

# High-titer production of aromatic amines in metabolically engineered

## *Escherichia coli*

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## ABSTRACT

Aromatic amines are widely used in the pharmaceutical industry. Here, we reported the establishment of a bacterial platform for synthesizing three types of aromatic amines, namely, tyramine, dopamine, and phenylethylamine. Firstly, we expressed aromatic amino acid decarboxylase from *Enterococcus faecium* (*pheDC*) in an *Escherichia coli* strain with an increased shikimate (SHK) pathway flux toward L-tyrosine or L-phenylalanine synthesis. We found that glycerol served as a better carbon source than glucose, resulting in  $940 \pm 46$  mg/L tyramine from 4% glycerol. Next, the genes of lactate dehydrogenase (*ldhA*), formate acetyltransferase (*pflB*), phosphate acetyltransferase (*pta*), and alcohol dehydrogenase (*adhE*) were deleted to mitigate the fermentation byproduct formation. The tyramine level was further increased to  $1.965 \pm 0.205$  g/L in shake flasks, corresponding to 2.1 times improvement compared with that of the parental strain. By using a similar strategy, we also managed to produce  $703 \pm 21$  mg/L dopamine and  $555 \pm 50$  mg/L

phenethylamine. In summary, we have demonstrated that the knockout of *ldhA-pflB-pta-adhE* is an effective strategy in improving aromatic amine productions, and achieved the highest aromatic amine titers in *E. coli* under shake flasks reported to date.

**Key points:** Aromatic amino acid decarboxylase from *E. faecium* was used for aromatic amine production; *ldhA*, *pflB*, *pta* together with *adhE* were deleted to mitigate the fermentation byproduct formation; Our work represented the best aromatic amine titers reported in *E. coli* under shake flasks.

**Keywords:** aromatic amino acid decarboxylase; tyramine; dopamine; phenylethylamine; shikimate pathway; metabolic engineering

## Introduction

Aromatic compounds represent a large and diverse class of chemicals that are widely used in manufacturing solvents, polymers, fine chemicals, feed and food additives, nutraceuticals, and medicines (Huccetogullari et al. 2019; Shen et al. 2020; Wang et al. 2018). Among them, aromatic amines with diverse physical characteristics are often employed as antioxidants, and precursors to pharmaceutical products (Masuo et al. 2016; Minami 2013). For example, tyramine is a high-value industrial product with widespread applications in medicine (Beltran et al. 2011). Dopamine acts as an intermediate in the biosynthesis of epinephrine and other drugs (Davie 2008; Jeong et al. 2018), and also can influence the physiological activity of plants and humans (Liang et al. 2018; Wise 2004). In addition, phenylethylamine is a precursor of antidepressants for addiction cessation (Brackins et al. 2011; Dwoskin et al. 2006). To

44 date, the biomanufacturing process of aromatic amines mainly relies on chemical  
45 synthesis (Corrigan et al. 1945; Epstein et al. 1964). However, the chemical method  
46 typically involves complicated steps, harsh reaction conditions, and non-renewable  
47 feedstock, which is considered an environment-unfriendly process.

48 With the fast advancements of synthetic biology and metabolic engineering, microbial  
49 synthesis of chemicals has made significant strides in recent years (Cho et al. 2015;  
50 Huccetogullari et al. 2019). For instance, *Escherichia coli* has been extensively  
51 utilized as the host for synthesizing many natural products, owing to its  
52 well-characterized genetic information and abundant molecular tools (Yang et al.  
53 2020). Aromatic amines typically use aromatic amino acids as precursors, which are  
54 synthesized primarily through the shikimate pathway (SHK) in microorganisms  
55 (Averesch and Kromer 2018; Shen et al. 2020). The SHK pathway is initiated by the  
56 condensation of phosphoenolpyruvate (PEP) from the glycolytic pathway and  
57 D-erythrose 4-phosphate (E4P) in the pentose phosphate (PP) pathway to produce  
58 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP), which is subsequently  
59 transformed to aromatic amino acids (Averesch and Kromer 2018; Cao et al. 2020)  
60 (Fig. 1). Koma *et al.* overexpressed a cluster of genes from the SHK pathway and a  
61 heterologous decarboxylase gene in *E. coli*, and the resulting strain produced 6.3 mM  
62 (863mg/L) tyramine (Koma et al. 2012). Heterologous expression of tyrosinase and  
63 decarboxylase in the L-tyrosine overproducing *E. coli* resulted in 260 mg/L dopamine  
64 (Nakagawa et al. 2011).

65 In this work, we aimed to develop an *E. coli* platform to improve the biosynthesis of

aromatic amines. As shown in Fig. 1, the main strategy used to improve aromatic amines production comprises the deletion of fermentation byproduct-related pathways to enhance the metabolic flux of the SHK pathway. In particular, we investigated the effect on aromatic amine biosynthesis by knockout of lactate dehydrogenase (*ldhA*), formate acetyltransferase (*pflB*), phosphate acetyltransferase (*pta*), and alcohol dehydrogenase (*adhE*).

## **Materials and methods**

### **Strains and reagents**

*E. coli* DH5 $\alpha$  was utilized to construct plasmids, and *E. coli* MG1655 (DE3) derived strain with  $\Delta$ tyrA and  $\Delta$ pheA (Lai et al. 2022) was employed as the chassis cell for aromatic amine production. LB medium containing 10 g/L tryptone, 5 g/L yeast extract, and 10 g/L NaCl was applied for cultivating *E. coli*. Appropriate antibiotics (100  $\mu$ g/mL ampicillin, 50  $\mu$ g/mL kanamycin, 34  $\mu$ g/mL chloramphenicol) were supplemented to maintain the plasmids when needed. Enzymes (high-fidelity phusion polymerase, *Bam*HI-HF, *Xho*I, *Bsa*I-HF, *Esp*3I-HF, and T4 DNA ligase) were purchased from New England Biolabs (Beverly, MA, USA). PCR purification kit, gel extraction kit, and plasmid DNA extraction kit were all purchased from BioFlux (Shanghai, China). The details of chemicals used in this study are provided in Supplementary materials.

### **Plasmid and strain construction**

All the genes were PCR amplified using high-fidelity phusion polymerase. The oligonucleotides used in this study are listed in Supplementary Table S1. The gene

88 encoding *pheDC* from *E. faecium* (Genbank: AJ783966.1) was codon optimized for *E.*  
 89 *coli* and synthesized by GenScript (Nanjing, China). The genes encoding *hpaBC*  
 90 (Genbank: Z37980) were obtained from the genomic DNA of *E. coli*.  
 91 The plasmid pET-AroG<sup>fbr</sup>-TyrA<sup>fbr</sup> and pET-AroG<sup>fbr</sup>-PheA<sup>fbr</sup> were constructed in a  
 92 similar way to our previous report (Lai et al. 2022). In brief, the genes encoding  
 93 *tyrA<sup>fbr</sup>*, *pheA<sup>fbr</sup>*, and *aroG<sup>fbr</sup>* were obtained from the genomic DNA of *E. coli* via  
 94 overlapping PCR amplification process, digested with *Esp3I*, and ligated into  
 95 pETDuet-1 between *Bam*HI and *Xho*I sites. For the plasmid pRSF-PheDC, the  
 96 synthesized *pheDC* gene was inserted into pRSFDuet-1 between *Bam*HI and *Xho*I  
 97 sites. For the plasmid pACYC-HpaBC, the *hpaBC* gene was amplified using *E. coli*  
 98 genomic DNA as a template, and inserted into pACYCDuet-1 between *Bam*HI and  
 99 *Xho*I sites. For constructing MG1655 (DE3) derived  $\Delta$ pflB- $\Delta$ ldhA- $\Delta$ pta- $\Delta$ adhE strain,  
 100 the gene knockout procedure was carried out via the CRISPR/Cas9 method (Cong et  
 101 al. 2013). The engineering strains with corresponding plasmids were obtained by  
 102 standard electroporation or heat-shock approach. All the details of plasmids and  
 103 strains are provided in Supplementary Table S2.

#### 104 **Shake flask cultivation**

105 Colonies were inoculated from solid agar plates into 10 mL test tubes containing 2  
 106 mL LB medium and cultivated at 37°C and 250 rpm to prepare the seed culture. The  
 107 next day, fresh overnight cultures (0.15 mL) were inoculated into 50 mL shake flasks  
 108 containing 15 mL modified M9 medium with appropriate antibiotics. The components  
 109 of the modified M9 medium were given in Supplementary Material. Upon the cell

density reaching 0.4-0.6, isopropyl  $\beta$ -D-1-thiogalactopyranoside (IPTG) was added to the media to a final concentration of 10  $\mu$ M for inducing the gene expressions. The cell cultures were shifted to 30°C and 250 rpm for the aromatic amine productions. Samples were periodically taken for monitoring the cell growth by using a microplate reader (Biotek, Synergy H1).

### HPLC analysis of aromatic amine levels

The samples were centrifuged at 14,000 rpm for 10 min to remove the cellular pellets. Shimadzu LC-20A system equipped with a photodiode array detector and a reversed-phase C18 column (150 mm  $\times$  4.6 mm  $\times$  5  $\mu$ m) was used for the quantitation of aromatic amines. The column was maintained at a temperature of 40 °C. To identify tyramine and phenethylamine, the mobile phase comprising 90% ultrapure H<sub>2</sub>O (supplemented with 0.1% trifluoroacetic acid) and 10% acetonitrile was used. For dopamine detection, the mobile phase containing 95% ultrapure H<sub>2</sub>O (supplemented with 0.1% trifluoroacetic acid) and 5% acetonitrile was used. The flow rate was maintained at 1.0 mL/min. The wavelengths used for detecting tyramine, dopamine and phenethylamine were set at 222 nm, 203 nm, and 208 nm, respectively. The retention times of tyramine, dopamine and phenethylamine were 3.5 min, 3.8 min and 8.3 min, respectively. The aromatic amine levels were quantitated using an external standard curve based on authentic standards.

## Results

### *De novo* production of tyramine in *E. coli*

As shown in Fig.1, aromatic amines such as tyramine and phenethylamine can be

132 produced by coupling the aromatic amino acid synthesis with heterologous expression  
133 of aromatic amino acid decarboxylase (AADC). In this study, we chose AADC from  
134 *E. faecium* (PheDC) as it has been functionally expressed in *E. coli*, resulting in  
135 L-phenylalanine and L-tyrosine decarboxylase activities (Marcobal et al. 2006). To  
136 increase the metabolic flux toward the SHK pathway, the feedback-resistant genes  
137 encoding 3-deoxy-D-arabinoheptulosonate-7-phosphate synthase (*aroG*) together  
138 with chorismate mutase/prephenate dehydratase (*pheA*) or chorismate  
139 mutase/prephenate dehydrogenase (*tyrA*) were overexpressed in MG1655 (DE3) with  
140  $\Delta tyrA \Delta pheA$ . As shown in Fig.2a, we constructed two plasmids for expressing  
141 *aroG<sup>fbr</sup>*, *tyrA<sup>fbr</sup>*, and *pheDC* for tyramine production in *E. coli*. To identify suitable  
142 carbon sources for the synthesis of aromatic amines, we compared the modified M9  
143 media with 4% (w/v) glycerol or glucose for tyramine productions. As shown in Fig.  
144 2b, the tyramine titer reached  $940 \pm 46$  mg/L when 4% glycerol was used, whereas  
145 only  $656 \pm 72$  mg/L tyramine was obtained in glucose-containing medium. The  
146 maximum levels of tyrpine were achieved around 36 h, and further cultivation did not  
147 obviously improve the titer.

#### 148 **Tyramine production by abolishing fermentation side-pathways**

149 When cultured in the absence of oxygen, *E. coli* undergoes a mixed acid fermentation  
150 (Forster and Gescher 2014), resulting in the production of ethanol, acetate, lactate,  
151 and formate (Clark 1989; Gonzalez et al. 2008). These fermentation byproducts are  
152 respectively mediated by lactate dehydrogenase (*ldhA*) and formate acetyltransferase  
153 (*pflB*) in the pyruvate catabolism and by phosphate acetyltransferase (*pta*) and alcohol

dehydrogenase (*adhE*) in the acetyl-CoA catabolism (Clark 1989; Trotter et al. 2011).

Even under aerobic circumstances, *E. coli* diverts a significant amount of carbon flow to fermentation byproducts as a consequence of glycolytic overflow (Kang et al. 2009). It was reported that the accumulation of by-products such as lactate, acetate, and formate would arrest the cell growth (Causey et al. 2004). In addition, James Liao's group has demonstrated that the knockout of genes that contribute to the fermentation byproduct formation such as *adhE*, *ldhA*, *pflB*, and *pta* could substantially improve isobutanol productions in *E. coli* (Atsumi et al. 2008). Therefore, the reduced formation of fermentation byproducts would theoretically channel more carbon flux to the products-of-interest, and maximize the productivity.

In this study, we further proceeded to engineer the *E. coli* metabolism by mitigating byproduct formations, so that more metabolic flux could be diverted to aromatic amines. In particular, lactate dehydrogenase (*ldhA*), formate acetyltransferase (*pflB*), phosphate acetyltransferase (*pta*), and alcohol dehydrogenase (*adhE*) were deleted by CRISPR/Cas9 mediated approach (Fig. 3a). According to Fig. 3b, the deletions of side pathway genes resulted in a slightly slower growth rate of strain TA3.0 than that of TA1.0 at the initial stage, indicating that disruption of fermentation related pathways would slightly affect the cells growth at the initial phase. However, strain TA3.0 surpassed the growth of strain TA1.0 after 12 h, probably because more carbon flux toward the TCA cycle enhances energy utilization, resulting in a higher cell density at the later phase. As shown in Fig. 3b, strain TA3.0 produced  $1.965 \pm 0.205$  g/L tyramine at 72 h, which is 2.1 times compared with that of strain TA1.0 ( $940 \pm 46$  mg/L),

176 confirming that disruption of fermentation related pathways could effectively increase  
177 more carbon flux toward aromatic amine synthesis.

### 178 **Dopamine production using the strains carrying the *hpaBC* gene**

179 For investigating the feasibility of this platform for producing other aromatic amines,  
180 we continued our efforts to synthesize dopamine, an important pharmaceutical  
181 compound. Endogenous 4-hydroxyphenylacetate 3-monooxygenase from *E. coli* is an  
182 enzyme with two components encoded by *hpaB* and *hpaC* genes that adds a second  
183 hydroxyl group at the *ortho* position to 4-hydroxyphenylacetate and L-tyrosine (Guo  
184 et al. 2021). As shown in Fig. 4a, L-tyrosine can be hydroxylated to form  
185 L-dihydroxyphenylalanine (L-DOPA) by HpaBC, which is further decarboxylated to  
186 dopamine. Alternatively, the hydroxylation step might also occur at  
187 4-hydroxyphenylacetate or tyramine. Overall, the enzyme cascade for dopamine  
188 production comprises AroG<sup>fbr</sup>, TyrA<sup>fbr</sup>, HpaBC, and PheDC (Fig. 4b). As shown in  
189 Fig. 4c, there was no significant difference in cell growth between strain DA1.0 and  
190 DA3.0 during dopamine synthesis. Notably, strain DA3.0 exhibited the highest level  
191 of dopamine production (703±21 mg/L) at 48 h, which is nearly 2.9 times than that of  
192 strain DA1.0 (242±24 mg/L). Therefore, we achieved a considerable improvement in  
193 dopamine production than the previous study (Nakagawa et al. 2011).

194 As mentioned above, L-tyrosine can also be first converted to tyramine under the  
195 action of PheDC (Fig. 4a). To this end, we also measured the accumulation of  
196 tyramine during dopamine production. As shown in Fig. 4d, both strain DA1.0 and  
197 DA3.0 accumulated a substantial amount of tyramine, indicating the HpaBC activity

198 toward tyramine hydroxylation might be not sufficient. Surprisingly, more tyramine  
199 was accumulated in the DA1.0 strain than that of strain DA3.0. We reasoned that the  
200 hydroxylation reaction requires a large amount of NADH and NADPH, critical  
201 cofactors for the flavin reduction by *hpaC*. Therefore, knockout of side-pathways that  
202 consume NADH and NADPH might favor the hydroxylation reaction with improved  
203 dopamine production in strain DA3.0.

#### 204 **Phenylethylamine production using the engineered *E. coli* strain**

205 Next, we also proceeded with phenylethylamine production in the engineered *E. coli*.  
206 Briefly, we replaced *aroG<sup>fbr</sup>-tyrA<sup>fbr</sup>* plasmid with *aroG<sup>fbr</sup>-pheA<sup>fbr</sup>* to switch the  
207 metabolic flux from L-tyrosine to L-phenylalanine synthesis. The two-plasmid system  
208 with enzyme cascade including *aroG<sup>fbr</sup>-pheA<sup>fbr</sup>* and *pheDC* was used to convert  
209 L-phenylalanine to phenylethylamine (Fig. 5a). As depicted in Fig. 5b, the maximum  
210 phenethylamine production of 555±50 mg/L was achieved by strain PEA3.0 after 72 h,  
211 which was increased by 2.28-fold compared with that of strain PEA1.0 (169±20  
212 mg/L). The phenethylamine level achieved in the shake flask by our study was also  
213 much higher than that of a recent report (Xu and Zhang 2020). In addition, we found  
214 that both strain PEA1.0 and PEA3.0 gave a similar growth profile (Fig. 5b). However,  
215 the final cell densities for phenylethylamine-producing strains were slightly lower  
216 than dopamine or tyramine-producing strains. Since strain PEA1.0 and 3.0 with  
217 different levels of phenylethylamine gave a similar growth rate, we concluded that  
218 phenylethylamine is not toxic to the cells at the current concentrations. Therefore, it is  
219 likely that the reduced biomass of phenylethylamine-producing strains was mainly

220 caused by  $\Delta tyrA$ .

## 221 Discussion

222 Aromatic amines have a wide range of applications in medicine, chemistry, and  
 223 biology. Rapid advances in synthetic biology have facilitated the study of aromatic  
 224 compound biosynthesis and offered an engineering framework for producing  
 225 high-value aromatic compounds. *E. coli* has been genetically engineered to boost the  
 226 productivity of aromatic compounds by removing or inhibiting undesirable genes and  
 227 increasing the expression of rate-limiting genes. In this study, we applied similar  
 228 strategies to increase the metabolic flux toward the SHK pathway. By introducing  
 229 feedback-resistant versions of *tyrA*<sup>fbr</sup>/*pheA*<sup>fbr</sup> and *aroG*<sup>fbr</sup>, the recombinant strains with  
 230 a further expression of *pheDC* from *E. faecium* were able to produce 940±46 mg/L  
 231 tyramine, 242±24 mg/L dopamine, and 169±20 mg/L phenylethylamine, respectively.  
 232 According to recent studies, decreasing mixed acid fermentation is proved to be  
 233 effective in increasing the production of 3-hydroxypropionate (3HP) (Liu et al. 2018),  
 234 polyhydroxyalkanoate (Jung et al. 2019),  $\beta$ -alanine (Zou et al. 2020), and  
 235 2,3-butanediol (2,3-BD) (Song et al. 2019). Therefore, we further constructed a high  
 236 titer aromatic amine production platform by abolishing fermentation side-pathways.  
 237 Namely, the genes of (i) *ldhA* and *pflB* (from pyruvate to lactate and formate) and (ii)  
 238 *pta* and *adhE* (from acetyl-CoA to acetate and ethanol) were deleted to improve the  
 239 metabolic flux from the carbon source to the SHK pathway. Finally, 1.965±0.205 g/L  
 240 tyramine, 703±21 mg/L dopamine, and 555±50 mg/L phenethylamine were obtained  
 241 from 40 g/L glycerol in shake flask cultivation. The titers achieved by us were much

242 higher than previous reports under shake flask conditions (Koma et al. 2012;  
243 Nakagawa et al. 2011; Xu and Zhang 2020).

244 During the dopamine biosynthesis, we observed that both strain DA1.0 and DA3.0  
245 accumulated a substantial amount of tyramine, suggesting that the HpaBC could not  
246 effectively hydroxylate tyramine to dopamine. Considering that a mutant HpaBC was  
247 recently identified with good activity toward the tyramine hydroxylation (Chen et al.  
248 2019), it will be possible to address tyramine accumulation by simply introducing the  
249 mutant HpaBC to our engineered *E. coli* strain. In addition, we were surprised to find  
250 out that less tyramine was accumulated in strain DA3.0 when compared to that of  
251 strain DA1.0. Since the hydroxylation reaction requires additional reducing power for  
252 flavin recycling, the knockout of fermentation side-pathways that consume NADH  
253 and NADPH would also improve dopamine synthesis. Therefore, it is likely that our  
254 engineered platform would be of great interest for hosting other biosynthetic  
255 pathways that require cofactors such as NADH and NADPH.

256 In summary, we have optimized the SHK pathway and eliminated the side-pathways  
257 involved in the mixed acid fermentation to enable high-titer generation of aromatic  
258 amines in metabolically modified *E. coli*. We demonstrated that the knockout of  
259 *ldhA-pflB-pta-adhE* is an effective strategy in improving aromatic amine productions.

260 Based on our findings, we believe that the *E. coli* system has great prospects for the  
261 future industrial-scale aromatic amine production. Moreover, these engineered strains  
262 might also be used to manufacture other aromatic compounds with pharmaceutical  
263 value.

## 264 **Author contributions**

265 J. Y. conceived and designed the project. T. Y. and P. W. constructed the plasmids,  
266 strains and collected the data. T. Y. and Y. Z. analyzed the data. T.Y., Y. Z. and J. Y.  
267 wrote the manuscript.

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## 271 **Compliance with ethical standards**

272 **Conflicts of interest** The authors declare that they have no competing interests.

273 **Ethical approval** This study does not contain any studies with human participants or  
274 animals performed by any of the authors.

275 **Data availability** All data generated or analyzed during this study are included in this  
276 published article [and its supplementary information files].

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# Figure Captions

**Fig. 1.** Schematic diagram of aromatic amine production from the shikimate (SHK)

pathway. PP pathway: pentose phosphate pathway; AroG, 3-deoxy-D-arabinoheptulosonate-7-phosphate synthase; TyrA, chorismate mutase/prephenate dehydrogenase; PheA, chorismate mutase/prephenate dehydratase; HapBC, 4-hydroxyphenylacetate 3-monooxygenase; AADC, aromatic amino acid decarboxylase. DHAP: Dihydroxyacetone phosphate; E4P: D-erythrose 4-phosphate; PEP: Phosphoenolpyruvate; DAHP: 3-deoxy-D-arabino-heptulosonate-7-phosphate; CHOR: Chorismate; PREPH: Prephenate; L-Tyr, L-tyrosine; L-Phe, L-phenylalanine. *ldhA* encodes D-lactate dehydrogenase; *pflB* encodes pyruvate formate lyase; *pta* encodes phosphotransacetylase; *adhE* encodes alcohol dehydrogenase. The red box highlights the fermentation byproduct related pathways. Dashed lines illustrate multiple steps.

**Fig. 2.** Engineering *E. coli* for tyramine overproduction. (a) The plasmids for

tyramine production. *AroG<sup>fbr</sup>*, feedback resistant 3-deoxy-d-arabinoheptulosonate-7-phosphate synthase; *TyrA<sup>fbr</sup>*, feedback resistant chorismate mutase/prephenate dehydrogenase; *PheDC*, aromatic amino acid decarboxylase from *E. faecium*. (b) Growth profile and tyramine production of strain

TA1.0 under shake flasks. All the experiments were carried out in 15 mL modified M9 medium containing 40 g/L glycerol or 40 g/L glucose. Experiments were performed in triplicate biological repeats and the data represent the mean value with standard deviation.

**Fig. 3.** Knockout of fermentation byproduct related pathways substantially improved tyramine production. (a) Agarose gel image for PCR verification of gene knockout events. Strain G1.0 strain with  $\Delta\text{tyrA}$ - $\Delta\text{pheA}$  was used as the control. Strain G3.0 with further deletion of *pflB*, *ldhA*, *adhE*, and *pta* was confirmed by diagnostic PCR. (b) Growth profile and tyramine production of strains TA1.0 and TA3.0. All the experiments were carried out in 15 mL modified M9 medium containing 40 g/L glycerol. Experiments were performed in triplicate biological repeats and the data represent the mean value with standard deviation.

**Fig. 4.** The dopamine production in shake flasks. (a) The proposed biosynthetic route toward dopamine synthesis. The dashed arrow of HpaBC indicates the poor activity of HpaBC in converting tyramine to dopamine. (b) The plasmids for dopamine production. (c) Growth profile and dopamine production of strains DA1.0 and DA3.0. (d) Tyramine accumulation in strains DA1.0 and DA3.0. All the experiments were carried out in 15 mL modified M9 medium containing 40 g/L glycerol. Experiments

431 were performed in triplicate biological repeats and the data represent the mean value

432 with standard deviation.

433 **Fig. 5.** The phenethylamine production in shake flasks. **(a)** The plasmids for

434 phenethylamine production. PheA<sup>fbr</sup>, feedback-resistant chorismate

435 mutase/prephenate dehydratase. **(b)** Growth profile and phenethylamine production of

436 strains PEA1.0 and PEA3.0. All the experiments were carried out in 15 mL of

437 modified M9 medium containing 40 g/L glycerol. Experiments were performed in

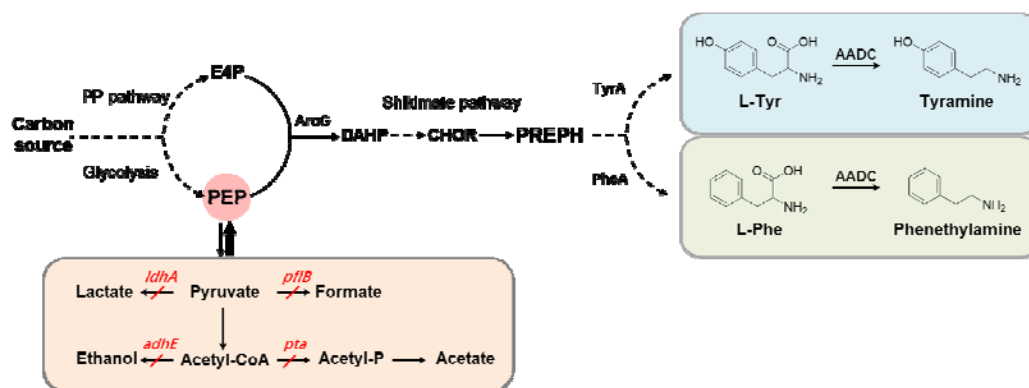
438 triplicate biological repeats and the data represent the mean value with standard

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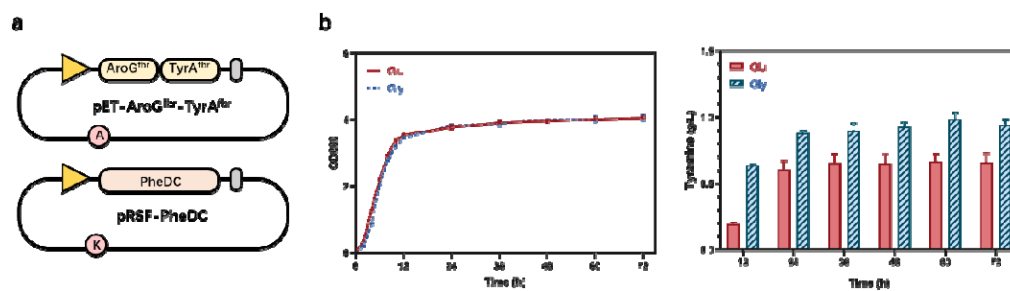


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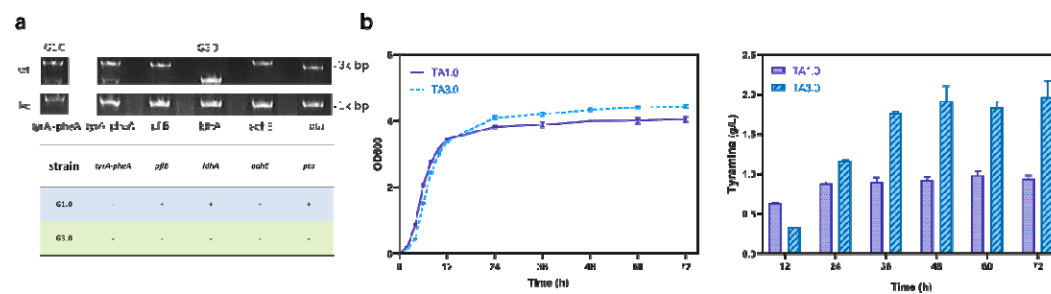
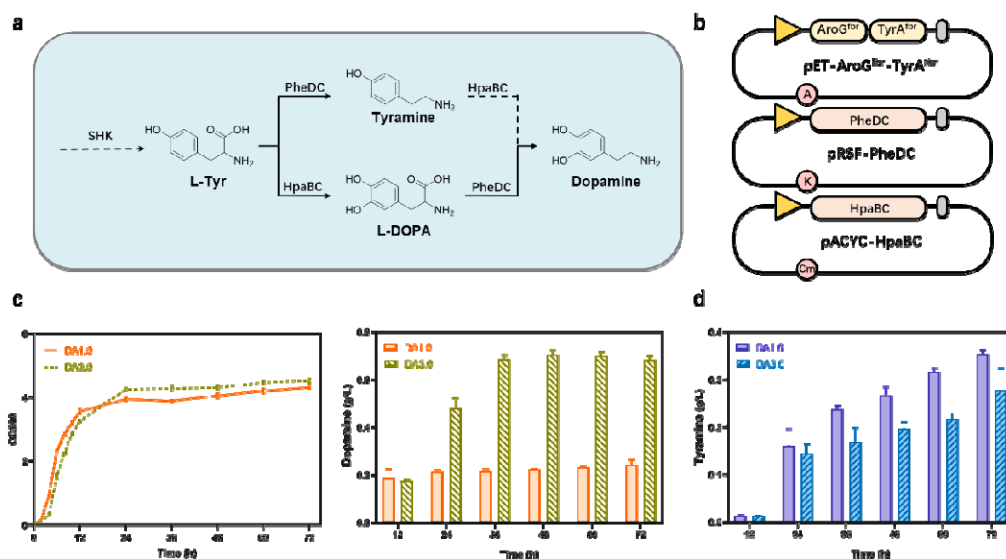


Fig. 3.

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455 Fig. 4.

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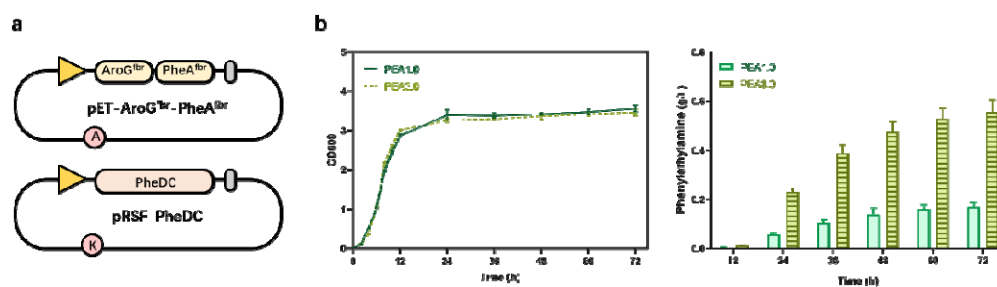
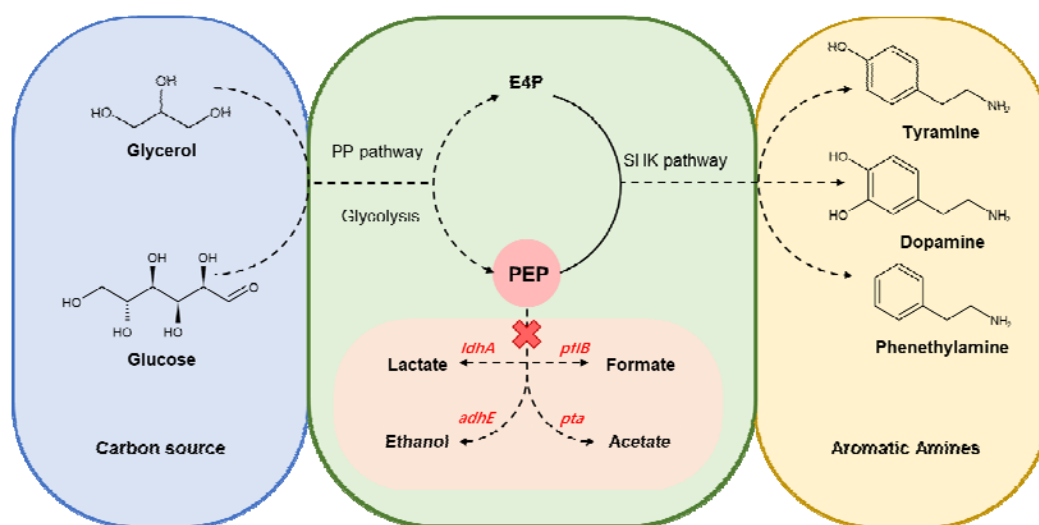


Fig. 5.



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