

1 **High-titer production of aromatic amines in metabolically engineered**

2 ***Escherichia coli***

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8 **ABSTRACT**

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

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

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

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

32 **Introduction**

33 Aromatic compounds represent a large and diverse class of chemicals that are widely
34 used in manufacturing solvents, polymers, fine chemicals, feed and food additives,
35 nutraceuticals, and medicines (Huccetogullari et al. 2019; Shen et al. 2020; Wang et al.
36 2018). Among them, aromatic amines with diverse physical characteristics are often
37 employed as antioxidants, and precursors to pharmaceutical products (Masuo et al.
38 2016; Minami 2013). For example, tyramine is a high-value industrial product with
39 widespread applications in medicine (Beltran et al. 2011). Dopamine acts as an
40 intermediate in the biosynthesis of epinephrine and other drugs (Davie 2008; Jeong et
41 al. 2018), and also can influence the physiological activity of plants and humans
42 (Liang et al. 2018; Wise 2004). In addition, phenylethylamine is a precursor of
43 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

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

72 **Materials and methods**

73 **Strains and reagents**

74 *E. coli* DH5 α was utilized to construct plasmids, and *E. coli* MG1655 (DE3) derived
75 strain with Δ tyrA and Δ pheA (Lai et al. 2022) was employed as the chassis cell for
76 aromatic amine production. LB medium containing 10 g/L tryptone, 5 g/L yeast
77 extract, and 10 g/L NaCl was applied for cultivating *E. coli*. Appropriate antibiotics
78 (100 μ g/mL ampicillin, 50 μ g/mL kanamycin, 34 μ g/mL chloramphenicol) were
79 supplemented to maintain the plasmids when needed. Enzymes (high-fidelity phusion
80 polymerase, *Bam*HI-HF, *Xho*I, *Bsa*I-HF, *Esp*3I-HF, and T4 DNA ligase) were
81 purchased from New England Biolabs (Beverly, MA, USA). PCR purification kit, gel
82 extraction kit, and plasmid DNA extraction kit were all purchased from BioFlux
83 (Shanghai, China). The details of chemicals used in this study are provided in
84 Supplementary materials.

85 **Plasmid and strain construction**

86 All the genes were PCR amplified using high-fidelity phusion polymerase. The
87 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^{fbr}-TyrA^{fbr} and pET-AroG^{fbr}-PheA^{fbr} were constructed in a
92 similar way to our previous report (Lai et al. 2022). In brief, the genes encoding
93 *tyrA^{fbr}*, *pheA^{fbr}*, and *aroG^{fbr}* 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 Δ pflB- Δ ldhA- Δ pta- Δ 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

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

115 **HPLC analysis of aromatic amine levels**

116 The samples were centrifuged at 14,000 rpm for 10 min to remove the cellular
117 pellets. Shimadzu LC-20A system equipped with a photodiode array detector and a
118 reversed-phase C18 column (150 mm \times 4.6 mm \times 5 μ m) was used for the quantitation
119 of aromatic amines. The column was maintained at a temperature of 40 °C. To
120 identify tyramine and phenethylamine, the mobile phase comprising 90% ultrapure
121 H₂O (supplemented with 0.1% trifluoroacetic acid) and 10% acetonitrile was used.
122 For dopamine detection, the mobile phase containing 95% ultrapure H₂O
123 (supplemented with 0.1% trifluoroacetic acid) and 5% acetonitrile was used. The flow
124 rate was maintained at 1.0 mL/min. The wavelengths used for detecting tyramine,
125 dopamine and phenethylamine were set at 222 nm, 203 nm, and 208 nm, respectively.
126 The retention times of tyramine, dopamine and phenethylamine were 3.5 min, 3.8 min
127 and 8.3 min, respectively. The aromatic amine levels were quantitated using an
128 external standard curve based on authentic standards.

129 **Results**

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

131 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 Δ *tyrA* Δ *pheA*. As shown in Fig.2a, we constructed two plasmids for expressing
141 *aroG^{fbr}*, *tyrA^{fbr}*, 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±46 mg/L when 4% glycerol was used, whereas
145 only 656±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

154 dehydrogenase (*adhE*) in the acetyl-CoA catabolism (Clark 1989; Trotter et al. 2011).
155 Even under aerobic circumstances, *E. coli* diverts a significant amount of carbon flow
156 to fermentation byproducts as a consequence of glycolytic overflow (Kang et al.
157 2009). It was reported that the accumulation of by-products such as lactate, acetate,
158 and formate would arrest the cell growth (Causey et al. 2004). In addition, James
159 Liao's group has demonstrated that the knockout of genes that contribute to the
160 fermentation byproduct formation such as *adhE*, *ldhA*, *pflB*, and *pta* could
161 substantially improve isobutanol productions in *E. coli* (Atsumi et al. 2008).
162 Therefore, the reduced formation of fermentation byproducts would theoretically
163 channel more carbon flux to the products-of-interest, and maximize the productivity.
164 In this study, we further proceeded to engineer the *E. coli* metabolism by mitigating
165 byproduct formations, so that more metabolic flux could be diverted to aromatic
166 amines. In particular, lactate dehydrogenase (*ldhA*), formate acetyltransferase (*pflB*),
167 phosphate acetyltransferase (*pta*), and alcohol dehydrogenase (*adhE*) were deleted by
168 CRISPR/Cas9 mediated approach (Fig. 3a). According to Fig. 3b, the deletions of side
169 pathway genes resulted in a slightly slower growth rate of strain TA3.0 than that of
170 TA1.0 at the initial stage, indicating that disruption of fermentation related pathways
171 would slightly affect the cells growth at the initial phase. However, strain TA3.0
172 surpassed the growth of strain TA1.0 after 12 h, probably because more carbon flux
173 toward the TCA cycle enhances energy utilization, resulting in a higher cell density at
174 the later phase. As shown in Fig. 3b, strain TA3.0 produced 1.965 ± 0.205 g/L tyramine
175 at 72 h, which is 2.1 times compared with that of strain TA1.0 (940 ± 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^{fbr}, TyrA^{fbr}, 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^{fbr}-tyrA^{fbr}* plasmid with *aroG^{fbr}-pheA^{fbr}* to switch the
207 metabolic flux from L-tyrosine to L-phenylalanine synthesis. The two-plasmid system
208 with enzyme cascade including *aroG^{fbr}-pheA^{fbr}* 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*^{fbr}/*pheA*^{fbr} and *aroG*^{fbr}, 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), β -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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395 **Figure Captions**

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

397 pathway. PP pathway: pentose phosphate pathway; AroG,

398 3-deoxy-D-arabinoheptulosonate-7-phosphate synthase; TyrA, chorismate

399 mutase/prephenate dehydrogenase; PheA, chorismate mutase/prephenate dehydratase;

400 HapBC, 4-hydroxyphenylacetate 3-monooxygenase; AADC, aromatic amino acid

401 decarboxylase. DHAP: Dihydroxyacetone phosphate; E4P: D-erythrose 4-phosphate;

402 PEP: Phosphoenolpyruvate; DAHP: 3-deoxy-D-arabino-heptulosonate-7-phosphate;

403 CHOR: Chorismate; PREPH: Prephenate; L-Tyr, L-tyrosine; L-Phe, L-phenylalanine.

404 *ldhA* encodes D-lactate dehydrogenase; *pflB* encodes pyruvate formate lyase; *pta*

405 encodes phosphotransacetylase; *adhE* encodes alcohol dehydrogenase. The red box

406 highlights the fermentation byproduct related pathways. Dashed lines illustrate

407 multiple steps.

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

409 tyramine production. *AroG^{fbr}*, feedback resistant

410 3-deoxy-d-arabinoheptulosonate-7-phosphate synthase; *TyrA^{fbr}*, feedback resistant

411 chorismate mutase/prephenate dehydrogenase; *PheDC*, aromatic amino acid

412 decarboxylase from *E. faecium*. (b) Growth profile and tyramine production of strain

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

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

425 **Fig. 4.** The dopamine production in shake flasks. (a) The proposed biosynthetic route
426 toward dopamine synthesis. The dashed arrow of HpaBC indicates the poor activity of
427 HpaBC in converting tyramine to dopamine. (b) The plasmids for dopamine
428 production. (c) Growth profile and dopamine production of strains DA1.0 and DA3.0.
429 (d) Tyramine accumulation in strains DA1.0 and DA3.0. All the experiments were
430 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^{fbr}, 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

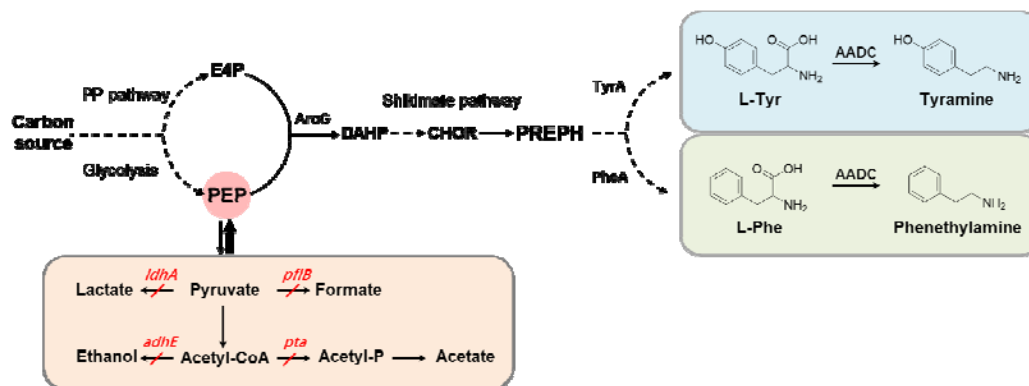
438 triplicate biological repeats and the data represent the mean value with standard

439 deviation.

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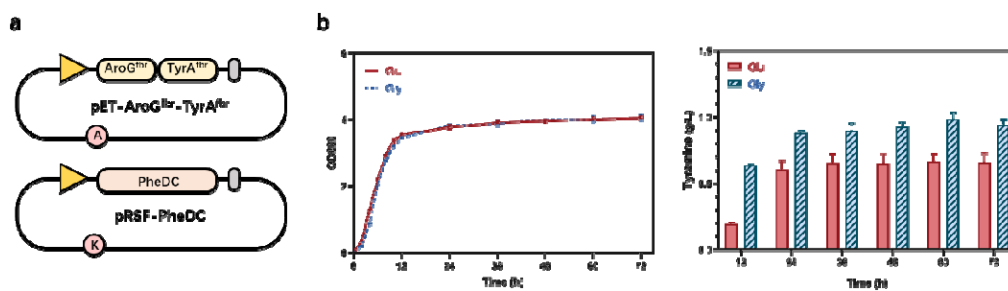


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444 Fig. 1.

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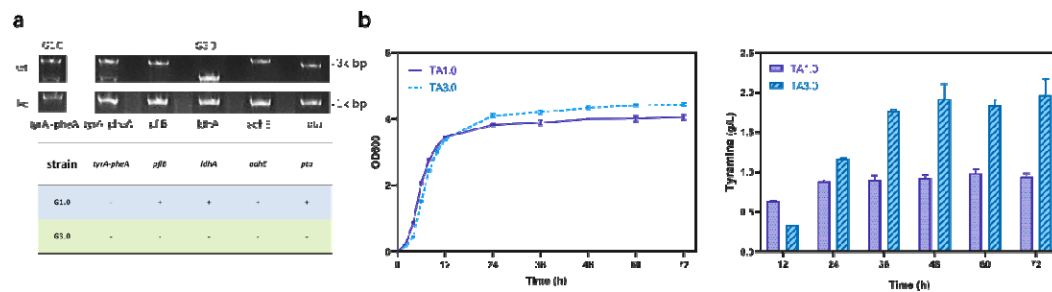
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448 Fig. 2.

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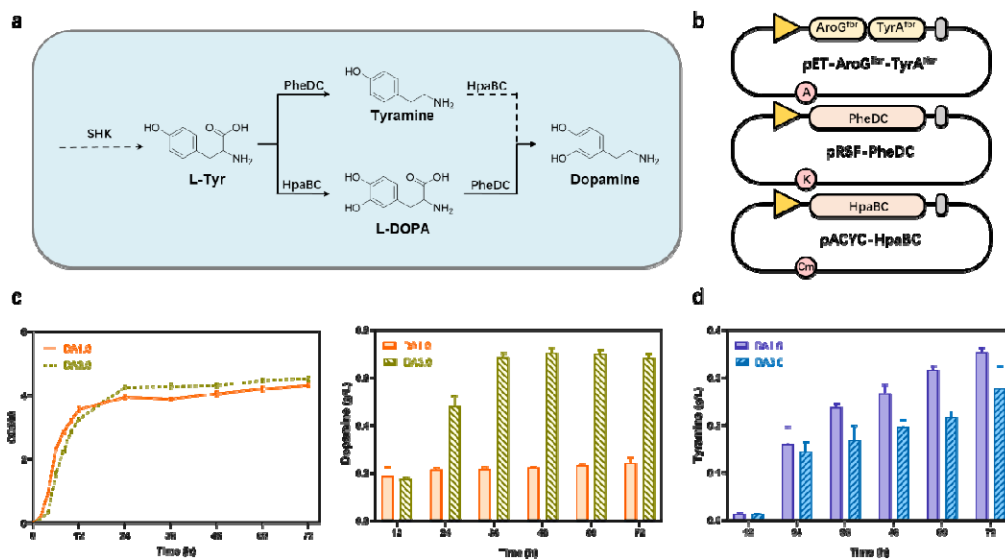


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451 Fig. 3.

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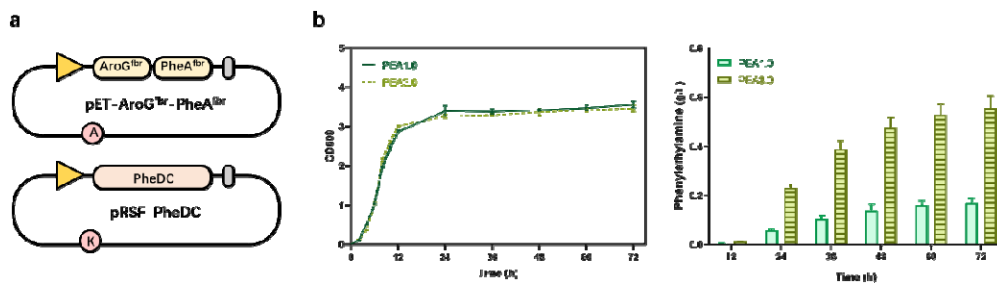
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455 Fig. 4.

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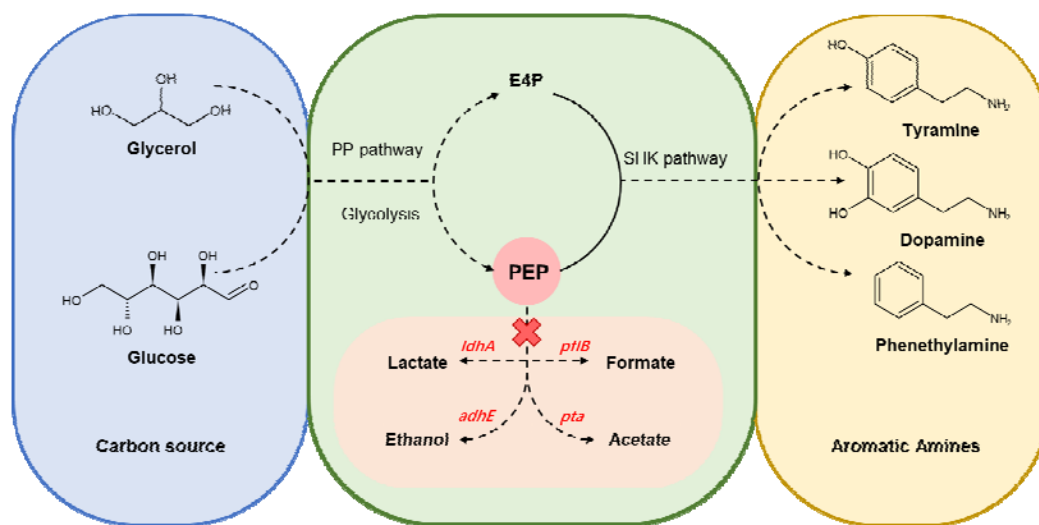


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461 Fig. 5.

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TOC