Fundamental Study on De Novo Biosynthesis of Dopamine in Escherichia Coli

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Abstract: Dopamine is an important endogenous organic compound that exerts its unique and crucial functions in the body through various mechanisms. Advancements in biotechnology have accelerated the shift toward biosynthetic production of functional compounds like dopamine. Although de novo dopamine synthesis has been achieved via molecular biology approaches, current production strains remain constrained by low yields and suboptimal efficiency, limiting broader applications. To address these limitations, this study implemented systematic molecular modifications in E. coli targeting three critical aspects: (i) key dopamine synthetic genes, (ii) precursor-supplying genes governing metabolic flux, and (iii) glucose transport systems regulating substrate uptake. These interventions achieved measurable enhancement of dopamine titers. Subsequent screening of the rate-limiting enzyme gene ddc coupled with temporal optimization of inducer administration further elevated dopamine production to 608 mg/L in shake-flask fermentation. This work thus provides actionable guidance for advancing microbial dopamine biosynthesis platforms.
Keywords: Dopamine; Biosynthesis; Synthetic biology

1. Introduction

Dopamine (DA, $C_8H_{11}NO_2$), an endogenous nitrogenous organic compound derived from the enzymatic hydroxylation and decarboxylation of tyrosine (an aromatic amino acid), serves as a critical intermediate in catecholamine biosynthesis [1]. Also known as 3,4-dihydroxyphenethylamine or hydroxytyramine, DA functions as a key neuromodulator involved in regulating mood, enhancing cognitive performance, modulating motor control, and maintaining neuroendocrine homeostasis. Structurally, DA shares the alkaline properties common to biogenic amines. It exhibits pH-dependent stability: protonation under acidic conditions confers relative stability, whereas exposure to oxygen or oxidants promotes degradation [2,3]. In alkaline environments, the deprotonated form demonstrates heightened reactivity. Notably, pharmaceutical formulations utilize dopamine hydrochloride salts to ensure stability and bioavailability. Physiologically, DA orchestrates fundamental neural processes including memory consolidation, locomotor coordination, and emotional regulation. Dysregulation of dopaminergic pathways is etiologically linked to neurodegenerative disorders and neuropsychiatric conditions [8]. Therapeutically, its clinical utility in treating hemodynamic instability and neurological deficits. Given expanding therapeutic demands and increasingly elucidated pathophysiological roles, DA continues to attract significant research interest as a pharmacologically indispensable molecule [4].

In 1957, Kathleen Montagu in the UK isolated dopamine's precursor, 3,4-dihydroxyphenylalanine, from the human brain and thus named it "dopamine" [5,6]. Current dopamine production primarily relies on two methods: animal extraction and plant extraction.

However, animal-based extraction incurs high costs, while plant extraction is hampered by low yields and high resource consumption [7]. In recent years, biological synthesis of dopamine has emerged as a focal direction for green chemistry and sustainable manufacturing. This biological approach utilizes renewable carbon sources (e.g., glucose, glycerol) as substrates and employs microbial cell factories for directed catalytic synthesis of dopamine, offering significant advantages including mild conditions, high stereoselectivity, and environmental friendliness. The primary modification involves reconstructing microbial tyrosine metabolic pathways by introducing heterologous tyrosine hydroxylase (TH) and dopa decarboxylase (DDC), enabling efficient conversion of tyrosine or simple carbon sources to L-DOPA, followed by decarboxylation to dopamine. In Das et al., [9] *E. coli* DH5α expressing genes encoding *HpaB/C* and wild boar *ddc* metabolized tyrosine to produce target compounds, with final yields of 26 mg/L L-DOPA and 27 mg/L dopamine. In contrast, Nakagawa et al. [10] utilized a tyrR-knockout *E. coli* BL21(DE3) strain with overexpression of key enzymatic genes in the metabolic pathway, ultimately producing >2 g/L dopamine through glycerol metabolism. In these existing studies, dopamine's metabolic pathway still has room for improvement, facing challenges including unstable metabolic flux, toxicity inhibition by intermediates, and inefficient cofactor regeneration, necessitating further development of high-efficienc production strains.

To enhance dopamine production, this study systematically investigated the dopamine synthesis pathway and implemented molecular modifications targeting key synthetase genes, precursor supply-related genes, and glucose transport systems, effectively improving biosynthetic capacity. Through subsequent screening of the critical decarboxylase gene *ddc* and optimization of its induction timing, a further breakthrough in yield was achieved, with dopamine production reaching 608 mg/L in shake-flask fermentation. This work offers valuable experimental reference for microbial dopamine synthesis.

2 Materials and Methods

2.1 Strains and Plasmids

The fermentation strain used was *E. coli* BL21(DE3), purchased from TransGen Biotech. For genetic operations, *E. coli* DH5α provided by the laboratory was self-prepared into competent cells. The plasmids employed were pCDFDdute and pETDute. All PCR enzymes used were sourced from Vazyme. Detailed information on strains and plasmids is provided in Table 1. The target genes enhanced in this study were primarily sourced from Escherichia coli K12. Vazyme Bacterial Genome Extraction Kit was employed for the extraction of genomic DNA and subsequently served as the template for PCR amplification. Regarding the ddc genes derived from *Sus scrofa* and *Homo sapiens*, codon-optimized versions were chemically synthesized by TsingKe Biological Technology.

Table1: Strains and Plasmids Involved.

ID	Description
Plasmids	
pCDFDdute	Basic empty plasmid
pCD-1	From pCDFDdute including the genes *ddc*, *tyrA*, and *hapB/C*, all derived from *E. coli*.
pCD-2	From pCD-1 including the gene *iolT1*
pCD-3	From pCD-2, the *ddc* gene within it was replaced with the *ddc* gene derived from *Sus scrofa*

pCD-4	From pCD-2, the *ddc* gene within it was replaced with the *ddc* gene derived from *Homo sapiens*
pETDute	Basic empty plasmid
pETD-1	From pCDFDdute including the gene *aroG*
pETD-2	From pCD-2 including the genes *gapA* and *tal*
Strains	
EWD-1	Introduced pCD-1
EWD-2	Introduced pCD-2
EWD-3a	Introduced pCD-2 and pETD-1
EWD-3b	Introduced pCD-2 and pETD-2
EWD-3b1	Introduced pCD-3 and pETD-2
EWD-3b2	Introduced pCD-4 and pETD-2

2.2 Culture and Culture Media

E. col cultivation conditions were uniformly maintained at 37°C and 180 rpm in a shaking incubator. Routine cultivation of bacterial strains is performed using LB medium. The fermentation medium was LBG, prepared by adding separately sterilized glucose stock solution (400 g/L) to LB base medium. Glucose was added to achieve an initial concentration of 20 g/L through calculated supplementation.

2.3 PCR and Colony PCR System

PCR using high-fidelity enzyme purchased from New England Biolabs, with the specific system outlined in the table below. Colony PCR using Taq enzyme from New England Biolabs, with the specific system outlined in the table below.

Table 2: PCR Systems Used in This Study.

PCR System (μL)	
DNA template	1
Primer-F	2
Primer-R	2
Enzyme	50
dd H_2O	45
Colony PCR System (μL)	
Single colony	Pick up with a pipette tip
Primer-F	1
Primer-R	1
Enzyme	10
dd H_2O	8

2.4 Preparation of Escherichia Coli Competent Cells

Inoculate a single *E. coli* colony into 3 mL LB medium and culture 12 h. Then, using a 1% inoculation volume, transfer 500 μL of the *E. coli* culture into 30 mL of fresh LB and culture for 1 h; place the cultured bacteria at 4°C for 15 min, then centrifuge at 4°C and 3500 rpm for 5 min, discarding the supernatant; add 30 mL of pre-chilled 0.1 M $CaCl_2$ and incubate in 4°C for 15 min;

centrifuge at 4°C and 3500 rpm for 10 min, discarding the supernatant; add 5 mL of 0.1 M $CaCl_2$ and 50% glycerol respectively, resuspend by pipetting; aliquot the prepared cells, 50 μl per tube, and store at -80°C.

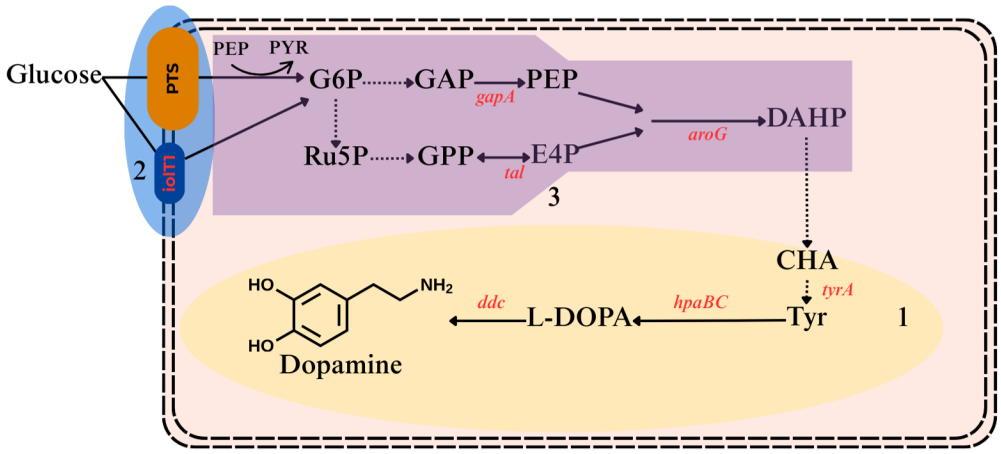
2.5 Analysis by HPLC

Fermentation broth samples underwent centrifugation at 8,000 rpm for 4 min. The filtered supernatant can then be used for detection. Analysis was conducted using an HPLC system featuring an Innoval C18 column use UV detector. The mobile phase, delivered at 0.8 mL/min, comprised a mixture of 100% methanol and a 0.1% phosphoric acid solution in a volumetric ratio of 20:80. Detection was performed at 30°C with wavelengths of 220 nm and 280 nm [7].

3 Results and Discussion

3.1 Introduction of Dopamine Production Pathway

Our goal for this study is to construct an efficient dopamine production pathway in microbial strains. Initially, we analyzed the endogenous pathways of conventional microbes and found a natural dopamine production pathway in *E. coli* [11, 15]. However, the yield was extremely low, almost undetectable. For rigorous research, we chose *E. coli* BL21 as the original strain for testing. Results indicated the absence of dopamine in the medium. Consequently, we introduced key synthetic enzymes in the dopamine production pathway. Firstly, we selected to overexpress the gene DOPA decarboxylase (*ddc*), which directly synthesizes dopamine from L-dopa in *E. coli*. Previous studies (NCBI) have highlighted the low efficiency of this enzyme, which was a significant reason for its overexpression in our study. Additionally, considering the abundance of studies on L-dopa production in the intermediate product research of this pathway, we further strengthened the genes *tyrA*: CHA mutase/prephenate dehydrogenase gene and genes *hpaB*/*C*: 4-hydroxyphenylacetate 3-hydroxylase gene to ensure an adequate supply of precursors for dopamine production (Figure 1). The introduction of these four genes aimed to reinforce the latter part of the pathway from glucose to dopamine production, as glucose metabolism exhibits high activity in microbial metabolic pathways. The strain carrying these four genes was named EWD-1. Subsequently, under the same conditions as the original strain, EWD-1 was tested, and trace amounts of dopamine accumulation were detected, marking a transition from absence to presence. Although the dopamine yield was only 37.5 mg/L (Figure 2), this initial success was encouraging. However, the yield was still significantly lower than expected, necessitating further pathway modifications from multiple perspectives to enhance dopamine production.



PTS: phosphotransferase system; E4P: D-erythrose 4-phosphate; PEP: phosphoenolpyruvate; G6P: glucose 6 phosphate; GAP: glyceraldehyde 3 phosphate; Ru5P: ribulose 5 phosphate; DAHP: 3-deoxy-arabino-heptulonate 7-phosphate; CHA: chorismate; Tyr: L-tyrosine; L-DOPA: levodopa; *gapA*: Glyceraldehyde-3-phosphate dehydrogenase; *ddc*: DOPA decarboxylase gene; *tal*: Transaldolase; *tyrA*: CHA mutase/prephenate dehydrogenase gene; *aroG*: DAHP synthase gene; *hpaBC*: 4-hydroxyphenylacetate 3-hydroxylase gene; iolT1: Myo-inositol import MFS transporter IolT1

Figure 1: Metabolic Pathways Involved in this Study.

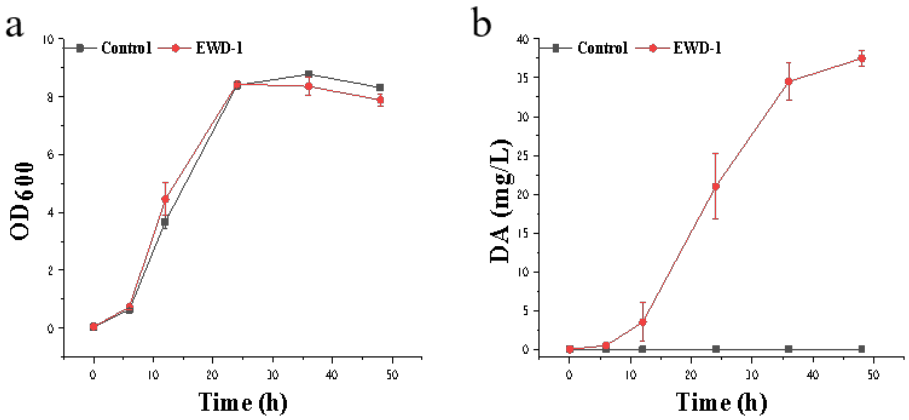


Figure 2: Growth and metabolism of strain EWD-1

3.2 Enhancing Glucose Uptake in the Strain Through the Introduction of Transport Proteins

E. coli relies on its PTS for glucose transport, which is an efficient and versatile sugar transport system in bacteria. It plays an important role in sugar uptake and metabolic regulation and signal transduction [12,13]. Within the PTS system is the enzyme Phosphoenolpyruvate-dependent Protein Kinase I, which converts phosphoenolpyruvate (PEP) to pyruvate, consuming a key precursor, PEP, in our dopamine production pathway. The presence of abundant PEP and E4P in the production pathway is beneficial for dopamine production. Therefore, we attempted to introduce an alternative glucose transport pathway into the strain by introducing the protein iolT1 (myo-inositol permease) in strain EWD-1, resulting in the strain EWD-2. The transport process of this protein belongs to secondary active transport and does not consume ATP. Subsequently, we compared the growth and metabolism of the two strains. Experimental results (Figure 3) showed that glucose consumption in

the strain was accelerated, with an increase of 13.03% compared to strain EWD-1. Additionally, dopamine production also slightly increased by 6.67%, reaching 40 mg/L. These results demonstrate the effectiveness of our modification idea and validate our theoretical hypothesis.

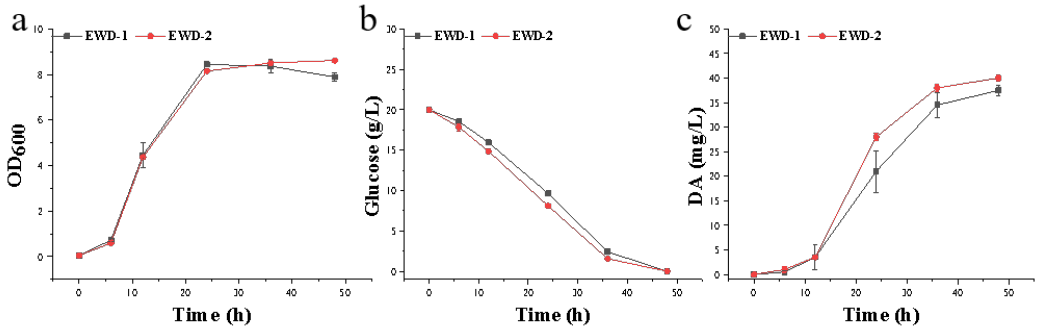


Figure 3: Growth and Metabolism Comparison of Strains after Introducing iolT1.

3.3 Enhancing carbon flux in synthetic pathways to boost target product production

After the overexpression of key synthetic genes and the introduction of transport proteins, there was some progress in dopamine production, but the productivity remained low. Further analysis of the production pathway revealed that although PEP and E4P have significant carbon flux in the organism, there are also multiple competing pathways for these intermediates [13]. In order to channel more carbon flux into our production pathway, we strengthened the gene *aroG* (DAHP synthase) from PEP and E4P to DAHP. Strengthening this gene theoretically increases the carbon flux of the entire production pathway, similar to reports in current research showing that overexpressing aroG and aroF can markedly increase the metabolic flux through the shikimate pathway, which is our desired outcome. We named the resulting strain EWD-3a and tested its growth and metabolism (Figure 4). The dopamine production of this strain far exceeded that of strain EWD-2, with a 52.6-fold increase, reaching 524.6 mg/L. The significant effect of strengthening the key precursor genes in the pathway was evident. Therefore, we attempted to further enhance the production of key intermediates in the production pathway. We sequentially introduced the gene *gapA*, which increases PEP production, and the gene *tal*, which increases E4P production, into the aforementioned strain, naming it EWD-3b. Subsequent fermentation tests showed (Figure 4) that the dopamine production of the modified strain further increased. Although the magnitude did not surpass the previous modification, it still increased by 14.97%, reaching 603 mg/L, which is a significant improvement.

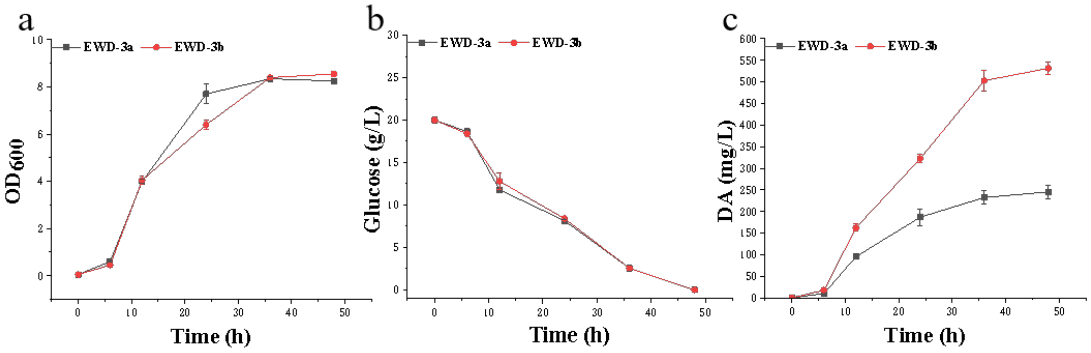


Figure 4: Growth and Metabolism Changes of the Strain After Strengthening Precursor Supply.

3.4 Screening for ddc Gene to Reduce Intermediate Accumulation

Literature analysis reveals that the biosynthesis of L-DOPA-a key precursor in our dopamine production pathway is well-established, with high-yielding microbial strains routinely reported. However, DA titers in our system remain substantially lower, prompting the hypothesis that the low activity of the *ddc* gene [7]. To validate this, we quantified intracellular L-DOPA accumulation at 48 h (Fig. 5a), detecting significant residual concentrations of 107.1 mg/L and 254.3 mg/L in strains EWD-3a and EWD-3b, respectively. This confirms inefficient *ddc*-mediated conversion.Therefore, we further screened ddc genes from two different sources, *Sus scrofa* and *Homo sapiens*, and named the strains EWD-3b1 and EWD-3b2, respectively. Experimental results, as shown in Figure 5d, revealed that the ddc gene from Homo sapiens had the most significant enhancement effect, with dopamine production reaching 603.7 mg/L. Through the screening of ddc gene sources, we obtained a superior result, achieving a more efficient dopamine production. However, noticeable experimental errors were observed in our results. Through the analysis of the experimental process, we speculate that the different production states of the strains after the addition of the inducer may have caused these discrepancies. Subsequently, we conducted optimization experiments on the optimal strain EWD-3b2 regarding the addition of the inducer.

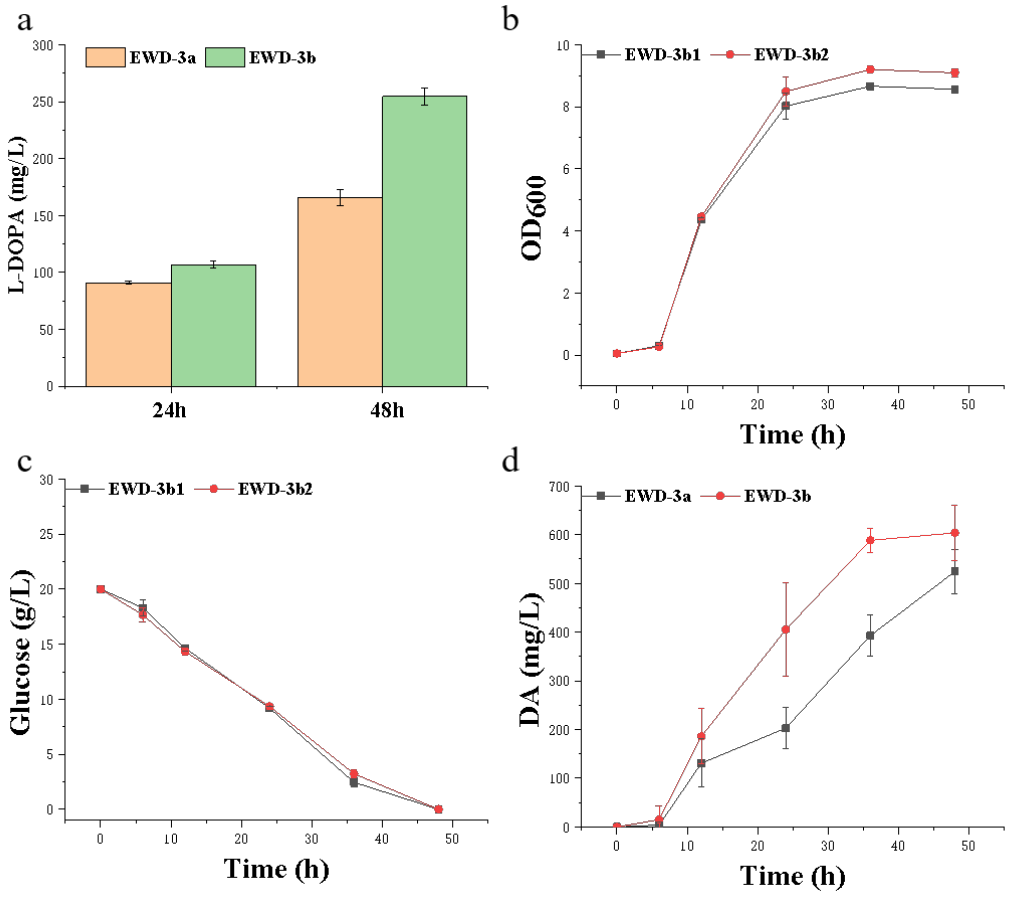


Figure 5: Residual L-DOPA Levels in the Strains Before Optimizing the *ddc* Gene and Growth Metabolism Status of the Optimized Strains.

3.5 Optimizing IPTG Addition Time to Enhance Production Stability

IPTG is an analog of lactose that can induce the transcription process of the lactose operon. We

chose to add IPTG at different growth stages of the microorganism, specifically at three time points: early logarithmic growth phase, when cells are fully recovered and advantageous for early initiation of exogenous gene expression; mid-logarithmic growth phase, when cells are in an active growth period with vigorous metabolism; and late logarithmic growth phase, when cell biomass is approaching maximization and individual cells are more abundant [14,16]. By examining the cell growth and metabolism under these three conditions (Figure 6a), we found that adding IPTG during the mid-logarithmic growth phase of the strain was more beneficial for product accumulation. At this stage, dopamine production reached 608.3mg/L, which was a 2.4% and 6.9% increase compared to the early and late logarithmic phases, respectively. The appropriate timing of IPTG addition has a better enhancing effect on the expression of genes on the plasmid in the strain [17,18].

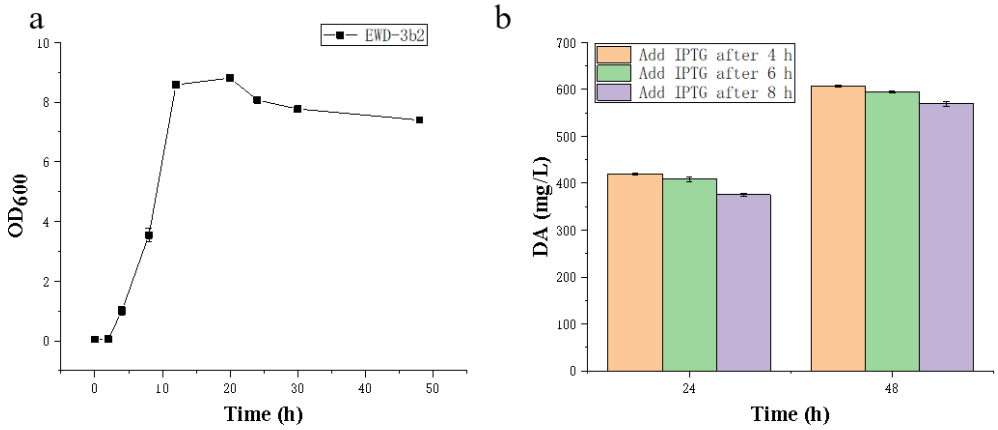


Figure 6: Production Yield of Strains with IPTG Added at Different Time Points.

4. Conclusion

Although natural microbial strains have pathways for dopamine production, their production capacity is insufficient to be commercially viable. In this study, we genetically modified key synthetic genes, critical precursor supply genes, and glucose transport systems in the dopamine production pathway, resulting in an improvement in dopamine production. Subsequently, we further screened key gene ddc and optimized the timing of inducer addition, leading to a further increase in dopamine production to 608.3 mg/L. Our experiments provide valuable insights for dopamine biosynthesis.

References

[1] Shen X M, Zhang F, Dryhurst G. Oxidation of dopamine in the presence of cysteine: characterization of new toxic products. Chemical research in toxicology, 1997, 10(2): 147-155.

[2] Chen A P F, Chen L, Kim T A, et al. Integrating the Roles of Midbrain Dopamine Circuits in Behavior and Neuropsychiatric Disease. Biomedicines, 2021(6). DOI:10.3390/BIOMEDICINES9060647.

[3] Wei T, Cheng B Y, Liu J Z. Genome engineering Escherichia coli for L-DOPA overproduction from glucose. Scientific Reports, 2016, 6:30080. DOI:10.1038/srep30080.

[4] Lee J Y, Jun H, Soma S, et al. Dopamine facilitates associative memory encoding in the entorhinal cortex. Nature Publishing Group, 2021(7880). DOI:10.1038/S41586-021-03948-8.

[5] Stanley, Fahn. The history of dopamine and levodopa in the treatment of Parkinson's disease. Movement Disorders, 2008, 23(S3): S497-S508. DOI:10.1002/mds.22028.

[6] Benes F M. Carlsson and the discovery of dopamine. Trends in Pharmacological Sciences, 2001, 22(1):46-47. DOI:10.1016/S0165-6147(00)01607-2.

[7] Fuqiang S, Wujiu C, Fengli W, et al. [Heterogeneous expression of DOPA decarboxylase to improve the production of dopamine in Escherichia coli]. Sheng wu gong cheng xue bao = Chinese journal of biotechnology,2021,37(12):4266-4276.

[8] Salamone J D, Mercè Correa. The Neurobiology of Activational Aspects of Motivation: Exertion of Effort, Effort-Based Decision Making, and the Role of Dopamine. Annual Review of Psychology, 2024, 75(1): 32.DOI:10.1146/annurev-psych-020223-012208.

[9] Das A, Verma A, Mukherjee K J. Synthesis of Dopamine in E. coli Using Plasmid Based Expression System and Its Marked Effect on Host Growth Profiles.Preparative Biochemistry & Biotechnology, 2017, 47(7). DOI:10.1080/10826068.2017.1320291.

[10] Nakagawa A, Matsuzaki C, Matsumura E, et al. (R, S)-Tetrahydropapaveroline production by stepwise fermentation using engineered Escherichia coli. Scientific Reports, 2017, 4:6695. DOI:10.1038/srep06695.

[11] Satoh Y, Tajima K, Munekata M, et al. Engineering of l-tyrosine oxidation in Escherichia coli and microbial production of hydroxytyrosol. Metabolic Engineering, 2012, 14(6). DOI:10.1016/j.ymben.2012.08.002.

[12] Rodriguez A, Martínez, Juan A, Báez-Viveros, José L, et al. Constitutive expression of selected genes from the pentose phosphate and aromatic pathways increases the shikimic acid yield in high-glucose batch cultures of an Escherichia coli strain lacking PTS and pykF. Microbial Cell Factories, 2013, 12(1):86-86. DOI:10.1186/1475-2859-12-86.

[13] Ou RW, Swofford CA, Zhong-Johnson EL, Li C, Sinskey AJ. The effect on shikimate production by deleting iolR and metabolic engineering in PTS deficient Corynebacterium glutamicum strain. Front Bioeng Biotechnol. 2025 Jun 26; 13:1616558. doi: 10.3389/fbioe.2025.1616558. PMID: 40642537; PMCID: PMC12241104.

[14] Rong Y, Jensen S I, Woodley J M, et al. Modulating metabolism through synthetic biology: Opportunities for two-stage fermentation. Biotechnology and Bioengineering, 2024, 121(10): 3001-3008.

[15] Zhao Y, Lv B, Feng X, et al. Perspective on Biotransformation and De Novo Biosynthesis of Licorice Constituents. J Agric Food Chem, 2017: 11147-11156.DOI:10.1021/acs.jafc.7b04470.

[16] Yan Z B, Liang J L, Niu F X, et al. Enhanced Production of Pterostilbene in Escherichia coli Through Directed Evolution and Host Strain Engineering. Frontiers in Microbiology, 2021, 12.DOI:10.3389/fmicb.2021.710405.

[17] Li X, Liu Y, Ma L, et al. Metabolic engineering of Escherichia coli for high-yield dopamine production via optimized fermentation strategies. Applied and Environmental Microbiology, 2025.DOI:10.1128/aem.00159-25.

[18] Kogure T , Kubota T , Suda M ,et al. Metabolic engineering of Corynebacterium glutamicum for shikimate overproduction by growth-arrested cell reaction. Metabolic Engineering, 2016, 38:204-216. DOI:10.1016/j.ymben.2016.08.005.