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A New Strategy to Improve the Efficiency and Sustainability of *Candida parapsilosis* Catalyzing Deracemization of (*R,S*)-1-Phenyl-1,2-Ethanediol Under Non-Growing Conditions: Increase of NADPH Availability

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Microbial oxidoreductive systems have been widely used in asymmetric syntheses of optically active alcohols. However, when reused in multi-batch reaction, the catalytic efficiency and sustainability of non-growing cells usually decreased because of continuous consumption of required cofactors during the reaction process. A novel method for NADPH regeneration in cells was proposed by using pentose metabolism in microorganisms. Addition of D-xylose, L-arabinose, or D-ribose to the reaction significantly improved the conversion efficiency of deracemization of racemic 1-phenyl-1,2-ethanediol to (*S*)-isomer by *Candida parapsilosis* cells already used once, which afforded the product with high optical purity over 97%e.e. in high yield over 85% under an increased substrate concentration of 15 g/l. Compared with reactions without xylose, xylose added to multi-batch reactions had no influence on the activity of the enzyme catalyzing the key step in deracemization, but performed a promoting effect on the recovery of the metabolic activity of the non-growing cells with its consumption in each batch. The detection of activities of xylose reductase and xylitol dehydrogenase from cell-free extract of *C. parapsilosis* made xylose metabolism feasible in cells, and the depression of the pentose phosphate pathway inhibitor to this reaction further indicated that xylose facilitated the NADPH-required deracemization through the pentose phosphate pathway in *C. parapsilosis*. moreover, by investigating the cofactor pool, the xylose addition in reaction batches giving more NADPH, compared with those without xylose, suggested that the higher catalytic efficiency and sustainability of *C. parapsilosis* non-growing cells had resulted from xylose metabolism recycling NADPH for the deracemization.

Keywords: *Candida parapsilosis*, deracemization, NADPH availability, pentose, sustainability

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Microbial redox systems involving stereospecific oxidoreductases have gained increasing relevance in catalyzing reactions of commercial interest and have many applications, for instance, in the preparation of chiral compounds for pharmaceutical and fine chemical industries [14, 17, 26, 28, 29]. As known, microbial cells are readily and inexpensively prepared, and have been most commonly used for applied purposes thanks to their diversity and ease of handling [7]. However, the practical applications of microbial oxidoreductive systems usually require necessary electron-donating cofactors, NAD(H)- or NADP(H), and the insufficiency of required cofactors, due to continuous consumption during the reaction process, is generally a bottleneck to limiting the reaction efficiency and decreasing the sustainability of biocatalyst for reuse [35]. The need to provide cofactors for oxidoreductases-involving systems is a critical problem to the efficiency and the reusability of whole-cell catalysts in either single or multiple batches reactions. Cofactor regeneration is, therefore, an important consideration when reaction processes involving cofactor-dependent oxidoreductases are to be applied in a commercial setting, and the development of efficient and economical regeneration approaches is of great necessity [16, 34, 35].

It should be noticed that, of the cofactor-dependent oxidoreductases, NADP(H)-dependent enzymes are less common than their NAD(H)-dependent counterparts and NADPH is more expensive and less stable than other coenzymes, so NADPH regeneration is best carried out in whole cells [35]. As a hot topic to whole-cell catalysts, in addtion, NADPH is generally involved as the reduction equivalent in deracemization, by which valuable enantiomerically pure products can be obtained in 100% yield and with 100% enantiomeric excess (e.e.) from a cheap racemic substrate [21, 30, 36]. Although *in vitro* enzyme-coupled strategies for NADPH regeneration have been developed by $NADP^+$-accepting enzymes, such as engineered formate dehydrogenase

[33], hydrogenase from *Pyrococcus furiosis* [22], and soluble pyridine nucleotide transhydrogenase [1, 6], these methods are usually conducted with cell-free or purified enzyme systems but are not suitable to whole-cell systems. Therefore, it is necessary to explore an efficient and economic cofactor regeneration system for whole-cell catalysts and make native cells more available.

For microbial cells, construction of a cofactor regeneration system by directly adding an inexpensive cosubstrate in whole-cell systems is widely adopted, avoiding additional regeneration enzymes and keeping biocatalysts more stable as native type [28]. Various sugars and alcohols, such as glucose, fructose, ethanol, 2-propanol, and 2-hexanol, have been used as sacrificial cosubstrates to supply redox equivalents [3, 5, 15, 23]. Of these cosubstrates, alcohols of high concentration unfortunately tend to inactivate the involved functional enzymes [8, 38], whereas sugars can regenerate reduction equivalents internally in the form of nicotinamide cofactors by the metabolism of non-growing cells [5, 37]. When using glucose as cosubstrate, however, it is generally converted into gluconic acid and then enters the pentose phosphate pathway to generate NADPH, which would make a pH shift, and additional adjustment of the pH value is necessary to maintain the catalytic activity of cells [3, 5]. It is well known that the metabolism of available pentose and its catabolic pathway (*i.e.*, the pentose phosphate pathway) are pertinent to NADPH generation in virtually all cellular organisms [10, 11, 27]. Thus, there is potential to regenerate NADPH from exogenously added available pentose. Although several sugars have been utilized as cosubstrates for cofactor regeneration in cells, few examples have been reported using pentose to drive the internal cofactor regeneration cycles in cells, and further to improve the sustainability of whole-cell systems for repetitive utilization.

In our previous study, *Candida parapsilosis* CCTCC M203011 was selected for efficiently catalyzing the deracemization of racemic 1-phenyl-1,2-ethanediol (PED) to (*S*)-enantiomer [24], which is a versatile chiral building block for the synthesis of pharmaceuticals, agrochemicals, and liquid crystals [9], and the key enzyme, NADPH-dependent CPADH, was discovered catalyzing the irreversible reduction from the intermediate of 2-hydroxyacetophenone to (*S*)-PED [25]. Recently, in a preliminary research, we found that xylose actually enhanced the efficiency of *C. parapsilosis* catalyzing deracemization, but only assumed that xylose provided the reducing equivalents without further proof and investigation [20]. Therefore, we attempted here to evaluate the activities of key enzymes involved in deracemization and the whole cells in multi-batch reactions, investigate the effect of pentose on the enzyme activity and the metabolic activity of non-growing cells, respectively, and explore the coupling relationship between pentose uptake in *C. parapsilosis* and required cofactor regeneration from the viewpoint of cell metabolism to improve the catalytic efficiency of whole cells.

Materials and Methods

Enzymes and Chemicals

(*R*)-PED, (*S*)-PED, (*R*,*S*)-PED, coenzymes including NAD(P)H and NAD(P)$^+$, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Sigma-Aldrich Chemical (U.S.A.). 2-Hydroxyacetophenone was prepared as per the method described by Liese *et al.* [18]. All other chemicals used in this work were of analytical grade and commercially available.

Microorganism and Cultivation

C. parapsilosis was deposited in the China Center for Type Culture Collection (CCTCC, Wuhan, China) under Accession No. CCTCC M203011. The growth medium contained 4% (w/v) glucose, 0.3% (w/v) yeast extract, 1.3% (w/v) $(NH_4)_2HPO_4$, 0.7% (w/v) KH_2PO_4, 0.08% (w/v) $MgSO_4 \cdot 7H_2O$, and 0.01% (w/v) NaCl (pH 7.0). After cultivation in a 5-l fermentor containing 3 l of medium at 30°C for 48 h, *C. parapsilosis* cells were harvested by centrifugation and stored at –20°C for further use.

Preparation of Cell-Free Extract and CPADH

All purification procedures were done at the temperature of 4°C, and 20 mM potassium phosphate buffer (pH 6.5) was used as the buffer. The collected cells suspended in buffer were disrupted by sonication, at 250 W for 15 min at 0°C, with an ultrasonic oscillator (Sonic Materials Co., U.S.A.). The cell debris was removed by centrifugation (15,000 ×*g*, 20 min) at 4°C, and the supernatant was used as the cell-free extract. CPADH was purified according to a procedure described previously [25].

Enzyme and Protein Assays

The standard assay mixture for 2-hydroxyacetophenone reductase activity comprised 0.1 M potassium phosphate buffer (pH 6.5), 0.5 mM NADPH, 5 mM 2-hydroxyacetophenone, and appropriate enzyme in a total volume of 250 µl. The assay mixture for xylose reductase activity comprised 0.05 M potassium phosphate buffer (pH 7.0), 0.2 mM NADPH, 50 mM D-xylose, and appropriate enzyme in a total volume of 250 µl. The assay mixture for xylitol dehydrogenase activity comprised 0.05 M Tris-HCl buffer (pH 9.0), 0.2 mM NAD$^+$, 50 mM xylitol, and appropriate enzyme in a total volume of 250 µl. The decrease or increase in the amount of the coenzyme was measured spectrophotometrically at 340 nm. One unit of enzyme activity was defined as the amount of enzyme catalyzing the oxidation/reduction of 1 µmol NADPH/NAD$^+$ per minute under the assay conditions.

The activity of D-xylose isomerase was determined in a reaction mixture containing 50 mM Tris-HCl buffer (pH 7.5), 1 mM $MnSO_4$, 0.5 mM DTT, 2 mM D-xylose, and an appropriate amount of the enzyme in a final volume of 1 ml. The reaction was initiated by the addition of D-xylose. After incubation at 30°C for 60 min, the reaction was terminated by the addition of 0.05 ml of 10% trichloroacetic acid. One unit of D-xylose isomerase is defined as the amount of enzyme converting 1 µmol of D-xylose to D-xylulose in 1 min [31].

The protein concentration was determined using Bradford reagents (Bio-Rad) with bovine serum albumin as a standard [2].

Evaluation of Metabolic Activity

After catalyzing asymmetric reactions, the harvested cells were added to the glucose solution (20 g/l) to a final cell concentration of 50 g/l for 4 h, and then the residual concentration of glucose was measured. The metabolic activity retention of the cells after reaction was used here to express the cell activity in the reaction mixture [12]. The metabolic activity retention is defined as: R%=(C1/C2)×100%.

R: metabolic activity retention (%);

C1: the amount of consumed glucose after 4 h culture in glucose solution by the cells already used in the reaction;

C2: the amount of consumed glucose after 4 h culture in glucose solution by the intact cells not used in the reaction.

Biotransformation Procedure

For the reactions with purified enzymes, the reaction mixture in 1 ml comprised 0.1 M potassium phosphate buffer (pH 6.5), 0.5 mg 2-hydroxyacetophenone, NADPH (10 μmol), and appropriate amount of the purified enzyme. To investigate the effect of xylose on conversion by enzyme, glucose-6-phosphate (3.5 mM) and glucose-6-phosphate dehydrogenase (10 U) and/or NADPH (10 μmol) were added to the reaction mixture with or without xylose. The reactions were carried out at 30°C for 8 h with shaking.

For the reactions with *C. parapsilosis* cells, the reaction mixture in 20 ml comprised 0.1 M potassium phosphate buffer (pH 6.5), 100 g/l wet cells, and 105 mM (1.5%) (*R,S*)-PED. Reactions were carried out at 30°C for 48 h with shaking. For cofactor regeneration, various sugars of 52 mM were separately added to the reaction mixture containing whole cells already used once. For reuse of cells, free cells were centrifuged at the end of each batch and resuspended in fresh reaction mixture containing raw material and pentose. To investigate the effect of a pentose phosphate pathway inhibitor, 6-aminonicotinamide of different concentration was added in the second batch reaction.

Analytic Methods

Xylose was measured by HPLC analysis using an Aminex HPX-87H column. The column was kept at 65°C and eluted with 5 mM H_2SO_4 at a flow rate of 0.5 ml/min. The compound was detected refractometrically (Waters 410 Differential Refractometer).

Pyridine nucleotides were measured using a single extract as per the method described by Zhang *et al.* [39]. Cells were suspended with 0.15 ml of extraction buffer containing 0.1 M Tris-HCl (pH 8.0), 0.01 M EDTA, and 0.05% (v/v) Triton X-100, and disrupted on ice using an ultrasonic oscillator (Sonic Materials Co., U.S.A.) for 8 min with a 30-s interval. Then, the solution was centrifuged at 12,000 ×*g* for 5 min and the supernatant was immediately analyzed for $NADP^+$ and NADPH. $NADP^+$ and NADPH were assayed spectrophotometrically based on the measurement of the absorbance of the reduced coenzyme at 340 nm. In both assay systems, reaction buffers without NADPH were measured to correct for a low level of background absorbance.

The reaction products were extracted with ethyl acetate and analyzed by HPLC on a Chiralcel OB-H column [24].

RESULTS AND DISCUSSION

Effects of Various Sugars on Reaction Efficiency of Single Batch

In our previous research, it has been found that adding xylose to the reaction mixture significantly increased the efficiency of *C. parapsilosis* catalyzing deracemization of racemic PED for the second time, resulting in an optically active product with high e.e. value and yield under a higher substrate concentration of 15 g/l, which is almost 2-folds of the initial level [20]. Encouraged by this consequence, various sugar cosubstrates, especially including D-xylose, L-arabinose, and D-ribose, the most widely used pentoses, were added to the reaction mixture, respectively. The whole cells already used once were collected and resuspended in the fresh reaction solution. As shown in Fig. 1, addition of D-xylose, L-arabinose, or D-ribose significantly improved the reaction efficiency of deracemization of (*R,S*)-PED to (*S*)-isomer by *C. parapsilosis*. From the above results, when xylose was added to the reaction, the optical purity and yield of the product were increased to 97.7%e.e. and 85.3%, respectively, with a higher substrate concentration of 15 g/l. The fact that D-xylose, L-arabinose, and D-ribose, which are all available pentoses for cellular organisms, facilitated *C. parapsilosis* cells-mediated deracemization involving NADPH-dependent alcohol dehydrogenase indicated that pentose uptake in cells could be associated with the proceeding of this reaction process and affords great benefit in driving the reaction to the desired product [3, 10]. In this study, glucose addition to the reaction did not significantly enhance the formation of product, which might result from the reason that glucose metabolism in a certain degree trends the flux from glucose-6-phosphate to fructose-6-phosphate and enters the route of the lower glycolysis with the citric acid cycle and the pyruvate branch-point, but not the pentose phosphate pathway for NADPH generation [27]. As the most abundant pentose and second only to glucose in natural abundance, xylose was thus selected for

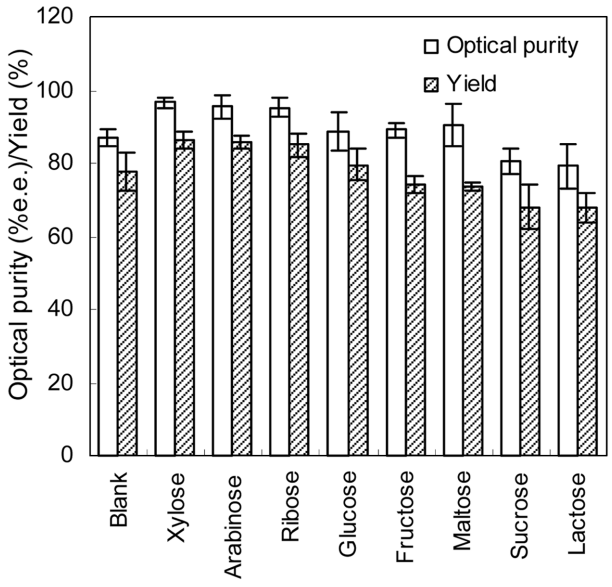

Fig. 1. Effects of various sugars on *C. parapsilosis* catalyzing deracemization of racemic PED in the second batch.

Table 1. Effect of xylose on the enzyme activity of NADPH-dependent alcohol dehydrogenase in the cell-free extract from *C. parapsilosis* and reaction catalyzed by the cells in each batch.

Reaction batch[a]	Xylose	Specific activity (U/g)	Optical purity (%e.e.)	Yield (%)
1	–	47.2	97.5	88.2
2	–	42.0	87.2	78.1
2	+	42.6	97.1	85.3
3	–	38.1	76.2	68.3
3	+	39.0	85.3	77.8
4	–	30.9	40.8	58.1
4	+	31.3	76.6	65.4
5	–	18.6	24.2	48.7
5	+	17.8	46.3	60.4

[a] The specific activity of the enzyme and the optical purity and yield of the product were measured after whole cells catalyzing each batch reaction for 48 h with and without xylose, respectively.

further research as the suitable cosubstrate in *C. parapsilosis* catalyzing deracemization.

Effect of Xylose on Catalytic Activity of Key Enzyme

As known, sugars can play a role to maintain the activity and stability of enzymes by holding protein in the right structure [32]. To demonstrate the actual function of xylose to this reaction, the effect of xylose on the enzyme activity of NADPH-dependent CPADH, catalyzing the key step of asymmetric reduction from 2-hydroxyacetophenone to (*S*)-PED in deracemization, was investigated using the cell-free extract from cells in each batch. As shown in Table 1, the profile change of the enzyme activity after catalyzing each batch reaction showed a descending trend, which might result from the long-period enzyme inactivation for existing in the reaction mixture. Compared with the systems without xylose, addition of xylose in each batch reaction exhibited no significant promotion to the enzyme activity, but improved the optical purity and yield of the product. To further demonstrate the effect of xylose on CPADH, the asymmetric reduction of 2-hydroxyacetophenone by CPADH was carried out with xylose and/or an NADPH-recycling system involving glucose-6-phosphate and glucose-6-phosphate dehydrogenase. From the results in Table 2, there was no obvious difference between the trials with and without xylose, indicating that xylose had no notable influence on the catalytic activity of the enzyme.

Associating the fact that xylose added to each batch was consumed during each reaction process, it was indicated that xylose did not act as a protein protector saccharide to the involved key enzyme, and its positive effect on this whole-cell catalyzing reaction was mainly realized through xylose utilization by non-growing cells.

Effect of Xylose on Metabolic Activity of Non-Growing Cells

By using whole cells for cofactor-requiring oxidoreductions, the metabolic activity of the employed cells would influence not only the catalytic activity and stability of cells soaked in the reaction mixture, but also the incellular regeneration of desired cofactor through metabolism cycles *in vivo* [37]. However, organism cells generally lose their metabolic activity to some extent when used in organic synthesis, which leads to limited availability only fit for somewhat low substrate concentrations in single-batch reactions and low sustainability of free cells not meeting well the requirement of repetitive utilization [12]. In this study, the change of metabolic activity of *C. parapsilosis* cells during the multi-batch conversion was investigated with and without xylose, respectively, and the reaction results in the same conversion process were also monitored (Fig. 2). The declining trend of the metabolic activity of cells was suggested to correspond to the decrease of optical purity and yield of product in the multi-batch reaction process; that is, the less the metabolic activity of cells, the lower the reaction efficiency. The metabolic activity of cells was recovered to a certain degree by adding xylose to the reaction, and accordingly, the reaction efficiency was improved compared with the reactions without xylose. Especially in the third batch, this promoting effect was more obvious. Simultaneously, the added xylose was consumed in each batch. Thus, it was proposed that when whole cells were reused for several times, their activity decreased as a result of to long-term exposure to non-cultured conditions, resulting in deficient

Table 2. Effect of xylose on reduction of 2-hydroxyacetophenone to (*S*)-PED catalyzed by NADPH-dependent alcohol dehydrogenase, and the enzyme activity[a].

	Xylose			Without xylose		
	NADPH	NADPH+Glu+GDH		NADPH	NADPH+Glu+GDH	
Optical purity (%e.e.)	98.1	97.6	–	97.9	97.3	–
Yield (%)	50.9	85.4	–	51.2	84.7	–
Specific activity (U/mg)	–	–	38.6	–	–	38.0

[a] The reaction mixture in 1 ml comprised 0.1 M potassium phosphate buffer (pH 6.5), 0.5 mg of 2-hydroxyacetophenone, and appropriate amount of the purified enzyme. To investigate the effect of xylose on conversion by enzyme, NADPH (10 μmol) or NADPH (10 μmol) with its regeneration system involving glucose-6-phosphate (Glu) (3.5 mM) and glucose-6-phosphate dehydrogenase (GDH) (10 U) was added to the reaction mixture with or without xylose. The reactions were carried out at 30°C for 8 h with shaking.

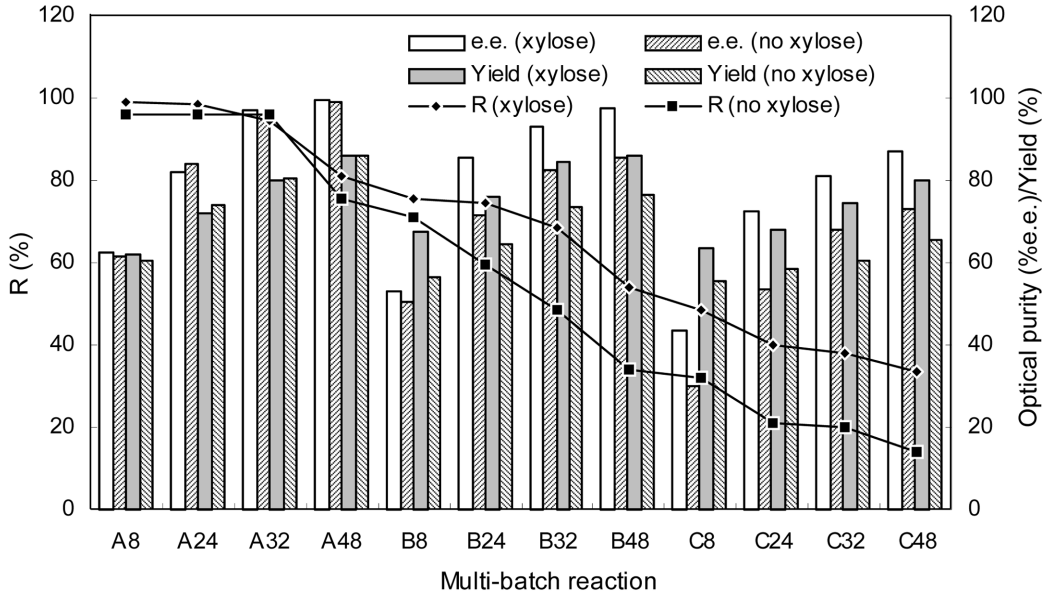

Fig. 2. Effect of xylose on the metabolic activity of *C. parapsilosis* cells in multi-batch conversion.
A, B, and C mean the first, the second, and the third batch reaction, respectively, and the numbers following A, B, and C are the reaction time in each batch.

supply of required cofactor from metablism, whereas xylose uptake promoted the cell activity and the biocatalytic efficiency was consequently developed.

Feasibility of Xylose Metabolism in *C. parapsilosis*

Xylose in all cellular organisms virtually enters the pentose phosphate pathway through xylulose. The conversion of xylose to xylulose goes by way of xylose isomerase in bacteria, whereas in fungi and other eukaryotes, this proceeds *via* a two-step reduction and oxidation mediated by NAD(P)H-dependent xylose reductase and NAD^+-dependent xylitol dehydrogenase, respectively [13, 19]. To confirm the feasibility of xylose metabolism in *C. parapsilosis*, the general enzymes, xylose reductase and xylitol dehydrogenase, responsible for xylose uptake in yeast were further assayed. The activities of xylose reductase and xylitol dehydrogenase were measured as 0.56 U/mg and 0.68 U/mg, respectively. The result that the activities of xylose reductase and xylitol dehydrogenase were both obviously detected in cell-free extract of *C. parapsilosis* suggested that these two enzymes both existed in the *C. parapsilosis* cell, which made xylose metabolism more feasible in the catalytic whole cells, so that xylose can be transformed to xylulose by xylose reductase and xylitol dehydrogenase to enter the pentose phosphate pathway for NADPH generation [10].

To further prove xylose metabolism through the pentose phosphate pathway in *C. parapsilosis*, based on the above result, 6-aminonicotinamide, the reported inhibitor of the pentose phosphate pathway [4], was added in the reaction systems with cells already used onces and xylose. As seen from the results in Fig. 3, the inhibitor indeed showed significantly negative effect on this reaction, giving product with much lower optical purity and yield than that from the trial only with xylose, and even inferior to that without xylose. Thus, when the inhibitor was used, the addition of xylose to the reaction was unable to perform a promoting effect on this reaction. This result suggested that the pentose phosphate pathway was of great importance to the

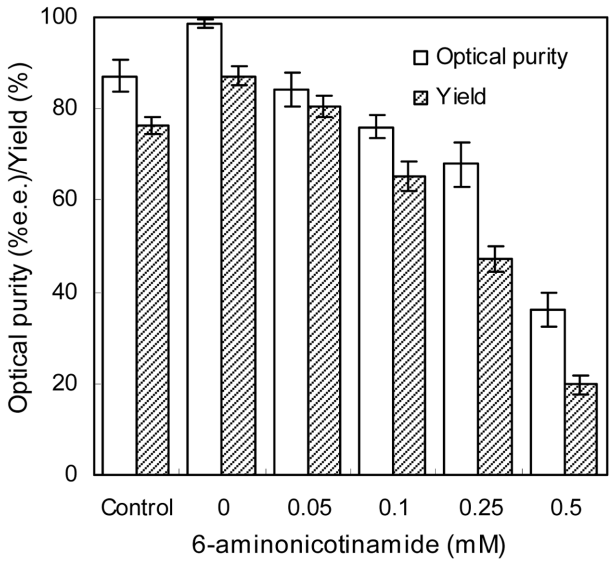

Fig. 3. Effect of a pentose phosphate pathway inhibitor on *C. parapsilosis* catalyzing deracemization of racemic PED with xylose in the second batch. In the control trial, neither xylose nor the inhibitor 6-aminonicotinamide was added in the reaction system.

proceeding of NADPH-requiring oxidoreduction, which recycles NADPH in organism cells, and the contribution of xylose to this reaction would be achieved by its metabolism through the pentose phosphate pathway in *C. parapsilosis* cells.

Effect of Xylose on Regeneration of Required Cofactor in Cell

Based on the above results, direst quantitation of the cofactor pool was further attempted to support our assumption of xylose metabolism affording NADPH regeneration in *C. parapsilosis*. As shown in Table 3, addition of xylose to reaction batches really offered much more NADPH for the reaction process compared with those without xylose. As expected, there was a correspondence between the quantity of the required cofactor and the efficiency of asymmetric reaction by cofactor-dependent oxidoreductase.

It is well known that most of the cytosolic NADPH is generated in the reactions from glucose-6-phosphate to ribulose-5-phosphate, a route known as the oxidative branch of the pentose phosphate pathway [27], in which glucose-6-phosphate was sequentially oxidized through glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, thus generating 2 mol of NADPH per mol of glucose-6-phosphate [10, 35]. Associating xylose metabolism with NADPH regeneration in *C. parapsilosis* cells, it was proposed that the higher catalytic efficiency and sustainability of *C. parapsilosis* non-growing cells resulted from xylose metabolism providing more NADPH for the deracemization process, in which the asymmetric reduction from 2-hydroxyacetophenone to (*S*)-PED was the key step driving the reaction equilibrium to the target product catalyzed by NADPH-dependent CPADH in cells.

In this study, widespread pentoses, including xylose, arabinose, and ribose, were applied as feedstocks for NADPH-required asymmetric oxidoreduction by taking advantage of cellular machinery. Based on the evidence from different aspects, the effect of pentose on this asymmetric oxidoreduction could be explained in that *in vivo* xylose metabolism took place through the pentose phosphate pathway, and NADPH was simultaneously generated from $NADP^+$ *via* this route and used for NADPH-dependent CPADH catalyzing asymmetric reduction, the key step in the deracemization of racemic PED. Apart from the scientific interest in the mechanism of manipulating cofactor regeneration by pentose addition, there is also interest from an application perspective. This "metabolism-coupled" cofactor regeneration using pentose would make the redox involving NADPH-dependent oxidoreductases more efficient and provide a fertile ground for further improvements.

Table 3. Effect of xylose addition to in the reaction on the regeneration of NADPH in *C. parapsilosis* cells for multi-batch conversion.

Reaction batch	Reaction time (h)	Xylose	NADPH/ $NADP^+$	Optical purity (%e.e.)	Yield (%)
2	48	–	0.095	88.2	78.6
2	48	+	0.125	97.8	86.1
3	24	–	0.466	58.3	60.8
3	24	+	0.538	72.5	65.6
3	48	–	0.278	76.4	67.6
3	48	+	0.416	85.6	78.2

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References

1. Boonstra, B., D. A. Rathbone, C. E. French, E. H. Walker, and N. C. Bruce. 2000. Cofactor regeneration by a soluble pyridine nucleotide transhydrogenase for biological production of hydromorphone. *Appl. Environ. Microbiol.* **66:** 5161–5166.
2. Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
3. Dahl, A. C. and J. O. Madsen. 1998. Baker's yeast: Production of D- and L-3-hydroxy esters. *Tetrahedr. Asymm.* **9:** 4395–4417.
4. Gupte, S. A., T. Okada, I. F. McMurtry, and M. Oka. 2006. Role of pentose phosphate pathway-derived NADPH in hypoxic pulmonary vasoconstriction. *Pulm. Pharmacol. Ther.* **19:** 303–309.
5. Haberland, J., A. Kriegesmann, E. Wolfram, W. Hummel, and A. Liese. 2002. Diastereoselective synthesis of optically active (*2R,5R*)-hexanediol. *Appl. Microbiol. Biotechnol.* **58:** 595–599.
6. Ichinose, H., N. Kamiya, and M. Goto. 2005. Enzymatic redox cofactor regeneration in organic media: Functionalization and application of glycerol dehydrogenase and soluble transhydrogenase in reverse micelles. *Biotechnol. Prog.* **21:** 1192–1197.
7. Ishige, T., K. Honda, and S. Shimizu. 2005. Whole organism biocatalysis. *Curr. Opin. Chem. Biol.* **9:** 174–180.
8. Itoh, N., M. Matsuda, M. Mabuchi, T. Dairi, and J. Wang. 2002. Chiral alcohol production by NADH-dependent phenylacetaldehyde reductase coupled with *in situ* regeneration of NADH. *Eur. J. Biochem.* **269:** 2394–2402.

9. Iwasaki, F., T. Maki, W. Nakashima, O. Onomura, and Y. Matsumura. 1999. Nonenzymatic kinetic resolution of 1,2-diols catalyzed by organotin compound. *Org. Lett.* **1:** 969–972.
10. Jeffries, T. W. 2006. Engineering yeasts for xylose metabolism. *Curr. Opin. Biotechnol.* **17:** 320–326.
11. Jeffries, T. W. and Y. S. Jin. 2004. Metabolic engineering for improved fermentation of pentoses by yeasts. *Appl. Microbiol. Biotechnol.* **63:** 495–509.
12. Jiang, Q., S. Yao, and L. Mei. 2002. Tolerance of immobilized Baker's yeast in organic solvents. *Enzyme Microb. Technol.* **30:** 721–725.
13. Karhumaa, K., R. Fromanger, B. Hahn-Hagerdal, and M. F. Gorwa-Grauslund. 2007. High activity of xylose reductase and xylitol dehydrogenase improves xylose fermentation by recombinant *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* **73:** 1039–1046.
14. Kataoka, M., K. Kita, M. Wada, Y. Yasohara, J. Hasegawa, and S. Shimizu. 2003. Novel bioreduction system for the production of chiral alcohols. *Appl. Microbiol. Biotechnol.* **62:** 437–445.
15. Kataoka, M., Y. Nomura, S. Shimizu, and H. Yamada. 1992. Enzymes involved in the NADPH regeneration system coupled with asymmetric reduction of carbonyl compounds in microorganisms. *Biosci. Biotech. Biochem.* **56:** 820–821.
16. Koeller, K. M. and C. H. Wong. 2001. Enzymes for chemical synthesis. *Nature* **409:** 232–240.
17. Kroutil, W., H. Mang, K. Edegger, and K. Faber. 2004. Recent advances in the biocatalytic reduction of ketones and oxidation of sec-alcohols. *Curr. Opin. Chem. Biol.* **8:** 120–126.
18. Liese, A., M. Karutz, J. Kamphuis, C. Wandrey, and U. Kragl. 1996. Resolution of 1-phenyl-1,2-ethanediol by enantioselective oxidation overcoming product inhibition by continuous extraction. *Biotechnol. Bioeng.* **51:** 544–550.
19. Lima, L. H. A., C. G. Pinheiro, L. M. P. de Moraes, S. M. de Freitas, and F. A. G. Torres. 2006. Xylitol dehydrogenase from *Candida tropicalis*: Molecular cloning of the gene and structural analysis of the protein. *Appl. Microbiol. Biotechnol.* **73:** 631–639.
20. Lv, T., Y. Xu, X. Mu, and Y. Nie. 2007. Promotion effect of xylose co-substrate on stability of catalytic system for asymmetric redox of (*R,S*)-1-phenyl-1,2-ethanediol to its (*S*)-enantiomer by *Candida parapsilosis*. *Chin. J. Catal.* **28:** 446–450.
21. Matsuyama, A., H. Yamamoto, N. Kawada, and Y. Kobayashi. 2001. Industrial production of (*R*)-1,3-butanediol by new biocatalysts. *J. Mol. Catal. B Enzym.* **11:** 513–521.
22. Mertens, R., L. Greine, E. C. D. van den Ban, H. B. C. M. Haaker, and A. Liese. 2003. Practical applications of hydrogenase I from *Pyrococcus furiosus* for NADPH generation and regeneration. *J. Mol. Catal. B Enzym.* **24-25:** 39–52.
23. Nakamura, K., Y. lnoue, and A. Ohno. 1995. Improvement of enantioselectivity of microbial reduction by using organic solvent redox coupler system. *Tetrahedron. Lett.* **36:** 265–266.
24. Nie, Y., Y. Xu, and X. Q. Mu. 2004. Highly enantioselective conversion of racemic 1-phenyl-1,2-ethanediol by stereoinversion involving a novel cofactor-dependent oxidoreduction system of *Candida parapsilosis* CCTCC M203011. *Org. Process Res. Dev.* **8:** 246–251.
25. Nie, Y., Y. Xu, X. Q. Mu, H. Y. Wang, M. Yang, and R. Xiao. 2007. Purification, characterization, gene cloning and expression of a novel alcohol dehydrogenase with anti-Prelog stereospecificity from *Candida parapsilosis*. *Appl. Environ. Microbiol.* **73:** 3759–3764.
26. Panke, S., M. Held, and M. Wubbolts. 2004. Trends and innovations in industrial biocatalysis for the production of fine chemicals. *Curr. Opin. Biotechnol.* **15:** 272–279.
27. Pitkanen, J. P., A. Aristidou, L. Salusjarvi, L. Ruohonen, and M. Penttila. 2003. Metabolic flux analysis of xylose metabolism in recombinant *Saccharomyces cerevisiae* using continuous culture. *Metab. Eng.* **5:** 16–31.
28. Schmid, A., J. S. Dordick, B. Hauer, A. Kiener, M. Wubbolts, and B. Witholt. 2001. Industrial biocatalysis today and tomorrow. *Nature* **409:** 258–268.
29. Schoemaker, H. E., D. Mink, and M. G. Wubbolts. 2003. Dispelling the myths–biocatalysis in industrial synthesis. *Science* **299:** 1694–1697.
30. Strauss, U. T., U. Felfer, and K. Faber. 1999. Biocatalytic transformation of racemates into chiral building blocks in 100% chemical yield and 100% enantiomeric excess. *Tetrahedr. Asymm.* **10:** 107–117.
31. Sultana, I., R. M. Mizanur, K. Takeshita, G. Takada, and K. Izumori. 2003. Direct production of D-arabinose from D-xylose by a coupling reaction using D-xylose isomerase, D-tagatose 3-epimerase and D-arabinose isomerase. *J. Biosci. Bioeng.* **95:** 342–347.
32. Timasheff, S. N. 1993. The control of protein stability and association by weak interactions with water: How do solvents affect these processes? *Annu. Rev. Biophys. Biomol. Struct.* **22:** 67–97.
33. Tishkov, V. I., A. G. Galkin, V. V. Fedorchuk, P. A. Savitsky, A. M. Rojkova, H. Gieren, and M. R. Kula. 1999. Pilot scale production and isolation of recombinant NAD^+- and $NADP^+$-specific formate dehydrogenases. *Biotechnol. Bioeng.* **64:** 187–193.
34. Urlacher, V. B. and R. D. Schmid. 2006. Recent advances in oxygenase-catalyzed biotransformations. *Curr. Opin. Chem. Biol.* **10:** 156–161.
35. van der Donk, W. A. and H. Zhao. 2003. Recent developments in pyridine nucleotide regeneration. *Curr. Opin. Biotechnol.* **14:** 421–426.
36. Voss, C. V., C. C. Gruber, and W. Kroutil. 2008. Deracemization of secondary alcohols through a concurrent tandem biocatalytic oxidation and reduction. *Angew. Chem. Int. Ed. Engl.* **47:** 741–745.
37. Walton, A. Z. and J. D. Stewart. 2002. An efficient enzymatic Baeyer-Villiger oxidation by engineered *Escherichia coli* cells under non-growing conditions. *Biotechnol. Prog.* **18:** 262–268.
38. Yamamoto, H., A. Matsuyama, and Y. Kobayashi. 2002. Synthesis of ethyl (*R*)-4-chloro-3-hydroxybutanoate with recombinant *Escherichia coli* cells expressing (*S*)-specific secondary alcohol dehydrogenase. *Biosci. Biotechnol. Biochem.* **66:** 481–483.
39. Zhang, Z., J. Yu, and R. C. Stanton. 2000. A method for determination of pyridine nucleotides using a single extract. *Anal. Biochem.* **285:** 163–167.