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研究报告

In vitro biosynthesis of serotonin and halogenated tryptamine through the heterologous expression of tryptophan decarboxylase

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Abstract: [Background] Tryptophan decarboxylases that catalyze tryptophan to tryptamine, have specificity to catalyze target substrate in the nature. A decarboxylase from marine *Bacillus atrophaeus* C89, involved in the biosynthesis of bacillamide C, is referred to as BaTDC. **[Objective]** We are aiming to characterize BaTDC and explore the substrate spectrum of BaTDC including halogenated tryptophans and hydroxytryptophan in order to provide new methods to produce novel and pharmaceutically vital tryptamine analogues. **[Methods]** A phylogenetic tree was constructed using protein sequences of several TDCs to understand the status of BaTDC in evolution. Its activity was assayed with various tryptophan derivatives and the products were detected by HPLC and UPLC-MS. **[Results]** Phylogenetic analysis revealed the similarity of BaTDC with that of the gut bacterium *Ruminococcus gnavus*. The optimum temperature and pH of the purified recombinant BaTDC enzyme was 40−45 °C and 8.0, respectively. BaTDC exhibited substrate broadness and catalytic efficiency with hydroxytryptophan and halogenated tryptophans including 4-fluorotryptophan, 5,6,7-chlorotryptophan and 4-bromotryptophan. **[Conclusion]** The study presents a comprehensive characterization of the BaTDC as a promising member of its enzyme family. BaTDC exhibits broad substrate tolerance to tryptophan derivatives, suggesting the potential of substrate-feeding approach in producing novel tryptamine analogs or complex secondary metabolite analogs through precursor-directed biosynthesis.

Keywords: Bacillamides, Halogenated derivatives, Substituted tryptamine, Tryptophan decarboxylase

宽泛底物谱色氨酸脱羧酶体外合成卤代色胺衍生物和血清素

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摘 要:【背景】色氨酸脱羧酶在自然界有高度特异性，行使催化色氨酸为色胺的功能。一个色氨酸

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脱羧酶 BaTDC 参与南海海绵共生菌 *Bacillus atrophaeus* C89 次级代谢产物 Bacillamides 的生物合成过程。【目的】探究 BaTDC 酶学特征和底物谱，建立体外合成色胺衍生物的方法。【方法】通过构建系统发育树揭示 BaTDC 在进化中的地位。在温度梯度和 pH 梯度下进行酶反应，利用不同的色氨酸衍生物为底物，通过 HPLC 和 UPLC-MS 检测酶反应过程，表征 BaTDC 活性。【结果】系统发育分析显示 BaTDC 与肠道菌 *Ruminococcus gnavus* 亲缘关系相近。纯化重组 BaTDC 的最适温度为 40−45 °C，最适 pH 值为 8.0。BaTDC 可以催化羟代色氨酸和卤代色氨酸包括 4-氟色氨酸和 5,6,7-氯色氨酸及 4-溴色氨酸，得到相应的卤代色胺衍生物和血清素。【结论】本研究分析了 BaTDC 的特性，发现 BaTDC 表现出宽泛的底物耐受性，可为前体喂养或定向生物合成新型药用色胺衍生物和下游复杂天然产物奠定基础。

关键词：Bacillamides，卤代衍生物，色胺衍生物，色氨酸脱羧酶

Tryptophan decarboxylases (TDCs) catalyze tryptophan to tryptamine. TDCs play a critical role in providing indole structures. Compounds with an indole structure, known as a privileged structure, have a wide range of bioactivities such as anticancer, antibacterial, antifungal, and antiangiogenic bioactivities[1].

In plants, TDCs have high substrate specificity and can thus discriminate indoles from phenolic substrates; however, TDCs have poor efficiency in differentiating unnatural substrates[2-4]. For example, 5-OH-tryptophan potently inhibits TDC activity in rice[5], and TDCs from *Catharanthus roseus* exhibit no activity toward 5-Cl-tryptophan[6].

Microbial TDCs require further investigation. Considerable attention has been given to TDCs in gut microbiota because they may influence the host brain and behavior through mediating microbe-host interactions[7-8]. Furthermore, *Bacillus atrophaeus*, *Xenorhabdus nematophilus*, and *Streptomyces griseofuscus* are the only bacterial species known to produce tryptamine scaffolds for the biosynthesis of large natural products (*S. griseofuscus* uses 5-OH-tryptophan as a substrate)[9-12].

Investigating tryptamine and its derivatives has maintained research interest for many reasons. First, tryptamine is a trace amine in low quantities in mammalian nervous systems[13]. Second, tryptamine is considered a precursor of many vital bioactive molecules such as serotonin (5-hydroxytryptamine) and melatonin[5,14]. Moreover, tryptamine is a biosynthetic intermediate to numerous complex pharmaceutic natural secondary metabolites (Figure 1).

As a neurotransmitter, serotonin plays a role in numerous critical physiological mechanisms such as sleep, mood disorders, appetite regulation, sexual behavior, cerebral blood flow regulation, and blood-brain barrier permeability in mammals[15]. It is biosynthesized through the catalyzation of tryptophan hydroxylase and pyridoxal-5-phosphatemono-hydrate-dependent aromatic L-amino acid decarboxylases[16]. In plants, serotonin is implicated in several physiological roles such as flowering, morphogenesis, adaptation to environmental changes, and the biosynthesis of serotonin from tryptophan through the catalyzation of TDC and tryptamine 5-hydroxylase (T5H)[17].

In recent years, serotonin synthesis in microorganisms has been reported. Park et al. revealed the biosynthesis of serotonin through the dual expression of TDC and T5H in *Escherichia coli*[18]. Tryptamine as a serotonin precursor is a biogenetic amine that belongs to the monoamine alkaloid family. Wang et al. developed *E. coli*-*E. coli* cocultures to biosynthesize tryptamine using glucose and glycerol as the carbon source[19]. McDonald et al. identified a TDC from the gut microbe *Ruminococcus gnavus* and found that the TDC could catalyze various substrates to diverse tryptamines[20].

As tryptamine is the starting point of various natural products, synthesizing a tryptamine substitute has attracted considerable attention. For example, in the last decade, scientists have attempted to establish methods for introducing halogens into compounds in drug design and development owing to their unique physicochemical properties[21-22]. However, producing tryptamine substitutes using enzymatic approaches has not advanced to the extent of organic synthesis approaches[20].

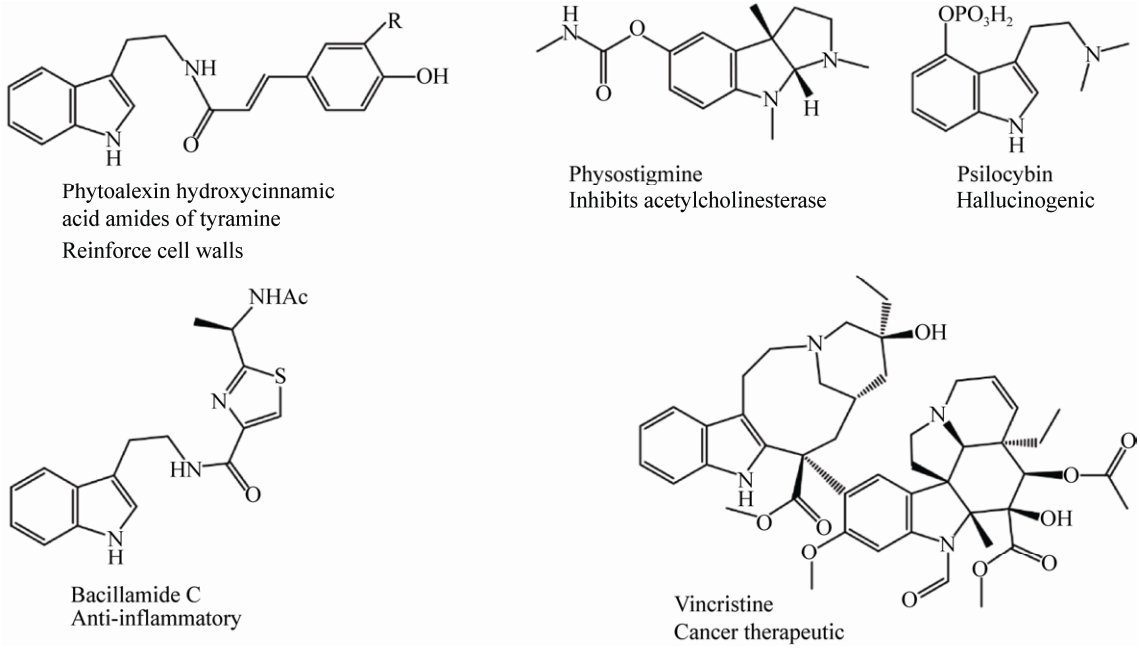

Figure 1 Important tryptamine derivatives in nature
图 1 重要的色胺衍生物

In relevant studies, a decarboxylase from marine *B. atrophaeus* C89 (GenBank accession No. JQ400024) was predicted to be responsible for decarboxylating L-tryptophan to tryptamine in the biosynthesis of bacillamide C[11]. In the present study, we report a comprehensive characterization of it (BaTDC) as a promising member of its enzyme family. We purified recombinant BaTDC by heterologous expression. The optimal temperature and pH for obtaining purified BaTDC to catalyze L-tryptophan was considered to be 40−45 °C and 8.0, respectively. Hydroxytryptophan and halogenated tryptophans were used to detect enzyme activities. Halogenated tryptophans at C-4, C-5, C-6, and C-7 were used to interrogate the steric constraints of the active site. The results showed that BaTDC exhibited broad substrate selectivity to tryptophan derivatives, thus laying a foundation to introduce a small atom or group into useful bioactive molecules in the future.

1 Materials and Methods

1.1 Main reagents and instruments

KOD-Plus DNA Polymerase, TOYOBO (Shanghai) Biotech Company Limited; Restricted enzymes, TaKaRa Biomedical Technology (Beijing) Company Limited; Plasmid Extraction Kit, Gel Extraction Kit, Tiangen Biotech (Beijing) Company Limited; Competent cell, Shanghai Weidi Biotechnology Company Limited; Pyridoxal-5-phosphatemonohydrate, Sigma-Aldrich Llc; 4-F-tryptophan, 5-Cl-tryptophan, 5-OH-tryptophan, 7-Cl-tryptophan, Shanghai Yuanye Biotechnology Company Limited; 4-Br-tryptophan, Bide Pharmatech Ltd; Isopropyl-β-D-thiogalactopyranoside, 6-Cl-tryptophan, Ark Pharm, Inc; 4-Br-tryptophan, Shanghai Macklin Biochemical Company Limited.

PCR instrument, Bio-Rad Company; ÄKTA Pure, HisTrap FF crude, GE Medical System China Company Limited; Amicon Ultra filter, Merck KGaA; High-performance liquid chromatography, Ultra-high-performance liquid chromatography-time of fligh mass spectrometer, Agilent Technologies Company.

1.2 Bacterial strains and plasmids

B. atrophaeus C89 was isolated from the South China Sea sponge *Dysidea avara* in previous study[23]. The plasmid SK(+) was used for cloning *tdc*, and pET-28a was used for expressing *tdc*. *E. coli* DH5α was used to propagate the plasmids. *E. coli* BL21(DE3) was a host for expressing the target gene. Cells were cultivated in Luria-Bertani (LB) medium at

37 °C and shaken at 200 r/min.

1.3 Construction

Forward primer BaTDC-F (5′-TATAGATCTAG CTAGCGTGAAACAAGTGTCGGAAAAC-3′, *Bgl* Ⅱ site is underlined) and reverse primer BaTDC-R (5′-AAAGTCGACTTATTCAGCAACGCATGGATA-3′, *Sal* I site is underlined) were used for the amplification of *tdc* according to its sequence in the genome of *B. atrophaeus* C89 (GenBank accession No. JQ687535). The polymerase chain reaction (PCR) mixture (50 μL) contained 0.2 mmol/L deoxy ribonucleoside triphosphates (dNTPs), 1 mmol/L $MgSO_4$, 0.2 mmol/L of each primer, 1 U KOD-Plus DNA polymerase, and 1 μL of *B. atrophaeus* genomic DNA as a template. *B. atrophaeus* C89 genomic DNA was isolated using a modified version of Marmur's method[24]. The purified PCR product was first ligated into the pSK(+) to screen for positive recombinants with the right sequence using T7 promoter and T7 terminator primers. Then, the target gene was ligated into pET-28a expression vectors digested with the same restriction enzymes. The recombinant plasmids containing *tdc* gene were transformed into competent cells of *E. coli* BL21(DE3).

1.4 Protein production and purification

A single transformed *E. coli* colony was cultivated overnight in LB (5 mL) medium with ampicillin (50 μg/mL) and used to inoculate LB (1 L) production culture amended with ampicillin (50 μg/mL). Cultures were incubated at 37 °C and shaken at 180 r/min on an orbital shaker to an OD_{600} of 0.4–0.6. Subsequently, gene expression was induced through the addition of isopropyl-β-D-thiogalactopyranoside (IPTG) and pyridoxal-5-phosphatemonohydrate (PLP) at final concentrations of 0.5 mmol/L and 0.1 mmol/L, respectively. The incubation was continued at 25 °C for another 9 h and the culture was centrifuged to obtain the biomass (4 000 r/min, 4 °C, 20 min). The biomass was resuspended in lysis buffer (1×phosphate buffer saline (PBS), 1 mmol/L phenylmethylsulfonyl fluoride (PMSF), 20 mmol/L imidazole, pH 7.4) and disrupted through sonication on ice (3 s for work and 3 s for break, 66 W, 12 min). The clear supernatant was harvested through centrifugation (12 000 r/min, 4 °C, 20 min). The supernatant containing soluble protein was purified on the ÄKTA Pure FPLC equipped with a 1 mL Ni^{2+} affinity column. Proteins were separated in an isocratic elution using buffer A (1×PBS, pH 7.4) and buffer B (1×PBS, 500 mmol/L imidazole, pH 7.4) at a flow rate of 1 mL/min. Protein concentration was detected by ultraviolet (UV) absorption at 280 nm (A_{280}). After loading the sample in 4% buffer B (20 mmol/L imidazole), unbound protein was washed with 8% buffer B (40 mmol/L imidazole, 6 mL) followed by washing with 12% buffer B (60 mmol/L imidazole, 6 mL), before elution with 40% buffer B (200 mmol/L imidazole, 15 mL). The eluent containing a soluble recombinant TDC enzyme was concentrated and desalted using a 15 mL 30 K Amicon Ultra filter to 1 mL with buffer (1×PBS, 10% glycine, 0.1% β-mercaptoethanol). The purified enzyme was monitored using sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) in a 10.0% (*W*/*V*) polyacrylamide gel and stored at −80 °C. Protein concentration was estimated using the bicinchoninic acid (BCA) method[25].

1.5 BaTDC activity

In vitro reactions were conducted in triplicate. The enzyme activity was assayed at 35 °C in a 200 μL mixture of purified protein (4 μg) containing 0.1 mol/L Tris-HCl buffer (pH 8.0), 1 mmol/L L-tryptophan, and 0.4 mmol/L PLP; then, 10 μL of 2 mol/L NaOH was added to stop the reaction[26]. The control group was prepared by placing the protein in boiling water for 5 min. The reaction product was extracted with a double-volume ethyl acetate mixture. The ethyl acetate phase was evaporated to dryness, followed by resuspension in 50 μL of methanol. The products were detected through reversed-phase high-performance liquid chromatography (HPLC) using an Agilent C18 column (4.5×150 μm) with a gradient elution of 20%–60% (*V*/*V*) acetonitrile in water that contained 0.2% formic acid. Samples were tested at the flow rate of 0.8 mL/min. This is the default condition except for special instruction.

1.6 Effects of pH and temperature on BaTDC activity and stability

The effect of temperature on BaTDC was measured using the following steps. The reaction system was incubated for 10 min in the same buffer described above at a pH of 8.0 with 4 μg of purified

protein at a specified temperature in the range of 25–70 °C. The activity of BaTDC was determined at 25, 30, 35, 40, 45, 50, 55, 60, 70 °C. The temperature stability of BaTDC was tested through preincubation of the enzyme at different temperatures for 30 min; the residual activity was measured immediately using the method described previously. The control was prepared by placing the protein in boiling water for 5 min.

The effect of pH on BaTDC was measured through the incubation of the reaction product in an appropriate buffer in a pH range from 4.0 to 10.0 for 10 min at 40 °C with 4 μg of purified protein. The buffers used for the experiments are as follows: acetate (pH 4.0–5.0), phosphate (pH 6.0–7.0), Tris-HCl (pH 8.0), and glycine-NaOH (pH 9.0–10.0). The pH stability was tested through a 30 min preincubation of the purified TDC in buffers at different pH values at 40 °C. The remaining activities were measured immediately after this treatment.

1.7 Activity detection and product identification of BaTDC with various tryptophan derivatives as substrates

The enzyme reaction was assayed at 40 °C in the Tris-HCl buffer. Substrates in individual reactions were (final concentration: 1 mmol/L) 5-OH-tryptophan, 4-F-L-tryptophan, 4-Br-L-tryptophan, 5-Cl-L-tryptophan, 6-Cl-L-tryptophan and 7-Cl-L-tryptophan. The incubation times were 30 s, 1 min, 3 min, 5 min, 10 min, 20 min, 40 min, and 60 min. The samples were first detected using HPLC. The product of 4-F-L-tryptophan and 4-Br-L-tryptophan were detected with a flow rate of 0.4 mL/min Then, the products were analyzed using an Agilent 1290 Infinity II ultra-high-performance liquid chromatography (UPLC) equipped with a ZORBAX Eclipse XDB-C18 column (4.6×150 mm, 5 μm), coupled to a 6230 time-of-flight mass spectrometry (TOF-MS) in which a mass spectrometer was operated in positive electrospray ionization mode. The solvents and procedure were the same as those described above, except that the flow rate was 0.4 mL/min. tryptamine and its derivates were detected by A_{280}. The UPLC-TOF-MS analysis was conducted in Core Facility and Technical Service Center for School of Life Science and Biotechnology (SLSB) with the help of Dr. ZHANG Wei.

2 Results

2.1 Phylogenetic analysis

A phylogenetic tree was constructed using MEGA version X software on the basis of the maximum likelihood method with 1 000 bootstrap replicates to understand the status of BaTDC in evolution[27]. Twenty-two sequences were chosen, including 10 orthologs of BaTDC, 3 sequences from bacteria, 2 sequences from fungi, 3 TDC sequences from plants, and 3 TDC sequences from animals. Orthologs were retrieved through the BLAST search, other sequences representing enzymes capable of catalyzing tryptophan were chosen through research reports.

The results showed that BaTDC (marked with a star) was clustered with other TDCs in *Bacillus* (Figure 2). All the other 10 TDC amino acid sequences (WP_098188440.1, WP_106034294.1, ATO26721.1, WP_017152000.1, WP_098337285.1, WP_111922184.1, WP_033678921.1, WP_070128405.1, WP_098133372.1, and EEM41597.1) are annotated by automated computational analysis and none of them have been characterized *in vivo*. No typical rules were found among bacterial sequences. TDCs in bacterium *R. gnavus* (RgnTDC, Swiss-Prot: A7B1V0.1) has a closer relationship with BaTDC. RgnTDC has characterized in X-ray crystallography, hence four structural components of it important for substrate binding were revealed[8]. We aligned the amino acid sequences of BaTDC with RgnTDC. A slight similarity was noted (data not shown), supporting the inference that the substrate specificity is more likely governed by the differences in the active site structures and the orientations of the substrates instead of some key amino acid residues.

2.2 Purification and catalytic activity of BaTDC

The BaTDC gene was cloned to create an N-terminally hexa-histidine-tagged fusion protein and purified using Ni^{2+} affinity chromatography. The protein purity was judged using SDS-PAGE analysis, which revealed an apparent molecular mass of approximately 56 kD (Figure 3A). To analyze the biological activity of purified proteins, TDC was incubated using PLP-containing Tris buffer and tryptophan for 10 min at 35 °C and with a pH of 8.0. Negative controls included heat-inactivated enzymes. HPLC analysis showed that L-tryptophan was decarboxylated to tryptamine within 5 min (Figure 3B).

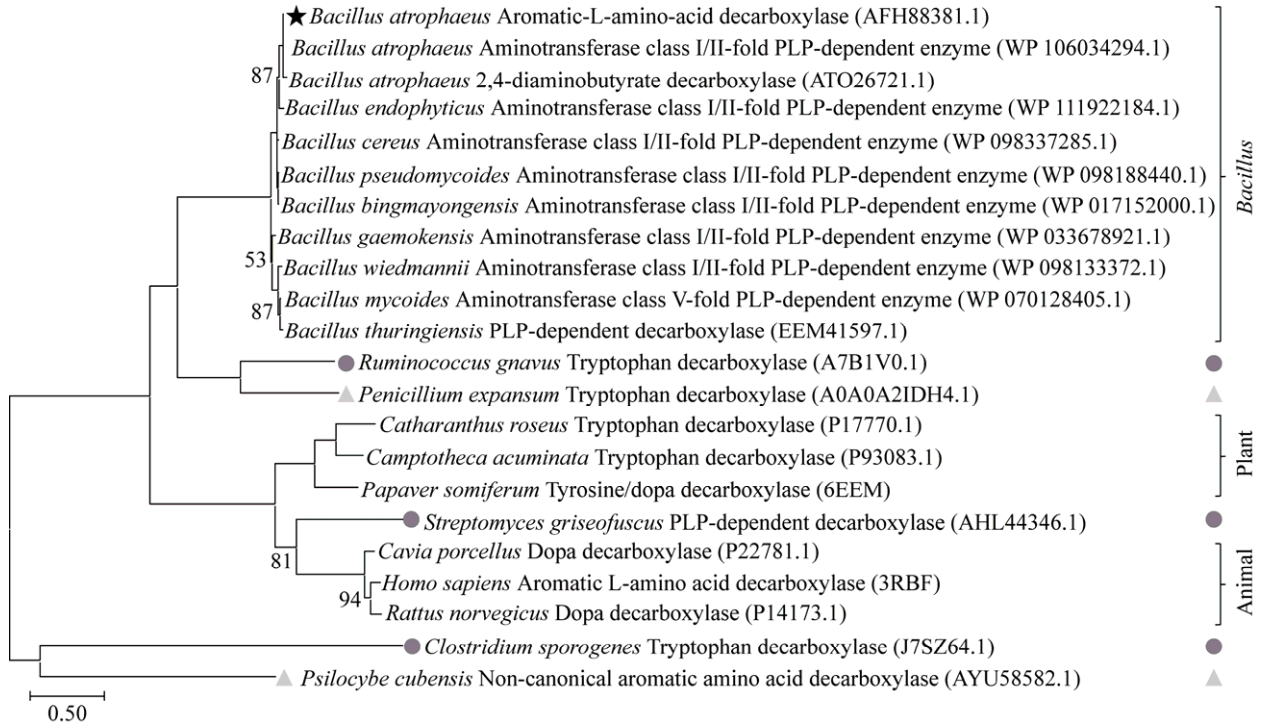

Figure 2 Phylogenetic tree of BaTDC from *B. atrophaeus* C89

图 2 *B. atrophaeus* C89 来源 BaTDC 蛋白的系统发育树

Note: The phylogenetic tree was drawn using the maximum likelihood method with 1 000 bootstrap replicates, those degree of confidence lower than 95% are marked. BaTDC is marked with a star, bacterial sequences other than sequences from *Bacillus* are marked with dots, and fungal sequences are marked with triangles. The phylogenetic tree listed in turn species names, NCBI accession numbers, protein names. Bar: 50% sequence divergence.

注：使用最大似然法，通过 1 000 次自检构建系统发育树，分支置信度低于 95 的被标出；BaTDC 用星标出，*Bacillus* 以外细菌来源序列用圆点标出，真菌来源序列用三角标出；发育树依次列出蛋白来源物种、NCBI 序列号和蛋白名称；标尺刻度：50%序列差异.

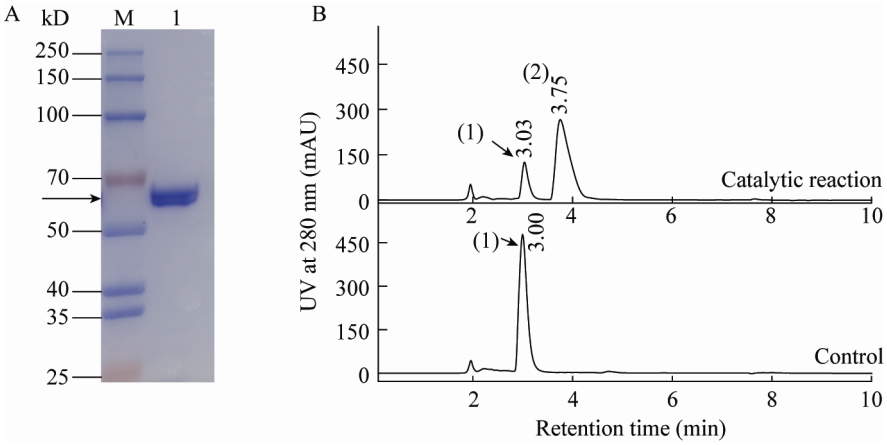

Figure 3 Purification and catalytic activity of BaTDC

图 3 BaTDC 的纯化与活性分析

Note: A: SDS-PAGE of purified recombinant decarboxylase. M: Protein Marker; 1: Purified enzyme. B: HPLC results showed that tryptophan was decarboxylated by the BaTDC enzyme. (1): L-tryptophan; (2): Tryptamine.

注：A：纯化重组蛋白的 SDS-PAGE 电泳图：M：蛋白 Marker；1：纯化蛋白. B：HPLC 结果表明 BaTDC 能使色氨酸脱羧：(1)：L-tryptophan；(2)：Tryptamine.

2.3 Optimum temperature and temperature stability

The optimum temperature range was from 40–45 °C (Figure 4A). The protein activity sharply declined when the temperature increased to more than 45 °C. BaTDC retained 80% activity when the reaction was maintained for 2 h at a temperature below 45 °C (Figure 4B), but the activity was lost when the reaction was maintained at a temperature above 45 °C for 2 hours. Thus, BaTDC activity is stable below 45 °C.

2.4 Optimum pH and pH stability

The optimum pH was relatively broad (6.0–9.0), and the maximum enzyme activity was detected at 8.0; a considerable loss of activity was noted on either side of the given pH range (Figure 4C). The activity profile of the enzyme was stable between pH values of 7.0 and 9.0. Thus, BaTDC exhibited a higher activity in an alkaline medium than in an acidic medium. The physicochemical properties of BaTDC are provided in Table 1.

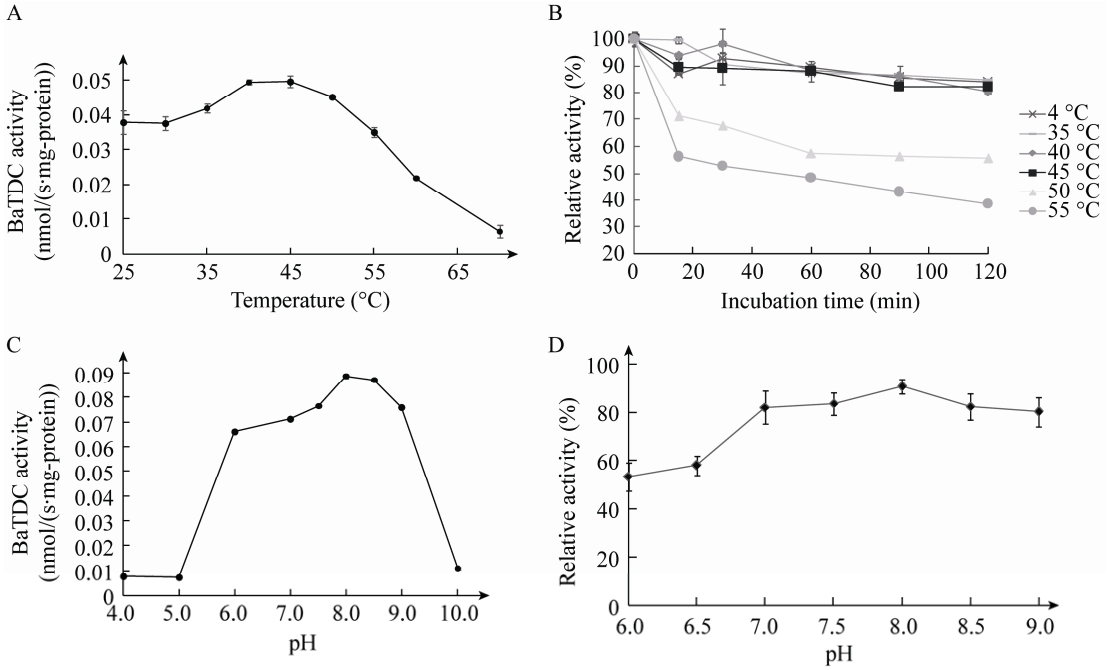

Figure 4 Catalytic activity of recombinant BaTDC
图 4 重组蛋白的活性分析

Note: A: Effect of temperature on BaTDC activity; B: Effect of temperature on BaTDC enzyme stability; C: Effect of pH on BaTDC activity; D: Effect of pH on BaTDC enzyme stability.

注：A：不同温度对 BaTDC 的酶活性影响；B：不同温度对 BaTDC 的酶热稳定性影响；C：不同 pH 对 BaTDC 的酶活性影响；D：不同 pH 对 BaTDC 的 pH 稳定性影响.

2.5 Enzyme activities and substrate spectrum of BaTDC

Using 4-F-L-tryptophan, 5-Cl-L-tryptophan, 6-Cl-L-tryptophan, 7-Cl-L-tryptophan, and 4-Br-L-tryptophan as substrates separately, the catalyzing potential of these substrates was determined.

After incubation with 0.1 mol/L Tris-HCl and 0.4 mmol/L PLP for 10 min, fluoro-tryptamine ($C_{10}H_{11}FN_2$, Figure 5A), with a calculated *m*/*z* of 179.097 9 $[M+H]^+$, was found at an *m*/*z* of 179.108 9

Table 1 Enzymatic properties of BaTDC
表 1 BaTDC 的酶学特性

Enzymatic parameter	Value
Apparent molecular mass (kD)	Approximately 56
K_m (mmol/L)	0.35[11]
V_{max} (IU/mg-protein)	1.02[11]
pH optimum	8.0
Temperature optimum (°C)	40–45

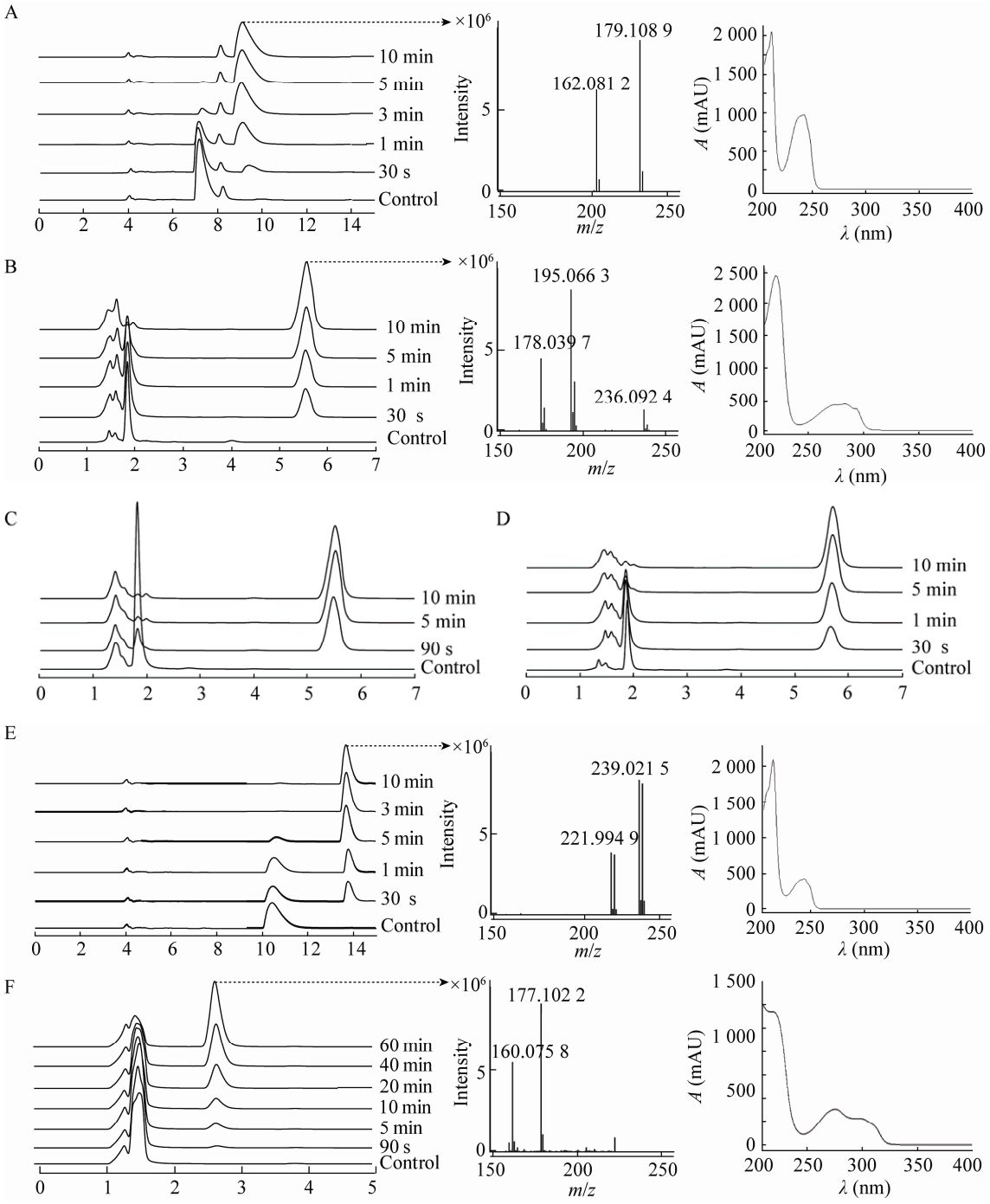

Figure 5 Chromatographic analysis of product formation by BaTDC

图 5 BaTDC 形成的产物色谱分析

Note: Panels show decarboxylase activity. A: 4-F-L-tryptophan; B: 5-Cl-L-tryptophan; C: 6-Cl-L-tryptophan; D: 7-Cl-L-tryptophan; E: 4-Br-L-tryptophan; F: 5-OH-L-tryptophan. Controls include reactions with heat-treated enzymes. Insets display high-resolution mass traces (electrospray ionization) and UV/Vis spectra.

注：结果显示不同底物下的脱羧酶活性. A：4-氟-L-色氨酸；B：5-氯-L-色氨酸；C：6-氯-L-色氨酸；D：7-氯-L-色氨酸；E：4-溴-L-色氨酸；F：5-羟基-L-色氨酸；对照为通过热处理的酶反应；图中包括产物的高分辨率电喷雾电离质谱图和紫外/可见光谱图.

$[M+H]^+$ at a retention time (R_t) of 9.108 min. Chloro-tryptamine was found at an *m/z* of 195.066 3 $[M+H]^+$ and a calculated *m/z* of 195.068 4 $[M+H]^+$ ($C_{10}H_{11}ClN_2$, Figure 5B), at a R_t of 5.542 min. Bromo-tryptamine was found at an *m/z* of 239.021 5 $[M+H]^+$ and a calculated *m/z* of 239.017 8 $[M+H]^+$ ($C_{10}H_{11}BrN_2$, Figure 4E), at a R_t of 13.737 min. HPLC and UPLC-TOFMS analyses showed that BaTDC accepted F-L-tryptophan, Cl-L-tryptophan, and Br-L-tryptophan as substrates, and halogenation at C-4, C-5, C-6, and C-7 did not affect the catalytic reactions. A peak occurred at R_t of 2.786 min for hydroxytryptamine, which was found at *m/z* 177.102 2 $[M+H]^+$, and the calculated *m/z* was 177.102 2 $[M+H]^+$ ($C_{10}H_{12}N_2O$, Figure 5F).

3 Discussion and Conclusion

The TDCs came from bacteria found in the National Center for Biotechnology Information (NCBI) database were reported in two gut-associated species named *R. gnavus* and *Clostridium sporogenes* in 2014. Additionally, no other TDCs were observed in marine organisms in the NCBI database.

The optimum pH and temperature suggest its optimal operation for *in vitro* application. BaTDC had optimum activity at 40–45 °C and was heat-stable below 45 °C. Alkalinity was optimal for BaTDC catalytic activity compared with neutral or slightly alkalescent conditions for the majority of plant TDCs. This is a reflection of the marine environment habitat and suggests that marine TDCs have considerable potential application in alkaline conditions. Other enzymes from marine sources have shown the same properties. Marine urease from *Sporobolomyces roseus* has an optimum activity at a temperature of 65 °C and pH of 8.5, and an alkaline protease produced by marine bacteria strain *Pseudoalteromonas* sp. 129-1 showed optimal activity at 50 °C and pH 8.0, whereas the enzyme activity of lipase purified form the marine bacterium *Oceanobacillus* sp. PUMB02 was optimal at 30 °C and pH 8.0[28-30].

BaTDC is notable for several reasons. First, with the continuous expansion of marine resources, potential secondary metabolites are constantly being explored. This represents a breakthrough for establishing a repository for marine TDCs. Second, precursor-directed biosynthesis can produce novel bacillamides. The introduction of halogen may improve the bioactivities of bacillamides and their derivatives, which exhibited anti-cell proliferation activity and anti-inflammatory activity as well as the ability to regulate harmful algal blooms[31-33]. The mechanism of bacillamide C biosynthesis has been preliminarily explored in our previous work[11,34-35]; we analyzed a putative pathway for synthesizing bacillamide derivatives in *B. atrophaeus* C89 by using a tryptophan substitute. Tryptophan substitutes as biosynthetic precursors are particularly useful because they are structurally similar to natural substrates (Figure 6).

Additionally, with the continuous development and improvement of new biotechnologies in bioengineering, BaTDC can participate in synthesizing tryptamine analogues and further complex secondary metabolites. BaTDC was tested for substrate selectivity among 17 types of natural amino acids by using thin-layer chromatography [11]. In this study, halogenated substrates were detected for the first time. Halogenated compounds generated from the hydrogen parents could have modulated pharmacokinetic properties. New properties and new functions had been noticed in two desferrioxamine B

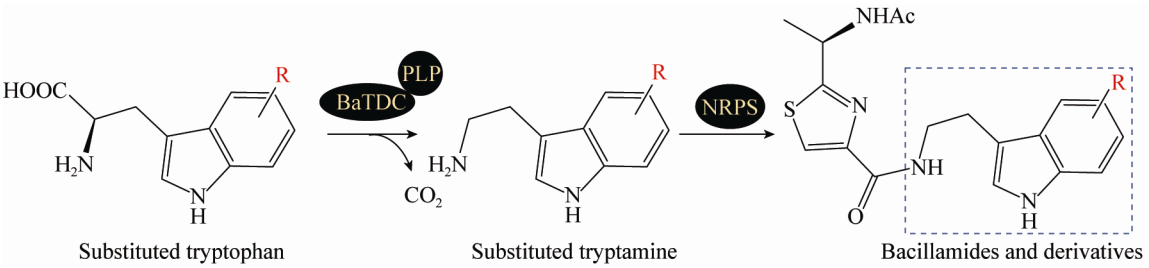

Figure 6 Scheme of Bacillamides and their derivatives
图 6 Bacillamides 以及衍生物的合成路线

analogues[36-37]. The fluoro-substituted analog was superior to the original one in identifying inhibitors of V-RAF murine sarcoma viral oncogene homologue B1 (BRAF) V600E kinase with improved pharmacokinetic properties and was advanced into clinical trials[38]. Novel halogenated chaetoglobosins have been detected with more preferable immunosuppressive activity[39]. McDonald et al. provided a strategy to produce tryptamine analogues with a two-step *in vitro* biocatalytic reaction using RgnTDC with substrate broadness to obtain tryptamine derivates[20]. Exlcuding BaTDC, RgnTDC is the only other characterized microbial TDC. The optimal temperature for BaTDC is 40–45 °C, whereas that for RgnTDC is 37 °C. Protein BLAST showed that the similarity between these two protein sequences is only 39%. Therefore, based on the analysis of the binding situation with the substrates of RgnTDC, different side-chain residues or different steric clashes with substrates can be inferred in the BaTDC structure. Therefore, BaTDC probably has different promiscuity and catalytic efficiency levels with substrates. Plant TDCs involved in the biosynthesis of many compounds affecting human health have been investigated[17,40-41]. Although plants are valuable sources of supplements containing these compounds, microbial production has advantages for mass production and straightforward purification. N-hydroxycinnamoyl tryptamine and serotonin were produced successfully in bioengineered *E. coli* through the introduction of BaTDC and other necessary enzymes[42].

In conclusion, with the broad substrate tolerance of BaTDC, we provide a useful enzyme of metabolic engineering in producing tryptamine derivates. We suggest the possibility of the substrate-feeding approach in producing novel tryptamine analogues or more complex secondary metabolic analogues through precursor-directed biosynthesis.

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