epimerase and mutarotase. By contrast, Klgal10mutΔ-carrying cells were specifically defective in mutarotase, but exhibited subnormal activity of epimerase. The lower than normal level may be due to the metabolic instability of the altered protein, because presumable degradation products were seen in polyacrylamide gel of metal-affinity purified protein (Fig. 2). The activity of epimerase and mutarotase in the partially purified enzyme is shown in Table 2.

### Growth behavior of mutarotase-deficient strains on various carbon sources

The transformants expressing either the wild-type or the mutarotase-deleted Gal10p were then tested for growth on galactose, lactose, and phenyl-β-d-galactose. As shown in Fig. 3, Klgal10mutΔ-carrying cells of JAgal10Δ gal80Δ did not grow on synthetic minimal medium containing either galactose or phenyl-β-d-galactose as the sole carbon source.

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**Table 1.** UDP-glucose-4-epimerase (epimerase) and aldose-1-epimerase (mutarotase) activity in various Kluyveromyces strains

<table>
<thead>
<tr>
<th>Yeast strain</th>
<th>Gene carried on plasmid</th>
<th>Carbon source</th>
<th>Epimerase</th>
<th>Mutarotase</th>
</tr>
</thead>
<tbody>
<tr>
<td>JA6</td>
<td>None</td>
<td>Glucose</td>
<td>&lt; 0.5</td>
<td>&lt; 2.0</td>
</tr>
<tr>
<td>JA6</td>
<td>None</td>
<td>Galactose</td>
<td>41 ± 1.0</td>
<td>34.6 ± 1.0</td>
</tr>
<tr>
<td>JA6 gal80Δ</td>
<td>None</td>
<td>Glucose</td>
<td>42 ± 1.0</td>
<td>25.6 ± 1.0</td>
</tr>
<tr>
<td>JA6gal10Δ</td>
<td>None</td>
<td>Glucose</td>
<td>&lt; 0.5</td>
<td>&lt; 2.0</td>
</tr>
<tr>
<td>JA6gal10Δ</td>
<td>KIGAL10</td>
<td>Glucose</td>
<td>58 ± 5</td>
<td>35.5</td>
</tr>
<tr>
<td>JA6gal10Δ</td>
<td>Klgal10mutΔ</td>
<td>Glucose</td>
<td>31 ± 3</td>
<td>&lt; 2.0</td>
</tr>
<tr>
<td>JA6gal10Δ</td>
<td>KIGAL10 + vacant vector</td>
<td>Galactose</td>
<td>125 ± 6</td>
<td>110 ± 4.0</td>
</tr>
<tr>
<td>JA6gal10Δ</td>
<td>Klgal10mutΔ + ScGAL2</td>
<td>Galactose</td>
<td>61 ± 5</td>
<td>&lt; 2.0</td>
</tr>
<tr>
<td>JA6gal10Δ</td>
<td>ScGAL10</td>
<td>Glucose</td>
<td>59.0</td>
<td>32.0</td>
</tr>
</tbody>
</table>

Epimerase activity was expressed as micromoles of UDP-glucose formed per hour per milligram of protein. Mutarotase activity was calculated from a first-order rate constant for catalyzed mutarotation of α-glucose subtracted with the rate constant of spontaneous mutarotation of α-glucose determined with a polarimeter (JASCO DIP36). The rate constant of catalyzed mutarotation reaction thus obtained was multiplied 1000 times for clarity in presentation. The values with ± are the average of two independent samples with deviations.

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**Fig. 2.** Polyacrylamide (8%)/SDS (0.1%) gel electrophoresis of metal-affinity purified KlGal10p and its deletion. Lanes contained mutarotase domain-deleted Gal10mutΔp (3.0 μg), Gal10p (2.6 μg), and M, a set of prestained size markers (SeeBlue Pre-stained, Invitrogen Co.), respectively. Enzyme samples were those described in Table 2. Markers are myosin (188 kDa), phosphorylase B (98 kDa), bovine serum albumin (62 kDa), glutamic dehydrogenase (49 kDa), and alcohol dehydrogenase (38 kDa). The gel was stained with a silver staining kit (ATTO Co.).

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on which KlGAL10-carrying cells of the same strain grew normally. Both strains grew well when glucose or lactose was present in the medium as the sole carbon source.

**Suppression of growth failure of mutarotase-deficient strains by ScGal2p**

Why, then, did the mutarotase-deficient *E. coli* K12 cells grow on galactose in the previous work (Bouffard et al., 1994)? In *E. coli*, galactose enters the cell by the galactose-specific transporter encoded by galP (Rotman et al., 1968) as well as by the β-galactoside-specific transporter encoded by lacY (Kennedy, 1970). We hypothesized that the galP-encoded transporter could mediate the transport of α-galactose, so that K12 cells were able to grow on galactose even in the absence of mutarotase. By contrast, only β-galactoscope could enter *K. lactis* JA6 cells, because of the lack of the α-galactose transporter. In *K. lactis*, the transport of galactose is mediated solely by the function of the β-galactoside transporter encoded by KILAC12 in some strains including the present strain JA6, because lac12 mutants derived thereof are unable to grow either on lactose or on galactose (Riley et al., 1987). In another strain like 2359, galactose transport is mediated by Lac12p, a low-affinity transporter, as well as by Hgt1p, a high-affinity transporter (Baruffini et al., 2006). On the other hand, galactose is transported into the cell solely by the function of Gal2p in *S. cerevisiae* (Tschoppe et al., 1986). Based on an assumption that both GAL2 and galLP encode the α-galactose transporter, expression of Gal2p in the mutarotase-deficient JA6 cells could lead to successful growth on galactose. This assumption was in conformity with the recent finding that partially purified *E. coli* galP protein prefers the α-anomer of $^{13}$C-glucose to its β-anomer in vitro binding judged by the solid-state nuclear magnetic resonance analysis (Patching et al., 2008). To test this hypothesis, we introduced an additional plasmid that expresses ScGal2p under the control of Gal4p into JA6gal10Δ bearing KlGAL10 or Klgal10mutΔ. The plasmid consists of one of the *K. lactis* centromeres, an autonomously replicating sequence, and URA3 as a selection marker in the backbone of an *E. coli* plasmid (Betina et al., 2001). In accordance with our hypothesis, the Klgal10mutΔ-carrying transformant was able to grow if ScGal2p was expressed concomitantly (Fig. 4). As the control, glucose was present in place of galactose in the medium, on which strains bearing either KlGAL10 or Klgal10mutΔ grew normally. In addition, we also tested KIHGT1, which is present only in some strains of *K. lactis*, but not in JA6 (Baruffini et al., 2006), for the ability to suppress the growth failure of mutarotase-deficient cells on galactose; the gene was previously identified as encoding a high-affinity glucose transporter (Billard et al., 1996), but has recently been demonstrated to encode the galactose transporter (Baruffini et al., 2006). Keep in mind that ScGal2p is also a high-affinity glucose transporter (Boles & Hollenberg, 1997), and that KIHgt1p allows a galactose transport-deficient *K. lactis* strain to grow on galactose to the same extent as ScGal2p (Baruffini et al., 2006). As can be clearly seen in the same figure, introduction of KIHGT1 did not restore the growth failure of the mutarotase-deficient yeast on galactose. Alternatively, one might argue that the difference observed between ScGal2p and KIHgt1p was due to the difference in the

**Table 2. UDP-glucose-4-epimerase activity of metal affinity-purified *Kluyveromyces* Gal10p or its mutarotase domain-deleted protein**

<table>
<thead>
<tr>
<th>Epimerase (U mg⁻¹ protein)</th>
<th>Mutarotase (U mg⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KlGAL10p</td>
<td>292</td>
</tr>
<tr>
<td>KlGAL10MutΔp</td>
<td>150</td>
</tr>
</tbody>
</table>

One unit of epimerase or mutarotase is defined as 1.0 μmol of UDP-glucose converted to UDP-galactose or 1.0 μmol of α-glucose converted to β-glucose per hour, respectively. Mutarotase is assayed enzymatically with β-glucose-specific dehydrogenase (Sigma/Aldrich Co.) according to the method described by Bouffard et al. (1994).

**Fig. 3.** Growth behavior of *Kluyveromyces lactis* strain JA6 gal10Δ gal80Δ carrying *TRP1*-selectable plasmids that express the indicated Gal10p on various sugars. Each plate was streaked with one-loop-full fresh cultures (1–2 × 10⁷ mL⁻¹) of the indicated strain grown in tryptophan-dropout synthetic medium containing 0.5% each of glycerol and sodium lactate. Streaks are a tryptophan-nonrequiring revertant of JA6gal10Δ gal80 without plasmid (none), JA6gal10Δ gal80Δ bearing a plasmid expressing the wild-type Gal10p (GA10), and two independent transformants with a plasmid expressing mutarotase-domain-deleted Gal10p (gal10mutΔ). Four plates of tryptophan-dropout synthetic agar contained 2% glucose, 0.25% galactose, 0.25% lactose, and 10 mM phenyl-β-D-galactose, which were incubated for 48 h at 30 °C and stored in a refrigerator before being photographed. Similar results were observed when the concentration of galactose or lactose was increased to 1.0% (data not shown).
such as Epimerase-deficient mutants of gram-negative bacteria, uptake was studied, which indicated that the kinetic parameters of galactose transport by these transporters is similar to each other. To eliminate such a possibility, the kinetics of galactose transport by these transporters (see Fig. S4).

Probable existence of another mutarotase gene in K. lactis

During the course of the present work, we found that JA6gal10Δ cells exhibit severe galactose sensitivity. The growth of these cells on nonfermentable carbon sources was completely arrested on addition of galactose to 0.1% (data not shown). The galactose sensitivity was suppressed if any epimerase-expressing plasmid was introduced into JA6gal10Δ cells (Fig. 5). It is known that deficiency of epimerase causes severe galactose sensitivity in many organisms including bacteria (Fukasawa & Nikaido, 1959a, b), yeast (Douglas & Hawthorne, 1964; Wasilenko & Fridovich-Keil, 2006), and humans (Holton et al., 1981). Epimerase-deficient mutants of gram-negative bacteria, such as E. coli or Salmonella, were killed due to cell lysis due to the presence of galactose in the medium (Fukasawa & Nikaido, 1959a). The galactose sensitivity of epimeraseless mutants is suppressed by an additional defect in galactokinase or any mutations that shut off other galactose-inducible genes, indicating that galactose must be metabolized to generate UDP-galactose in order for the galactose sensitivity to be seen (Fukasawa & Nikaido, 1959b; Nogi et al., 1977; Mumma et al., 2008). Thus, as can be seen in Fig. 5, galactose-resistant colonies outgrew epimeraseless colonies during the prolonged incubation due to second mutations, which would include any mutations that prohibit formation of UDP-galactose in the cell. The observed galactose sensitivity of gal10-deletion mutants of JA6 strongly suggests the existence of another mutarotase, which escaped our polarimetric assay due to the low sensitivity. In fact, we detected a low activity of mutarotase activity in a concentrated crude extract from JA6gal10Δ gal80Δ cells as well as in its precipitates with ammonium sulfate by the enzymatic assay.

Table 3. Ammonium sulfate fractionation of aldose-1-epimerase (mutarotase) from crude extracts of JA6gal10Δ gal80Δ

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Total protein (mg)</th>
<th>Total activity (U)</th>
<th>Specific activity (U mg⁻¹)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude extract</td>
<td>710</td>
<td>10 800</td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>0–55% precipitates</td>
<td>47</td>
<td>5580</td>
<td>119</td>
<td>52</td>
</tr>
<tr>
<td>55–80% precipitates</td>
<td>25</td>
<td>210</td>
<td>8.4</td>
<td>2</td>
</tr>
</tbody>
</table>

One unit of enzyme was defined as the activity of 1.0 μmol of α-glucose converted to β-glucose per hour. The assay was carried out using β-glucose dehydrogenase (Sigma/Aldrich Co.) according to the method described by Bouffard et al. (1994). The experiment on which these values are based is shown in Fig. S5.

Fig. 4. Suppression of growth failure of mutarotase-deficient Kluyveromyces lactis cells by ScGal2p. Cells of JA6gal10Δ carrying a set of the indicated plasmids were grown to the late-log phase (1–2 × 10⁶ cells mL⁻¹) in tryptophan- and uracil-dropout synthetic minimal medium containing 0.5% each of glycerol and sodium lactate. The cells were washed once with an equal volume of water, and resuspended in one-tenth the volume of the same medium without a carbon source. One-loop full of the indicated cells was streaked on the synthetic minimal medium containing 1% each of galactose or glucose. The plates were incubated at 30 °C for 72 h. The left and the right halves of each plate were streaked with cells expressing the wild-type Gal10p (GAL10) and those expressing mutarotase-domain-deleted Gal10p (gal10mutΔ), respectively. Each streak contained the cells expressing the additional plasmid as indicated outside the plate. Vector represents KCp491, a vacant vector.

Fig. 5. Galactose sensitivity of Kluyveromyces lactis strains expressing Gal10p from various yeasts. Cells of strain JA6gal10Δ gal80Δ carrying various plasmids were grown to 4–5 × 10⁶ mL⁻¹ in tryptophan-dropout synthetic medium containing 1% each of sodium lactate and glycerol. The cells were streaked on a pair of agar plates of the same medium with or without galactose (0.1%). The plasmids were expressing Schizosaccharomyces pombe Gal10p (SpGal10), Saccharomyces cerevisiae Gal10p (ScGal10), Kluyveromyces lactis Gal10p (KlGal10), and mutarotase-domain deletion of K. lactis Gal10p (Klgal10mutΔ), respectively. None represents a tryptophan-nonrequiring revertant from JA6gal10Δ gal80Δ without a plasmid. Note that galactose-resistant colonies are overgrowing on the streak.
(Table 3 and Fig. S5). Trials to purify the mutarotase in question have so far been unsuccessful, not only due to the low abundance in the starting materials but also due to the instability of the enzyme. We conclude, therefore, that the level of the second mutarotase is not high enough to support the growth of the gal10 deletion on galactose, but to cause the galactose sensitivity.

**Discussion**

It has long been known that there exist disaccharide transporters capable of distinguishing the α/β linkage in the sugar, for example the β-galactoside transporter (permease) for lactose, that is, β-D-galactopyranosyl β-D-glucopyranose, or the α-galactoside transporter for melibiose, that is, α-D-galactopyranosyl α-D-glucopyranose (Kennedy, 1970). For monosaccharide transporters, however, little attention has been paid to the recognition specificity of the α/β-anomer of substrates. The present results strongly suggest that Gal2p of *S. cerevisiae* or Hgt1p of *K. lactis* are specific for α-galactose or β-galactose, respectively. *Kluyveromyces lactis* possesses the ability to use lactose, for which the necessary enzymes are under the control of the Gal4p/Gal80p system as well (see Schaffrath & Breunig, 2000). By contrast, some strains of *S. cerevisiae* are able to use melibiose by the function of the α-galactosidase transporter and α-galactosidase encoded by *MEL1*, which is also controlled by the combination of Gal4p/Gal80 (see Bhat & Murthy, 2001). Sugar-metabolizing systems would have evolved depending on the available sugars in a given ecological niche at an evolutionary stage. Original habitats of *S. cerevisiae* are bark and sap exudates of oak trees, which are rich in saccharose and less in raffinose (Phaff, 1986). By contrast, some laboratory strains of *K. lactis* were originally isolated from milk-derived products, in which lactose is the major carbon source (see Schaffrath & Breunig, 2000). A question then arises: is the *K. lactis* ORF, YMR099c, is known in *S. cerevisiae* to code for hexose-6-phosphate mutarotase, which does not rotate hexoses at all, however (Graille et al., 2006). A highly conserved homolog of YMR099c is identified in *K. lactis*, which is referred to as KLLA0C1430p without biochemical information (http://cbi.labri.fr/Genolevures/). It remains to be determined whether or not this ORF is responsible for the observed activity of mutarotase in *JAgal10D*.

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**References**


**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Construction of Gal4p-dependent expression plasmid for Gal10p in *Kluyveromyces lactis*.

**Fig. S2.** Restriction map of *Kluyveromyces lactis* GAL10 and its deletions.

**Fig. S3.** Linearity of polarimetric assay of mutarotase.
Fig. S4. Lineweaver–Burk plot showing galactose uptake in the strain 2359lac12Δ hgt1Δ (Baruffini et al., 2006) transformed with either KCp491-HGT1 (black line and squares) or KCp491-GAL2 (grey line and squares).

Fig. S5. GAL10-independent mutarotase activity in concentrated crude extracts and ammonium sulfate precipitates from JAgal10Δgal80Δ.

Table S1. Molecular size and characteristics of uridine diphosphate glucose 4-epimerase purified from various sources

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