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Production of xylitol by *Candida peltata*

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The ability of *Candida peltata* NRRL Y-6888 to ferment xylose to xylitol was evaluated under different fermentation conditions such as pH, temperature, aeration, substrate concentration and in the presence of glucose, arabinose, ethanol, methanol and organic acids. Maximum xylitol yield of 0.56 g g^{-1} xylose was obtained when the yeast was cultivated at pH 6.0, 28°C and 200 rpm on 50 g L^{-1} xylose. The yeast produced ethanol (0.41 g g^{-1} in 40 h) from glucose (50 g L^{-1}) and arabitol (0.55 g g^{-1} in 87 h) from arabinose (50 g L^{-1}). It preferentially utilized glucose > xylose > arabinose from mixed substrates. Glucose (10 g L^{-1}), ethanol (7.5 g L^{-1}) and acetate (5 g L^{-1}) inhibited xylitol production by 61, 84 and 68%, respectively. Arabinose (10 g L^{-1}) had no inhibitory effect on xylitol production.

Keywords: xylitol production; xylose fermentation; mixed sugars utilization; *Candida peltata*

Introduction

Xylitol, a five-carbon sugar alcohol, has attracted much attention because of its potential as a natural food sweetener, a dental caries reducer and a sugar substitute for diabetics [6]. It is currently produced by chemical reduction in alkaline conditions of xylose derived mainly from wood hydrolyzate [13]. The recovery of xylitol from the xylan fraction is about 50–60% or 8–15% of the raw material employed [10,23]. The value depends on the xylan content of the raw material. Drawbacks of the chemical process are the requirements of high pressure and temperature, use of an expensive catalyst and use of extensive separation and purification steps to remove the by-products that are mainly derived from the hemicellulose hydrolyzate [14]. The bulk of xylitol produced is consumed in food products such as chewing gum, candy, soft drinks and ice cream [17]. It gives a pleasant cool and fresh sensation due to its high negative heat of solution.

The production of xylitol by fermentation is becoming more attractive because of the problems associated with its production chemically. Many yeasts and mycelial fungi possess NADPH-dependent xylose reductase (EC 1.1.1.21) which catalyzes the reduction of xylose to xylitol as a first step in xylose metabolism [3]. Xylitol can be subsequently oxidized to xylulose by the action of xylitol dehydrogenase, which preferentially uses NAD as an acceptor [7]. In xylose fermenting yeasts, the initial reactions of xylose metabolism appear to be rate-limiting [15]. This results in accumulation of xylitol in the culture medium, the degree varying with the culture conditions and the yeast strain used [21]. The factors that regulate the production and excretion of xylitol have not been clearly established [19,20,23].

While screening for yeasts to ferment xylose and arabinose to ethanol from the ARS Culture Collection, we found that *Candida peltata* NRRL Y-6888 grows very well on xylose and is a good producer of xylitol in comparison with that (0.43–0.51 g g^{-1} xylose) produced by *C. entomaea* NRRL Y-7785 and *Pichia guilliermondii* NRRL Y-2075 [18]. In this paper, we describe the fermentation conditions necessary for production of xylitol from xylose separately and in sugar mixtures by *C. peltata* NRRL Y-6888.

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Materials and methods

Yeast strain, medium and fermentation conditions

C. peltata NRRL Y-6888 was obtained from the ARS culture collection, NCAUR, Peoria, IL, USA. The growth medium designated as YMP contained 3 g yeast extract, 3 g malt extract and 3 g peptone per liter. The medium and the substrates dissolved or suspended in water were sterilized separately at 121°C for 15 min. Ethanol and methanol were filter sterilized. The pH was adjusted to 5.0 with 1 M HCl prior to inoculation. A 125-ml Erlenmeyer flask containing 50 ml medium with xylose (1%, w/v) was inoculated with a loopful of cells taken from a stock slant and incubated at 28°C on a rotary shaker (200 rpm) for 3 days. Fermentation flasks (125-ml Erlenmeyer flasks containing 50 ml medium) were inoculated with 2 ml of this starter culture and cultivated on a rotary shaker (200 rpm) at 28°C. Samples were withdrawn periodically to determine cell growth, residual substrate, and product yield.

Materials

Xylose, xylitol, glucose, l-arabinose, l-arabitol, chitin and Triton X-100 were purchased from Sigma Chemical Company, St Louis, MO, USA. An Aminex HPX-87C column and Carbo-C guard cartridge were purchased from Bio-Rad Laboratories, Hercules, CA, USA.

Analytical methods

Culture or cell growth was monitored by measuring the optical density of the appropriately diluted culture broth at 660 nm. Samples were clarified by centrifugation at 12 000 × *g* for 15 min for product and residual sugar analyses. Supernatant solutions were stored at –20°C before

Table 1 Effect of temperature and initial pH on growth and xylitol production from xylose by *Candida peltata* NRRL Y-6888[a]

Condition	Time of maximum xylitol yield (h)	pH at maximum xylitol yield	Growth at maximum xylitol yield (A660)	Maximum xylitol (g L^{-1})	Xylitol yield (g g^{-1} xylose)
Temperature of growth (°C)					
22	98	4.0 ± 0.0	19.4 ± 1.0	17.0 ± 0.0	0.34 ± 0.0
28	70	3.7 ± 0.1	12.9 ± 0.0	25.9 ± 0.0	0.52 ± 0.0
34	70	3.7 ± 0.0	10.6 ± 0.2	23.3 ± 1.3	0.47 ± 0.0
pH					
4.0	78	3.3 ± 0.0	12.5 ± 0.0	21.4 ± 0.0	0.43 ± 0.0
5.0	70	3.7 ± 0.1	12.9 ± 0.0	25.9 ± 0.4	0.52 ± 0.0
6.0	78	4.5 ± 0.3	14.7 ± 0.5	27.9 ± 0.7	0.56 ± 0.0
7.0	78	4.0 ± 0.0	15.4 ± 0.0	21.5 ± 0.0	0.43 ± 0.0

[a]Values reported are from duplicate experiments for each temperature (pH 5.0, 200 rpm) and pH (28°C, 200 rpm). Xylose used, 50 g L^{-1}.

analysis. Sugars and alcohols were determined by high pressure liquid chromatography (HPLC). The separation system consisted of a multi-solvent delivery system (Spectra system P4000, Spectra-Physics, San Jose, CA, USA) equipped with an auto sampler (717, Waters Chromatography Division, Millipore Corp, Milford, MA, USA), a refractive index detector (410 differential refractometer, Waters) and an integrator (HP 3396 series II, Hewlett-Packard Company, Wilmington, DE, USA). An ion moderated partition chromatography column (Aminex HPX-87C) fitted with a Carbo-C guard cartridge was used. The column was maintained at 85°C, and the sugars and alcohols were eluted with Milli-Q water at a flow rate of 0.6 ml min^{-1}. Peaks were identified and quantified by comparison with retention times of authentic standards (xylose, xylitol, arabinose, arabitol, glucose and ethanol).

Results and discussion

Factors affecting xylitol production from xylose

The effects of temperature (22–40°C) and pH (4.0–7.0) on growth and xylitol production were examined in shake flasks at 200 rpm on 50 g L^{-1} xylose in YMP medium. The results are summarized in Table 1. Growth was absent at 40°C. Xylitol production was optimal at 28°C while growth was best at 22°C. Yields of xylitol were 0.34, 0.52, 0.47 g per g xylose at 22, 28, 34°C, respectively. Production rates

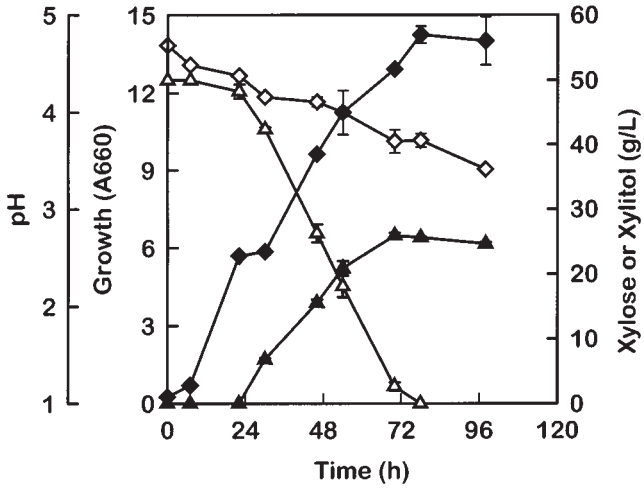

Figure 1 Time course of xylitol production from xylose by *Candida peltata* NRRL Y-6888 at pH 5.0, 28°C and 200 rpm. Substrate used, 50 g L^{-1}. Values reported are from duplicate experiments. ◇, pH; ◆, growth (A660); △, xylose; ▲, xylitol.

of xylitol on 50 g L^{-1} xylose were 0.17, 0.37, 0.47 g L^{-1} h^{-1} at 22, 28, 34°C, respectively. Consequently, a temperature of 28°C was selected for subsequent fermentations.

The initial pH of the culture medium influenced production of xylitol (Table 1). Yields of xylitol from xylose by *C. peltata* were 0.43–0.56 g g^{-1} over the pH range of 4.0–7.0. An optimal yield of xylitol (0.56 g g^{-1}) with a productivity of 0.36 g L^{-1} h^{-1} was obtained at the initial pH of 6.0. In all cases, the pH of the culture medium decreased gradually to 3.3–4.5. Rates of xylitol production were 0.27, 0.37, 0.36 and 0.28 g L^{-1} h^{-1} at pH 4.0, 5.0, 6.0 and 7.0, respectively. Time courses of xylose consumption, cell growth, change in culture pH and xylitol production at pH 5.0 and 28°C are shown in Figure 1. Cell density and xylitol concentration increased with xylose consumption and remained almost unchanged after xylose was exhausted from the medium. These results suggest that this yeast does not consume xylitol during fermentation and offers an advantage over another xylitol-producing yeast, *Pichia guilliermondii* [12]. *C. peltata* produced a maximum of 0.52 g xylitol per g xylose in 70 h when grown on 50 g L^{-1} xylose. The pH of the culture medium decreased to 3.7 at the time of maximum xylitol production.

Xylitol production increased from 0.43 to 0.53 g g^{-1} xylose when the yeast was grown at 200 rpm for 24 h and

Table 2 Fermentation of xylose in the presence of organic acids, methanol, chitin and Triton X-100 by *Candida peltata* NRRL Y-6888[a]

Condition	Time of maximum xylitol yield (h)	pH at maximum xylitol yield	Growth at maximum xylitol yield (A660)	Maximum xylitol (g L^{-1})	Xylitol yield (g g^{-1} xylose)
Xylose	72	4.3 ± 0.0	14.7 ± 0.2	23.6 ± 0.3	0.47 ± 0.0
Xylose plus acetate (5 g L^{-1})	96	5.2 ± 0.0	8.8 ± 0.3	7.7 ± 0.4	0.15 ± 0.0
Xylose plus lactate (5 g L^{-1})	72	4.5 ± 0.0	11.1 ± 1.3	18.4 ± 0.5	0.37 ± 0.0
Xylose plus succinate (5 g L^{-1})	72	5.3 ± 0.0	14.0 ± 0.1	25.2 ± 1.0	0.50 ± 0.0
Xylose plus methanol (10 g L^{-1})	72	4.2 ± 0.0	11.7 ± 0.2	17.6 ± 0.4	0.35 ± 0.0
Xylose plus chitin (4 g L^{-1})	48	5.3 ± 0.0	nd	20.2 ± 1.0	0.40 ± 0.0
Xylose plus Triton X-100 (1 g L^{-1}) added after 24 h growth	87	4.1 ± 0.0	12.1 ± 0.6	8.2 ± 0.2	0.16 ± 0.0

[a]Values reported are from duplicate experiments performed at pH 5.0, 28°C and 200 rpm. Xylose used, 50 g L^{-1}. nd, not determined.

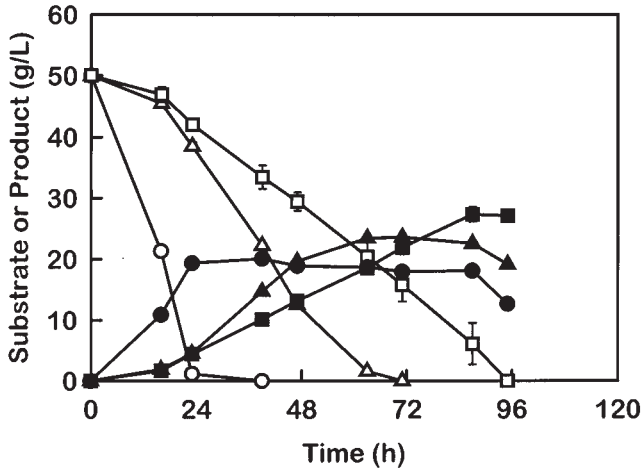

Figure 2 Comparative fermentations of substrates (glucose, xylose and arabinose) to respective products (ethanol, xylitol and arabitol) by *Candida peltata* NRRL Y-6888 at pH 5.0, 28°C and 200 rpm. Substrate used, 50 g L^{-1}. Values reported are from duplicate experiments. ○, Glucose; ●, ethanol; △, xylose; ▲, xylitol; □, arabinose; ■, arabitol.

then at 100 rpm for the rest of the fermentation, rather than maintaining 200 rpm throughout the fermentation. Fermentation time remained the same (72 h) even though growth of the yeast decreased at the lower agitation rate (A660, 9.4 *vs* 13.8 in 72 h). Thus, for optimum yield it may be important to maintain a high level of aeration during the growth phase and then limit aeration during the production phase. Similar observations have been reported for xylitol production from xylose by *C. guilliermondii* [1], *C. tropicalis* [8] and *Debaryomyces hansenii* [16]. An increase in the initial xylose concentration from 50 g L^{-1} to 100 g L^{-1} led to an increase in xylitol production rates (from 0.33 g L^{-1} h^{-1} to 0.60 g L^{-1} h^{-1}) and yields (from 0.47 g g^{-1} xylose to 0.52 g g^{-1} xylose).

The effects of chitin, organic acids (acetate, lactate and succinate), methanol and Triton X-100 on xylitol production are presented in Table 2. Acetate (5 g L^{-1}) severely inhibited xylitol production (84%) while lactate (5 g L^{-1}), methanol (10 ml L^{-1}) and chitin (4 g L^{-1}) reduced xylitol production by 21, 25 and 15%, respectively. Fermentation time required for maximum xylitol production decreased from 72 h to 48 h in the presence of chitin. Succinate (5 g L^{-1}) did not affect xylitol production. Methanol failed to increase production of xylitol from xylose by *C. peltata*, while others have reported increased xylitol production by *C. boidinni* [22] and *Petromyces albertensis* [4] in the presence of methanol. The addition of Triton X-100 (1 g L^{-1}) after 24 h growth, decreased xylitol yield by 66%, although growth of the yeast was affected by only 18%. Addition of mineral salts [18] and urea (0.64%) did not improve xylitol productivity by the yeast. Barbosa *et al* [1] reported that the use of urea led to higher xylitol productivity by *C. guilliermondii*.

Substrate preferences and tolerance

Utilization of xylose, glucose and arabinose separately and in combination at pH 5.0, 28°C and 200 rpm by a xylose-grown inoculum were investigated. The yeast utilized glucose, xylose and arabinose when grown on each sugar separately with the rate of utilization of these sugars being glucose > xylose > arabinose. *C. peltata* produced 0.41 g ethanol in 39 h, 0.47 g xylitol in 70 h and 0.55 g arabitol in 87 h per g of glucose, xylose and arabinose, respectively (Figure 2). No ethanol was detected in the culture broth when the yeast was grown on either xylose or arabinose. When grown on mixed sugars (xylose, glucose and arabinose), the yeast preferentially utilized glucose, then xylose and finally arabinose. Xylose was utilized from the medium only after glucose was totally depleted. Arabinose was not utilized during glucose and xylose consumption. This type of sequential utilization of multiple sugar substrates is probably due to catabolite inhibition [2,9,11,18]. Xylitol production was severely reduced in mixed sugar fermentations (0.10–0.14 g g^{-1} xylose, Table 3). During mixed sugar fermentation of glucose (10 g L^{-1}) and xylose (50 g L^{-1}), glucose was first exhausted and ethanol (4.54 g L^{-1}) was produced. Xylitol production was severely inhibited (0.17 g g^{-1} xylose) even though xylose was utilized. Ethanol added at 7.5 g L^{-1} caused 84% inhibition of xylitol production (0.07 g g^{-1} xylose). The enzymes essential for conversion of xylose to xylitol may be inhibited by ethanol produced from glucose, resulting in a low yield of product. In the case of mixed sugar fermentation of xylose (50 g L^{-1}) and arabinose (10 g L^{-1}), xylose was consumed first and xylitol production was unaffected (0.50 g g^{-1} xylose). This suggests that the presence of arabinose was not inhibitory to the enzyme system essential for conversion of xylose to xylitol.

Table 3 Effect of sugars on xylitol production from xylose by *Candida peltata* NRRL Y-6888[a]

Sugar (g L^{-1})	Time of maximum xylitol yield (h)	pH at maximum xylitol yield	Growth at maximum xylitol yield (A660)	Maximum xylitol (g L^{-1})	Xylitol yield (g g^{-1} xylose)
Xylose (50 g L^{-1})	72	4.5 ± 0.3	13.8 ± 0.6	21.4 ± 2.5	0.43 ± 0.0
Mixture A (50 g L^{-1}) (xyl, glu, ara, 2:1:1)	64	3.8 ± 0.0	7.6 ± 0.1	3.6 ± 0.2	0.14 ± 0.0
Mixture B (50 g L^{-1}) (xyl, glu, ara, 1:1:1)	88	4.0 ± 0.1	9.1 ± 0.6	1.6 ± 0.3	0.10 ± 0.0
Xylose (50 g L^{-1}) plus glucose (10 g L^{-1})	96	3.8 ± 0.0	9.9 ± 0.2	8.6 ± 0.2	0.17 ± 0.0
Xylose (50 g L^{-1}) plus arabinose (10 g L^{-1})	64	4.5 ± 0.0	14.6 ± 0.1	25.1 ± 0.8	0.50 ± 0.0
Xylose (50 g L^{-1}) plus ethanol (7.5 g L^{-1})	72	3.7 ± 0.0	8.9 ± 0.1	3.3 ± 0.2	0.07 ± 0.0

[a]Values reported are from duplicate experiments performed at pH 5.0, 28°C and 200 rpm. Xyl, xylose; glu, glucose; ara, arabinose.

Candida boidinii No. 2201 produced 0.49 g xylitol per g xylose when the medium was supplemented with 2% methanol [22]. Dahiya reported a xylitol yield of 0.4 g per g xylose produced by *Petromyces albertensis* [4], while *C. guilliermondii* FTI-20037 produced 0.59 g xylitol per g xylose on 40 g L^{-1} xylose [1]. *D. hansenii* Y-7426 produced about 0.56 g xylitol per g xylose present in detoxified wood hydrolyzates [16]. Horitsu *et al* [8] reported a maximum yield (0.64 g g^{-1} xylose) of xylitol after optimization of the production rate. To our knowledge, this is the first report of the production of xylitol from xylose by *C. peltata*. The yeast appears to be a promising candidate for the production of xylitol from xylose since it produced 0.56 g xylitol per g xylose when grown on xylose (50 g L^{-1}) and did not consume xylitol. Further optimization of the medium components and other process parameters such as aeration and pH-control should improve xylitol production [5,8].

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