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Tyrosine decarboxylase from *Lactobacillus brevis*: Soluble expression and characterization

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ABSTRACT

Tyrosine decarboxylase (TDC, EC 4.1.1.25) is an enzyme that catalyzes the decarboxylation of L-tyrosine to produce tyramine and CO_2. In this study, a 1881-bp *tdc* gene from *Lactobacillus brevis* was cloned and heterologously expressed in *Escherichia coli* BL21 (DE3). Glucose was discovered to play an important role in the soluble expression of rLbTDC. After optimization, recombinant TDC (rLbTDC) was achieved in excellent solubility and a yield of 224 mg rLbTDC/L broth. The C-terminal His-Tagged rLbTDC was one-step purified with 90% recovery. Based on SDS–PAGE and gel filtration analysis, rLbTDC is a dimer composed of two identical subunits of approximately 70 kDa. Using L-tyrosine as substrate, the specific activity of rLbTDC was determined to be 133.5 U/mg in the presence of 0.2 mM pyridoxal-5′-phosphate at 40 °C and pH 5.0. The K_m and V_{max} values of rLbTDC were 0.59 mM and 147.1 μmol min^{-1} mg^{-1}, respectively. In addition to L-tyrosine, rLbTDC also exhibited decarboxylase activity towards L-DOPA. This study has demonstrated, for the first time, the soluble expression of *tdc* gene from *L. brevis* in heterologous host.

Introduction

Tyrosine decarboxylase (TDC)[1], is a member of group II pyridoxal-5′-phosphate (PLP)-dependent decarboxylase catalyzing the decarboxylation of L-tyrosine to tyramine [1] (Fig. 1). Tyramine is a naturally occurred amine which acts as an indirect sympathomimetic in mammals [2]. Tyramine is also a biochemical precursor for a number of key neurotransmitters, including synephrine [3], salidroside [4], epinephrine [5], octopamine [6], and hordenine [7] (Supplementary Fig. S1).

TDC has been identified in several insects and plants, such as *Arabidopsis thaliana* [8], *Papaver somniferum* [9,10], *Rhodiola sachalinensis* [4,11], *Phormia regina* [12], and *Drosophila melanogaster* [13], as well as some microorganisms including *Methanocaldococcus jannaschii* [14], *Lactobacillus brevis* [15] etc. Previous studies showed that activity of microbial TDCs is much higher than those from plants or insects, among which TDC purified from *Lb. brevis* IOEB 9809 gave the highest enzyme activity (V_{max} of 998.6 μmol min^{-1} mg^{-1} protein) [15]. So far, a few *tdc* genes originated from plants have been functionally expressed in heterologous hosts, including *A. thaliana* [8], *P. somniferum* [9], *R. sachalinensis* [11] etc., and recombinant TDC from *A. thaliana* demonstrated the highest V_{max} of 3.68 μmol min^{-1} mg^{-1} protein. In Peng's study, TDC from *Rhamnus crenulata* was expressed in *Escherichia coli* with an extremely low solubility [16]. The only successful report on recombinant expression of microbial *tdc* gene is that from *M. jannaschii*, characterized by K_m of 1.6 mM and a rather low specific activity of 1.1 U/mg [14]. Consequently, the heterologous expression of *tdc* genes has been a challenging task. It is speculated that the toxicity of tyramine (catalytic product of TDC) to the host cells is mainly responsible for the observations.

In this study, highly soluble expression of *tdc* from *Lb. brevis* was achieved in *E. coli* BL21 (DE3) by adding 1% glucose. After optimization, a maximum recombinant TDC (rLbTDC) production of 224 mg/L was reached. Using L-tyrosine as substrate, rLbTDC exhibits a K_m of 0.59 mM and V_{max} of 147.1 μmol min^{-1} mg^{-1} protein. Additionally, rLbTDC also catalyzes the decarboxylation of L-DOPA (L-3,4-dihydroxyphenylalanine) to produce dopamine, a key reaction in the treatment of Parkinson's disease. Moreover, rLbTDC has a much higher activity toward L-DOPA (58.6 U/mg) compared with DOPA decarboxylases (DDC) from *Ceratitis capitata* (9.4 U/mg) [17] and rat (7.5 U/mg) [18]. This work allows crystallization of rLbTDC and its protein structure–function relationship study. Importantly, it could provide molecular basis for developing efficient therapeutical approaches for Parkinson's disease.

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[1] *Abbreviations used:* TDC, tyrosine decarboxylase; PLP, pyridoxal-5′-phosphate; rLbTDC, recombinant TDC; L-DOPA, L-3,4-dihydroxyphenylalanine; DDC, DOPA decarboxylases; LB, Luria–Bertani; IPTG, isopropyl β-D-1-thiogalactopyranoside; CBB, Coomassie Brilliant Blue; pI, isoelectric point; ORF, open reading frame; LbTDC, TDC from *Lb. brevis*; GAD, 3 glutamate decarboxylase; AADC, aromatic L-amino acid decarboxylase; SeMet, selenomethionine-substituted.

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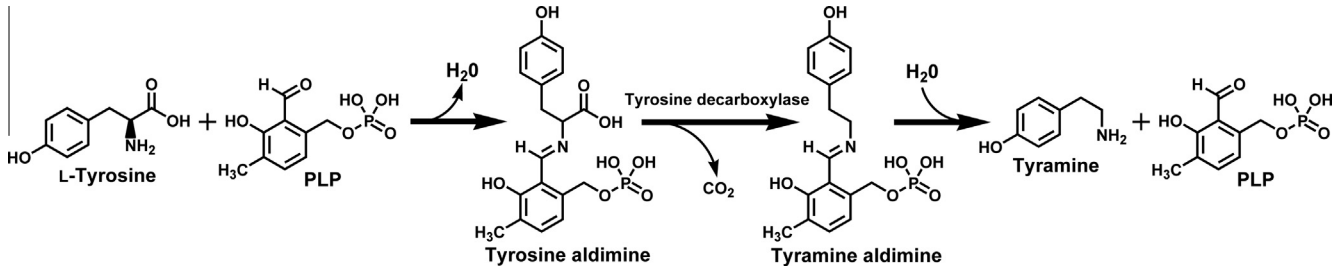

Fig. 1. Reaction catalyzed by tyrosine decarboxylase.

Methods

Cloning and sequence analysis of TDC

To amplify *tdc* gene from genomic DNA of *L. brevis* CGMCC 1.2028, two primers were designed based on the nucleotide sequence of *tyrdc* (GenBank No. EU195891.1) [19]. Forward primer tdc-F is: 5′-CTAGCTAGCATGGAAAAAAGTAATCGCTCAC-3′ (*Nhe* I site underlined); reverse primer tdc-R is: 5′-CCGCTCGAGAACATTTTCCTTTTGATTAAC-3′ (*Xho* I site underlined). PCR amplification was performed using *Ex-Taq* DNA polymerase (TaKaRa, Japan) on a PTC-200 thermocycler (Bio-Rad, USA), under a temperature profile of 94 °C for 10 min, followed by 30 cycles of 94 °C for 1 min, 57 °C for 1 min and 72 °C for 2 min, and a final extension at 72 °C for 10 min.

The PCR fragment of *tdc* gene was inserted into pMD18-T vector (TaKaRa, China) to create recombinant plasmid pMD18-tdc, and the nucleotide sequence of *tdc* was determined by Sangon Biotech Co. (Shanghai, China).

Recombinant expression of LbTDC

To facilitate the purification procedure, pET-24a (Novagen, USA) was selected as the expression vector to incorporate a His-Tag at C-terminal of recombinant TDC. *E. coli* BL21 (DE3) was used as the expression host.

Both plasmids pMD18-tdc and pET-24a were double-digested with *Nhe* I and *Xho* I (TaKaRa, China), and then, the resulted *tdc* gene and linearized pET-24a fragments were ligated by T4 DNA ligase (TaKaRa, China) to generate plasmid pET24-tdc, which was then transformed into *E. coli* BL21 (DE3). The positive clones were selected and confirmed by colony PCR and restriction enzyme digestion.

Recombinant *E. coli* BL21 (DE3) carrying pET24-tdc was cultured in a 250-mL flask containing 50 mL Luria–Bertani (LB) medium supplemented with 50 μg/mL kanamycin (Kan) at 37 °C. When OD_{600} reached 0.5, isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.2 mM for induction, and the culture medium was further incubated at 30 °C for 4 h before harvesting.

Enhancing soluble expression of rLbTDC

Recombinant *E. coli* strain was maintained in liquid or solid (2% agar) SOB medium supplemented with 50 μg/mL Kan and 1% glucose at 37 °C.

For protein expression, the recombinant *E. coli* strain was cultured in LBG (LB medium plus 1% glucose) medium containing 50 μg/mL Kan at 37 °C in a rotary shaker (200 rpm) for 12 h. The culture was then inoculated (2%, v/v) into same medium and incubated at 37 °C. Recombinant expression of rLbTDC was induced by 0.2 mM IPTG at 0.5 OD_{600}, and the temperature was shifted to 25 °C for its soluble expression.

The recombinant protein expression was also performed in a 3-L stirred tank bioreactor. The fermentation was started by inoculating 2% (v/v) overnight culture into 1 L LBG medium supplemented with 50 μg/mL Kan following by incubation under 37 °C with 1 vvm air flow and 300 rpm agitation. Expression of rLbTDC was induced with 0.2 mM IPTG when OD_{600} reached 1.0, and the temperature was shifted to 25 °C.

Protein purification and molecular weight of rLbTDC

Protein purification was carried out using an ÄKTA avant 25 system (GE Healthcare, Sweden) at 6 °C. Cells harvested by centrifugation (10,000*g*, 10 min) were resuspended in 25 mM Tris–HCl buffer (pH 7.4) followed by sonication in an ice bath. Cell debris was removed by centrifugation at 10,000*g* for 30 min at 4 °C. The supernatant was loaded onto a 5-mL HisTrap FF Crude column (GE Healthcare, Sweden), which had been pre-equilibrated with buffer A (25 mM Tris–HCl, pH 7.4, 20 mM imidazole, 300 mM NaCl). The recombinant protein was eluted with buffer B (25 mM Tris–HCl, pH 7.4, 280 mM imidazole, 300 mM NaCl) at a rate of 2 mL/min. The purity of the protein was confirmed by SDS–PAGE, followed by staining with Coomassie Brilliant Blue (CBB) R-250.

The molecular weight of rLbTDC was determined by Superdex 200 gel filtration column chromatography (10/300 GL, GE Healthcare, Sweden). The rLbTDC purified by Ni–NTA column was concentrated with Amicon Ultra-15 (30 kDa, Millipore, USA) to a final protein concentration of around 10 mg/mL, and then 0.5 mL protein solution was injected onto Superdex 200 column, and eluted with 1.5-column volume of buffer C (25 mM Tris–HCl, pH 7.4, 150 mM NaCl) at a rate of 0.5 mL/min. The middle fraction of rLbTDC peak was collected and used to determine its isoelectric point (pI) by PhastSystem (GE Healthcare, USA). All enzymatic properties of rLbTDC were determined using purified enzyme after gel filtration chromatography.

Enzymatic activity assay

The enzymatic activity of TDC was determined in a reaction mixture containing 5.5 mM L-tyrosine and 0.2 mM PLP in sodium acetate buffer (0.2 M, pH 5.0). To start the reaction, 10 μL enzyme solution (0.01–0.02 mg rLbTDC/mL) was added to the reaction mixture in a final volume of 1 mL. The reaction mixture was incubated at 40 °C for 10 min, and then placed in a boiling water bath for 10 min to inactivate the enzyme. After centrifugation (12,000*g*, 3 min) and filtration (0.22 μm), the sample was analyzed by HPLC.

HPLC analysis was performed with Shimadzu 20A system (Shimadzu, Japan) equipped with Diamonsil C18 column (5 μm, 250 × 4.6 mm, DIKMA, China). Methanol/water/acetic acid (10:90:0.01) was used as the mobile phase at a flow rate of 1 mL/min. An injection volume of 10 μL and DAD detection at 220 nm were used for all samples. One unit of rLbTDC activity was defined as the amount of enzyme required for the decarboxylation of 1.0 μmol substrate per min under above assay conditions.

The protein concentration was determined using Bradford protein assay kit (Sangon Biotech Co., Shanghai, China). Other substrates including L-DOPA, L-phenylalanine, L-tryptophan and L-glutamate were used to determine the substrate specificity of rLbTDC. And γ-amminobutyric acid (GABA, decarboxylation product of L-glutamate) was determined using pre-column derivatization method [20].

Effect of metal ions and EDTA was determined by pre-incubating the enzyme with various chemicals at a final concentration of 1 mM for 1 h at 4 °C, and the activity was measured under above assay conditions.

Dosage of coenzyme

Enzyme activity with respect to PLP dependence was also examined. Briefly, 10 μL enzyme solution (0.01–0.02 mg rLbTDC/mL) was added to reaction mixture (5.5 mM L-tyrosine, 0.2 M sodium acetate buffer, pH 5.0) in a final volume of 1 mL supplemented with PLP of 0–1.0 mM, and the reaction was incubated at 40 °C for 10 min.

Effect of pH and temperature on rLbTDC

Effect of pH on rLbTDC was determined in various buffers including 0.2 M sodium acetate buffer (pH 3.5–6.0), 0.2 M sodium phosphate buffer (pH 6.0–8.0), and 0.2 M Tris–HCl buffer (pH 8.0–9.0). For pH stability, purified enzyme was pre-incubated in buffers (0.2 M) with different pH values at 4 °C for 7 d. The residual activity was measured under assay conditions. Optimal temperature of rLbTDC was determined under standard conditions at 4–80 °C, and the reaction mixtures were pre-incubated under respective assay temperatures. Thermal stability was determined by pre-incubating the purified enzyme at 4–60 °C for 3 h followed by measuring the residual activity under assay conditions.

Kinetic parameters of rLbTDC

To determine the kinetic parameters such as K_m, V_{max} and k_{cat}, the activity of purified enzyme was determined at substrate concentrations of 0.2–8.0 mM under standard conditions. K_m and V_{max} were calculated using Lineweaver–Burk linear plot method.

Results and discussion

Nucleotide sequence and homology analysis of tdc

Nucleotide sequence of *tdc* from *Lb. brevis* CGMCC 1.2028 comprises an open reading frame (ORF) of 1881 bp encoding 626 amino acids. TDC from *Lb. brevis* (LbTDC) shares high amino acid sequence identities (99%, 76%, 73%, and 65%) with those from other microorganisms including *Lactobacillus plantarum* (GenBank No. AFQ52525.1), *Enterococcus faecium* (GenBank No. ELB45185.1), *Staphylococcus epidermidis* (GenBank No. EJE28330.1) and *Bacillus cereus* (GenBank No. EJQ77249.1) (Supplementary Fig. S2), while less than 20% homologies with those from plants and insects, such as *R. sachalinensis* (GenBank No. ABF06560.1), *D. melanogaster* (GenBank No. AAM70812.1). The nucleotide sequence of *tdc* from *Lb. brevis* CGMCC 1.2028 and its corresponding protein sequence have been deposited in GenBank under accession Nos. JX204286.1 and AFP73381.1.

Enhanced soluble expression of rLbTDC

In previous studies, the soluble expression of microbial *tdc* genes has been a highly challenging task. Peng reported the recombinant expression of *tdc* from *R. crenulata* in *E. coli* with an extremely low solubility, and most protein was formed as inclusion body which gave low activity recovery after renaturation [16]. Fan studied the cloning and expression of 3 glutamate decarboxylase (GAD) encoding genes from *Lb. brevis*. Among which, one GAD (GenBank No. ABJ65263.1), sharing 100% amino acid sequence identity with *tdc* in this work, showed no soluble expression even after optimization [21]. Our study on substrate specificity of rLbTDC however indicates that this "glutamate decarboxylase (ABJ65263.1)" is assumably an incorrect annotation since no decarboxylation activity was detected towards glutamate (in Section 3.5). In our preliminary studies, rLbTDC also showed little soluble expression in *E. coli* BL21 (DE3) (1.3 U/mL broth). Therefore, the induction conditions (such as IPTG concentration, temperature, time, etc.) were optimized to improve the soluble expression of rLbTDC. Whereafter, rLbTDC activity of 2.6 U/mL broth was attained, and appreciable amount of soluble rLbTDC was observed on SDS–PAGE (Fig. 2A, Lane 1). Nevertheless, majority of rLbTDC was expressed as insoluble inclusion bodies (Fig. 2A, Lane 3).

When using *E. coli* Rosetta (DE3) as expression host, solubility of rLbTDC was greatly improved. Under optimum conditions, no inclusion body was formed as confirmed by SDS–PAGE (data not shown). However, the expression level of rLbTDC was decreased to 1.9 U/mL broth, which is even lower than that in *E. coli* BL21 (DE3) host.

Unexpectedly, the inclusion body and low soluble expression issues were solved by addition of glucose to culture medium in this study (Fig. 2B, Lane 1). Almost no inclusion bodies were produced in the first 6 h of induction. The enzymatic activity of rLbTDC in fermentation broth was greatly enhanced by adding 1% glucose (Fig. 3). The optimal IPTG concentration was determined to be 0.2 mM, lower or higher dosages could result in either low expression level or inclusion bodies (Supplementary Fig. S3). The maximum enzymatic activity of 26 U/mL broth was obtained after 6 h induction at 25 °C (Fig. 4). In a 3-L stirred tank bioreactor, similar enzyme production of 30 U/mL broth (224 mg rLbTDC/L broth) could be achieved (data not shown). Cells began to autolyze after 6 h induction due to the toxicity of rLbTDC to host cells, mainly its catalytic product tyramine. Similar phenomena were reported by Humbert [22] and Ren [23], in which the soluble expression of streptavidin and AgaB protein was improved by addition of 12% and 1% glucose.

The production of toxic recombinant proteins in *E. coli* often leads to either low production level or inclusion body which is difficult to renature, such as streptavidin [22] and TolAI-β-lactamase fusion protein [24]. Due to the small amount of lactose exisited in LB medium, the rLbTDC expression in *E. coli* BL21 (DE3) using

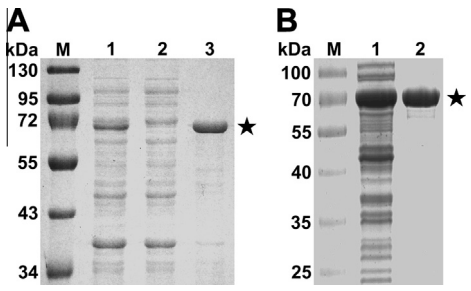

Fig. 2. SDS–PAGE analysis of the expression of rLbTDC in LB/LBG media and its purification. (A) Lane M, molecular weight marker; Lane 1, soluble extract from induced recombinant *E. coli* in LB medium; Lane 2, cell lysates of uninduced recombinant *E. coli*; Lane 3, insoluble extract from induced recombinant *E. coli* in LB medium. (B) Lane M, molecular weight marker; Lane 1, soluble extract from induced recombinant *E. coli* in LBG medium; Lane 2, eluted fraction from Ni–NTA column.

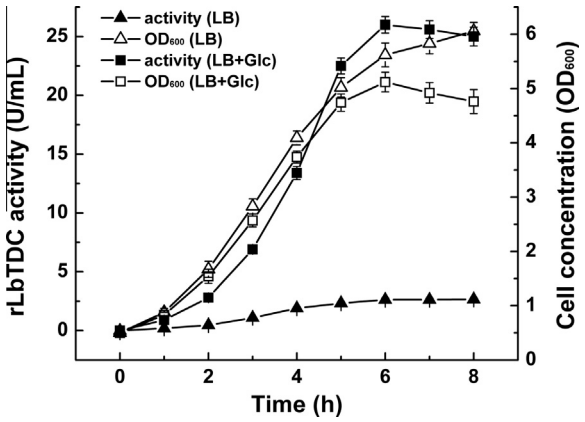

Fig. 3. Effect of 1% glucose on the soluble expression of rLbTDC. The recombinant *E. coli* was incubated in LB medium with or without glucose, induced with 0.2 mM IPTG when OD_{600} reached 0.5, and then the temperature was shifted to 25 °C.

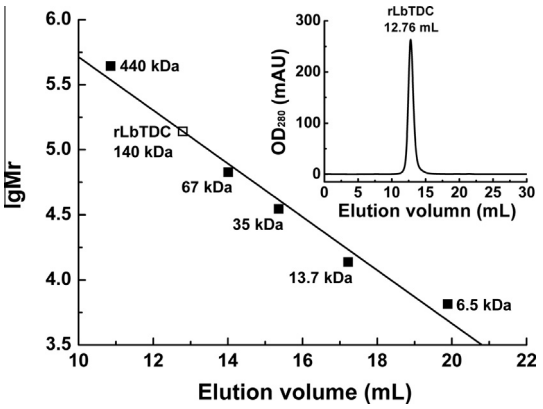

Fig. 5. Native molecular weight determination of rLbTDC by Superdex 200 gel filtration. The standard proteins used were ferritin (440 kDa), bovine serum albumin (67 kDa), β-lactoglobulin (35 kDa), ribonuclease A (13.7 kDa) and aprotinin (6.5 kDa). Elution profile of rLbTDC on Superdex 200 column by OD_{280} is shown in the inset.

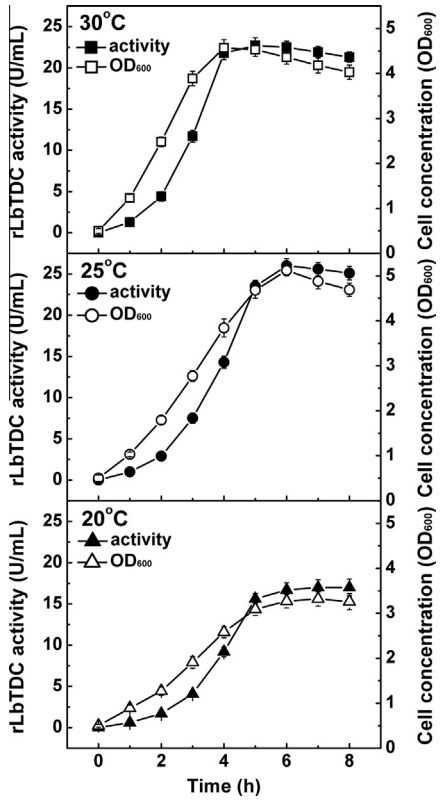

Fig. 4. Effects of induction time and temperature on cell growth and rLbTDC expression. The recombinant *E. coli* was incubated in LBG medium (250-mL flasks containing 50 mL medium supplemented with 50 μg/mL Kan) at 200 rpm and 37 °C, induced with 0.2 mM IPTG when OD_{600} reached 0.5, and further incubated at 20 °C, 25 °C and 30 °C for 8 h.

pET-24 vector may not be tightly regulated, and some basal expression was actually detected after 3–4 h of cell growth without IPTG induction (data not shown). Consequently, the host cells could easily be poisoned, either stop growing (even cell lysis) or producing inclusion bodies. To confirm this speculation, the recombinant strain was incubated on LB agar plate pre-coated with 0.2 mM IPTG for earlier induction. As expected, no cell growth was observed after 24 h of incubation. It was also noted that once the activity of rLbTDC reached over 20 U/mL broth, the recombinant cells started autolysis (Figs. 3 and 4). As shown in Supplementary Fig. S4, the recombinant *E. coli* strain was able to produce around 0.25 mg/mL inclusion body in LB medium without glucose for over 6 generations. However, rLbTDC activity decreased evidently after each generation, and less than 0.2 U/mL activity was remained after 6 generations. Pan reported that repeated growth of recombinant *E. coli* BL21 (DE3) in TB medium clearly leads to plasmid (pET-GFPuv) loss compared with growth in TB medium containing 1% glucose [25]. In this study, the extraordinary function of glucose in the soluble expression of rLbTDC might due to the "glucose effect" in *E. coli* cells, where transcription of *lac* operon was inhibited to some extent in the presence of glucose, especially at the beginning of cell growth. It is therefore believed that the addition of glucose could alleviate the early production of toxic protein, and in turn, improve the cell growth and protein expression throughout the fermentation process. Further study should be carried out to completely understand the mechanism of glucose on the soluble expression of rLbTDC.

Molecular weight and specific activity of purified rLbTDC

Cell-free extract of rLbTDC was purified for 5-fold by a 5-mL Ni–NTA column to a specific activity of 130.7 U/mg with 90% yield. As confirmed by SDS–PAGE, a single band at approximate 70 kDa was observed (Fig. 2B, Lane 2). After further purification by Superdex

Table 1
Purification of rLbTDC from recombinant *E. coli*.

Purification steps	Total activity (U)	Protein (mg)	Specific activity (U/mg)	Purification fold	Recovery (%)[a]
Cell-free extract	4153	159.7	26.0	1	100
HisTrap FF Crude	3738	28.6	130.7	5.0	90.0
Superdex 200	3044	22.8	133.5	5.1	73.3 [a]

[a] Recovery of the Superdex 200-purification is often close to 100%. Loss of activity is mainly due to sample concentration before loading onto Superdex 200 column.

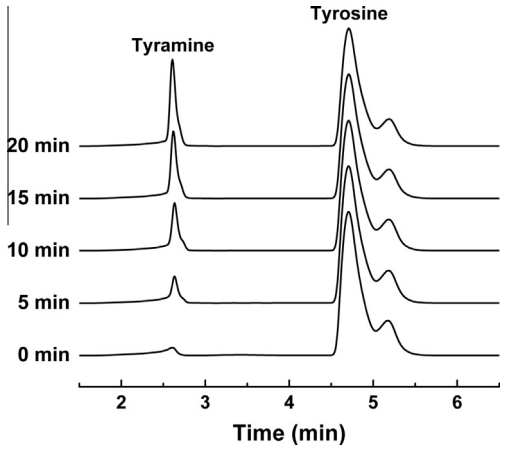

Fig. 6. HPLC analysis of the product tyramine and substrate L-tyrosine during TDC catalyzed reaction.

200 gel filtration, the specific activity was slightly enhanced to 133.5 U/mg (Table 1).

The molecular weight of rLbTDC was estimated to be 140 kDa by Superdex 200 gel filtration (Fig. 5). SDS–PAGE and gel filtration results indicate that rLbTDC is a dimer composed of two identical subunits of approximately 70 kDa. And isoelectric point (pI) of rLbTDC was determined to be 5.5. As rLbTDC is a PLP-dependent decarboxylase, purification by gel filtration led to a complete loss of the activity towards L-tyrosine. Over 50% of activity was restored upon addition of 0.5 μM PLP. And the maximum specific activity was determined at 0.2 mM PLP under standard conditions (Supplementary Fig. S5).

In previous reports, CO_2 electrode method had been utilized for TDC activity assay, which may fluctuate due to the volatility of CO_2 and inconstant CO_2 solubility under various temperatures and air pressures [15]. In this study, HPLC method was established for more accurate TDC activity assay by measuring the concentrations of substrate and product. As shown in Fig. 6, stable accumulation of product tyramine was observed in the first 20 min of reaction. The reaction rate gradually slowed down after substrate conversion ratio reached 20% (data not shown).

Enzymatic properties of rLbTDC

The maximum enzyme activity was observed at pH 5.0 in sodium acetate buffer (0.2 M). TDC from *Streptococcus faecalis* showed similar pH optimum of 5.5 [26], while those from *Eschscholtzia californica* and *Thalictrum rugosum* showed basic pH optima of 8.4 [27]. At pH below 3.5 or above 9.0, no rLbTDC activity could be detected. rLbTDC remained more than 90% of activity over pH range from 5.0 to 6.0, and decreased rapidly at pH below 5.0 or higher than 6.0 (Fig. 7A). The enzyme maintained more than 95% of its activity over pH range from 5.0 to 8.0 after incubation at 4 °C for 24 h. At pH less than 5.0 and greater than 8.0, enzyme stability was

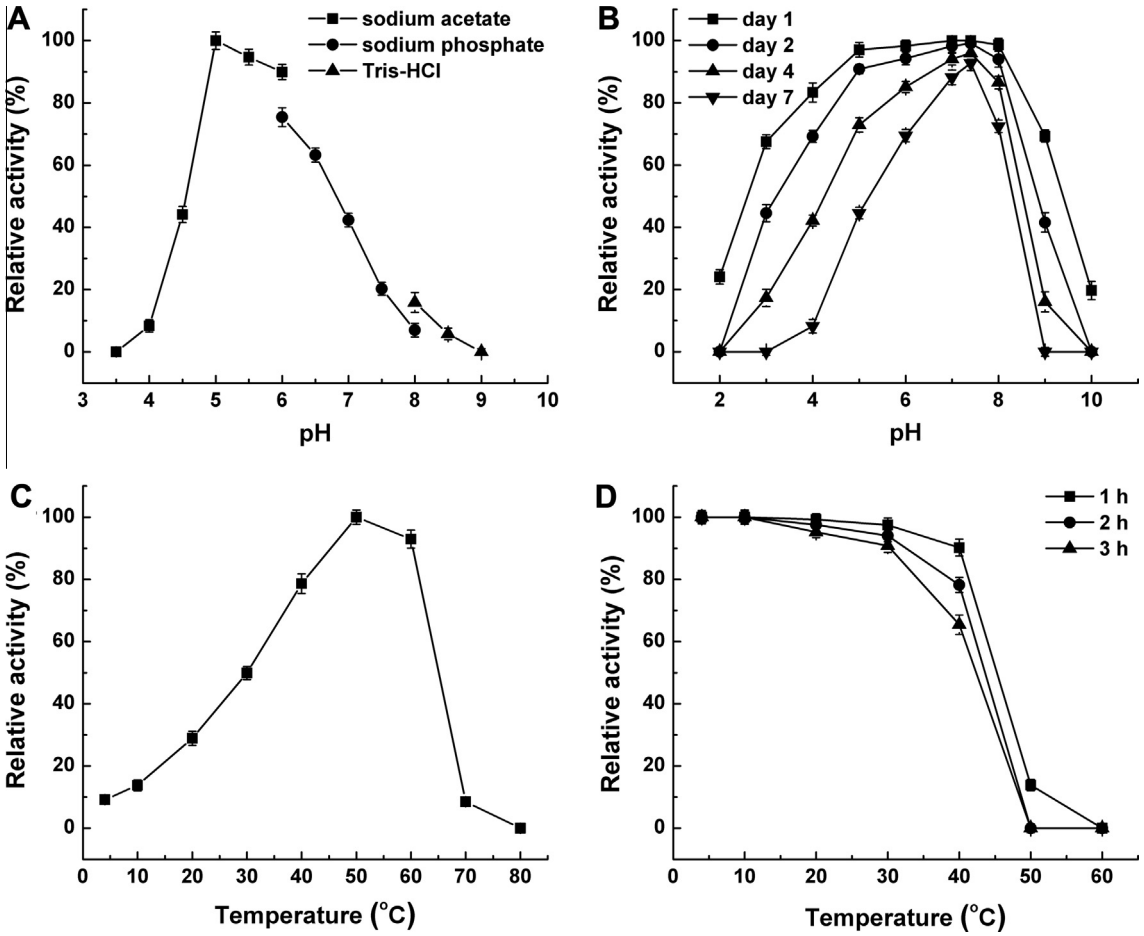

Fig. 7. Effect of pH and temperature on activity and stability of rLbTDC. (A) Effect of pH on rLbTDC activity. (B) Stability of rLbTDC under different pHs. (C) Effect of temperature on rLbTDC activity. (D) Thermostability of rLbTDC.

significantly decreased. The enzyme was quite stable at pH 7.4, 92% activity was retained after incubation for 7 days (Fig. 7B).

The effect of temperature on purified rLbTDC was also determined. rLbTDC exhibited a continuous rise in activity from 4 to 50 °C, and then decreased rapidly to undetectable level at 80 °C and above. Temperature optimum was determined to be 50 °C and only 8.5% of activity was remained at 70 °C (Fig. 7C). However, rLbTDC showed poor stability at 50 °C, the relative activity dropped to 14% after 1 h of incubation (Fig. 7D). Therefore, temperature as high as 50 °C could lead to compromised enzyme stability, and 40 °C was chosen as the standard condition for activity assay.

For cell-free extract, 100% of enzyme activity was lost within 3 weeks at 4 °C. Purified rLbTDC (5 mg/mL) was relatively stable in storage buffer (25 mM Tris–HCl, pH 7.4, 150 mM NaCl) at 4 °C, reserving over 80% of activity after 4 weeks.

Most of metal ions have moderate effect on rLbTDC activity, except that Cu^{2+}, Fe^{2+} and Al^{3+} severely diminished the activity. The metal-chelating reagent EDTA did not reduce the activity, indicating rLbTDC might not require metal ions for its enzymatic activity (Table 2). It was noted that, in Fig. 7A, lower rLbTDC activity was determined in sodium phosphate buffer than sodium acetate buffer at same pH of 6.0. To confirm this observation, we further investigated the effect of two sodium phosphate salts on activity of rLbTDC. Our result indicates that rLbTDC activity was reduced to 67% in the presence of 1 mM disodium hydrogen phosphate, whereas sodium dihydrogen phosphate has no influence at all (Table 2).

Substrate specificity of rLbTDC

In addition to tyrosine, rLbTDC exhibited decarboxylase activity towards L-DOPA, in which 43.9% (58.6 U/mg) of activity was detected compared with 133.5 U/mg using L-tyrosine as substrate. Furthermore, rLbTDC showed a much higher activity toward L-DOPA (58.6 U/mg) compared with DOPA decarboxylases (DDC) from *Ceratitis capitata* (9.4 U/mg) [17] and rat (7.5 U/mg) [18]. As the decarboxylation of L-DOPA to dopamine is a key reaction in the treatment of Parkinson's disease, our rLbTDC could potentially serve as a model enzyme in its therapeutical studies [28].

Other aromatic L-amino acids substrates like L-phenylalanine and L-tryptophan were also tested, and no decarboxylation activity was discovered. Mammalian aromatic L-amino acid decarboxylase (AADC, EC 4.1.1.28), however, could catalyze decarboxylation of L-phenylalanine and L-tryptophan, and its enzymatic activity (0.71 U/mg) is much lower compared with microbial TDCs [26].

BLAST analysis reveals that rLbTDC shares 100% amino acid sequence identity to glutamate decarboxylase (GAD, GenBank No. ABJ65263.1) from *Lb. brevis* ATCC 367, which was studied by Fan [21], Shi and Li [29]. In this study, however, no decarboxylation activity was detected when L-glutamate was tested as substrate. Presumably, TDC from *Lb. brevis* ATCC 367 has been incorrectly annotated as GAD in its complete genome sequence (GenBank No. CP000416.1).

Table 2
Effect of metal ions, EDTA, and salts on rLbTDC activity.

Metal ion (1 mM)	Relative activity (%)
Control	100
$Al_2(SO_4)_3$	ND
$BaCl_2$	84.8 ± 2.3
$CaCl_2$	93.9 ± 1.8
$CoCl_2$	98.6 ± 3.9
$CuCl_2$	9.2 ± 1.3
$FeSO_4$	5.7 ± 1.1
$MgCl_2$	84.5 ± 1.7
$MnSO_4$	86.9 ± 2.5
$NiSO_4$	64.3 ± 3.2
$ZnCl_2$	85.7 ± 2.1
EDTA	95.5 ± 2.2
NaH_2PO_4	98.3 ± 2.4
Na_2HPO_4	67.2 ± 2.9

Control enzyme activity was assayed in the absence of any test compounds and was taken as 100%. ND, not detected.

Kinetic parameters of rLbTDC

The kinetic parameters of purified rLbTDC were estimated by Lineweaver–Burk Plot using L-tyrosine as substrate (Supplementary Fig. S6). K_m, V_{max}, k_{cat}, and k_{cat}/K_m values were calculated to be 0.59 mM, 147.1 μmol min^{-1} mg^{-1} protein, 343.1 S^{-1}, and 583.3 mM^{-1} S^{-1}. Compared with rLbTDC in this study, TDCs from *S. faecalis* [30], *E. californica*, and *T. rugosum* [27] showed a bit lower K_m of 0.36 mM, 0.25 mM and 0.27 mM. Among various recombinant TDCs from *A. Thaliana* (V_{max} of 3.68 μmol min^{-1} mg^{-1} protein) [8], *P. Somniferum* (V_{max} of 35.4 pM min^{-1} mg^{-1} protein) [9], and *R. Sachalinensis* (V_{max} of 16.5 nM min^{-1} mg^{-1} protein) [11], rLbTDC possesses superior catalytic properties due to its stunning V_{max} of 147.1 μmol min^{-1} mg^{-1} protein.

Conclusion

This study, for the first time, reported the high-level and functional expression of *tdc* from *Lb. brevis* in heterologous host. After optimization, a high expression level of 30 U/mL broth was attained. Glucose plays an important role in improving the solubility of rLbTDC. Although the molecular mechanisms of glucose in soluble expression is not completely understood, it is speculated that glucose has an inhibitory effect on transcription of *lac* operon in earlier cell growth phase, which is advantageous for the expression of toxic proteins like TDC. The availability of large amount of soluble TDC is also favorable to its crystallization and further interpretation of protein structure–functional properties relationships. So far, TDC from *M. jannaschii* is the only microbial TDC with resolved protein crystal structure (PDB: 3F9T), which shares less than 30% homology with LbTDC in this study. Currently, our study on crystallization of selenomethionine-substituted (SeMet) rLbTDC is in progress.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pep.2013.10.018.

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