Synthesis of three major auxins from glucose in Engineered *Escherichia coli*

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ABSTRACT

Indole-3-acetic acid (IAA) is considered the most common and important naturally occurring auxin in plants and a major regulator of plant growth and development. In addition, phenylacetic acid (PAA) and 4-hydroxyphenylacetic acid (4HPA) can also play a role as auxin in some plants. In recent years, several microbes have been metabolically engineered to produce IAA from L-tryptophan. In this study, we showed that aminotransferase *aro8* and decarboxylase *kdc* from *Saccharomyces cerevisiae*, and aldehyde dehydrogenase *aldH* from *Escherichia coli* have broad substrate ranges and can catalyze the conversion of three kinds of aromatic amino acids (L-tryptophan, L-tyrosine or L-phenylalanine) to the corresponding IAA, 4HPA and PAA. Subsequently, three de novo biosynthetic pathways for the production of IAA, PAA and 4HPA from glucose were constructed in *E. coli* through strengthening the shikimate pathway. This study described here shows the way for the development of agricultural microorganism for biosynthesis of plant auxin and promoting plant growth in the future.

Keywords: metabolic engineering; *Escherichia coli*; auxin.

45 INTRODUCTION

46 Indole-3-acetic acid (IAA) is the most common plant auxin and it plays a central
 47 role in modulating plant growth and development ¹. Phenylacetic acid (PAA) and
 48 4-Hydroxyphenylacetic acid (4HPA) can also play a role as plant auxin in some plants
 49 ²⁻⁵. Many rhizosphere microbes can synthesize IAA which acts as a signal molecule to
 50 promote plant growth ^{6,7}. IAA synthesis in microbes can regulate the growth of plants
 51 by changing the plant auxin pool^{8,9}. Moreover, microbiological IAA could
 52 significantly increases root surface area and length, and therefore strengthen the
 53 absorption of water and nutrient by plants, which in turn stimulate the growth of
 54 microbes ^{10,11}.

55 Multiple IAA biosynthetic pathways have been described in microbes, most of
 56 which use L-tryptophan as the starting substrate. These L-tryptophan-dependent
 57 pathways include indole-3-acetamide, indole-3-pyruvate, tryptamine, tryptophan
 58 side-chain oxidase and indole-3-acetonitrile pathways¹². The most studied is the
 59 indole-3-pyruvic acid pathway, which involves three steps. L-tryptophan is first
 60 converted to indole-3-pyruvic acid by an aminotransferase. Subsequently, a
 61 decarboxylase enzyme converts indole-3-pyruvic acid into indole-3-acetylaldehyde.
 62 Finally, indole-3-acetylaldehyde is converted to IAA by aldehyde dehydrogenase. In
 63 the past decades, great efforts have been made in identifying the key genes of the
 64 indole-3-pyruvic acid pathway. Aminotransferases for the conversion of the
 65 L-tryptophan to indole-3-pyruvic acid has been reported in several strains^{13,14}. The
 66 gene encoding indole-3-pyruvic acid decarboxylase for the conversion of the

indole-3-pyruvic acid to indole-3-acetylaldehyde has been identified in *Azospirillum*
brasilense, *Enterobacter cloacae*, and *Pseudomonas putida*¹⁵⁻¹⁸. Putative
indole-3-acetylaldehyde dehydrogenase genes have been identified in the genomes of
Bacillus amyloliquefaciens FXB42, *Azospirillum brasilense* Yu62 and *Neurospora*
*crassa*¹⁹⁻²¹.

In recent years, many wild microbes have been evaluated the capacity of synthetic
IAA from L-tryptophan^{19,22-28}. Further, several microbes have been metabolically
engineered to produce IAA from L-tryptophan^{29,30}. However, the de novo synthesis
of IAA from glucose by engineered micro-organisms is lacking. In this study, we
showed that aminotransferase *aro8* and decarboxylase *kdc* from *Saccharomyces*
cerevisiae, and aldehyde dehydrogenase *aldH* from *Escherichia coli* have broad
substrate ranges and can catalyze the conversion of three kinds of aromatic amino
acids (tryptophan, tyrosine or phenylalanine) to the corresponding IAA, 4HPA and
PAA. Subsequently, three de novo biosynthetic pathways for the production of IAA,
PAA and 4HPA from glucose were constructed in *E. coli* through strengthening the
shikimate pathway (Figure 1). This study described here shows the way for the
development of agricultural microorganism for biosynthesis of plant auxin and
promoting plant growth in the future.

MATERIALS AND METHODS

Enzymes, chemicals, and strains

Restriction endonucleases and DNA polymerases were purchased from New
England Biolabs. T4 DNA ligase, plasmid extraction kits, PCR purification kits and

89 DNA extraction kits were purchased from Fermentas (Burlington, Canada). Plasmid
90 construction and screening were performed in *E. coli* DH1013. A reduced aromatic
91 aldehyde reduction *E. coli* strain RARE whose seven aldehyde reductase genes (*dkgB*,
92 *yeaE*, *yqhC*, *yqhD*, *dkgA*, *yahK*, *yjgB*) have been deleted, got the aromatic aldehydes
93 as end products could be accumulated, was used as a host for fermentation to
94 synthesize PAA and 4HPA. *E. coli* strain DG101 which was constructed by deleting
95 the *trpR* gene using the CRISPR/Cas9 technology in strain RARE by Genewiz
96 Biotech Co. Ltd, was used as a host for fermentation to synthesize IAA.

97 **Plasmid construction**

98 Plasmid pDG11 for expression *aroG^{fbr}* and pDG14 for co-expression *aroG^{fbr}* and
99 *pheA^{fbr}* gene from *E. coli* was constructed in our previous study³¹. *TyrA^{fbr}*
100 (M53I/A354V, GenBank NP_417091.1) was synthesized by Genewiz Biotech Co. Ltd
101 and amplified by PCR using primers *tyrA^{fbr}-XbaI* and *tyrA^{fbr}-SpeI-Sall*, and ligated
102 into pET28a(+) via *XbaI* and *Sall* to yield plasmid pDG20. The *XbaI-Sall* fragments
103 of *tyrA^{fbr}* from pDG20 was inserted into *SpeI* and *Sall* sites of pDG11 to give pDG21.
104 The *XbaI-Sall* fragments of *aroG^{fbr}* and *tyrA^{fbr}* from pDG21 was inserted into *XbaI*-
105 and *XhoI* sites of pBBRMCS1 to give pDG22.

106 *TrpE^{fbr}* (S40F, GenBank NP_415780.1) was synthesized by Genewiz Biotech Co.
107 Ltd and amplified by PCR using primers *trpE^{fbr}-XbaI* and *trpE^{fbr}-SpeI-BamHI* and
108 ligated into pET28a(+) via *XbaI* and *BamHI* to yield plasmid pDG23. *SerA^{fbr}*
109 (H344A/N364A, GenBank NP_417388.1) was synthesized by Genewiz Biotech Co.
110 Ltd and amplified by PCR using primers *serA^{fbr}-XbaI* and *serA^{fbr}-SpeI-Sall* and

111 ligated into pET28a(+) via *XbaI* and *Sall* to yield plasmid pDG24. The *XbaI-Sall*
 112 fragments of *trpE^{fbr}* from pDG23 was inserted into *SpeI* and *Sall* sites of pDG11 to
 113 give pDG25. The *XbaI-Sall* fragments of *serA^{fbr}* from pDG24 was inserted into *SpeI*
 114 and *Sall* sites of pDG25 to give pDG26. The *XbaI-Sall* fragments of *aroG^{fbr}*, *tyrA^{fbr}*
 115 and *serA^{fbr}* from pDG26 was inserted into *XbaI*- and *XhoI* sites of pBBRMCS1 to give
 116 pDG27.

117 Plasmid pDG9 for co-expression *aro8* and *kdc* gene from *S. cerevisiae* YPH499,
 118 and *aldH* gene from *E. coli* was constructed in our previous study³². *TrpABCD* was
 119 amplified by PCR using primers *trpABCD-XbaI* and *trpABCD-XhoI* and ligated into
 120 pDG9 via *NheI* and *XhoI* to yield plasmid pDG28. All the primers used in this study
 121 are listed in Table 1. The bacterial strains and plasmids used in this study are listed in
 122 Table 2.

123 **Shake flask cultures**

124 Experiments of shaking flask culture were performed at 30 °C in 100 mL modified
 125 M9 medium as previously described by Guo³³. 36 mg/L chloromycetin and 50 mg/L
 126 kanamycin were added to the medium, when needed. When the OD₆₀₀ reached about
 127 0.8, IPTG was added to the medium for induction of gene expression at a final
 128 concentration of 0.1 mM.

129 **Analytical methods**

130 Glass beads were added to 10 mL culture broth for breaking the cells. The pH of
 131 the culture broth was adjusted to 3.0 with 3 M HCl. Ethyl acetate (15 mL) is used to
 132 extract PAA, 4HPA and IAA. After the centrifuge stratification, the upper ethyl

133 acetate phase was withdrawn, evaporated to dryness. These extracted PAA and 4HPA
134 was redissolved in 0.6 mL ethyl acetate. A 1 μ L portion of the ethyl acetate phase was
135 analysed after a split 10:1 injection on an Agilent 7890A GC equipped with an Agilent
136 5975 MS detector and an Agilent DB-5MS capillary column. The following
137 temperature program was applied: 80 °C for 1 min, an increase of 20 °C/min to
138 260 °C, and 260 °C for 5 min.

139 When IAA is directly detected by GC/MS, IAA is partially decomposed into
140 3-Methylindole (Figure 2), probably due to its intolerance to high temperature
141 gasification. To achieve better detection of IAA, IAA was converted to more stable
142 methyl indole-3-acetate ³⁴. The extracted IAA was esterified by redissolving in 1 mL
143 5% H₂SO₄ in methanol and incubating at 90 °C for 2 hr. After the reaction, 1 mL 0.9%
144 NaCl was added into each sample, and then the methyl indole-3-acetate was extracted
145 using 1 mL ethyl acetate for subsequent GC/MS analysis. A 1 μ L portion of the
146 n-hexane phase was analysed after a split 20:1 injection on a HP-5MS capillary
147 column. The following temperature program was applied: 80 °C for 1 min, an
148 increase of 20 °C/min to 250 °C, and 250 °C for 8 min.

149 RESULTS

150 The biosynthesis of IAA and 4HPA from tryptophan and tyrosine in *E. coli*.

151 Several aldehyde dehydrogenases (*feaB*, *aldB* and *aldH*) were reported to have
152 the ability of oxidation phenylacetaldehyde or benzaldehyde in vitro ³⁵⁻³⁷.
153 Recently, we have compared the activity of these aldehyde dehydrogenases for the
154 oxidation of phenylacetaldehyde ³². We found that only *E.coli* AldH is an efficient

155 aldehyde dehydrogenase for oxidation of phenylacetaldehyde to PAA in vivo.
 156 Subsequently, we showed metabolic engineering of *E. coli* to high efficient synthesis
 157 PAA from L-phenylalanine through overexpression of an aminotransferase *aro8* for
 158 the conversion of L-phenylalanine to phenylpyruvate , a keto acid decarboxylase *kdc*
 159 from *S. cerevisiae* for the decarboxylation of phenylpyruvate to phenylacetaldehyde
 160 and an aldehyde dehydrogenase *aldH* from *E.coli* for the oxidation of
 161 phenylacetaldehyde to the corresponding PAA.

162 To determine whether *aro8* and *kdc* from *S. cerevisiae*, and *aldH* from *E.coli* have
 163 broad substrate ranges and can catalyze the conversion of other two kinds of aromatic
 164 amino acids (L-tryptophan or L-tyrosine) to the corresponding IAA and 4HPA. *E.*
 165 *coli* RARE strain harboring a plasmid pDG9 for co-expression *aro8*, *kdc* and *aldH*
 166 gene was grown in modified M9 medium with 0.4 g/L of L-tryptophan (the solubility
 167 of L-tryptophan in water is about 479 mg/L) or 1 g/L L-tyrosine. The resulting *E. coli*
 168 strain DG102 produced up to 303 ± 14.1 mg/L IAA from 0.4 g/L of L-tryptophan with
 169 the molar yield of 0.91 mol/mol or 726 ± 28.3 mg/L 4HPA from 1 g/L of L-tyrosine
 170 with the molar yield of 0.86 mol/mol (Table 3). This proves that *aro8*, *kdc* and *aldH*
 171 can catalyze effectively conversion of L-tryptophan or L-tyrosine to the
 172 corresponding IAA and 4HPA.

173 **Creation of a synthetic pathway for PAA in *E. coli* from glucose.**

174 The L-phenylalanine can be de novo biosynthesized from glucose via
 175 L-phenylalanine pathway. However the efficiency is quite low because of feedback
 176 inhibition in L-phenylalanine pathway. Overexpression of feedback resistant mutant

177 of 3-deoxy-D-arabinoheptulosonate-7-phosphate synthase (*aroG^{fbr}*) and the
 178 bifunctional enzyme chorismate mutase/prephenate dehydratase (*pheA^{fbr}*) has proven
 179 to be an effective strategy to overproduce L-phenylalanine from glucose³⁸⁻⁴⁰. In this
 180 study, a PAA artificial biosynthesis pathway was constructed in *E. coli* strain RARE
 181 by co-expression of two feedback-resistant mutants *aroG^{fbr}* and *pheA^{fbr}* for the
 182 efficient overproduction of L-phenylalanine, aminotransferase *aro8*, decarboxylase
 183 *kdc* and aldehyde dehydrogenase *aldH* for conversion of L-phenylalanine to PAA.
 184 The resulting *E. coli* strain DG103 was cultured in modified M9 medium with 20 g/L
 185 of glucose and produced up to 1217 ± 45.2 mg/L PAA within 28 h (Figure 2). PAA
 186 yield and productivity were calculated to be 60.8 mg/g glucose and 43.7 mg/L/h,
 187 respectively (Table 4). This proves that the designed PAA synthetic pathway was
 188 functionally expressed in *E. coli*.

189 **Creation of a synthetic pathway for 4HPA from glucose.**

190 After successfully demonstrating the biosynthesis of 4HPA from L-tyrosine in *E.*
 191 *coli*, we further designed the biosynthesis of 4HPA from glucose. The L-tyrosine can
 192 be de novo biosynthesized from glucose via L-tyrosine pathway^{41,42}. Overexpression
 193 of feedback resistant mutant of *aroG^{fbr}* and chorismate mutase/prephenate
 194 dehydrogenase *tyrA^{fbr}* is an essential strategy to overproduce L-tyrosine^{43,44}. In this
 195 study, a 4HPA biosynthesis pathway was constructed in *E. coli* strain RARE by
 196 co-expression of two feedback-resistant mutants (*aroG^{fbr}* and *tyrA^{fbr}*) for the efficient
 197 overproduction of L-tyrosine, *aro8*, *kdc* and *aldH* for conversion of L-tyrosine to
 198 4HPA. The resulting *E. coli* strain DG104 produced up to 991 ± 48.8 mg/L 4HPA

199 within 28 h (Figure 2). 4HPA yield and productivity were calculated to be 49.5 mg/g
200 glucose and 35.4 mg/L/h, respectively (Table 4). This proves that the designed 4HPA
201 synthetic pathway was functionally expressed in *E. coli*.

202 **Creation of a synthetic pathway for IAA from glucose.**

203 After successfully confirming the biosynthesis of IAA from L-tryptophan in *E.*
204 *coli*, we further designed the biosynthesis of IAA from glucose. To obtain IAA from
205 glucose, candidate strains need have a strong capacity for the L-tryptophan synthesis.
206 Recently, great efforts have been made on engineered *E. coli* with the goal of efficient
207 production of L-tryptophan from glucose^{45,46}. Indole and L-serine are two precursor
208 substrates for the biosynthesis of L-tryptophan. Therefore, increasing the availability
209 of indole and L-serine is requisite for efficient biosynthesis of L-tryptophan.

210 In order to overproduce precursor substrate L-tryptophan, several strategies have
211 been implemented in this study, such as knocking out L-tryptophan transcriptional
212 repressor *trpR* to eliminate transcription regulation of the genes in L-tryptophan
213 pathway, overexpression of feedback resistant derivatives of *aroG^{fbr}* and anthranilate
214 synthase *trpE^{fbr}* to increase the carbon flow from glucose to indole, overexpression of
215 feedback resistant derivatives of *serA^{fbr}* to increase the availability of L-serine, and
216 overexpression of *trpABCD* for the efficient synthesis of L-tryptophan from indole
217 and L-serine. Subsequently, a complete IAA biosynthesis pathway was established by
218 further co-expression of *aro8*, *kdc* and *aldH* for conversion of L-tryptophan to IAA.
219 The resulting *E. coli* strain DG105 produced up to 1265 ± 87.8 mg/L IAA within 28 h
220 (Figure 3). IAA yield and productivity were calculated to be 63.3 mg/g glucose and

221 45.2 mg/L/h, respectively (Table 4). This proves that the designed IAA synthetic
222 pathway from glucose was functionally expressed in *E. coli*.

223 **DISCUSSION**

224 Recently, great efforts have been made in developing sustainable methods that
225 could replace traditional chemical synthesis to produce valuable compounds ⁴⁷⁻⁵⁰.
226 Based on synthetic biology technology, varieties of engineered microbes have been
227 made to produce fuels, bulk chemicals and pharmaceuticals ⁵¹⁻⁵⁵. The production of
228 aromatic molecules which derived from the common shikimate pathway is a key
229 research area and has attracted a great deal of attention in recent years ⁵⁶⁻⁵⁹. In this
230 pathway, two glycometabolic intermediates erythrose 4-phosphate and
231 phosphoenolpyruvate are first assembled into
232 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP), which is then converted to
233 chorismate. Subsequently, chorismate can be easily converted into a variety of
234 aromatic molecules via a limited number of steps ³⁵. These aromatic molecules are
235 widely used in the paper and dye manufacture, pharmaceuticals, cosmetics, and food
236 industries.

237 The large-scale use of chemical fertilizers and pesticides has caused serious
238 environmental pollution. Great efforts have been made on the use of microorganisms
239 instead of chemical fertilizers and pesticides to support plant growth through direct
240 and indirect mechanisms. Among these beneficial microbes, the auxin-producing
241 strain has played an important role in promoting plant growth. Aromatic molecules
242 IAA, PAA and 4HPA are three major types of auxin in plants. By improving the levels

of auxin produced by microbe through synthetic biology technology, it is possible to develop an agricultural microorganism to promote plant growth. Great efforts have been made on engineered microbe for the biosynthesis of auxin from L-tryptophan. Shao et al. reported co-expression of the aminotransferase *patB*, decarboxylase *yclC*, indole-3-acetaldehyde dehydrogenase *dhaS* in *Bacillus amyloliquefaciens* SQR9 and *Bacillus subtilis* 168 produced up to 39 mg/L and 22 mg/L of IAA from 3 mM tryptophan, respectively. Overexpression of putative IAA biosynthesis genes from *Pseudomonas* sp UW4 in the wild-type strain resulted in a significant increase of IAA production from tryptophan³⁰. However, compared with L-tryptophan, cheaper substrate should be used to reduce the cost of production of auxin.

In this study, three de novo biosynthetic pathways for the production of IAA, PAA and 4HPA from glucose were constructed in *E. coli* through strengthening the shikimate pathway. The overall pathway includes the upstream pathway from glucose to aromatic amino acids and the downstream pathway from aromatic amino acids to auxin. Microorganic synthesis of auxin is impeded by the formation of by-product aromatic alcohols due to quick and endogenous conversion of aromatic aldehydes to their corresponding aromatic alcohols in *E. coli*. Kunjapur constructed an *E. coli* strain RARE that can accumulate aromatic aldehydes as end products by deleting seven aldehyde reductase genes⁶⁰. In this study, *E. coli* strain RARE was used as the host to prompt more PAA, 4HPA and IAA biosynthesis.

CONCLUSION

In summary, we report the discovery of *S. cerevisiae* aminotransferase *aro8*,

265 decarboxylase *kdc* and *E. coli* aldehyde dehydrogenase *aldH* from have broad
266 substrate ranges. We successfully generated engineered *E. coli* strains capable of
267 producing three main kinds of auxins from glucose and showed the way for the
268 development of agricultural microorganism for biosynthesis of auxin and promoting
269 plant growth in the future.

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273 Competing financial interests

274 The authors declare that they have no competing interests.

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Table 1. Primers used in this study

Primer name	Sequence (5'-3')
<i>tyrA^{fbr}-XbaI</i>	GTATCTAGAAAGAGGAGATATAATGGTTGCTGAATTGA CCGCAT
<i>tyrA^{fbr}-SpeI-SalI</i>	ATATGTCGACACTAGTTTACTGGCGATTGTCATTCGCC
<i>trpE^{fbr}-XbaI</i>	GTATCTAGAAAGAGGAGATATAATGCAAACACAAAAA CCGACTCTC
<i>trpE^{fbr}-SpeI-BamHI</i>	TATGGATCCACTAGTTCAGAAAGTCTCCTGTGCATGAT G
<i>serA^{fbr}-XbaI</i>	GTATCTAGAAAGAGGAGATATAATGGCAAAGGTATCG CTGGAG
<i>serA^{fbr}-SpeI-SalI</i>	ATATGTCGACACTAGTTTAGTACAGCAGACGGGCGC
<i>trpABCD-XbaI</i>	CCATCTAGATTTAAGAGGAGATATAATGGCTGACATTC
<i>trpABCD-XhoI</i>	TGCTGCTCGATAGATTCTCGAGTTAACTGCGCGTCGCC GCT

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Table 2. Plasmids and strains used in this study

Plasmids/strains	Properties	Source
Plasmids		
pDG9	pET28a; pT7: <i>aro8</i> , <i>kdc</i> and <i>aldH</i>	31
pDG11	pET28a; pT7: <i>aroG</i> ^{fbr}	This study
pDG12	pET28a; pT7: <i>pheA</i> ^{fbr}	This study
pDG13	pET28a; pT7: <i>aroG</i> ^{fbr} and <i>pheA</i> ^{fbr}	This study
pDG14	pBBRMCS1; pT7: <i>aroG</i> ^{fbr} and <i>pheA</i> ^{fbr}	This study
pDG20	pET28a; pT7: <i>tyrA</i> ^{fbr}	This study
pDG21	pET28a; pT7: <i>aroG</i> ^{fbr} and <i>tyrA</i> ^{fbr}	This study
pDG22	pBBRMCS1; pT7: <i>aroG</i> ^{fbr} and <i>tyrA</i> ^{fbr}	This study
pDG23	pET28a; pT7: <i>trpE</i> ^{fbr}	This study
pDG24	pET28a; pT7: <i>serA</i> ^{fbr}	This study
pDG25	pET28a; pT7: <i>aroG</i> ^{fbr} and <i>trpE</i> ^{fbr}	This study
pDG26	pET28a; pT7: <i>aroG</i> ^{fbr} , <i>trpE</i> ^{fbr} and <i>serA</i> ^{fbr}	This study
pDG27	pBBRMCS1; pT7: <i>aroG</i> ^{fbr} , <i>trpE</i> ^{fbr} and <i>serA</i> ^{fbr}	This study
pDG28	pET28a; pT7: <i>aro8</i> , <i>kdc</i> , <i>aldH</i> and <i>trpABCD</i>	This study
Strains		
RARE	MG1655 derivative; $\Delta dkgB$, $\Delta yeaE$, $\Delta yqhC$, $\Delta yqhD$, $\Delta dkgA$, $\Delta yahK$ and $\Delta yjgB$	59
DG101	RARE derivative; $\Delta trpR$	This study
DG102	RARE/pDG9	This study

DG103	RARE/pDG9/ pDG14	This study
DG104	RARE/pDG9/ pDG22	This study
DG105	DG101/pDG27/ pDG28	This study

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386 **Table 3.** The biosynthesis of IAA and 4HPA from L-tryptophan and L-tyrosine in *E.*

387 *coli* DG102. Data are the mean values of three experiments $\pm$ SD.

Strains	Culture medium	Titer (mg/L)	molar yield (mol/mol)
DG102	M9 + 0.4 g/L tryptophan	303 $\pm$ 14.1	0.91
DG102	M9 + 1 g/L tyrosine	726 $\pm$ 28.3	0.86

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Table 4. Titer, yield and productivity of PAA, 4HPA and IAA by engineered *E. coli* strains in shake flasks for 28 h. Yield calculated by milligrams PAA, 4HPA or IAA produced divided by grams glucose consumed. Productivity calculated by concentration of PAA, 4HPA or IAA produced per hour. Data are the mean values of three experiments $\pm$ SD.

Strains	product	Titer (mg/L)	Yield (mg/g)	Productivity (mg/L/h)
DG103	PAA	1217 $\pm$ 45.2	60.8 $\pm$ 2.26	43.7 $\pm$ 1.62
DG104	4HPA	991 $\pm$ 48.8	49.5 $\pm$ 2.44	35.4 $\pm$ 1.74
DG105	IAA	1265 $\pm$ 87.8	63.3 $\pm$ 4.38	45.2 $\pm$ 3.13

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Figure 1. Engineered pathways for production of IAA, PAA and 4HPA from glucose.

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427 **Figure 2.** GC/MS analyses of PAA, 4HPA and IAA from glucose in engineered E.

428 coli strains DG103, DG104 and DG105. Identified substances: 1, benzoic acid

429 (internal standard); 2, PAA; 3, 3-Methylindole; 4, 4HPA, 5, IAA.

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Figure 3. The extracted IAA from engineered *E. coli* strains DG105 was esterified and detected by GC/MS. Identified substances: 1, methyl benzoate (internal standard); 2, methyl phenylacetate; 3, methyl 4-hydroxyphenylacetate; 4, methyl indole-3-acetate.