Exceptional hexose-fermenting ability of the xylitol-producing yeast Candida guilliermondii FTI 20037

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Exceptional hexose-fermenting ability of the xylitol-producing yeast Candida guilliermondii FTI 20037

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The yeast Candida guilliermondii FTI 20037 is well-known for its ability to produce xylitol from xylose. Recently, this strain was found to produce greater than 5% (w/v) ethanol from glucose. This level of ethanol is typically not exceeded by wild-type strains of other native pentose-fermenting yeasts. This prompted the current study to examine the ability of C. guilliermondii FTI 20037 to utilize and ferment high concentrations of each of the hexoses commonly found in lignocellulosic hydrolysates. In defined media, FTI 20037 fermented 14.4%–25.9% (w/v) of glucose, mannose or galactose individually to ethanol in concentrations ranging from 6% to 9.3% (w/v). Fermentation was completed within 36 h (for glucose) to 100 h (for galactose). In 25.9% (w/v) glucose, FTI 20037 produced 9.3% (w/v) ethanol within 40 h. FTI 20037 produced xylitol exclusively when xylose was given as the sole carbon source. The strain utilized arabino-bose poorly. Under the same fermentation conditions, an industrial Saccharomyces cerevisiae strain produced slightly higher levels of ethanol [9.9% (w/v)] from 25.0% (w/v) glucose. Another pentose-fermenting yeast Pachysolen tannophilus also fermented high concentrations of glucose and mannose to produce relatively high peak ethanol concentrations; however, this yeast required considerably longer to completely consume these hexoses. The ability of FTI 20037 to produce high level of ethanol rapidly from glucose is remarkable. To our knowledge, this is the first known instance of a non-modified native xylose-fermenting yeast strain able to produce such high levels of ethanol from glucose as rapidly as S. cerevisiae in a defined medium.

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Key words: Ethanol; Fermentation; Hexose; Xylitol; Xylose; Yeast

Xylose is an aldopentose that occurs primarily in polymer form as xylan in the hemicellulose portion of plant cell walls. Next to glucose, xylose is the second most abundant renewable sugar in nature, forming up to 25% of the total dry weight of some forestry and agricultural residues. The efficient utilization of pentose sugars is therefore, important in the overall bioconversion of plant biomass for the production of liquid fuels and chemicals. However, xylose is not as readily fermented as glucose by yeasts. Native strains of the fermentative yeast Saccharomyces cerevisiae are unable to utilize xylose as a sole carbon source (1). In this connection, the discovery of xylose-fermenting yeasts in the early 1980s (2–4) was considered a milestone as it lends hope that xylose can also be fermented (5). Since this initial discovery, numerous xylose-fermenting yeasts have been reported. Among the better xylose-fermenting yeasts, three categories are known: (i) those such as Scheffersomyces (Candida) shehatae and Scheffersomyces (Pichia) stipitis that ferment xylose almost exclusively to ethanol (5); (ii) those such as Candida guilliermondii (6,7) and some Candida tropicalis strains (8) that produce mostly xylitol from xylose; and (iii) Pachysolen tannophilus that produces a mixture of ethanol and xylitol (7). The reason(s) for the preferential production of ethanol or xylitol by these yeasts is not known.

C. guilliermondii FTI 20037 is a naturally occurring yeast strain that was maintained at the culture collection of the Foundation for Industrial Technology (FTI), São Paulo, Brazil. The strain was first deposited at the National Research Council Canada (NRCC) Culture Collection in 1985 and designated as NRC 5578. In the early 1990s, one of us (H. Lee) deposited this strain in the American Type Culture Collection (ATCC) where it is currently designated as C. guilliermondii ATCC 201935 and is available to researchers worldwide.

In a yeast screening study carried out at NRCC, strain FTI 20037 was found to be an excellent xylitol producer from xylose (6). Since this discovery in the late 1980s, many papers have been published on various aspects of xylose-to-xylitol fermentation by FTI 20037 in both defined media and xylose-rich lignocellulosic hydrolysates. In some studies, researchers have noted that low amounts of ethanol were produced by C. guilliermondii, likely from the small amount of hexoses present (9–12). However, given the almost exclusive focus on xylitol production by C. guilliermondii FTI 20037, its ability to produce a small amount of ethanol was not considered significant and typically not investigated further.

Recently, we assessed the fermentative performance of C. guilliermondii FTI 20037 in several lignocellulosic hydrolysates.

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One of the hydrolysates contained considerably higher hexose content than pentoses. Interestingly, greater than 5% (w/v) ethanol was produced rapidly by FTI 20037 in this hydrolysate (data not shown). This level is considered to be near the high end of peak ethanol concentrations reported to be produced by some well-known native pentose-fermenting yeasts such as *S. stipitis*, *S. shehatae* (13,14) and *P. tannophilus* (15). This led to a series of experiments described below to specifically examine the ability of

FIG. 1. Sugar fermentation by *C. guilliermondii* FTI 20037. (A) Glucose fermentation. The initial concentrations were 15.3% (round dotted line), 20.1% (dash line) and 25.9% (w/v) (solid line). (B) Mannose fermentation. The initial concentrations were 15.6% (dash line) and 20.3% (w/v) (solid line). (C) Galactose fermentation. The initial concentrations were 14.4% (dash line) and 19.6% (w/v) (solid line). (D) Xylose fermentation. The initial concentration was 10.5% (w/v). Values shown are means ± SEM of three independent experiments. Crosses indicate sugar utilization trend; closed circles indicate ethanol production.

FIG. 2. Sugar fermentation by *S. cerevisiae* T2. (A) Glucose fermentation. The initial concentrations were 14.4% (round dotted line), 18.3% (dash line) and 25.0% (w/v) (solid line). (B) Mannose fermentation. The initial concentrations were 15.4% (dash line) and 20.6% (w/v) (solid line). (C) Galactose fermentation. The initial concentrations were 17.7% (dash line) and 22.6% (w/v) (solid line). Values shown are means ± SEM of three independent experiments. Crosses indicate sugar utilization trend; closed circles indicate ethanol production.

One of the hydrolysates contained considerably higher hexose content than pentoses. Interestingly, greater than 5% (w/v) ethanol was produced rapidly by FTI 20037 in this hydrolysate (data not shown). This level is considered to be near the high end of peak ethanol concentrations reported to be produced by some well-known native pentose-fermenting yeasts such as *S. stipitis*, *S. shehatae* (13,14) and *P. tannophilus* (15). This led to a series of experiments described below to specifically examine the ability of experiments. Crosses indicate sugar utilization trend; closed circles indicate ethanol production and open triangles indicate xylitol production (dotted line).
FTI 20037 to ferment high concentrations of three hexoses (glucose, mannose and galactose) in defined liquid medium. These three hexoses are commonly found in lignocellulosic hydrolysates. Our results established the remarkable ability of strain FTI 20037 to produce high concentrations of ethanol from each of these hexose sugars. S. cerevisiae strain T2, used for fermentation of spent sulfite liquor (SSL) at the Tembec Alcohol Plant, as well as three other native pentose-fermenting yeasts, P. tannophilus NRRL Y-2460, S. shehatae NRRL Y-2886 and S. stipitis NRRL Y-7124, were tested under the same conditions for comparison.

FIG. 3. Sugar fermentation by P. tannophilus NRRL Y-2460. (A) Glucose fermentation. The initial concentrations were 15.9% (round dotted line), 20.5% (dash line) and 26.6% (w/v) (solid line). (B) Mannose fermentation. The initial concentrations were 15.0% (dash line) and 20.5% (w/v) (solid line). (C) Galactose fermentation. The initial concentrations were 15.7% (dash line) and 20.8% (w/v) (solid line). (D) Xylose fermentation. The initial concentration was 10.3% (w/v). Values shown are mean ± SEM of three independent experiments. Crosses indicate sugar utilization trend; closed circles indicate ethanol production and open triangles indicate xylitol production (dotted line).

MATERIALS AND METHODS

Microorganisms and media C. guilliermondii FTI 20037 (ATCC 201935, NRC 5578), S. shehatae NRRL Y-2886 (ATCC 34887; NRRL Y-12858) and S. stipitis NRRL Y-7124 (NRC 2548; ATCC 58376) were obtained from the NRCC Culture Collection (Ottawa, ON, Canada). P. tannophilus NRRL Y-2460 (ATCC 32691) was kindly provided by Cletus Kurtzman (USDA, Peoria, IL, USA). A SSL-adapted S. cerevisiae T2 strain used in SSL fermentation at the Tembec Alcohol Plant (16), was kindly provided by Juraj Strmenc (formerly of Tembec, Témiscaming, QC, Canada). All sugars were purchased from Sigma–Aldrich (Oakville, ON, Canada). All culture media components were purchased from Thermo Fisher Scientific Company (Ottawa, ON, Canada).

Culture maintenance and inoculum preparation Yeast strains were maintained individually on YEPD (yeast extract, peptone, dextrose) agar plates at 4°C and subcultured periodically. For inoculum preparation, a loopful of cells from a distinct colony grown on the YEPD plate was used to inoculate 100 mL of defined media containing 0.67% (w/v) yeast nitrogen base (YNB) without amino acid or ammonium sulphate supplemented with 0.225% (w/v) urea and 2% (w/v) of either xylose for C. guilliermondii and other pentose-fermenting yeasts or glucose for S. cerevisiae in 250-mL Erlenmeyer flasks. The cultures were incubated at 28 ± 2°C for 48 h with gyration shaking at 180 rpm as described by Bajwa et al. (17). The OD600 values of these cultures, measured using an Ultraspec 3100 Pro UV/Visible spectrophotometer (Biochrom Ltd., UK), were about 7–8 (1.7–1.9 g dry cell weight/L).

Fermentation experiments The methods used for fermentation studies were as described by Bajwa et al. (17,18). Briefly, cells from the 48-h inoculum culture were centrifuged at 3700 g for 15 min at 21 ± 2°C in a Sorvall centrifuge (Thermo Fisher Scientific). The cell pellet was washed with sterile deionized water and re-suspended in 100 mL of defined media supplemented with varying concentrations of one of the test sugars (glucose, mannose, galactose, arabinose or xylose) in a 250-mL Erlenmeyer flask. The sugar concentrations tested ranged from 10% to 25.9% (w/v). Xylose fermentation was performed as a control to confirm the ability of strain FTI 20037 and other pentose-fermenting yeasts to convert xylose to ethanol and/or xylitol. The cultures were incubated at 28 ± 2°C for 48 h with gyration shaking at 180 rpm as described by Bajwa et al. (17). The OD600 values of these cultures, measured using an Ultraspec 3100 Pro UV/Visible spectrophotometer (Biochrom Ltd., UK), were about 7–8 (1.7–1.9 g dry cell weight/L).

Analytical methods Sugar and ethanol concentrations were analyzed using an Agilent-1200 HPLC system equipped with a BioRad Aminex HPX-87H (300 × 7.8 mm) column with 5 mM sulphuric acid as the mobile phase running at 0.6 mL/min at 40°C as previously described (19). Sugar samples were diluted before HPLC analysis when the initial sugar concentration was above 10% (w/v).

RESULTS AND DISCUSSION

Native xylose-fermenting yeasts can ferment the dominant hexose and pentose sugars in lignocellulosic hydrolysates individually to ethanol. However, the rate and yield of ethanol produced from pentoses are considerably lower compared to hexose fermentation by native strains of the fermentative yeast S. cerevisiae (5). Moreover, some well-known native xylose-fermenting yeasts exhibit low ethanol tolerance (19) which can limit the efficient fermentation of sugars in lignocellulosic hydrolysates.

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hydrolysates to ethanol. As an example, *S. stipitis* NRRL Y-7124 was reported to produce a peak ethanol concentration of 5.7% (w/v) from 20% (w/v) xylose in a yeast extract-containing medium, while several other pentose-fermenting yeast strains such as *S. shehatae* NRRL Y-12856 and NRRL Y-12857 and *S. stipitis* NRRL Y-11545 could not completely consume the sugar at this high initial concentration and produced lower peak ethanol levels of 1.6%—2.6% (w/v) (20). Furthermore, fermentation by some strains of *S. shehatae* and *S. stipitis* was inhibited by concentrations of ethanol as low as 4% (w/v) (14). *P. tannophilus* NRRL Y-2460 was reported to produce 5.5% (w/v) ethanol from 12% (w/v) glucose (15). In another study, the peak ethanol concentrations produced by *Spathaspora passalidarum* from 10% (w/v) xylose or 10% (w/v) glucose in a defined medium were reported to be 3.74% and 3.14% (w/v), respectively (21). Thus, it is generally thought that the maximum concentrations of ethanol produced from any sugars by native pentose-fermenting yeasts do not exceed 6% (w/v).

**Glucose fermentation** *C. guilliermondii* FTI 20037 is known to produce high levels of xylitol from xylose. To our knowledge, its ability to ferment high concentrations of hexoses has not been examined. In this study, we specifically examined the ability of FTI 20037 to ferment high concentrations of three hexoses (glucose, mannose and galactose) in defined liquid medium.

In defined medium, glucose was readily consumed by FTI 20037 and fermented to high levels of ethanol. The strain completely consumed 15.3% (w/v) glucose in 21 h, producing a peak ethanol concentration of 5.3% (w/v) (Fig. 1A). Higher concentrations of glucose [20.1% and 25.9% (w/v)] were also completely utilized in 27 and 36 h, respectively, and high maximum ethanol concentrations [7.3% and 9.3% (w/v), respectively] were produced. The yields were 0.35, 0.36 and 0.36 g ethanol/g glucose consumed for 15.3%, 20.1% and 25.9% (w/v) glucose, respectively.

Interestingly, the remarkable glucose-fermenting ability of *C. guilliermondii* was comparable to that of *S. cerevisiae* T2, with respect to ethanol production from the highest glucose concentrations tested in this study. Glucose at an initial concentration of 24.9% (w/v) was completely utilized by *S. cerevisiae* T2 within 24 h (Fig. 2A). A maximum ethanol concentration of 9.9% (w/v) was obtained from 24.9% (w/v) of glucose; and this corresponded to a yield of 0.40 g/g.

Three other well-known pentose-fermenting yeasts, *P. tannophilus* NRRL Y-2460, *S. shehatae* NRC 2886 and *S. stipitis* NRRL Y-7124, were also tested for fermentation of high concentrations of glucose under similar conditions. *P. tannophilus* completely consumed 26.6% (w/v) glucose in 120 h, producing a peak ethanol concentration of 8.9% (w/v) (Fig. 3A). However, *S. shehatae* (Fig. 4A) and *S. stipitis* (Fig. 5A) were unable to completely utilize 25.6% and 25.7% (w/v) glucose, respectively, over 144 h of incubation. The peak ethanol concentrations produced by the two yeasts were less than 6% (w/v). Amongst the pentose-fermenting yeasts tested, *C. guilliermondii* was the best at fermenting glucose based on the peak ethanol concentration [9.3% (w/v)] achieved, shortest time required for glucose utilization (36 h) and complete consumption of glucose at the highest concentration [26.9% (w/v)] tested. The glucose fermentation performance parameters for these yeasts are summarized in Table 1 for comparison.

**Mannose fermentation** Mannose is another hexose sugar found in lignocellulosic biomass and may represent up to 15% of the
total sugars in some softwood hydrolysates (22). In defined medium, mannose fermentation took slightly longer than glucose by all the yeast strains tested. Mannose at 15.6% (w/v) was completely utilized by *C. guilliermondii* FTI 20037 in 21 h (Fig. 1B). The maximum ethanol concentration produced was 5.3% (w/v), with a corresponding yield of 0.35 g/g. *C. guilliermondii* utilized 20.3% (w/v) mannose in 54 h, producing a peak ethanol concentration of 6.7% (w/v) with an ethanol yield of 0.33 g/g (Table 2).

Mannose at 15.4% and 20.6% (w/v) was completely utilized within 18 and 24 h, respectively, by *S. cerevisiae* T2 (Fig. 2B). The maximum ethanol concentrations produced were 5.9% and 7.7% (w/v), respectively. These corresponded to an ethanol yield of 0.38 g/g at both mannose concentrations. The results showed that *S. cerevisiae* fermented mannose slightly better than *C. guilliermondii* at the lower concentration, but considerably faster at the higher concentration.

Interestingly, *P. tannophilus* fermented 15.0% and 20.5% (w/v) mannose effectively to produce peak ethanol concentrations of 5.8% and 7.8% (w/v), respectively (Fig. 3B), with corresponding ethanol yields of 0.37 and 0.38 g/g, respectively. These levels are similar to those produced by *S. cerevisiae* and better than those produced by *C. guilliermondii*. However, *P. tannophilus* took 78 h to completely consume 20.5% (w/v) mannose; this time frame was considerably longer than either *S. cerevisiae* or *C. guilliermondii*.

*S. shehatae* (Fig. 4B) and *S. stipitis* (Fig. 5B) completely utilized 15.2% and 14.9% (w/v) mannose, respectively, within 72 h. However, these yeasts were unable to completely utilize higher concentrations of mannose. From the initial 21.1% and 19.8% (w/v) mannose, 1.6% and 4.6% (w/v) mannose remained unused by *S. shehatae* and *S. stipitis*, respectively, after 144 h of incubation. The peak ethanol concentrations produced by the two yeasts were under 5% (w/v) for all mannose concentrations tested.
Table 2. Fermentation performance parameters of selected yeasts in high concentrations of mannose in defined media.

<table>
<thead>
<tr>
<th>Yeast</th>
<th>[Mannose] (g% w/v)</th>
<th>[EtOH]max (g% w/v)</th>
<th>YE (g/g)</th>
<th>YE2 (g/g)</th>
<th>t (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. guilliermondii</td>
<td>15.6 ± 0.1</td>
<td>5.3 ± 0.1</td>
<td>0.35 (69%)</td>
<td>0.34</td>
<td>24 ± 0</td>
</tr>
<tr>
<td>P. tannophilus</td>
<td>20.3 ± 0.1</td>
<td>6.7 ± 0.0</td>
<td>0.33 (65%)</td>
<td>0.33</td>
<td>78 ± 0</td>
</tr>
<tr>
<td>S. shehatae</td>
<td>15.0 ± 0.1</td>
<td>5.8 ± 0.2</td>
<td>0.39 (76%)</td>
<td>0.39</td>
<td>48 ± 3</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>20.5 ± 0.2</td>
<td>7.8 ± 0.2</td>
<td>0.38 (75%)</td>
<td>0.38</td>
<td>78 ± 5</td>
</tr>
<tr>
<td>S. stipitis</td>
<td>15.2 ± 0.2</td>
<td>4.3 ± 0.1</td>
<td>0.28 (55%)</td>
<td>0.28</td>
<td>60 ± 7</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>14.9 ± 0.3</td>
<td>4.9 ± 0.1</td>
<td>0.33 (65%)</td>
<td>0.33</td>
<td>72 ± 3</td>
</tr>
<tr>
<td></td>
<td>19.8 ± 0.8</td>
<td>4.3 ± 0.1</td>
<td>0.28 (55%)</td>
<td>0.22</td>
<td>INC</td>
</tr>
<tr>
<td></td>
<td>20.6 ± 0.0</td>
<td>7.7 ± 0.0</td>
<td>0.38 (75%)</td>
<td>0.38</td>
<td>24 ± 6</td>
</tr>
</tbody>
</table>

Table 3. Fermentation performance parameters of selected yeasts in high concentrations of galactose in defined media.

<table>
<thead>
<tr>
<th>Yeast</th>
<th>[Galactose] (g% w/v)</th>
<th>[EtOH]max (g% w/v)</th>
<th>YE (g/g)</th>
<th>YE2 (g/g)</th>
<th>t (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. guilliermondii</td>
<td>14.4 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>0.32 (63%)</td>
<td>0.22</td>
<td>72 ± 3</td>
</tr>
<tr>
<td>P. tannophilus</td>
<td>19.6 ± 0.1</td>
<td>4.4 ± 0.1</td>
<td>0.27 (53%)</td>
<td>0.22</td>
<td>INC</td>
</tr>
<tr>
<td>S. shehatae</td>
<td>15.7 ± 0.3</td>
<td>0.2 ± 0.0</td>
<td>0.01 (2%)</td>
<td>0.01</td>
<td>INC</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>20.8 ± 0.8</td>
<td>0.1 ± 0.0</td>
<td>0.01 (2%)</td>
<td>0.01</td>
<td>INC</td>
</tr>
<tr>
<td>S. stipitis</td>
<td>15.0 ± 0.1</td>
<td>3.4 ± 0.3</td>
<td>0.23 (45%)</td>
<td>0.23</td>
<td>96 ± 14</td>
</tr>
<tr>
<td>S. cerevisiae</td>
<td>20.3 ± 1.6</td>
<td>3.2 ± 0.2</td>
<td>0.18 (35%)</td>
<td>0.16</td>
<td>INC</td>
</tr>
<tr>
<td></td>
<td>15.1 ± 0.3</td>
<td>4.7 ± 0.1</td>
<td>0.31 (61%)</td>
<td>0.31</td>
<td>78 ± 0</td>
</tr>
<tr>
<td></td>
<td>20.2 ± 0.6</td>
<td>3.9 ± 0.2</td>
<td>0.29 (57%)</td>
<td>0.19</td>
<td>INC</td>
</tr>
<tr>
<td></td>
<td>17.7 ± 1.6</td>
<td>5.3 ± 0.1</td>
<td>0.30 (59%)</td>
<td>0.30</td>
<td>60 ± 5</td>
</tr>
<tr>
<td></td>
<td>22.6 ± 1.6</td>
<td>7.3 ± 0.1</td>
<td>0.32 (63%)</td>
<td>0.32</td>
<td>108 ± 6</td>
</tr>
</tbody>
</table>

All abbreviations are as in Table 1.

Galactose fermentation

Compared to glucose and mannose, galactose constitutes a smaller proportion of the overall sugar composition ranging from 0% to 4% in agricultural and softwood biomass and less than 2% of the total carbohydrate found in hardwood (23). Galactose was not as readily or well fermented as glucose or mannose by the yeasts tested in this study. This was seen by the longer times required by the yeasts to consume this sugar and the lower ethanol yields produced.

Galactose at 14.4% (w/v) was completely consumed in 72 h by C. guilliermondii FTI 20037 to produce a peak ethanol concentration of 4.7% (w/v) (yield = 0.32 g/g) (Fig. 1C). However, C. guilliermondii could not completely utilize 19.6% (w/v) galactose over 144 h of incubation. C. guilliermondii consumed approximately 84% of 19.6% (w/v) galactose to produce a peak ethanol concentration of 4.4% (w/v), representing a yield of 0.27 g/g (Table 3).

S. cerevisiae T2 was slightly better at fermenting galactose, requiring 60 and 108 h to completely utilize 17.7% and 22.6% (w/v) galactose, respectively (Fig. 2C). The maximum ethanol concentrations produced were 5.3% and 7.3% (w/v), respectively, and these corresponded to ethanol yields of 0.30 g and 0.32 g/g, respectively (Table 3).

Similar to C. guilliermondii, 20% (w/v) galactose was not completely consumed by S. stipitis and S. shehatae (Figs. 4C and 5C). The peak ethanol concentrations produced from galactose were 3.2% and 3.9% (w/v) by S. shehatae and S. stipitis, respectively. Of particular note was the very slow galactose utilization by P. tannophilus. This yeast consumed about 59% and 55% of the initial 15.7% and 20.8% (w/v) galactose, respectively, after 144 h of incubation and produced negligible amount of ethanol [about 0.1% (w/v)] (Fig. 3C).

Overall, C. guilliermondii FTI 20037 exhibited excellent glucose-fermenting ability which was the best amongst the pentose-fermenting yeasts and comparable to that of S. cerevisiae T2. However, mannose and galactose were not fermented as efficiently by C. guilliermondii as S. cerevisiae. Of the other pentose-fermenting yeasts tested, P. tannophilus also fermented glucose and mannose well to yield high peak ethanol concentrations which rivalled those produced by C. guilliermondii and S. cerevisiae. However, this strain took considerably longer to ferment these two hexoses. Moreover, it utilized galactose poorly and did not produce any ethanol from this sugar. S. shehatae and S. stipitis were relatively poor at fermenting the hexose sugars.

**Pentose fermentation**

Xylose fermentation was conducted as control experiments to ascertain the ability of pentose-fermenting yeasts to produce ethanol and/or xylitol from this sugar. C. guilliermondii FTI 20037 efficiently fermented 10.5% (w/v) xylose to produce 6.44% (w/v) xylitol in 72 h (Fig. 1D). Arabinose was poorly utilized by C. guilliermondii FTI 20037, and no ethanol was produced during 114 h of fermentation (data not shown). The other pentose-fermenting yeasts produced mostly ethanol [S. shehatae (Fig. 4D) and S. stipitis (Fig. 5D)] or both ethanol and xylitol [P. tannophilus (Fig. 3D)] from xylose. This is consistent with their known xylose-fermenting abilities.

As expected, S. cerevisiae was unable to utilize xylose or arabinose over 144 h of incubation and neither ethanol nor xylitol was produced (data not shown).

In summary, C. guilliermondii FTI 20037 was found to ferment high concentrations of glucose rapidly in a defined medium to yield very high peak ethanol concentrations. To our knowledge, the uncharacteristically high peak ethanol concentrations achieved (9.3%, w/v) has not been seen in any other unmodified native pentose-fermenting yeasts. This remarkable glucose-fermenting ability was similar to that of an industrial S. cerevisiae T2 strain. C. guilliermondii also fermented mannose and galactose to ethanol, although less efficiently than glucose. P. tannophilus fermented high concentrations of glucose to ethanol; however this yeast took considerably longer compared to C. guilliermondii or S. cerevisiae. In comparison, S. shehatae and S. stipitis were relatively poor at hexose fermentation. The results are of considerable interest in that C. guilliermondii is known to produce high levels of xylitol from xylose, but its exceptional hexose-fermenting ability has not been reported to date.

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**References**


