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 redox systems involving stereospecific oxidoreductases 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-xylene, 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 oxidoreductase-involving systems is a critical problem to the efficiency and the reusability of whole-cell catalysts in either single or multiple batch 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 addition, 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...
require cofactor regeneration from the viewpoint of coupling relationship between pentose uptake in effect of pentose on the enzyme activity and the metabolic evaluate the activities of key enzymes involved in deracemization and investigation [20]. Therefore, we attempted here to provide the reducing equivalents without further proof PED [25]. Recently, in a preliminary research, we found CPADH, was discovered catalyzing the irreversible reduction from the intermediate of 2-hydroxyacetophenone to (S and liquid crystals [9], and the key enzym NADPH-dependent block for the synthesis of pharmaceuticals, agrochemicals, available pentose and its catabolic pathway (i.e., the pentose phosphate pathway to generate NADPH, it is generally converted into gluconic acid and then enters the pentose phosphate pathway to generate NADPH, which would make a pH shift, and an 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 activity 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\(_4\))\(_2\)HPO\(_4\), 0.7% (w/v) KH\(_2\)PO\(_4\), 0.08% (w/v) MgSO\(_4\)\(\cdot\)7H\(_2\)O, 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 xyitol dehydrogenase activity comprised 0.05 M Tris-HCl buffer (pH 9.0), 0.2 mM NAD\(^{+}\), 50 mM xyitol, 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 30 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-xyulose in 1 min [31].

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