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Two-step enzymatic synthesis of tyramine from raw pyruvate fermentation broth

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ABSTRACT

Tyramine, as a metabolite of tyrosine, is an important intermediate in synthesizing some drugs and medicinal materials. In this study, an efficient method for producing tyramine was developed by a two-step biocatalytic reaction with recombinant tyrosine phenol-lyase whole cells and tyrosine decarboxylase immobilized cells. Raw pyruvate fermentation broth was used as substrate of tyrosine phenol-lyase to economically produce L-tyrosine. L-tyrosine was catalyzed by immobilized tyrosine decarboxylase cells to effectively synthesize tyramine. The conditions of two-step enzymatic catalysis reactions were optimized separately, and the influence of immobilization on tyrosine decarboxylase activity was investigated. In a scale up study, 94.3% L-tyrosine was obtained from raw pyruvate fermentation broth under the optimal conditions. L-Tyrosine was decarboxylated to tyramine with a high yield 91.2%. The total yield of tyramine could reach approximately 86% by this two-step biocatalytic reaction. This study provides us with a green strategy for efficient preparation of tyramine.

1. Introduction

Tyramine [2-(*p*-hydroxyphenyl) ethylamine], a significant metabolite of tyrosine, has gained tremendous interest in recent years as a catecholamine drug and an intermediate of medicinal materials and some drugs [1–6]. Tyramine is mainly prepared through chemical synthesis [6–11]. Compared with chemical synthesis, enzymatic synthesis has its advantages for its mild reaction conditions, eco-friendly and high enantio-, regio- and chemoselectivity. Tyramine could be produced by enzymatic decarboxylation of L-tyrosine, a reaction catalyzed by tyrosine decarboxylase (TDC, EC 4.1.1.25) [12–19]. Nevertheless, based on tyramine as the metabolite of L-tyrosine, this enzymatically synthetic route was more utilized to study the metabolic diversion of tyramine in plant [12], and the effect of tyramine on food safety and spoilage due to the health problem caused by high quantities tyramine [13–17]. By far, there is no detailed reference to investigate the enzymatic synthesis of tyramine by TDC immobilized cells, neither for the industrial production with the method. Furthermore, the efforts in cost reduction of L-tyrosine and enzyme should be made in order to utilize this green method, because L-tyrosine is expensive and purified enzyme as catalyst is another cost-added factor [20]. Consequently, pyruvate fermentation broth and recombinant whole cells were employed to prepare tyramine by two-step biocatalytic reaction in the present work.

Tyrosine can be produced from phenol, pyruvic acid and ammonia by tyrosine phenol-lyase (TPL, EC 4.1.99.2) [21–24]. The cost of pyruvic acid accounts for a considerable proportion in enzymatic synthesis of L-tyrosine. Fermentative production of pyruvic acid as one of the most competitive method has many advantages such as cheap source of glucose, mild condition, environmental and low cost [25,26]. However, it is still difficult to purify pyruvic acid from pyruvate fermentation broth. Pyruvic acid is generally separated with filtration, decolorization, concentration and distillation, and ion exchange resin is always used for further purification [25]. If the fermentation broth could be directly used to manufacture downstream products to avoid the tedious operations, it will greatly decrease the cost and stimulate the development of pyruvate fermentation production.

On the other hand, immobilization of whole cells could further improve the potential of biocatalyst for application. Since immobilized cells can easily be retrieved from the reaction mixture and reused, biotransformation with immobilized cells superiorly

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performed in cell recovery and greatly declined the cost [27,28]. In L-tyrosine decarboxylation reaction, immobilized recombinant cells were consequently used as biocatalysts because they are very competitive and operationally stable compared with free cells.

In the present study, enzymatic synthesis of tyramine was established by two-step biocatalytic reaction (Scheme 1). Firstly, L-tyrosine was prepared from raw pyruvate fermentation broth under the catalysis of TPL. Pyruvate fermentation broth was simply centrifuged, and then the supernatant was diluted and used as substrate to produce L-tyrosine. Secondly, L-tyrosine was collected as starting material to synthesize tyramine by immobilized TDC cells from *Lactobacillus brevis*. Pyridoxal phosphate was the common coenzyme for these two enzymes.

In order to explore the feasibility of bioconversion, the optimization of enzymatic synthesis conditions of L-tyrosine such as pH, temperature, substrate concentration and surfactant were considered. During the production of tyramine, the reaction conditions were optimized with immobilized cells and the advantages of immobilized cells were investigated in comparison with free cells. Bioconversion efficiency and tyramine yield were simultaneously determined under the optimal conditions.

2. Materials and methods

2.1. Chemicals

Pyruvate fermentation broth (pH 6) as raw material was provided by Shandong Yangcheng Biotech Co. Ltd., (Shangdong, China). Pyruvate fermentation broth was only disposed by centrifugation and pyruvate content in the light yellow supernatant is 6.1% (m/v) by HPLC. Pyridoxal-5′-phosphate (PLP) was purchased from Sigma (St. Louis, MO, USA). All other chemicals and reagents used in this work were of analytical grade and purchased from Sinopharm Chemical Reagent Co. Ltd., (Shanghai, China).

2.2. Microorganisms and shake flask fermentation

According to the protocols established by Zhang et al. [29], all the enzyme genes were amplified by PCR and inserted into corresponding plasmids, and the recombinant cells were constructed by cloning plasmids into *Escherichia coli* BL21 (DE3). The tyrosine phenol-lyase gene from *Citrobacter koseri* was cloned using plasmid pETDuet as vector. Plasmid pET28a was utilized as vector to carry the tyrosine decarboxylase gene from *L. brevis*.

Based on the protocols developed by Zhang et al. [29], the recombinant cells were inoculated in LB medium and fermented in the sterilized fermentation media. Fermented broth was collected at the end of fermentation process and centrifuged at 2800× *g*, 4 °C for 10 min to obtain wet cells in the pellet, which were then lyophilized for a minimum of two days (Martin Christ GmbH, Germany) and stored at −20 °C.

2.3. Optimization for synthesis of L-tyrosine

The general procedure of synthesizing L-tyrosine was shown as follows: TPL cells (50 mg) were added to capped falcon tube and mixed with reaction mixture containing pyruvate fermentation broth (3%, m/v, 10 mL), 350 mM ammonium chloride, phenol (71 mM), 20 μL PLP (1%, m/v) and 20 μL Triton X-100 (10%, v/v). The remnant phenol was added at intervals of 1 h and total phenol reached 284 mM (4 × 71 mM). The reactions were carried out at pH 7, 37 °C and 200 rpm for 4 h after all of phenol was added. When the influential factors were investigated, the enzymatic activities were tested with varying each bioconversion condition one at a time. The influential factors including pH (6–11), temperature (25–60 °C), pyruvate substrate concentration (1.0–6.0%, m/v) and ammonium chloride concentrations (0–1500 mM) were optimized respectively. The effects of four kinds of surfactants on enzymatic activity, Triton X-100, Tween-80, cetyltrimethyl ammonium bromide (CTAB) and Emulsifier OP-10, were examined at pH 7 and 37 °C. Concentrations of Triton X-100, Tween-80, CTAB and OP ranged from 0 to 0.05% (v/v) respectively. All assays were performed in triplicates. TPL activity was determined by measuring L-tyrosine generated from the reaction by HPLC.

2.4. Optimization of L-tyrosine decarboxylation

2.4.1. Preparation of immobilized TDC cells

Based on the protocol of our lab [30], immobilized cells were prepared as follows: 2 g lyophilized cells were mixed with 100 mL sodium alginate water solution (3%, m/v), the mixture were stirred thoroughly and then extruded into 500 mL 2% (m/v) stirring $CaCl_2$ solution via a syringe to form calcium alginate beads from a 10 cm height. The beads were immersed in the $CaCl_2$ solution to harden for 5 h at 4 °C. The beads were washed with sodium acetate buffer (0.2 M, pH 5.5) three times and stored in buffer at 4 °C. The bead size varied with flow rates and always kept at 2.6 ± 0.2 mm. When the immobilized cells were used, they were taken out and blotted with tissue to remove the excess buffer on the surface of beads. The weight of immobilized beads for 2 g lyophilized cells was generally maintained at 38.6 ± 1.5 g.

2.4.2. Effect of pH, temperature and L-tyrosine load

TDC activity was detected by measuring tyramine via HPLC. When one influential factor was inspected, the other conditions were fixed.

TDC immobilized cells (2 g) were added to the reaction mixture (final volume, 10 mL) containing L-tyrosine, 20 μL PLP (1%, m/v), 20 μL TritonX-100 (10%, v/v). L-Tyrosine was added by five batches at intervals of 0.5 h. The reactions proceeded for 4 h after L-tyrosine was added completely. When L-tyrosine concentration was investigated, it was varied as follows: 0.3 g, 0.5 g, 0.7 g, 0.9 g, 1.1 g. The mixture was shaken at 50 rpm and 40 °C for determination of the optimal pH at pH 3, 4, 5, 5.5, 6, 7, 8 respectively. And then, the mixture was shaken at pH 5.5 for determination of the optimal temperature at 30 °C, 37 °C, 40 °C, 45 °C, 50 °C, 60 °C respectively. All of the reaction solutions were collected and determined by HPLC.

2.4.3. Comparison of enzymatic activity before and after immobilization

Enzymatic activities were determined to assess the difference between immobilized cells and free cells. L-Tyrosine (0.7 g), 20 μL PLP (1%, m/v) and 20 μL TritonX-100 (10%, v/v) were added into sodium acetate buffer (10 mL, pH 5.5) at 40 °C under the catalysis of 100 mg lyophilized free cells and 2 g immobilized cells respectively. L-Tyrosine was added by five batches at intervals of 0.5 h.

2.4.4. Repeated batch bioconversion for immobilized cells and free cells

The experiments were operated as follows: after each cycle of 6 h conversion, the beads and free cells were collected and washed with sodium acetate buffer (0.2 M, pH 5.5) three times, the catalysts were reused in a subsequent cycle and reacted with the substrates at the same conditions. Enzymatic activities of each cycle were detected by HPLC.

2.4.5. Immobilized biocatalyst stability testing

The stability of the immobilized biocatalyst for long-term storage at 4 °C in sodium acetate buffer (0.2 M, pH 5.5) was measured. The stability testing was performed in standard biotransformation at 2, 4, 6, 8, 12 and 14 weeks respectively. The immobilized