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Improving Soluble Expression of Tyrosine Decarboxylase from *Lactobacillus brevis* for Tyramine Synthesis with High Total Turnover Number

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Abstract

The soluble expression of tyrosine decarboxylase (TDC) in heterologous host is often challenging. Here, acidic condition was found to be favorable for improving the soluble expression of TDC from *Lactobacillus brevis* in *Escherichia coli*, while addition of carbohydrates (such as glucose, arabinose, and fructose) was vital for decreasing the insoluble fraction. By simple pH control and addition of glucose, the specific activity of TDC in crude extract was enhanced to 46.3 U mg^{-1}, 3.67-fold of that produced from LB medium. Optimization of the reaction conditions revealed that Tween-80 was effective in improving the tyramine production catalyzed by TDC, especially at high tyrosine loadings. As much as 400 mM tyrosine could be completely converted into tyramine with a substrate to catalyst ratio of 29.0 g g^{-1} and total turnover number of 23,300. This study provides efficient strategies for the highly soluble expression of TDC and biocatalytic production of tyramine.

Keywords Soluble expression · Tyrosine decarboxylase · Tyramine · Glucose · Tween-80

Introduction

Biogenic amines are natural compounds synthesized during normal metabolism of microorganisms, plants, and animals. Tyramine is a main metabolite of tyrosine and has important

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physiological functions and medicinal relevance. As a result, tyramine and its derivatives have been widely used in medicine, health products, and cosmetics industry, such as Bezafibrate for hyperlipidemia [1, 2], dopamine for Parkinson's disease [3], octopamine as sympathomimetic drug [4, 5], synephrine for prevention of type II diabetes [6, 7], and salidroside for immunity regulation and anti-fatigue [8, 9]. Tyramine could be produced through chemical methods employing photochemical decomposition, microwave-assisted deacylation, curtius rearrangement or reduction of hydroxyl mandelonitrile etc. [10–14]. However, enzymatic synthesis of tyramines has emerged as a promising alternative due to its high stereoselectivity, mild reaction conditions, environmental friendliness, and high catalytic efficiency.

Tyrosine decarboxylase (EC4.1.1.25, TDC) is a pyridoxal 5′-phosphate dependent decarboxylase, which could catalyze the decarboxylation of L-tyrosine into tyramine [15]. There are a number of tyrosine decarboxylases identified from microorganisms, plants, animals, and insects, such as *Papaver somniferum* [16, 17], *Rhodiola sachalinensis* [18, 19], *Phormia regina* [20], as well as *Methanocaldococcus jannaschii* [21], *Enterococcus* sp. [22] and *Lactobacillus brevis* [23] etc. The specific activity of TDC purified from different sources falls in the range of 3.5×10^{-5}–998 U mg^{-1} [17–23], and the activity of TDC from bacterial origin is significantly higher than that of TDC derived from plant and archaea. However, the enzyme production capacity of wild-type bacteria is limited, and the purification of TDC from wild-type bacteria is complicated and the recovery rate is low. Borresen and Moreno-Arribas had purified TDC from *Enterococcus faecalis* and *Lactobacillus brevis* with a viable recovery rate of 3.4 and 24%, respectively [22, 24]. Heterogeneous overexpression of TDC encoding genes by platform bacteria is regarded as a promising strategy for producing TDC at high level. Various TDC coding genes from different sources have been heterogeneously expressed in *Escherichia coli* however with little soluble fraction due to the formation of inclusion body, even lower than that produced by wild-type strains. Kezmarsky et al. had successfully expressed *tdc* of *Methanococcus jannaschii* in *E. coli* with a specific activity of 1.1 U mg^{-1} [21]. Many TDC coding genes, such as *tdc* of *Rhodiola rosea*, were mainly expressed as inclusion bodies in *E. coli* [25]. Similar phenomenon was also found in other decarboxylases, such as lysine decarboxylase. Refolding of inclusion bodies is one solution, however, usually suffered from low recovery rate and inevitably great loss of enzyme activity. In summary, the soluble expression of TDC is still challenging. To achieve of highly soluble and economic expression of TDC is critical for further application in industrial biosynthesis of tyramine.

In our previous study, a TDC coding gene has been identified from *Lactobacillus brevis*, and heterologously expressed in *E. coli* [26]. However, the soluble fraction of recombinant TDC was low. In spite of various strategies were attempted including codon optimization, promoter optimization, and adoption of *E. coli* Rosseta etc., the soluble expression level of *tdc* was still very low. It was interesting to note that the addition of 1% glucose in the medium resulted in the increase of soluble expression, which was speculated to be ascribed to the repression of glucose on the *lac* operon. We thus carried out systematic optimization to get insight into the possible mechanism of the positive effect, then rationally improve the enzyme production and specific activity of TDC in an economic way. Various factors including IPTG, metabolites, different carbohydrates, and molecular chaperones which might influence the expression status of TDC were investigated and compared. The production and specific activity of TDC were remarkably improved under the optimized condition. Furthermore, the biotransformation of tyrosine was also optimized for efficient synthesis of tyramine with high substrate to catalyst ratio and total turnover frequency.

Materials and Methods

Chemical Reagents, Plasmids, and Strains

Tryptone and yeast extract were purchased from Oxoid. Carbohydrates including glucose, fructose, arabinose, etc. were purchased from Sinopharm Inc. Pyridoxal-5′-phosphate (PLP) was purchased from Sigma. Plasmid extraction kits, gel extraction kits, etc. were purchased from Generay biotechnology. All chemical reagents are of analytical grade. Plasmids including pET24a, pET21a, and pQE80L were used for cloning of genes encoding for tyrosine decarboxylase and chaperones. *Escherichia coli* BL21(DE3) was employed for the expression of recombinant tyrosine decarboxylase.

Strain Cultivation and Protein Expression

Recombinant *E. coli* BL21(DE3) with pET24a-*tdc* was inoculated into LB medium supplemented with 50 μg mL^{-1} kanamycin and other ingredients including carbohydrates and metabolites. Strains were cultivated at 37 °C and 180 rpm until OD_{600} reached 0.6, and then, 0.2 mM isopropyl-β-D-thiogalactopyraniside (IPTG) was added for induction at 25 °C for 6 h. Afterwards, the cells were harvested by centrifugation at 8000×*g* for 10 min, washed with physiological saline, and resuspended in phosphate sodium buffer (pH 6.0, 20 mM), then disrupted by high-pressure homogenizer (ATS BASIC-II, Shanghai). The cell lysate was centrifuged at 12,000×*g* and 4 °C for 10 min to remove the cell debris. The resultant supernatant and precipitant were analyzed by SDS-PAGE.

Tyrosine Decarboxylase Activity Determination

Decarboxylase activity of TDC toward tyrosine was determined as previously reported [26]. The assay mixture was 1 mL and consisted of 2.0 mM L-tyrosine, 0.02 mM PLP and appropriate amount of enzyme in sodium acetate buffer (pH 5.0, 200 mM), and was conducted at 40 °C for 10 min and terminated by boiling at 100 °C for 10 min. Tyrosine and tyramine were quantified by HPLC (Agilent 1260) equipped with Diamonsil C18 column (150 mm × 4.6 mm × 5 μm, DIKMA) using water/methanol/trifluoroacetic acid (90/10/0.1) at 1.0 mL min^{-1} and 30 °C, and detected at 220 nm. One unit of activity was defined as the amount of enzyme that required for the production of 1.0 μmol tyramine per minute under above condition. All assay was performed in triplicate.

Quantitative PCR

Recombinant *E. coli* harboring pET24a-TDC was cultivated in LB medium supplemented with or without 1.0% glucose, fructose, arabinose, and mannose, and for quantitative analysis of chaperone expression level. Total RNA for quantitative PCR (qPCR) was isolated from cultures with and without pretreatment of 1% glucose and other aforementioned carbohydrates using the Simply P Total RNA Extraction Kit (BioFlux, Japan). Reverse transcription PCR step was carried out using Rayscript cDNA Synthesis Kit (Generay, China) with random primer mixture. The cDNA product was diluted into 50-fold for quantitative PCR (qPCR). qPCR was performed on the LightCycler®480 System

using 2 μL of diluted cDNA, SYBR® Premix Ex Taq II (Takara, Japan), and 0.4 μM primers. 16S rRNA gene was used as the housekeeping gene in qPCR. qPCR procedure was set as follows: 95 °C for 30 s, 40 cycles of 95 °C for 5 s, and 55 °C for 30 s. $2^{-\Delta\Delta Ct}$ method was adopted to evaluate the fold change in gene expression level. All assay was performed in triplicate.

Cloning and Co-expression of Molecular Chaperones

Various molecular chaperone genes identified in quantitative PCR were amplified by PCR and ligated into pQE80L by Exanse II (Vazyme, Nanjing). TDC coding gene was sub-cloned from pET24a into pET21a. The recombinant plasmids pET21a-*tdc* and pQE80L with chaperone genes were transformed into *E. coli* BL21(DE3) and screened against ampicillin and kanamycin, to achieve the co-expression of TDC and molecular chaperones. All the recombinant strains were inoculated in LB medium and cultivated as above described. The expression level was determined by SDS-PAGE.

Fermentation and Preparation of Crude Enzyme Powder

Fermentation was conducted in a 5-L bioreactor with 20 g L^{-1} tryptone, 10 g L^{-1} yeast extract, 20 g L^{-1} NaCl, and 10 g L^{-1} glucose as medium. When the OD_{600} reached 6.0, 0.2 mM IPTG was added and further cultivated at 25 °C for 6 h. The cells were harvested by centrifugation, re-suspended, and disrupted as above described. The supernatant was lyophilized under vacuum to form the crude enzyme powder and stored at 4 °C for further use.

Effect of pH, Temperature, Co-solvents, and Organic Phases on the Decarboxylation of Tyrosine

Various factors that might influence the initial velocity and conversion of tyrosine decarboxylation were investigated. Reaction was performed in 10-mL reactor with 40 mM tyrosine, 0.05 g L^{-1} TDC, different reaction buffers (NaAc-HAc (200 mM, pH 4.5, 5.0, 5.5, 6.0), Na_2HPO4-KH_2PO_4 (200 mM, pH 5.0, 5.5, 6.0), and Na_2HPO_4-NaH_2PO_4 (200 mM, pH 6.0, 6.5, 7.0, 7.5)), temperatures (25, 30, 35, 40, and 45 °C), 5% (*v*/*v*) surfactants (PEG200, PEG400, Triton X-100, Tween-20, Tween-60, Tween-80, CTAB, TBAB, and *β*-hydroxycyclodextrin), and/or 10% (*v*/*v*) organic solvents (toluene, butyl acetate, cyclohexane, n-heptane, isooctanol, 1-octanol, and MTBE). At 0.5 and 6 h, samples were withdrawn to determine the initial velocity and conversion by HPLC.

Biotransformation of Tyrosine for the Synthesis of Tyramine

For the preparation of tyramine, 25-mL reaction was performed with 100 to 400 mM tyrosine, 0.125 to 2.5 g L^{-1} TDC, Na_2HPO_4-KH_2PO_4 buffer (pH 5.5, 200 mM) at 40 °C and 120 rpm. After reaction, taking 1 mL of the reaction solution was centrifuged at 12,000×*g* for 10 min to remove reaction impurities. All these samples were filtered through Millipore filtering membrane (0.22-μm pore size) before HPLC analysis.

Results and Discussion

Factors Influencing the Soluble Expression of Tyrosine Decarboxylase

It is widely accepted that the existence of glucose in the medium might lead to the repression effect on *lac* operon and further result in reduced and slow expression of recombinant proteins, which is favorable for the correct folding of TDC from *L. brevis*. As a result, the IPTG concentrations for induction of *tdc* were reduced to 0.01 mM in order to lower the transcriptional level and allow correct folding of recombinant TDC. According to Fig. 1a, the specific activities of TDC under 0.01, 0.05, and 0.20 mM IPTG were 1.38, 1.95, and 1.92 U mg^{-1} crude extract, respectively. Similar activities under different IPTG were in coincidence with the similar soluble expression level (Fig. 1a). However, the insoluble parts significantly varied with the different IPTG concentrations (Fig. S1). The increased addition of IPTG resulted in the increased inclusion bodies and had little influence on the soluble expression level. It can also be concluded that the improved soluble expression level of TDC was not caused by the repression effect of glucose on *lac* operon. Meanwhile, the result suggests that 0.05 mM IPTG was enough for the induction and expression of TDC.

Furthermore, the effect of different glucose concentrations on the soluble expression of TDC was also investigated and illustrated in Fig. 1b and Fig. S2. The addition of glucose apparently influenced the expression level of TDC. The soluble fraction of TDC increased with glucose concentration at 1.0–2.5 g L^{-1} and decreased at 2.5–20 g L^{-1} (Fig. 1b). Recombinant TDC exhibited the highest specific activity at 2.5 g L^{-1} glucose, which was 2.60-fold of the control and 10% higher than that at 10 g L^{-1} glucose, which was adopted in our previous study [26]. It is interesting to note that the insoluble parts were decreased along with the increase of glucose. At 10 or 20 g L^{-1} glucose, almost none inclusion bodies was found. And 2.5 g L^{-1} was selected as the optimum glucose loading for the highly soluble expression of TDC.

Different metabolites, such as tyrosine, glutamate, arginine, acetate, lactate, succinate, and pyruvate, were also investigated for their effects on the soluble expression level of TDC (Fig. S3). The activity of TDC was improved with the addition of tyrosine, acetate, succinate, and pyruvate (Fig. 1c). Naturally, tyrosine decarboxylase is evolved to resist against acidic environment by producing basic tyramine [27]. The addition of tyrosine could cause a reduction in pH of medium. After induction of TDC expression, tyrosine was converted into tyramine, then led to increase of pH. Several metabolic intermediates in the carbon utilization such as acetate, succinate, and pyruvate were found to be important for the soluble expression of TDC. And TDC displayed the highest relative activity of 236% when supplemented with acetate; the insoluble part was, however, not decreased (Fig. 1c), which was different from the result of glucose.

Then, various carbohydrates including fructose, arabinose, lactose, mannose, starch, sucrose, xylose, glycerol, and sorbitol were supplemented into LB medium to test their effect on the expression of TDC (Fig. 1d and Fig. S4). Besides glucose, other sugars including fructose, arabinose, and mannose could also improve the soluble expression of TDC, with relative activities of 207, 257, and 214%, respectively. The highest soluble expression level and specific activity of TDC were observed in the presence of arabinose. Only little insoluble parts were found in LB medium supplemented with fructose and arabinose, while similar

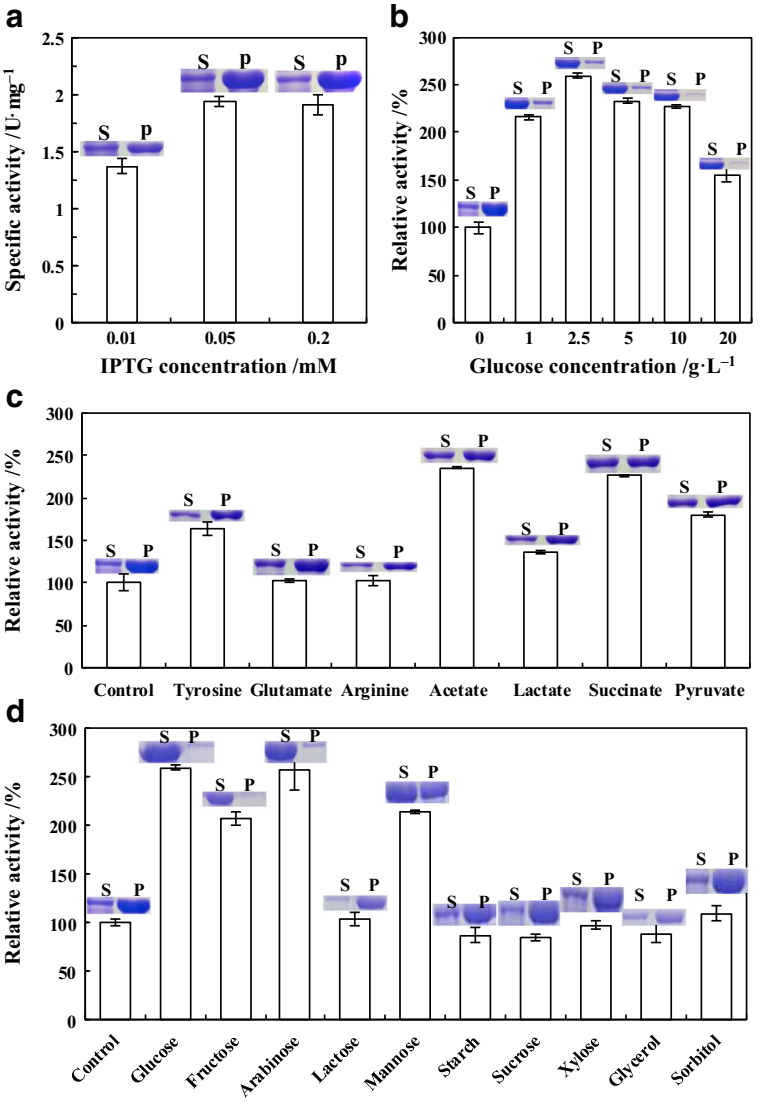

Fig. 1 Factors influencing the soluble expression of TDC. **a** IPTG, **b** glucose concentrations, **c** LB medium supplemented with 5 mM metabolites, **d** LB medium supplemented with 2.5 g L^{-1} carbohydrates. SDS-PAGE analysis results were shown above the column, S: supernatant, P: precipitant

amount of soluble and insoluble parts was detected in mannose (Fig. 1d). Addition of starch, succinate, xylose, and sorbitol led to the dramatically improved insoluble expression. To understand the mechanisms behind the positive effect of carbohydrates, the cell growth, pH, and residual sugars were monitored in LB medium supplemented with 2.5 g L^{-1} glucose, arabinose, mannose, and xylose. As shown in Fig. 2a, the pH of LB medium was increased from 7.0 to 7.5 after 6 h of induction, and the OD_{600} could reach 3.74. While in the LB medium supplemented with glucose, the pH was decreased from 6.6 to 5.5 and the OD_{600} was 2.92 (Fig. 2b), lower than that of LB medium due to the acidic condition caused by the glucose metabolism. The residual sugar quickly decreased in the first 3 h and stabilized afterwards.

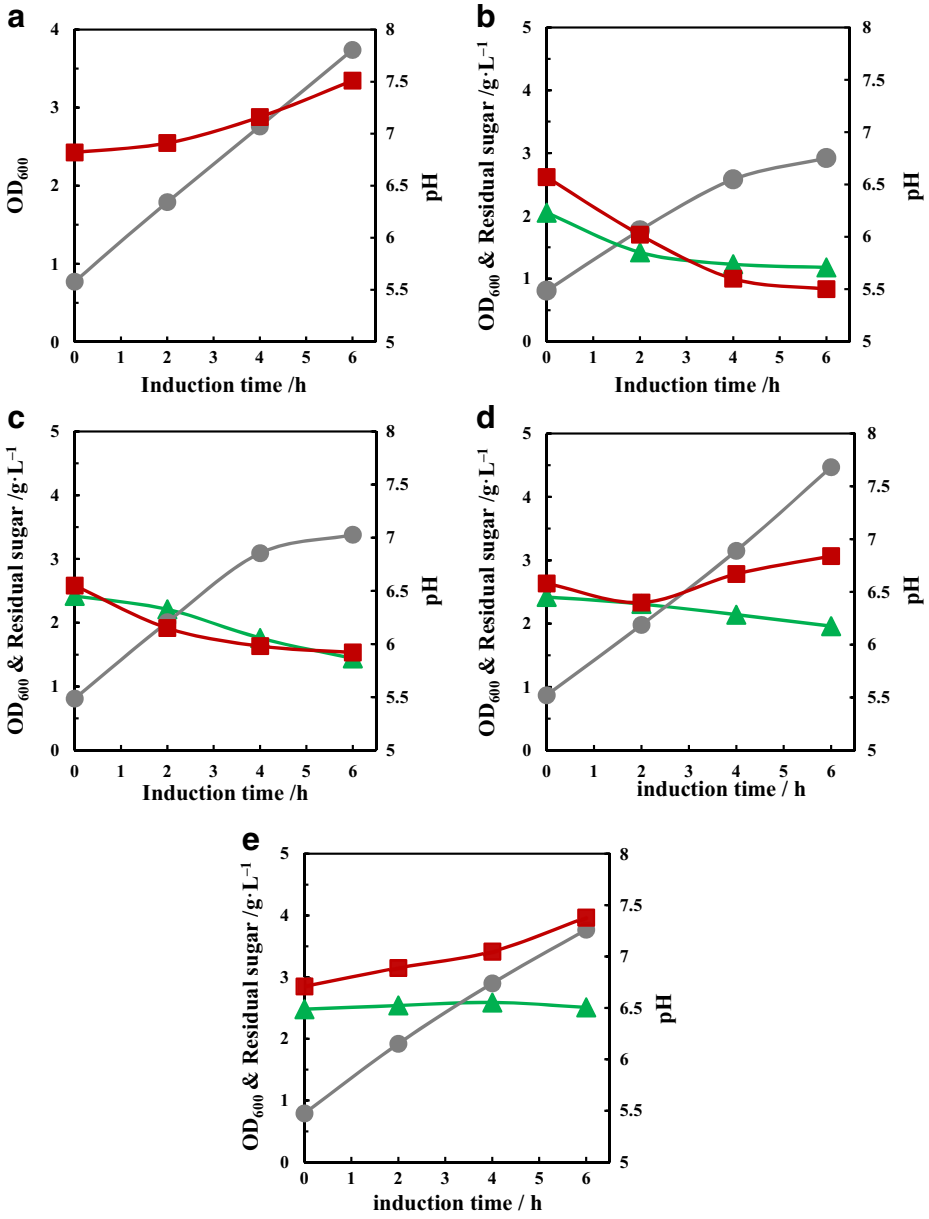

Fig. 2 Growth, pH, and residual sugar curves of recombinant *E. coli* harboring TDC cultivated in LB medium supplemented with different carbohydrates. **a** LB medium, **b** LB medium with 2.5 g L^{-1} glucose, **c** LB medium with 2.5 g L^{-1} arabinose, **d** LB medium with 2.5 g L^{-1} mannose, **e** LB medium with 2.5 g L^{-1} xylose. (●) OD_{600}, (■) pH, (▲) residual sugar

Similar phenomenon was also observed with arabinose (Fig. 2c). The pH and OD_{600} reached 5.92 and 3.38. While the mannose was slowly utilized by *E. coli*, and the OD_{600} increased to as high as 4.47, 0.73 higher than control. However, the pH was decreased in the first 2 h, and then

increased to 6.84 (Fig. 2d). With regard to xylose, it could not be utilized by *E. coli* during the cultivation. As a result, the pH increased from 6.71 to 7.38, and the OD_{600} was increased from 0.79 to 3.77, which was similar to the LB medium (Fig. 2e). Most of the recombinant TDC was located in the precipitants. According to the SDS-PAGE, recombinant TDC displayed the highest solubility in glucose and arabinose, and also, little inclusion bodies were found (Fig. S4), which was in coincidence with the activity analysis (Fig. 1d). It is speculated that the inclusion bodies were transformed into soluble fraction. It has been widely accepted that TDC is naturally responsible for the biosynthesis of tyramine from tyrosine in order to resist against the acidic conditions [27]. Here, pH is presumed to be a key factor influencing the soluble expression of TDC, since improved soluble expression was observed in all experiments with pH less than 7.0. However, the soluble fraction of TDC in mannose was as high as the insoluble part (Fig. S4). In addition, due to the lower pH, the OD_{600} in arabinose and glucose was also lower than that in control, mannose, and xylose. Another control experiment was performed with LB medium and maintaining at acidic condition (Fig. S5(A)). The soluble part was increased, along with the increase of insoluble part, which was different from the effect of glucose and arabinose. It can be proposed that carbohydrates have double (or multiple) roles in manipulating the soluble and insoluble expression of TDC. The glucose or arabinose led to the decreasing of insoluble part, and the lower pH caused by metabolites led to the increase of soluble fraction.

It has been reported that molecular chaperones, including IbpA, IbpB, GroEL, GroES, ClpB, DnaK, DnaJ, GrpE, HscC, HcHA, HslO, and Tig, mainly participate in assisting the correct folding of proteins in *E. coli* [28]. It was supposed that the sugars might activate some of them and prompted the soluble expression of TDC. To acquire in-depth knowledge of the potential mechanism, the fold changes in the transcription level of these molecular chaperones with or without addition of glucose, fructose, arabinose, and mannose were determined by quantitative PCR. As illustrated in Fig. S6, chaperones IbpA, IbpB, GroES, HslO, and Tig were differentially expressed in all the tested carbohydrates with 1.11–4.95-fold enhancements. Co-expression of above five molecular chaperones and TDC in *E. coli* was also conducted. However, the soluble expression of TDC was not improved apparently by the expression of these individual chaperones. Multiple chaperones might contribute to the soluble expression of TDC as reported [29].

Fed-Batch Fermentation of TDC

Based on the important roles of pH and carbohydrates, the fermentation was performed to prepare recombinant TDC for the preparation of tyramine. As mentioned above, the acidic condition was favorable for the soluble expression of TDC, while the insoluble part was also increased. In addition, lower pH (< 6.5) was not suitable for the growth of *E. coli*. As a result, fed-batch fermentation of TDC was carried out in both LB and LB medium supplemented with glucose (LBG medium) (Fig. 3), and the pH was maintained at pH 6.8. The growth curves of recombinant *E. coli* in LB and LBG were similar. After about 2 h of lagging phase, the growth entered exponential phase and the highest OD_{600} could reach 15.9 and 13.6, respectively. When the OD_{600} achieved 6.0, 0.2 mM IPTG was added to induce the expression of TDC. The production of TDC in LBG medium was 30.7-kU L^{-1} fermentation broth, higher than 22.0 kU L^{-1} in LB medium. After cell disruption and freeze drying, the specific activities of TDC produced in LB and LBG medium were calculated to be 12.6 and 46.3 U mg^{-1} lyophilized crude enzyme powder, respectively. According to the SDS-PAGE in Fig. S5, some

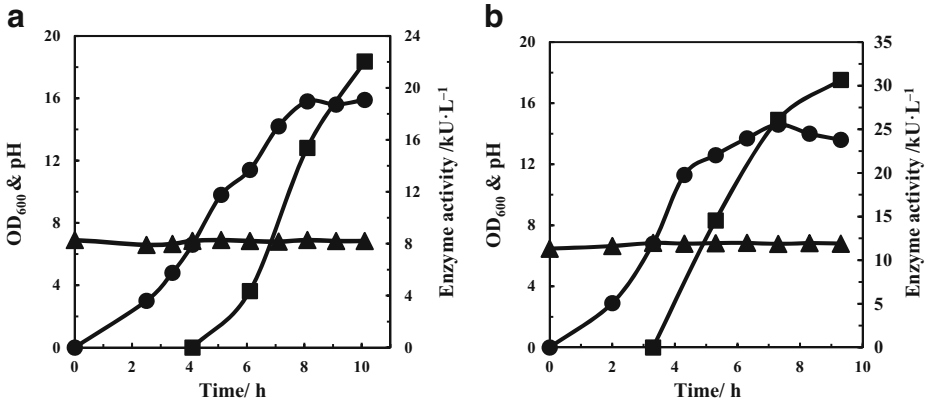

Fig. 3 Fed-batch fermentation of TDC in **a** LB medium and **b** LBG medium. (▲) pH, (●) OD_{600}, (■) enzyme activity

inclusion bodies were produced in LB medium at pH 6.8, while most TDC was produced as soluble protein in LBG medium under the same condition. The total enzyme production and specific activity of TDC were improved by 1.40- and 3.67-fold through simple addition of glucose and maintaining the pH at acidic condition.

Optimization of Decarboxylation Reaction Catalyzed by TDC

Various factors including reaction buffer, temperature, surfactants, and organic phases were evaluated for their effects on initial reaction rate and conversion ratio in the decarboxylation of tyrosine catalyzed by TDC. Various buffer including NaAc-HAc (200 mM, pH 4.5–6.0), Na_2HPO_4-KH_2PO_4 (200 mM, pH 5.0–6.0), and Na_2HPO_4-NaH_2PO_4 (200 mM, pH 6.0–7.5) were selected as shown in Fig. 4a. In NaAc-HAc buffer, the highest initial reaction rate was reached at pH 5.5. However, the conversion was lower than that in Na_2HPO_4-KH_2PO_4 buffer (pH 5.5). TDC displayed higher activity in NaAc-HAc buffer, whereas higher stability in

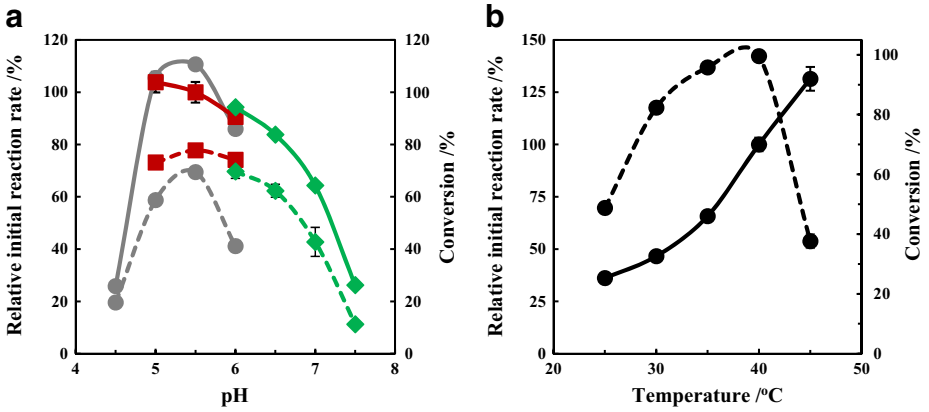

Fig. 4 Effect of reaction buffers and temperatures on the decarboxylation of tyrosine catalyzed by TDC. **a** Reaction buffer: (●) NaAc-HAc buffer, (■) Na_2HPO_4-KH_2PO_4 buffer, (◆) K_2HPO_4-KH_2PO_4 buffer, (dotted line) conversion ratio, (solid line) relative initial reaction rate. **b** Temperature: (dotted line) conversion rate, (solid line) relative initial reaction rate

Na_2HPO_4-KH_2PO_4 buffer, which accounted for the decreased conversion ratio in NaAc-HAc. Along with the increase of reaction pH to 7.5, the initial reaction rate and conversion ratio decreased. Different temperatures including 25, 30, 35, 40, and 45 °C were also investigated (Fig. 4b). The initial reaction rate increased with the increasing temperature, while the highest conversion was obtained at 40 °C. As a result, Na_2HPO_4-KH_2PO_4 (pH 6.5) and 40 °C were determined to be the optimum conditions for the preparation of tyramine.

Due to the low solubility of tyrosine, various surfactants including PEG200, PEG400, Triton X-100, Tween-20, Tween-60, Tween-80, cetyltrimethyl ammonium bromide (CTAB), tetrabutylammonium bromide (TBAB), and hydroxy-β-cyclodextrin (HCD) were evaluated at 40 mM substrate loading (Fig. 5a). The CTAB has severe inhibitory effect on the decarboxylation reaction. Although the TBAB displayed a positive effect in enhancing the initial reaction rate, the conversion was only 88.5%. The highest conversion was found with Tween-80, with relative initial reaction rate of 108%. PEG200, Tween-60, and HCD could also promote the reaction rate to some extent. Furthermore, different organic phases were also

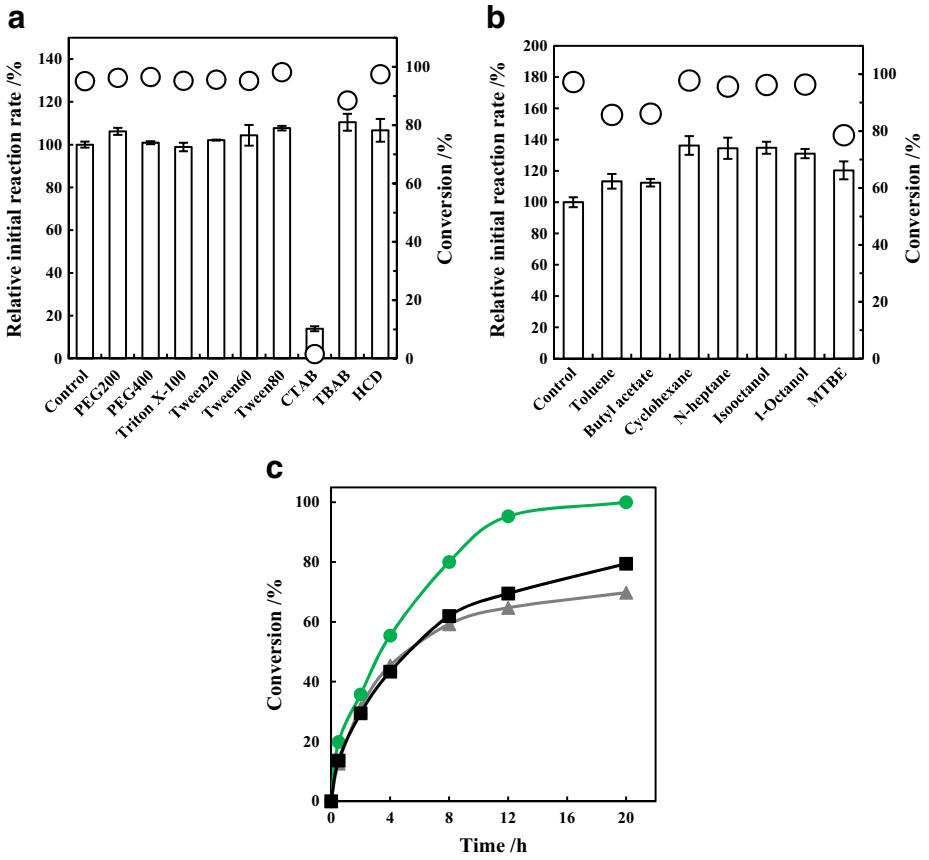

Fig. 5 Effect of surfactants and organic solvents on the decarboxylation of tyrosine catalyzed by TDC. **a** Surfactants: (□) relative initial reaction rate, (○) conversion rate. **b** Organic solvents (□) relative initial reaction rate, (○) conversion ratio. **c** Bioconversion curves of 100 mM tyrosine: (▲) aqueous phase, (●) Tween-80, (■) 1-octanol and Tween-80. Reaction was performed in 25-mL reaction mixture consisted of 100 mM tyrosine, 0.125 g L^{-1} TDC in Na_2HPO_4-KH_2PO_4 buffer at 40 °C and 120 rpm

investigated, including toluene, butyl acetate, cyclohexane, n-heptane, isooctanol, n-octanol, and methyl tert-butyl ether (MTBE) (Fig. 5b). The initial reaction rate was improved in most organic phases compared with the control; however, the final conversion ratio was similar or even lower than the control, due to the deactivation of TDC by organic solvents. However, due to the low solubility of tyrosine, the effect of surfactants and organic phases might prompt the reaction at higher substrate loadings. As a result, Tween-80 and 1-octanol were further evaluated at 100 mM tyrosine. As shown in Fig. 5c, only 69.8 mM tyramine was produced at 20 h, while tyrosine was completely transformed into tyramine with the assistance of 5% Tween-80. However, the addition of 10% 1-octanol in the reaction mixture with 5% Tween-80 resulted in the decreased conversion to 79.5%. In view of high initial reaction rate and conversion at high substrate loading, Tween-80 could reduce the mass-transferring resistance caused by the low solubility of tyrosine and was chosen as the best surfactant.

Biotransformation of Tyrosine into Tyramine

Different substrate loadings were attempted in the biotransformation of tyrosine into tyramine. Initially, 100 mM tyrosine was fully converted with 0.125 g L^{-1} TDC (lyophilized crude enzyme) within 20 h. The substrate to catalyst ratio (S/C) was calculated to be 145 g g^{-1}. When calculating the total turnover number (TTN), we convert the specific activity of crude enzyme (46.3 U mg^{-1}) into purified enzyme (96 U mg^{-1}) based on the total activity in the reaction system. And the TTN was calculated to be 116,000. When substrate and catalyst loadings were increased to 200 mM tyrosine and 0.5 g L^{-1} TDC (lyophilized crude enzyme), conversion of > 99.9% was achieved within 24 h, with S/C of 72.5 g g^{-1} and TTN of 58,200 (Fig. 6a). Higher tyrosine loadings could lead to deactivation of TDC, as well as compromised mass-transfer efficiency due to the low solubility of tyrosine. When the substrate loading was further increased to 400 mM, about 2.5 g L^{-1} TDC (lyophilized crude enzyme) was required to achieve > 99.9% conversion within 24 h, with S/C of 29.0 g g^{-1} and TTN of 23,300 (Fig. 6b). It has been reported that 70 g L^{-1} tyrosine could be fully converted into tyramine employing 5 g L^{-1} TDC (wet whole cell) with S/C of 14 g g^{-1} [30]. However, when tyrosine loading

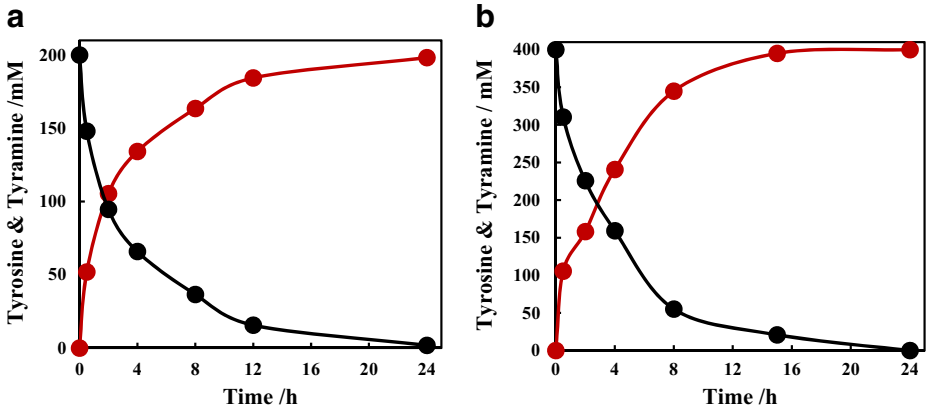

Fig. 6 Biotransformation of tyrosine into tyramine catalyzed by TDC at different substrate loading. **a** 200 mM tyrosine, **b** 400 mM tyrosine. (●) concentration of tyramine, (●) concentration of tyrosine. Reaction was performed in 25-mL reaction mixture consisted of 200 or 400 mM tyrosine, 0.5 or 2.5 g L^{-1} TDC in Na_2HPO_4-KH_2PO_4 buffer at 40 °C and 120 rpm

increased to 90 g L^{-1}, the conversion was slightly reduced to 98%. In summary, tyrosine could be efficiently decarboxylated into tyramine employing TDC in this study. In comparison with reported biotransformation strategies for the preparation of tyramine, the highly soluble recombinant TDC from *Lactobacillus brevis* displayed the highest S/C and TTN.

Conclusion

In this study, various factors influencing the soluble expression of tyrosine decarboxylase were investigated. The acidic condition was favorable for the soluble expression of TDC, while the addition of glucose, arabinose, or fructose could reduce the insoluble expression of TDC. Molecular chaperones Tig, IbpA, IbpB, GroES, and HslO exhibited higher transcription levels under glucose, arabinose, fructose, or mannose and might participate in the correct folding of TDC. Highly soluble expression of TDC was achieved by pH control at acidic condition and addition of glucose. The specific activity of TDC in LBG medium was 46.3 U mg^{-1}, 3.67-fold of that produced in LB medium. Tween-80 is beneficial to the biotransformation especially at high tyrosine concentration. As much as 400 mM tyrosine could be completely converted into tyramine with substrate to catalyst ratio of 29.0 g g^{-1} and total turnover frequency of 23,300. This study provides feasible strategies for the highly soluble expression of TDC and efficient preparation of tyramine from tyrosine employing recombinant TDC.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflicts of interest.

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