STRAIN ISOLATION AND STUDY ON PROCESS PARAMETERS FOR XYLOSE-TO-XYLITOL BIOCONVERSION

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ABSTRACT
A xylitol producing yeast was isolated from soil and identified as Candida tropicalis W103 by 18S rDNA gene sequence analysis and physiological characteristics. The optimal fermentation conditions for Candida tropicalis W103 were: 35°C, pH 4.5 and 120 g l⁻¹ initial xylose concentration. However, the maximum yield and productivity of xylitol were obtained at K_a 16.5 h⁻¹ and 18.3 h⁻¹, respectively. A two-stage aeration strategy (0-24 h, K_a 18.3 h⁻¹; after 24 h, shift K_a to 16.5 min⁻¹) was applied in the fermentation to get higher xylitol yield and productivity. After 60 h in batch fermentations, both the xylitol concentration and xylose consumption reached the maximum, obtaining 87.1 g l⁻¹ of xylitol with 1.45 g l⁻¹ h⁻¹ productivity and 0.72 g g⁻¹ xylose yield. Fed-batch fermentation with 120 g l⁻¹ initial xylose and regulating xylose concentration to 40-55 g l⁻¹ during 24-96 h was performed to reach a higher productivity of 1.82 g l⁻¹ h⁻¹, and xylitol concentration of 218.7 g l⁻¹.

Keywords: Candida tropicalis, fermentation, isolation, xylitol, xylose
Abbreviations: K_a: oxygen transfer coefficient; XR: xylose reductase; XD: xylitol dehydrogenase


Introduction
Xylitol is a natural polyol with particular physico-chemical properties, which permit its use either in foods like chewing gum, bakery and chocolate as a sweeter with anticariogenic properties, or in medicines as a sugar substitute for people with erythrocytic glucose-6-phosphate dehydrogenase deficiency (1, 8, 10, 27). Traditional chemical conversion of xylene into xylitol is rather difficult, has a low product yield and the product recovery requires various expensive operations for separation. The microbial conversion of xylose to xylitol is particularly attractive because the process does not need toxic catalyst and is relatively easy and environmentally safe (21, 26).

Microbial species are of importance for xylitol production. Xylose can be converted to xylitol by several microorganisms including Pichia (17), Debaryomyces (6), Pachysolen (4), recombinant Escherichia coli (25), recombinant Saccharomyces cerevisiae (18) and also Candida (5, 13, 20). Among these, Candida ferments xylose to xylitol with high yield and productivity (12, 14, 16, 23). Xylitol production involves complicated metabolic regulation including xylose transport, key enzymes formation, and cofactor regeneration (9). To achieve efficient xylitol production from xylose, high concentration, rapid production rate and high yield are desirable. Screening of high efficient xylitol producing microorganisms is very important for industrial application of xylose-to-xylitol microbial conversion. To enhance the feasibility of the xylitol biotechnological process, it is necessary to optimize several process parameters, such as temperature, pH, and oxygen transfer coefficient (K_a), in xylitol production. Despite many papers published in recent years, many published data about K_a are controversial. Some studies reported that optimum K_a values for xylitol production were close to 100 h⁻¹ (1), whereas others described only 47 h⁻¹ K_a values for optimum (28). Furthermore, few studies were carried out to investigate effect of temperature on cell growth and xylitol production.

The present study screened a high xylitol-production yeast, identified as C. tropicalis W103 by 18S rDNA gene sequence analysis and physiological characteristics. In addition, some of the parameters which influence the xylitol production by the selected C. tropicalis strain are investigated. A two-stage aeration fed-batch culture was developed to improve the final xylitol concentration and productivity.

Materials and Methods
Enrichment and isolation of the strains
The soil samples were collected at Harbin in Heilongjiang province in 2007. The basic medium used for enrichment, isolation and cultivation of xylitol-producing strains was prepared as follows: 5 g l⁻¹ KH₂PO₄, 2 g l⁻¹ (NH₄)₂SO₄, 4 g l⁻¹ yeast extract, 0.5 g l⁻¹ MgSO₄·7H₂O, 20 g l⁻¹ xylose. The initial pH was adjusted by 1M NaOH to 5.5. Xylose in medium was used as substrate to favor the growth of xylose-utilizing microorganisms, because xylose was the only compound that could be used as substrate and converted to xylitol by nature microorganisms. One gram of soil sample was inoculated to a 250 ml flask with 50 ml enrichment medium and then incubated at 35°C and 150 rpm in a rotary shaker. After 24 h of incubation, 1 ml of the culture broth was transferred to another flask with the same enrichment medium and incubated for 24 h. After 3 rounds of such enrichment operation, 1 ml of

1606
Batch flask fermentation

Screened strains were firstly cultured in flasks to identify if xylitol was a major metabolic product of the strains. The medium used for batch flask fermentation contained the following components: 60 g l\(^{-1}\) xylose, 5 g l\(^{-1}\) \((NH_4)_2SO_4\), 2 g l\(^{-1}\) KH\(_2PO_4\), 5 g l\(^{-1}\) yeast extract, 1 g l\(^{-1}\) peptone, 0.5 g l\(^{-1}\) MgSO\(_4\)·7H\(_2\)O. One loop of cells was transferred into a 250 ml flask containing 100 ml medium and 0.5 g CaCO\(_3\) and then incubated in a rotary shaker at 35°C and 150 rpm with pH that was not controlled for 2 days.

Batch and fed-batch fermentation in bioreactor

The strain W103 which showed the highest xylitol producing potential was selected for further fermentation investigation. The composition of preculture medium was the following: 2 g l\(^{-1}\) KH\(_2PO_4\), 5 g l\(^{-1}\) \((NH_4)_2SO_4\), 4 g l\(^{-1}\) yeast extract, 0.5 g l\(^{-1}\) MgSO\(_4\)·7H\(_2\)O, 20 g l\(^{-1}\) xylose. The selected colonies were transferred into a 250 ml flask with 50 ml preculture medium and incubated in a rotary shaker at 35°C and 150 rpm for 14 h. The medium used for batch fermentation contained the following components: 60-160 g l\(^{-1}\) xylose, 2 g l\(^{-1}\) KH\(_2PO_4\), 5 g l\(^{-1}\) \((NH_4)_2SO_4\), 0.5 g l\(^{-1}\) MgSO\(_4\)·7H\(_2\)O, 1 g l\(^{-1}\) peptone, 5 g l\(^{-1}\) yeast extract. The biomass concentration in bioreactor at the beginning of the fermentation ranged from 0.29 g l\(^{-1}\) to 0.31 g l\(^{-1}\). The batch or fed-batch cultivations were performed in four 1-liter stirred-vessel bioreactors (Biostat Q1000, B.Braun, Germany) containing 750 ml fermentation medium under 0.3-0.8 vvm air flow. All fermentation experiments were carried out at 35°C and 500 rpm and the broth was sampled every 6-12 h to monitor the xylose and xylitol concentrations. In fed-batch fermentation, after the xylose content in the medium was below 50 g l\(^{-1}\), a solution containing 500 g l\(^{-1}\) xylose was continuously fed to the bioreactor with a peristaltic pump. The data are average of triplicate experiments.

Enzymatic assays

Cell extracts for enzyme assays were prepared using YeastBuster™ reagent (Novagen). Protein concentrations were measured according to the Bradford method. Enzyme activity of XR was measured according to the procedure previously described (7). The activity is expressed as units (U) per mg protein, one unit being equivalent to the amount of enzyme needed to consume or produce one mmol of NAD(P)H per minute.

Analytical methods

Xylose and xylitol were analyzed by HPLC (Shimadzu Corp., Kyoto, Japan) with a refractive index detector (RID-10A) and Aminex HPX-87H column (300×7.8 mm, Bio-Rad, USA) at 65°C with 5 mM H\(_2\)SO\(_4\) as mobile phase at 0.8 ml min\(^{-1}\). Cell growth was monitored at 600 nm (Spectrophotometer, Techcomp Instruments, Shanghai, China) and converted to cell dry weight (CDW) by a correlational calibration curve.
a strain of *C. tropicalis*, which was coincident with the results of 18S rDNA genes analysis. Thus, it allows us to classify the W103 strain as one that belongs to the *C. tropicalis*.

**Batch fermentation for xylitol production using C. tropicalis W103: effect of initial xylose concentration on xylitol production**

The initial xylose concentration was varied from 60 to 160 g l\(^{-1}\) to evaluate the effect of the initial xylose concentration on the fermentation parameters of *C. tropicalis* W103 (Table 1). Increasing the initial xylose concentration, the final xylitol concentration was also increased. Maximum specific growth rate of 0.2 h\(^{-1}\) was the highest at 80 g l\(^{-1}\) xylose. Sirisansaneeyakul et al. reported that increasing xylose concentration resulted in increasing rates of xylose uptake and xylitol formation at similar specific growth rate (24). The volumetric xylitol productivity and yield were maximal at 120 g l\(^{-1}\) initial xylose concentration. However, increasing further the xylose concentration to 160 g l\(^{-1}\), led to a drastic decrease in cell growth and volumetric productivity. This phenomenon can be explained by substrate inhibition and/or osmotic pressure (12).

**Batch fermentation for xylitol production using C. tropicalis W103: effects of temperature, pH and oxygen mass transfer coefficient (K\(_{La}\)) on xylitol production**

As shown in Table 2A, with the increase of the culture temperature, the biomass decreased gradually. However, the xylitol concentration increased gradually when the culture temperature was increased from 30 to 35\(^{\circ}\)C. According to previous reports (25), xylose reductase (XR) activity is increasing from 25 to 50\(^{\circ}\)C. Higher temperature results in higher xylitol production because of higher XR activity. This could be one reason for the observed increase in xylitol production when the temperature of media was maintained at 30-35\(^{\circ}\)C. Although the XR activity is high at 45\(^{\circ}\)C, due to then an obvious decrease in biomass, there was an obvious decrease in xylitol volumetric productivity. Thus, the optimum temperature for xylitol production by the strain *C. tropicalis* W103 was around 35\(^{\circ}\)C.

The influence of pH on cell growth of *C. tropicalis* in the pH controlled fermentation bioprocess was significant (Table 2B). The low pH inhibited the cell growth and xylitol production. At pH 2.5, little cell growth (3.91 g l\(^{-1}\)) and little xylitol (54.2 g l\(^{-1}\)) were produced. At pH 6.5, the similar phenomena were observed. The maximum concentration of xylitol was obtained at pH 4.5 media, and the volumetric productivity and yield were 1.37 g l\(^{-1}\) h\(^{-1}\) and 0.73 g g\(^{-1}\), respectively.

In xylose–xylitol bioconversion a key operational parameter is the available oxygen for the microbial cell growth. Therefore, batch fermentations under different initial K\(_{La}\) values were carried out to study the influence of K\(_{La}\) on xylitol production. At the lowest K\(_{La}\) of 10.8 h\(^{-1}\), due to limitation of oxygen supply, the biomass was only 4.71 g l\(^{-1}\), thereby decreasing the xylitol productivity. With increased K\(_{La}\), xylose consumption rate was enhanced and more biomass was produced but xylitol production yield was gradually decreased. The maximum yield (0.73 g g\(^{-1}\)) and volumetric productivity (1.43 g l\(^{-1}\) h\(^{-1}\)) of xylitol were obtained at K\(_{La}\) 16.5 h\(^{-1}\) and 18 h\(^{-1}\), respectively. Further increasing to K\(_{La}\) 20 h\(^{-1}\), both xylitol productivity and yield were decreased, indicating that high dissolved oxygen condition is detrimental. To keep the high xylitol productivity and yield, the reasonable K\(_{La}\) should range from 16.5 h\(^{-1}\) to 18 h\(^{-1}\).

**Batch fermentation with two-stage aeration strategy**

Based on the above single factor experiments, batch fermentations were performed at pH 4.5, 35\(^{\circ}\)C. In this case, two-stage aeration strategy was applied. In the first 24 h fermentation, K\(_{La}\) 18 h\(^{-1}\) was applied for more vigorous cell growth. After 24 h, the K\(_{La}\) was shifted to 16.5 h\(^{-1}\). The time courses of xylitol production by *C. tropicalis* W103 with two-stage aeration is presented on Fig. 1. The xylose was completely consumed in 60 h and the concentration of xylitol was up to maximum (72.2 g l\(^{-1}\)). The xylitol yield was 0.73 g g\(^{-1}\), similar to that at K\(_{La}\) 16.5 h\(^{-1}\). The volumetric productivity of the whole process was about 1.45 g l\(^{-1}\) h\(^{-1}\), higher than that under either K\(_{La}\) 18 h\(^{-1}\) or K\(_{La}\) 16.5 h\(^{-1}\) conditions.

**Xylitol production by fed-batch fermentation**

In view of the high cost of xylitol recovery from aqueous solution, an economical production of xylitol from xylose requires not only to improve product concentration and productivity, but also to keep a low residual xylose in fermentation broth (22). To get a high productivity and high final xylitol concentration, fed-batch fermentations were performed under optimal conditions and two-stage aeration strategy. The substrate xylose levels were regulated to 40-55 g l\(^{-1}\) during 24-96 h in fermentation.

As indicated on Fig. 2 that the concentration of xylitol in fermentation broth increased quickly in 6-36 h and the concentration at 36 h was 69.6 g l\(^{-1}\). The fed-batch fermentation had the highest productivity (approximately 2.5 g l\(^{-1}\) h\(^{-1}\)) between 24 and 36 h. After 36 h, the xylitol productivity decreased gradually. XR specific activity increased during
the course of fermentation to reach a maximum at 36 h, then decreased until the end of the process. Although the biomass is still increasing after 36 h, due to then a decrease in XR specific activity, there was a decrease in xylitol volumetric productivity. At the end of the process, the concentration of xylitol was 218.7 g l⁻¹, with a whole productivity of 1.82 g l⁻¹ h⁻¹, and a yield of 0.73 g g⁻¹ xylose.

Fig. 2. Concentration profiles during xylose fed-batch fermentation by C. tropicalis W103 under two-stage aeration strategy. Symbols: xylose(●), xylitol(♦), CDW(○), productivity(▲), XR (▼). The values were the means of three independent samples.

One aim of this work was to isolate microorganisms which were suitable for producing xylitol from xylose with high production. Generally, an increase in the initial sugar concentration leads to increases in the productivity and yield in a batch process if the microorganisms can tolerate a higher concentration of sugar and a higher osmotic pressure. However, the xylose inhibition on the growth of C. tropicalis was even more severe than that on C. parapsilosis KFCC 10875 and C. tropicalis HY200, which are able to grow up to 150 g l⁻¹ or 200 g l⁻¹ xylose respectively (12, 14).

The profiles of xylitol bioproduction and the main enzymes (XR and XD) involved in xylose assimilation to xylitol were greatly influenced by the available oxygen (3). Kim et al., using Candida parapsilosis, studied the effect of different dissolved oxygen on xylitol production, XR and XD specific activities.

They observed that high level of activity of XR (reduction of xylose to xylitol) gave a high level of xylitol production. The activity of XR reached the maximum at a dissolved oxygen concentration of 0.7%. However the activity of XD always increased with increasing dissolved oxygen concentration. The high level of activity of XD led to most of xylitol being converted to xylulose, which was further metabolized to cell material; thus less xylitol but more cells were accumulated (14). As can be deduced from our data obtained, the overall metabolism was accelerated and biomass production was increased with an increasing K₉ₐ from 10.8 h⁻¹ to 16.5 h⁻¹. Further increasing K₉ₐ to 18.3 h⁻¹, xylose consumption rate was still enhanced and more biomass was produced but xylitol yield decreased. This could be explained by higher XD activity at K₉ₐ 18.3 h⁻¹, which led to some xylitol conversion to xylulose and consequent cells. Compared with single stage aeration strategy, a two-stage aeration strategy (0-24 h, K₉ₐ 18.3 h⁻¹; after 24 h, shift K₉ₐ to 16.5 min⁻¹) is effective for improving final xylitol concentration and productivity due that higher K₉ₐ in 0-24 h favors producing more biomass and lower K₉ₐ after 24 h benefits to lower the XD activity.

Ikeuchi et al. reported a xylitol production of 256 g l⁻¹ using Candida sp. over a period of 276 h in a flask culture, which is the highest previous reported xylitol yield from xylose using fed-batch fermentation (11). The xylitol production rate of C. tropicalis W103 in present work was 1.8 times higher than that of Candida sp. used (Table 3). By controlling oxygen supply in the range from 0.8 to 1.2% in C. parapsilosis KFCC 10875, Kim et al. obtained a xylitol concentration of 210 g l⁻¹ from 300 g l⁻¹ xylose in 66 h (14). Kang et al. reported a 154 g l⁻¹ xylitol production from 200 g l⁻¹ xylose in 60 h using C. tropicalis HY200 (12). Kim et al. got 237 g l⁻¹ xylitol from 270 g l⁻¹ xylose after 120 h by C. tropicalis KCTC 7221 using a chemically defined medium that included urea and various vitamins (15). It should be pointed out that C. parapsilosis KFCC 10875, C. tropicalis KCTC 7221 and C. tropicalis HY200 grew faster and easily reached a higher biomass concentration (12 g l⁻¹ and 33.3 g l⁻¹, respectively) under high K₉ₐ conditions. As shown in Fig. 2, the biomass for C. tropicalis W103 is only 7.29 g l⁻¹. Though the xylitol volumetric productivity of C.

Effect of initial xylose concentration on xylitol production by C. tropicalis W103. The fermentations were performed at 35°C, K₉ₐ 16.5 h⁻¹ and pH 4.5. The values were the means of three independent samples.

### Table 1

<table>
<thead>
<tr>
<th>Initial xylose (g l⁻¹)</th>
<th>Time (h)</th>
<th>DCW (g l⁻¹)</th>
<th>Xylitol (g l⁻¹)</th>
<th>Yield (g g⁻¹)</th>
<th>Volumetric productivity (g l⁻¹ h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>40</td>
<td>5.15±0.02</td>
<td>41.6±1.2</td>
<td>0.69±0.01</td>
<td>1.04±0.03</td>
</tr>
<tr>
<td>80</td>
<td>50</td>
<td>6.19±0.01</td>
<td>57.6±0.5</td>
<td>0.72±0.01</td>
<td>1.15±0.01</td>
</tr>
<tr>
<td>100</td>
<td>58</td>
<td>6.97±0.01</td>
<td>72.3±1.4</td>
<td>0.72±0.02</td>
<td>1.25±0.02</td>
</tr>
<tr>
<td>120</td>
<td>64</td>
<td>7.03±0.02</td>
<td>87.6±1.7</td>
<td>0.73±0.02</td>
<td>1.37±0.02</td>
</tr>
<tr>
<td>140</td>
<td>82</td>
<td>6.43±0.02</td>
<td>97.4±0.6</td>
<td>0.70±0.01</td>
<td>1.19±0.02</td>
</tr>
<tr>
<td>160</td>
<td>100</td>
<td>6.14±0.01</td>
<td>102.9±2.1</td>
<td>0.64±0.02</td>
<td>1.03±0.02</td>
</tr>
</tbody>
</table>
Effects of temperature, pH and K\textsubscript{a} on biomass and product formation in xylose fermentation by \textit{C. tropicalis} W103. All fermentation experiments were carried out at 120 g l\textsuperscript{-1} initial xylose. The values were the means of three independent samples.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Maximal DCW (g l\textsuperscript{-1})</th>
<th>Xyitol</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Concentration (g l\textsuperscript{-1})</td>
<td>Volumetric productivity (g l\textsuperscript{-1} h\textsuperscript{-1})</td>
<td>Specific productivity (g l\textsuperscript{-1} h\textsuperscript{-1} g cells\textsuperscript{-1})</td>
<td>Yield (g g\textsuperscript{-1})</td>
</tr>
<tr>
<td>A Temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30°C</td>
<td>6.79±0.04</td>
<td>75.3±0.6</td>
<td>1.30±0.01</td>
<td>0.19±0.005</td>
<td>0.63±0.01</td>
</tr>
<tr>
<td>33°C</td>
<td>6.31±0.07</td>
<td>84.2±0.4</td>
<td>1.36±0.01</td>
<td>0.22±0.003</td>
<td>0.70±0.02</td>
</tr>
<tr>
<td>35°C</td>
<td>5.93±0.09</td>
<td>87.6±1.8</td>
<td>1.37±0.01</td>
<td>0.23±0.010</td>
<td>0.73±0.02</td>
</tr>
<tr>
<td>37°C</td>
<td>5.24±0.05</td>
<td>85.3±1.1</td>
<td>1.25±0.02</td>
<td>0.24±0.007</td>
<td>0.71±0.02</td>
</tr>
<tr>
<td>40°C</td>
<td>4.24±0.01</td>
<td>80.4±0.5</td>
<td>1.06±0.02</td>
<td>0.25±0.009</td>
<td>0.67±0.01</td>
</tr>
<tr>
<td>B pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>3.91±0.01</td>
<td>54.2±0.9</td>
<td>0.54±0.02</td>
<td>0.14±0.009</td>
<td>0.45±0.01</td>
</tr>
<tr>
<td>3.5</td>
<td>5.43±0.04</td>
<td>79.4±1.2</td>
<td>1.06±0.02</td>
<td>0.19±0.011</td>
<td>0.66±0.03</td>
</tr>
<tr>
<td>4.5</td>
<td>5.93±0.03</td>
<td>87.6±0.5</td>
<td>1.37±0.01</td>
<td>0.23±0.012</td>
<td>0.73±0.02</td>
</tr>
<tr>
<td>5.5</td>
<td>5.77±0.02</td>
<td>85.1±1.6</td>
<td>1.22±0.03</td>
<td>0.21±0.011</td>
<td>0.71±0.03</td>
</tr>
<tr>
<td>6.5</td>
<td>5.65±0.04</td>
<td>83.2±0.4</td>
<td>0.92±0.01</td>
<td>0.16±0.013</td>
<td>0.69±0.02</td>
</tr>
<tr>
<td>C K\textsubscript{a}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.8 h\textsuperscript{-1}</td>
<td>4.71±0.02</td>
<td>67.4±0.2</td>
<td>0.75±0.01</td>
<td>0.16±0.002</td>
<td>0.56±0.02</td>
</tr>
<tr>
<td>14.7 h\textsuperscript{-1}</td>
<td>5.47±0.05</td>
<td>80.2±1.0</td>
<td>1.11±0.02</td>
<td>0.20±0.003</td>
<td>0.67±0.04</td>
</tr>
<tr>
<td>16.5 h\textsuperscript{-1}</td>
<td>5.93±0.02</td>
<td>87.6±0.5</td>
<td>1.37±0.01</td>
<td>0.23±0.003</td>
<td>0.73±0.01</td>
</tr>
<tr>
<td>18.3 h\textsuperscript{-1}</td>
<td>6.24±0.03</td>
<td>85.8±0.4</td>
<td>1.43±0.01</td>
<td>0.23±0.005</td>
<td>0.72±0.01</td>
</tr>
<tr>
<td>20.1 h\textsuperscript{-1}</td>
<td>6.77±0.03</td>
<td>77.3±0.4</td>
<td>1.38±0.01</td>
<td>0.20±0.007</td>
<td>0.64±0.02</td>
</tr>
</tbody>
</table>

The fermentations in A part were performed at K\textsubscript{a} 16.5 h\textsuperscript{-1} and pH 4.5. The fermentations in B part were performed at K\textsubscript{a} 16.5 h\textsuperscript{-1} and 35°C. The fermentations in C part were performed at 35°C and pH 4.5.

Comparison of xylitol production by some \textit{Candida} strains

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Maximal DCW (g l\textsuperscript{-1})</th>
<th>Concentration (g l\textsuperscript{-1})</th>
<th>Volumetric productivity (g l\textsuperscript{-1} h\textsuperscript{-1})</th>
<th>Specific productivity (g l\textsuperscript{-1} h\textsuperscript{-1} g cells\textsuperscript{-1})</th>
<th>Yield (g g\textsuperscript{-1})</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Candida} sp. 559-9</td>
<td>9</td>
<td>256</td>
<td>0.93</td>
<td>0.1</td>
<td>0.76</td>
<td>(11)</td>
</tr>
<tr>
<td>\textit{C. parapsilosis} KFCC 10875</td>
<td>33.3</td>
<td>210</td>
<td>3.18</td>
<td>0.1</td>
<td>0.7</td>
<td>(14)</td>
</tr>
<tr>
<td>\textit{C. tropicalis} HY200</td>
<td>12</td>
<td>154</td>
<td>2.57</td>
<td>0.21</td>
<td>0.77</td>
<td>(12)</td>
</tr>
<tr>
<td>\textit{C. tropicalis} KCTC 7221</td>
<td>17</td>
<td>237</td>
<td>2</td>
<td>0.12</td>
<td>0.89</td>
<td>(15)</td>
</tr>
<tr>
<td>\textit{C. tropicalis} W103</td>
<td>7.3</td>
<td>218.7</td>
<td>1.82</td>
<td>0.29</td>
<td>0.73</td>
<td>This study</td>
</tr>
</tbody>
</table>

tropicalis W103 is lower than that of \textit{C. parapsilosis} KFCC 10875, \textit{C. tropicalis} KCTC 7221 or \textit{C. tropicalis} HY200, the xylitol specific productivity of \textit{C. tropicalis} W103 (0.23 g g\textsuperscript{-1} h\textsuperscript{-1}) is the highest, which means the highest XR specific activities. Therefore, the level of volumetric productivity of the \textit{C. tropicalis} W103 can be further improved by increasing the biomass concentration and optimizing the production conditions. Separation of the growth phase and the production phase should be advantageous in designing more flexible processes.

Conclusions
In summary, we reported herein a novel yeast \textit{C. tropicalis} W103 that provides high level production of xylitol at high
specific productivity. A two-stage aeration strategy was
developed for improving final xylitol concentration and
productivity, thus indicating a possibility of application for
industrial use. Moreover, the research on selected strain will
contribute to a better understanding of regular properties of
xylose metabolism in different yeasts.

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