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n-Butylamine production from glucose using a
 transaminase-mediated synthetic pathway in *Escherichia coli*

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16 Running title

17 • *n*-Butylamine production from glucose in *E. coli*

18

19 Highlights

- 20 •*n*-Butanol was converted to *n*-butylamine by a three enzyme-mediated cascade.
- •The cascade was combined with part of the *n*-butanol-producing pathway.
- 22 •*n*-Butylamine was produced in *Escherichia coli* from glucose as a carbon source.

24 ABSTRACT

25 Bioamination methods using microorganisms have attracted much attention because of the 26 increasing demand for environmentally friendly bioprocesses. *n*-Butylamine production from 27 glucose in *Escherichia coli* was demonstrated in this study, which has never been reported because 28 of the absence of *n*-butylamine-producing pathway in nature. We focused on a transaminase-29 mediated cascade for bioamination from an alcohol or aldehyde. The cascade can convert an 30 alcohol or an aldehyde to the corresponding amine with L-alanine as an amine donor. Here, n-31 butyraldehyde, which is a metabolic intermediate in the *n*-butanol producing pathway, is a 32 potential intermediate for producing *n*-butylamine using this cascade. Hence, the *n*-butanol-33 producing pathway and the transaminase-mediated cascade were combined into a synthetic 34 metabolic pathway for producing *n*-butylamine from glucose. Firstly, we demonstrated the 35 conversion of *n*-butanol to *n*-butylamine using a three enzyme-mediated cascade. *n*-Butanol was 36 successfully converted to *n*-butylamine in 92% yield in the presence of L-alanine and ammonium chloride. Then, the *n*-butanol-producing pathway and transaminase-mediated cascade were 37 38 introduced into E. coli. Using this system, n-butylamine was successfully produced from glucose 39 as a carbon source at a concentration of 53.2 mg L⁻¹ after 96 h cultivation using a ppc 40 (phosphoenolpyruvate carboxylase)-deficient strain. To the best of our knowledge, this is the first 41 report of the direct production of *n*-butylamine from glucose, and may provide a starting point for 42 the development of microbial methods to produce other bioamines.

43

44 Keywords: transaminase, cascade, *n*-butylamine, metabolic engineering, *Escherichia coli*

46 Introduction

Microbial bioproduction has gained increasing attention because of environmental and 47 48 energy concerns. Microbial bioproduction is performed under mild conditions contrary to chemical 49 methods, which are often performed at relatively high pressure and temperature using an organic 50 solvent. Microorganisms such as Escherichia coli and Saccharomyces cerevisiae, which use 51 biomass as a carbon source and produce a variety of chemicals, have been employed for 52 bioproduction (1-3). Recent progress in genome engineering tools has facilitated the development 53 of microbial bioproduction at an accelerated rate (4, 5). Amines are one of the essential building 54 blocks in the chemical, pharmaceutical and agrochemical industries. While the conversion of 55 amines from carbonyl compounds is usually catalyzed by a metal catalyst (6-9), bioamination 56 processes using microorganisms have attracted much attention because of the increasing demand 57 for environmentally friendly bioprocesses.

58 Three enzyme-mediated cascades have been previously used to synthesize amine 59 compounds using alcohol as the substrate (10-12). Firstly, the alcohol is oxidized to an aldehyde 60 by alcohol dehydrogenase (ADH), and then the transamination between the aldehyde and L-61 alanine, the amine donor, is catalyzed by transaminase (TA). At the same time, the NAD⁺ 62 consumed by alcohol dehydrogenase is regenerated to NADH by alanine dehydrogenase 63 (ALADH), resulting in a cascade that is redox-neutral (Fig. 1). Therefore, only the supplementation of ammonium sources for the regeneration of L-alanine from pyruvate is required 64 65 for the cascade. Although a variety of amines, including alkylamine, diamine and aromatic amines, 66 have been synthesized using three enzyme-mediated cascades or whole-cell biocatalysts (10-12), 67 only alcohols, aldehydes or alkanes are used as substrates for bioamination and these substrates 68 are often toxic to enzymes or cells thus limiting the effectiveness of bioamination. Therefore, we

focused on bioamination using other carbohydrates as the carbon source, such as glucose, which can be assimilated. Several diamines or aminocarboxylic acids have been produced from glucose using *E. coli*, such as cadaverine (13), putrescine (14), gamma-amino butyric acid (15), and 5aminovalerate (16). Although *E. coli* can be engineered to produce these amines by genetically introducing naturally occurring exogenous metabolic pathways, it is usually challenging to engineer *E. coli* to produce other amines using metabolic pathways that are not present in nature.

75 Herein, we demonstrate alkylamine production from glucose based on a synthetic 76 metabolic pathway inspired from an enzyme cascade. To produce alkylamine with a synthetic 77 pathway, alkylaldehyde is required as a metabolic intermediate. We focused on *n*-butylamine as 78 the target product, which can be converted from *n*-butanol using a transaminase-mediated cascade. 79 The production of *n*-butylamine from glucose by metabolically engineered microorganisms has 80 not been reported because a pathway for the direct metabolism of glucose to *n*-butylamine does 81 not exist in nature. n-Butyraldehyde is one potential substrate that can be converted into n-82 butylamine by transaminase. *n*-Butanol production has been achieved by metabolically engineered 83 E. coli to contain an exogenous metabolic pathway that converts acetyl-CoA into n-butanol 84 through *n*-butyraldehyde as a metabolic intermediate (17, 18). In this study, *n*-butanol producing 85 pathway and transaminase-mediated cascade were combined in E. coli for direct production of nbutylamine from glucose. This study is the first report of direct *n*-butylamine production from 86 87 glucose in E. coli.

88

89 Materials and methods

90 Bacterial Strains and Growth Conditions

91	The bacterial strains used in this study are listed in Table 1. E. coli NovaBlue cells
92	(Novagen Inc., Madison, WI, USA) were used for DNA manipulations. E. coli BL21(DE3) cells
93	and MG1655 (National BioResource Project) cells were used as the base strain for <i>n</i> -butylamine
94	production. Cells were precultivated with 100 μ g mL ⁻¹ of ampicillin and 20 μ g mL ⁻¹ of kanamycin
95	containing 5 mL of Luria-Bertani (LB) medium in a test tube overnight. For the <i>n</i> -butylamine
96	production from <i>n</i> -butanol, cells were grown in 5 mL of LB medium at 37 °C, 220 rpm in test
97	tubes (initial optical density at 600 nm: 0.1). After 3 h of cultivation, protein expression was
98	induced by the addition of isopropyl β -D-1-thiogalactopyranoside (IPTG; final concentration = 0.5
99	mM). Then, <i>n</i> -butanol and NH ₄ Cl or L-alanine (final concentration = 20 mM, 40 mM or 20 mM)
100	were also added and then the cultivation temperature was decreased to 30 °C. The reaction was
101	carried out for 24 h.

For the production of *n*-butylamine from glucose, cells were grown in 5 mL of 20 g L⁻¹ glucose containing M9Y medium (5 g L⁻¹ yeast extract containing M9 minimal medium) at 37 °C and 220 rpm in test tubes (initial optical density at 600 nm: 0.1). After 3 h of cultivation, protein expression was induced by the addition of IPTG (final concentration = 0.5 mM). Then NH₄Cl or L-alanine (final concentration = 40 mM or 20 mM) was also added and the cultivation temperature was decreased to 30°C.

108 Plasmid Construction and Gene Disruption

The plasmids used in this study are listed in Table 1, the primers used in this study are described in Table S1, and the sequence of synthetic genes are shown in Fig.S1-6. The polymerase chain reaction (PCR) was performed with KOD FX polymerase (TOYOBO CO., Ltd., Osaka, Japan). Vectors and inserts were ligated with the In-Fusion HD Cloning Kit following the manufacturer's

113 protocol (TAKARA BIO INC., Shiga, Japan). Primer 1-12 were used for amplifying fragments 114 including atoB from E. coli, ter from Treponema denticola (optimized for E. coli), phaB from 115 *Ralstonia eutropha* and *phaJ* from *Aeromonas caviae*. The amplified fragment by using primer 1, 116 2 and the amplified fragment by using primer 3, 4 were conjugated with overlap extension PCR. 117 Then, the conjugated fragment was inserted into NcoI/NotI site of the pETDuet-1 vector and the 118 resulting plasmid was named *atoBter*-pETd. The amplified fragment by using primer 5, 6 and the 119 amplified fragment by using primer 7, 8 were also conjugated with overlap extension PCR. Then 120 the conjugated fragment was inserted into *NdeI/AvrII* site of the *atoBter*-pETd and the resulting 121 plasmid was named *atoBter-phaBphaJ*-pETd. The amplified fragment by using primer 13~15 with 122 pET32-Gly5-RFP (19) as a template was inserted into the *XhoI/XbaI* site of the pZE12-luc or 123 pZA23-MCS vector (Expressys). The resulting plasmids were named pMR1 or pMR2, which 124 include a red fluorescent protein under the M1-93 promoter in pZE12-luc or pZA23-MCS (20). 125 Then, the fragments amplified by using primer 18, 19 or primer 20, 21 with atoBter-phaBphaJ-126 pETd as a template were inserted into the *KpnI/XbaI* site of pMR1 vector and the resulting plasmid 127 was named *atoBter*-pMR1 or *phaBphaJ*-pMR1. The amplified fragment by using primer 26, 27 128 with *phaBphaJ*-pMR1 as a template was inserted into *Avr*II site of *atoBter*-pMR1 and the resulting 129 plasmid was named *atoBter-phaBphaJ*-pMR1. Finally, ATPP-pZE12 was constructed by using 130 primer 28-31, atoBter-phaBphaJ-pMR1 as a template and the pZE12-MCS vector. Primer 32-35 131 was used for amplifying fragments including csbld from Clostridium saccharoperbutylacetonicum 132 and cvta from Chromobacterium violaceum. The amplified fragment was inserted into the 133 *KpnI/XbaI* site of pMR2 vector and the resulting plasmid was named *csbldcvta*-pMR2. The 134 amplified fragment by using primer 36, 37 with *csbldcvta*-pMR2 as a template and the amplified 135 fragment by using primer 38, 39 were conjugated with overlap extension PCR and inserted into

136 the KpnI/HindIII site of pZA23-MCS. The resulting plasmid was named BCA-pZA23. Similarly, 137 primer 40-45, pET-22b(+) and pCOLA Duet-1 were used for constructing pET22-bsadh and 138 pCOLA-bsaladh-cvta. The deletion of endogenous genes (phosphoenolpyruvate carboxylase (ppc) 139 and malate synthase G (glcB)) was performed by the gRNA-containing plasmid (pTargetT-ppc 140 and pTargetT-glcB, respectively) and pCas. The gRNA-containing plasmids were prepared with 141 pTartgetF and primer 46-57. The E. coli MG1655 genes, ppc and glcB, were deleted using the 142 CRISPR-Cas9 system as described in a previous report (21). Confirmation of gene deletions was 143 carried out by colony PCR. The resulting strains are shown in Table 1.

144 Metabolite Analysis

145 The concentration of glucose was determined by high-performance liquid 146 chromatography (Shimadzu Co., Kyoto, Japan; solvent delivery system, LC-20AB; column, 147 Shodex SUGAR KS-801; column temperature, 50 °C; detector, RID-10A). Ultra-pure water was used as the mobile phase. Chromatography was carried out at a flow rate of 0.8 mL min⁻¹. The 148 149 concentration of *n*-butylamine was determined using a gas chromatograph equipped with a flame 150 ionization detector (Shimzadzu Co., Kyoto, Japan; gas chromatography, GC-2025; auto-injector, 151 AOC-20i/s; column, SH-Stabiliwax). The culture (50 µL) was dissolved in 500 µL benzene, and 152 then 0.05 M of trimethylamine containing benzene (100 µL) was added. Then heptafluorobutyric 153 acid anhydride (Merck KGaA, Darmstadt, Germany) (5 µL) was added to the sample and 154 incubated at 50 °C for 15 min. Ice-cooled 5% aqueous ammonia (500 µL) was added and mixed. 155 The sample (1 µL) from the upper layer was injected at a split ratio of 1:50. The initial oven 156 temperature was 40 °C for 1 min and then was increased at a rate of 10 °C/min to 150 °C and at a 157 rate of 35 °C/min until 220 °C, where it was held for 2 min. Helium was used as the carrier gas at 158 a linear velocity of 39.2 cm/sec. The detector was maintained at 250 °C.

159

160 **Results and discussion**

161 *n*-Butylamine production from *n*-butanol using a three enzyme-mediated cascade

162 A three enzyme-mediated cascade was examined for *n*-butylamine conversion from *n*-163 butanol. Sattler et al. showed that the use of ADH from Bacillus stearothermophilus (bsADH), TA 164 from Chromobacterium violaceum (cvTA) and ALADH from Bacillus subtilis (bsALADH) 165 converted primary alcohols, such as 1-hexanol and 1-octanol, to their corresponding amines with 166 high efficiency. Although elongating the chain length of the substrate alcohols led to lower 167 conversion, the addition of a co-solvent partly improved the yield (10). In this study, we examined 168 the conversion of *n*-butanol to *n*-butylamine using bsADH, cvTA, and bsALADH. A BL21(DE3) 169 and pET system were used for the substrate feeding experiments. A total of 5.92 mM of n-170 butylamine was synthesized from 20 mM *n*-butanol using the cascade (the yield of the conversion 171 was about 30%). This result indicates that the cascade was capable of converting *n*-butanol to *n*-172 butylamine. As shown in Fig. 1, the cascade is totally redox-neutral, requiring only a supply of 173 ammonium. However, there have been several reports that the residual addition of L-alanine or 174 ammonium salt as an amine donor facilitated the conversion (10-12). Hence the influence of the 175 addition of L-alanine or ammonium chloride on the cascade was evaluated. The conversion was 176 clearly improved when either L-alanine or ammonium chloride was added, and moreover, the 177 addition of both L-alanine and ammonium chloride was most effective contributing to 92% of the 178 yield in the substrate-feeding experiments (Fig. 2). These results imply that the transamination by 179 cvTA was the rate-limiting step and improvement of this step by the addition of NH₄⁺ source 180 directly increased the conversion. In addition, the elementary reactions on transaminase-mediated 181 cascade are reversible and concentration-driven. The addition of L-alanine probably was effective 182 because the delta-G value of *n*-butylamine production was smaller than the value of *n*-183 butyraldehyde and pyruvate. Therefore, the supplementation of both L-alanine and ammonium 184 chloride was determined to be optimal in this study.

185 Direct *n*-butylamine production from glucose through the synthetic metabolic pathway

186 To the best of our knowledge, there have been no reports demonstrating direct *n*-187 butylamine production from glucose because of the absence of *n*-butylamine-producing pathway 188 in nature. By harnessing the cascade in this study, *n*-butylamine was successfully produced from 189 *n*-butanol as a substrate. Here, we focused on *n*-butyraldehyde, which was an intermediate in the 190 reaction. *n*-Butanol production from glucose was demonstrated in previous reports (17-18), some 191 of which show that *n*-butyraldehyde is a metabolic intermediate in the synthetic pathway (17). In 192 this study, part of the *n*-butanol-producing pathway and the enzyme-mediated cascade were 193 combined to directly produce *n*-butylamine from glucose (Fig. 3). Glucose was converted into *n*-194 butyraldehyde through 2 acetyl-CoA, and subsequently, n-butyraldehyde was aminated by 195 transaminase using L-alanine as the amine donor. The synthetic pathway is partly redox-neutral 196 because residual NADH was used for the regeneration of L-alanine from pyruvate. The MG1655 197 strain was used for producing *n*-butylamine from glucose. Only MG1655 harboring ATPP-pZE 198 and BCA-pZA produced *n*-butylamine from glucose (3.55 mg L⁻¹), whereas the other strains which 199 were lack of n-butanol-producing pathway or transaminase-mediated cascade did not produce *n*-200 butylamine (Fig. 4A), indicating that the synthetic combined pathway was functional. Additionally, 201 the production of *n*-butylamine was decreased when the reaction was not supplemented with L-202 alanine or ammonium chloride (Fig. 4B). This implies that the supplementation of both L-alanine 203 and ammonium chloride was important for producing *n*-butylamine in the synthetic pathway.

204 The effect of knocking out a gene for accumulating metabolites

205 To enhance the production of *n*-butylamine, pyruvate needs to accumulate for the 206 regeneration of alanine by bsALADH. However, pyruvate is usually a catabolite in the endogenous 207 metabolic pathway in *E. coli*. Blocking pyruvate-catabolizing pathways has been shown as an 208 effective approach for amination in a whole-cell biocatalyst (11). Therefore, one of the pyruvate 209 catabolizing pathways in MG1655 was blocked by the deletion of the relevant gene (ppc) using 210 the CRISPR-Cas9 system. The deletion of the ppc gene is known to result in the accumulation of 211 pyruvate (22). As predicted, the production of *n*-butylamine in a ppc-deficient strain was clearly increased compared with wild-type strain at 53.2 mg L⁻¹ after 96 h cultivation (Fig. 5). Additionally, 212 213 the effect of deletion of the glcB gene, which is involved in acetyl-CoA catabolism in the 214 glyoxylate shunt, was also determined as the intracellular acetyl-CoA level is critical for enhancing 215 the production of *n*-butanol from glucose (18). As shown in Fig. 5, the production of *n*-butylamine 216 by the *glcB*-deficient strain was also increased compared with wild-type strain at 34.8 mg L^{-1} after 217 72 h of cultivation. The deletion of both ppc and glcB resulted in a slightly lower production of nbutylamine (32.0 mg L⁻¹ after 96 h cultivation) compared with the *ppc*-deficient strain. These 218 219 results indicate that blocking the pyruvate- or acetyl-CoA-catabolizing pathway is one effective 220 approach for producing *n*-butylamine. In addition, produced n-butylamine was decreased in 221 several strains maybe because the transamination by cvTA step was reversible. Although most of 222 the synthetic pathways used in this study are identical with the *n*-butanol-producing pathway, the 223 amount of *n*-butylamine produced was relatively less than the *n*-butanol previously generated (17, 224 18). This is probably because the *n*-butanol-producing pathway employed herein was not fully 225 optimized because *n*-butyraldehyde was not detected in culture as a byproduct. In addition, *n*-226 butanol-producing pathway in this study was employed *phaB* for the conversion of acetoacetylCoA to 3-hydroxybutyryl-CoA. PhaB only utilizes NADPH as a cofactor, thereby occurring redox
unbalance in the synthetic pathway. Therefore, further improvement of the strain, such as deleting
competing endogenous pathways or enhancing the production pathway for accumulating
intracellular acetyl-CoA or L-alanine, should increase the production of *n*-butylamine.

231 In summary, we demonstrated direct *n*-butylamine production from glucose using a 232 transaminase synthetic pathway in E. coli. The conversion of n-butanol from n-butylamine using 233 a three-enzyme cascade was successfully demonstrated as shown by the high reaction rate (92% 234 under the optimized condition). After combining the cascade with part of the *n*-butanol-producing 235 pathway, the resulting strains directly produced *n*-butylamine from glucose. To the best of our 236 knowledge, this study is the first report of *n*-butylamine production from glucose and may provide 237 inspiration for producing other bioamines, such as short-chain or long-chain alkylamines and 238 aromatic amines.

239

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246 REFERENCES

247	1.	Matsumoto, T., Tanaka, T., and Kondo, A.: Engineering metabolic pathways in
248		Escherichia coli for constructing a "microbial chassis" for biochemical production, Bioresour.
249		Technol., 245 , 1362-1368 (2017).
250	2.	Hong. K.K., and Nielsen. J.: Metabolic engineering of Saccharomyces cerevisiae: a key cell
251		factory platform for future biorefineries, Cell Mol. Life Sci., 69, 2671-2690 (2012).
252	3.	Liao, J.C., Mi, L., Pontrelli, S., and Luo, S.: Fuelling the future: microbial engineering for
253		the production of sustainable biofuels, Nat. Rev. Microbiol., 14, 288-304 (2016).
254	4.	Lian, J., Mishra, S., and Zhao, H.: Recent advances in metabolic engineering of
255		Saccharomyces cerevisiae: New tools and their applications, Metab. Eng., 50 , 85-108 (2018).
256	5.	Jakočiūnas, T., Jensen, M.K., and Keasling, J.D.: CRISPR/Cas9 advances engineering of
257		microbial cell factories, Metab. Eng., 34 , 44-59 (2016).
258	6.	Imm, S., Bähn, S., Neubert, L., Neumann, H., and Beller, M.: An efficient and general
259		synthesis of primary amines by ruthenium-catalyzed amination of secondary alcohols with
260		ammonia, Angew. Chem. Int. Ed. Engl., 49, 8126-8129 (2010).
261	7.	Pingen, D., Müller, C., and Vogt, D.: Direct amination of secondary alcohols using ammonia,
262		Angew. Chem. Int. Ed. Engl., 49, 8130-8133 (2010).
263	8.	Imm S1, Bähn S, Zhang M, Neubert L, Neumann H, Klasovsky F, Pfeffer J, Haas T, and
264		Beller M. : Improved ruthenium-catalyzed amination of alcohols with ammonia: synthesis of
265		diamines and amino esters, Angew. Chem. Int. Ed. Engl., 50, 7599-7603 (2011).

266	9.	Lafrance, M., Roggen, M., and Carreira, E.M.: Direct, enantioselective iridium-catalyzed
267		allylic amination of racemic allylic alcohols, Angew. Chem. Int. Ed. Engl., 51, 3470-3473
268		(2012).
269	10.	Sattler, J.H., Fuchs, M., Tauber, K., Mutti, F.G., Faber, K., Pfeffer, J., Haas, T., and
270		Kroutil, W.: Redox self-sufficient biocatalyst network for the amination of primary alcohols,
271		Angew. Chem. Int. Ed. Engl., 51 , 9156-9159 (2012).
272	11.	Klatte, S., and Wendisch, V.F.: Role of L-alanine for redox self-sufficient amination of
273		alcohols, Microb. Cell. Fact., 14, 9 (2015)
274	12.	Ladkau, N., Assmann, M., Schrewe, M., Julsing, M.K., Schmid, A., and Bühler, B.:
275		Efficient production of the Nylon 12 monomer ω-aminododecanoic acid methyl ester from
276		renewable dodecanoic acid methyl ester with engineered Escherichia coli, Metab. Eng., 36,
277		1-9. (2016)
278	13.	Qian, Z.G., Xia, X.X., Lee, S.Y.: Metabolic engineering of Escherichia coli for the
279		production of cadaverine: a five carbon diamine, Biotechnol. Bioeng., 108 , 93-103 (2011).
280	14.	Qian, Z.G., Xia, X.X., Lee, S.Y.: Metabolic engineering of Escherichia coli for the
281		production of putrescine: a four carbon diamine, Biotechnol. Bioeng., 104, 651-662 (2009).
282	15.	Dung Pham, V., Somasundaram, S., Lee, S.H., Park, S.J., and Hong, S.H.: Efficient
283		production of gamma-aminobutyric acid using Escherichia coli by co-localization of
284		glutamate synthase, glutamate decarboxylase, and GABA transporter, J. Ind. Microbiol.
285		Biotechnol., 43, 79-86 (2016).

286	16. Park, S.J., Kim, E.Y., Noh, W., Park, H.M., Oh, Y.H., Lee, S.H., Song, B.K., Jegal, J.,
287	and Lee, S.Y.: Metabolic engineering of Escherichia coli for the production of 5-
288	aminovalerate and glutarate as C5 platform chemicals, Metab. Eng., 16, 42-47 (2013).
289	17. Kataoka, N., Vangnai, A.S., Pongtharangkul, T., Tajima, T., Yakushi, T., Matsushita,
290	K., Kato, J.: Construction of CoA-dependent 1-butanol synthetic pathway functions under
291	aerobic conditions in <i>Escherichia coli</i> , J. Biotechnol., 204 , 25-32 (2015).
292	18. Atsumi, S., Cann, A.F., Connor, M.R., Shen, C.R., Smith, K.M., Brynildsen, M.P., Chou,
293	K.J., Hanai, T., and Liao, J.C.: Metabolic engineering of Escherichia coli for 1-butanol
294	production, Metab. Eng., 10, 305-311. (2008).
295	19. Matsumoto, T., Tanaka, T., Kondo, A.: Sortase A-catalyzed site-specific coimmobilization
296	on microparticles via streptavidin., Langmuir, 28, 3553-3557 (2012).
297	20. Lu, J., Tang, J., Liu, Y., Zhu, X., Zhang, T., Zhang, X.: Combinatorial modulation of galP
298	and glk gene expression for improved alternative glucose utilization., Appl. Microbiol.
299	Biotechnol., 93 , 2455-2462 (2012).
300	21. Jiang, Y., Chen, B., Duan, C., Sun, B., Yang, J., Yang, S.: Multigene editing in the
301	Escherichia coli genome via the CRISPR-Cas9 system, Appl. Environ. Microbiol., 81, 2506-
302	2514 (2015).
303	22. Zhu, J., Shimizu, K.: Effect of a single-gene knockout on the metabolic regulation in
304	Escherichia coli for D-lactate production under microaerobic condition, Metab. Eng., 7, 104-
305	115 (2005).

306 Figure legends

Figure 1. A three enzyme-mediated cascade for the conversion of *n*-butanol to *n*-butylamine.
Abbreviations: bsADH, alcohol dehydrogenase from *Bacillus stearothermophilus*; bsALADH,
alanine dehydrogenase from *Bacillus subtilis* subsp. *subtilis str.168*; cvTA, ω-transaminase from *Chromobacterium violaceum*.

Figure 2. *n*-Butylamine production in the presence of various additives in BL21(DE3) harboring pET22-*bsadh* and pCOLA-*bsaladh-cvta*. Control (1), NH4Cl (2), L-alanine (3), NH4Cl and Lalanine (4). Data are presented as the average of three independent experiments and error bars represent the standard deviation.

Figure 3. Metabolic pathways used in this study. Gene abbreviations: atoB, acetyl-CoA acetyl
transferase; phaB, Acetoacetyl-CoA reductase; phaJ, (R)-specific enoyl-CoA hydratase; ter, transenoyl-CoA reductase; bld, butyraldehyde dehydrogenase; bsaladh, alanine dehydrogenase; cvta,
ω-transaminase. phaB, phaJ, ter, bld, bsaladh and cvta are derived from *R. eutropha, A. caviae, T. denticola, C. saccharoperbutylacetonicum, B. subtilis* and *B. stearothermophilus*, respectively.

Figure 4. (A) *n*-Butylamine production by MG1655 harboring different combinations of plasmids.

321 pZE12-MCS and pZA23-MCS (1), pZE12-MCS and BCA-pZA (2), ATPP-pZE and pZA23-MCS

322 (3), ATPP-pZE and BCA-pZA (4). (B) *n*-Butylamine production of MG1655 harboring ATPP-

323 pZE and BCA-pZA in the presence of various additives. Control (1), NH₄Cl (2), L-alanine (3),

324 NH₄Cl and L-alanine (4). Data are presented as the average of three independent experiments and

325 error bars represent the standard deviation.

Figure 5. The production of *n*-butylamine (A) and the consumption of glucose (B) in various strains. Symbols represent MG1655 Δppc circle; MG1655 $\Delta glcB$, triangle; and MG1655 $\Delta ppc\Delta glcB$, square, harboring ATPP-pZE12 and BCA-pZA23. Data are presented as the average of three independent experiments and error bars represent the standard deviation.



Figure 1



Figure 2



Transaminase-mediated cascade

Figure 3



Figure 4





1 Table

Table 1. Strains and plasmids used in this study

Strains or plasmids	Characteristics	Source of reference
Strain		
E. coli NovaBlue	endA1 hsdR17 (rk ⁻ mk ⁺) supE44 thi-1 gyrA96 relA1 lac recA1/F'	Novagen
	$[proAB \ lacI^{q} \ Z \ \Delta M15 \ Tn10(tet^{r})]$	
E. coli BL21(DE3)	$F^- \text{ ompT hsdS}_B(r_B^-, m_B^-) \text{ gal dcm } \lambda(DE3)$	Novagen
<i>E. coli</i> MG1655	K-12; F ⁻ λ ⁻ rph-1	NBRP
MG1655∆ <i>ppc</i>	as MG1655, but Δppc	This study
MG1655∆ <i>glcB</i>	as MG1655, but $\Delta glc B$	This study
$MG1655\Delta ppc\Delta glcB$	as MG1655, but $\Delta ppc \Delta glc B$	This study
Plasmid		
pET-22b(+)	pBR322 ori; Amp ^R ; P _{T7} ::MCS	Novagen
pCOLA Duet-1	ColA ori; Km ^R ; P _{T7-1} ::MCS; P _{T7-2} ::MCS	Novagen
pET22-bsadh	pBR322 ori; Amp ^R ; P _{T7} ::bsadh	This study
pCOLA-bsaladh-cvta	ColA ori; Km ^R ; P _{T7-1} ::bsaladh; P _{T7-2} ::cvta	This study
pZE12MCS	ColE1 ori; Amp ^R ; P _{LlacO-1} ::MCS	Expressys
pZA23MCS	p15A ori; Km ^R ; P _{A1lacO-1} ::MCS	Expressys
ATPP-pZE12	ColE1 ori; Amp ^R ; P _{LlacO-1} :: <i>atoB ter phaB phaJ</i>	This study
BCA-pZA23	p15A ori; Km ^R ; P _{A1lacO-1} ::bld cvta bsaladh	This study
pCas	repA101 (Ts) ori; Km ^R ; P _{cas} :: <i>cas9</i> ; P _{araB} :: <i>Red</i> ; lacI ^q ; P _{trc} ::sgRNA-pMB1	Addgene (21)
pTargetF	pMB1 ori; Spe ^R ; P _{J23199} :::sgRNA	Addgene (21)
pTargetT- ppc	pMB1 ori; Spe ^R ; P _{J23199} :::sgRNA-ppc; Donor-ppc	This study
pTargetT-glcB	pMB1 ori; Spe ^R ; P _{J23199} :::sgRNA-glcB; Donor-glcB	This study

ATGATCAAAGATACCCTGGTGAGCATTACCAAAGACCTGAAAACCGAATGTGGAAAAACGCCAACCTGAAAAACT ATAAAGATGACAGCAGCTGTTTTGGCGTGTTTGAAAAACGTTGAAAAATGCCATTAGCAATGCAGTTCATGCCCAGAAAATTCT ${\tt GAGCCTGCATTATACCAAAGAACAGCGCGAAAAAATCATTACCGAAATTCGTAAAGCAGCCCTGGAAAACAAAGAAATTCT}$ GGCAACCATGATTCTGGAAGAAAACCCACATGGGTCGTTATGAAGATAAAATCCTGAAACATGAACTGGTGGCCAAATATAC ACCGGGTACAGAGGATCTGACCACCACCGCATGGTCAGGTGATAATGGTCTGACCGTTGTTGAAATGAGCCCGTATGGTGTT ATTGGTGCAATTACCCCGAGCACCAATCCGACCGAAACCGTTATTTGTAATAGCATTGGTATGATTGCAGCCGGTAATACCG TTGTGTTTAATGGTCATCCGGGTGCCAAAAAATGTGTTGCATTTGCAGTGGAAATGATCAACAAAGCCATTATTAGCTGTGG TGGTCCGGAAAATCTGGTTACCACCATTAAAAACCCGACAATGGATAGCCTGGATGCCATTATCAAACATCCGAGCATTAA ACTGCTGTGTGGCACAGGCGGTCCGGGTATGGTTAAAACCCTGCTGAATAGCGGTAAAAAAGCAATTGGTGCCGGTGCAGG TAATCCGCCTGTTATTGTTGATGATACCGCAGATATTGAGAAAGCCGGTAAAAGCATTATTGAAGGCTGCAGCTTTGATAAT AATCTGCCGTGTATTGCCGAAAAAGAGGTGTTTGTTTTTGAGAATGTTGCCGATGATCTGATCAGCAACATGCTGAAAAATA GCATCAACAAAAATGGGTGGGCAAAGATGCAAAACTGTTTCTGGATGAAATTGATGTTGAAAGCCCGAGCAGCGTGAAAT GTATTATTTGTGAAGTTAGCGCAAGCCACCCGTTTGTTATGACCGAACTGATGCCGATTCTGCCGATTGTTCGTGTTAAA GATATTGATGAAGCCATCGAGTATGCCAAAAATTGCAGAACAGAATCGTAAACACAGCGCCTATATCTACAGCAAAAACATC GATAATCTGAACCGCTTTGAACGCGAAATTGATACCACCATCTTTGTGAAAAACGCGAAAAGCTTTGCCGGTGTTGGTTATG AAGCAGAAGGTTTTACCACCTTTACCATTGCAGGTAGCACCGGTGAAGGTATTACCAGCGCACGTAATTTTACCCGTCAGCG TCGTTGTGTTCTGGCAGGT

- 1
- 2 Fig. S1. Sequence of synthetic gene: BLD from *Clostridium saccharoperbutylacetonicum*.

5 Fig. S2. Sequence of synthetic gene: TER from *Treponema denticola*.

atgCAGAAACAGCGTACCACCTCTCAGTGGCGTGAACTGGATGCAGCACCATCATCTGCATCCGTTTACCGATACCGCAAGCCTGAATCAGGCAGGTGCACGTGTTATGACCCGTGGTGAAGGTGTTTATCTGTGGGATAGCGAAGGCAACAAAATTATTGATG GTATGGCAGGTCTGTGGTGTGTTAATGTTGGTTATGGTCGCAAAGATTTTGCAGAAGCAGCACGTCGTCAGATGGAAGAACT GCCGTTTTATAATACCTTTTTTTAAAACCACCCATCCGGCAGTTGTTGAACTGAGCAGCCTGCTGGCCGAAGTTACACCGGCA ${\tt GGTTTTGATCGTGTGTTTTATACCAATAGCGGTAGCGAAAGCGTTGATACCATGATTCGCATGGTTCGTCGTTATTGGGATGT}$ TCAGGGCAAACCGGAAAAAAAAACCCTGATCGGTCGTTGGAATGGTTATCATGGTAGCACCATTGGTGGTGCCAGCCTGGG TGGTATGAAATATATGCATGAACAGGGTGATCTGCCGATTCCGGGTATGGCACATATTGAACAGCCGTGGTGGTATAAACAT GGCAAAGATATGACACCGGATGAATTTGGTGTTGTTGCAGCACGTTGGCTGGAAGAAAAAATTCTGGAAATTGGTGCCGAT AAAGTTGCAGCATTTGTGGGTGAACCGATTCAGGGTGCAGGTGGTGGTGTTATTGTTCCGCCTGCAACCTATTGGCCTGAAATTG AACGTATCTGCCGCAAAATATGATGTTCTGCTGGTTGCCGATGAAGTTATTTGTGGTTTTGGTCGTACCGGTGAATGGTTTGGT ${\sf CATCAGCATTTTGGTTTTCAGCCGGACCTGTTTACCGCAGCCAAAGGCTTATCTTCTGGCTATCTGCCGATTGGTGCAGTTTT}$ TGTTGGTAAACGTGTTGCAGAAGGTCTGATTGCAGGCGGTGATTTTAATCATGGCTTTACCTATAGCGGTCATCCGGTTTGTG CAGCAGTTGCACATGCAAATGTTGCAGCACTGCGTGATGAAGGTATTGTTCAGCGCGTGAAAGATGATATTGGTCCGTATAT ${\tt GCAGAAACGTTGGCGTGAAACCTTTAGCCGTTTTGAACATGTTGATGATGTTCGTGGTGTTGGTATGGTTCAGGCATTTACC}$ ATAATCTGATTATGCGTGCCTGTGGTGATCACATTGTTAGCGCACCGCCTCTGGTGATGACCCGTGCCGAAGTTGATGAAAT GCTGGCCGTTGCAGAACGCTGTCTGGAAGAATTTGAACAGACCCTGAAAGCACGTGGTCTGGCCtaa

7

8 Fig. S3. Sequence of synthetic gene: TA from *Chromobacterium violaceum*.

atgAAAGCAGCAGTTGTGGAACAGTTTAAAGAACCGCTGAAAATTAAAGAAGTGGAAAAACCGACCATTAGCTATGGTGAAAGTTCTGGTTCGTATTAAAGCCTGTGGTGTTTGTCATACCGATGCAGCAGCAGCACATGGTGATTGGCCTGTTAAACCGAAACTGCCGCTGATTCCGGGGTCATGAAGGTGTTGGTATTGTTGAAGAAGTTGGTCCTGGCGGTACCCATCTGAAAAGTTGGTGGTACGTGTTGGTATTCCGTGGCTGTATAGCGCATGTGGTCATTGTGGAGATTATTGTCTGAGCGGTCAAGAAACCCTGTGCGAACATCAGAAAAATGCAGGTTATAGCGTGGATGGTGGTTATGCAGAATATTGTCGTGCAGCAGCAGCAGAATTATGTTGTGAAAAATTCCGGATAATCTGAGCTTTGAAGAAGCAGCACCGATTTTTTGTGCCGGTGTTACCACCTATAAAGCACTGAAAGTTACCGGTGCAAAACCGGGTGAATGGGTTGCAATTTATGGTAGTGGTGGTCTGGGCCATGTTGCAGCAGATCTGGTTGTTAATCCGCTGAAAGACGGGTGAATGGGATATTGGTGGATGAAAACTGGAACTGGCAAAAGAACTGGGTGCAGATCTGGTTGTTAATCCGCTGAAAAGAGATGCAGCCAAATTTATGGTAGTGGTGGTGGTGGTGTCATGCAGCAGTTGTTACCGCAGGTTGCAAAACCGGCATTTCAGAGCGCATATAATAGCATTCGTCGTGGTGGTGGTGGTGTCTGGGCCACCGTAAAAAATTCGCAAAAGCACTGCAGTTTGCAGCAGAAAGGTAAAGGTAAAACCATTATTGGTAGCAATGTGGGGCACCCGTAAAAAATTAATGAAGTGTTGACGCATGCAAGAGGTAGATTAATGGTCGTGTTCTGACCCTGGAAGATAAAATTAATGAAGTGTTTGATCGCATGCTGAAAGGTCAGATTAATGGTCGTGTTGTTCTGACCCTGGAAGAAAAATTAATGAAGTGTTTGATCGCATGCTGAAAGGTCAGATTAATGGTCGTGTTGTTCTGACCCTGGAAGATAAAATTAATGAAGTGTTTGATCGCATGCAAAGGTC

10

11 Fig. S4. Sequence of synthetic gene: ADH from *Bacillus stearothermophilus*.

- 14 Fig. S5. Sequence of synthetic gene: ALADH from *Bacillus subtilis* subsp. *subtilis str.168*.

ATGAGCGCACAGAGCCTGGAAGTTGGTCAGAAAGCACGTCTGAGCAAACGTTTTGGTGCAGCAGAAGTTGCAGCATTTGC AGCACTGAGCGAAGATTTTAATCCGCTGCATCTGGACCCTGCATTTGCCGCAACCACCGCATTTGAACGTCCGATTGTTCAT GGTATGCTGCTGGCAAGCCTGTTTAGCGGTCTGCTGGGTCAGCAGCTGCCTGGTAAAGGTAGCATTTATCTGGGTCAGAGCC TGTCATTTAAACTGCCGGTTTTTGTTGGTGATGAAGTTACCGCAGAAGTGGAAGTTACAGCACTGCGTGAAGATAAACCGAT TGCAACCCTGACCACCCGTATTTTTACCCAGGGTGGTGCACTGGCAGTGCAGGTGGAAGCAGTTGTGAAACTGCCGTAA

- 16
- 17 Fig. S6. Sequence of synthetic gene: PHAJ from *Aeromonas caviae*.

Table S1 Primers used in this study

No.	Primer name	
1	NcoI pETd atoB for	ggagatataccatggcgATGAAAAATTGTGTCATCGTCAGTGCGGTA
2	atoB RBS re	ggtaccCTAGTTTGTCCCCTCTTTCgaattettattaATTCAACCGTTCAATCACCATCGCAATTCC
3	RBS ter for	taataagaattcGAAAGAGGGGACAAACTAGggtaccATGATCGTTAAACCGATGGTGCGCAATAAC
4	NotI pETd ter re	gcattatgcggccgcTTAGATGCGGTCAAAACGTTCCACTTCTGC
5	NdeI pETd phaB for	ggagatatacatatgatgactcagcgcattgcgtatgtgaccggc
6	phaB RBS re	ggtaccCTAGTTTGTCCCCTCTTTCgaattettattagcccatatgcaggccgccgttgagcga
7	RBS phaJ for	taataagaattcGAAAGAGGGGACAAACTAGggtaccATGAGCGCACAGAGCCTGGAAGTTGGTCAG
8	AvrII pETd phaJ re	tggcagcagcctaggTTACGGCAGTTTCACAACTGCTTCACCGGT
9	NcoI pETd R	ccatggtatatctccttattaaagttaaacaaaat
10	NotI pETd F	gcggccgcataatgcttaagtc
11	NdeI pETd R	catatgtatatctccttcttatacttaactaatatactaag
12	AvrII pETd F	cctaggctgctgccaccgctga
13	XhoI pZE12 M1-93 F	CGTCTTCACCTCGAGTTATCTCTGGCGGTGTTGACAAGAGATAACAACGTTGATATAATTGAGCCCGTATTGTTAGC ATGTACG
14	M1-93 rbs RFP F	AGCCCGTATTGTTAGCATGTACGTTTAAACGAATTCATTAAAGAGGAGAAAGGTACCATGGACAACACCGAGGACG TC
15	XbaI pZE12 RFP F	TTTGATGCCTCTAGAttaACTGGGAGCCGGAGTGGCGGG
16	XbaI pZE12 F	TCTAGAGGCATCAAATAAAAC
17	XhoI pZE12 R	CTCGAGGTGAAGACGAAAGGG
18	KpnI pMR1 phaA F	GAGGAGAAAGGTACCATGACTGACGTTGTCATCGTATC
19	XbaI pMR1 ter R	TTTGATGCCTCTAGAttaGATGCGGTCAAAACGTTCCACTTC
20	KpnI pMR1 phaB F	GAGGAGAAAGGTACCATGactcagcgcattgcgtatgtg
21	XbaI pMR1 phaJ R	TTTGATGCCTCTAGAttaCGGCAGTTTCACAACTGCTTCA
22	KpnI pMR1 R	GGTACCTTTCTCCTCTTTAATGAATTCGTTTAAAC
23	XbaI pMR1 F	TCTAGAGGCATCAAATAAAACGAAAGGCTCAG
24	AvrII pMR op2 F	CTAGGCGTTCGGCTGCGGCGAGCGGTATCAGCTCACTCAA
25	AvrII pMR op2 R	CTAGGTCTAGGGCGGCGGATTTGTCCTACTCAGGAGAGCG
26	XbaI ter IF M1-93 F	CCGCATCtaaTCTAGTTATCTCTGGCGGTGTTGACAAGA
27	XbaI IF Term R	ATTTGATGCCTCTAGTCTAGGGCGGCGGATTTGT
28	AvrII IF M1-93 F	CCGCCCTAGACCTAGTTATCTCTGGCGGTGTTGACAA
29	AvrII IF Term R	CAGCCGAACGCCTAGgTCTAGGGCGGCGGATTTG
30	inv pMR1 ABJt 1cassette F	aagcttAGGAGGGTACCatgactcagcgcattgcgtatgtgaccggc
31	inv pMR1 ABJt 1cassette R	GGTACCCTCCTaagettttaGATGCGGTCAAAACGTTCCACTTCTGC
32	Xho1 atoB fo	CGGGCCCCCCCCGAGatgaaaaattgtgtcatcgtcagtgcggta
33	Xma1 phaJ re	CATGGGATCCCCCGGGttaCGGCAGTTTCACAACTGCTTCACCGGT
34	KpnI pMR2 csbld F	GAGGAGAAAGGTACCATGATCAAAGATACCCTGGTGA

35	cxbld rbs R	ggtaccCTAGTTTGTCCCCTCTTTCgaattcttattaACCTGCCAGAACACAACG
36	rbs cvta F	taataagaattcGAAAGAGGGGACAAACTAGggtaccatgCAGAAACAGCGTACCAC
37	XbaI pMR2 cvta R	TTTGATGCCTCTAGAttaGGCCAGACCACGTG
38	kpn1 bld for	ATTAAAGAGGAGAAAGGTACCATGATCAAAGATACCCTGGTGAGCATTAC
39	cvta sodo-rbs2 re	GGTACCTTTCTCCTCTTTAATGAATTCGTTttaGGCCAGACCACGTGCTTTCAGGGTCTG
40	sodo-rbs2 aladh for	AACGAATTCATTAAAGAGGAGAAAAGGTACCatgATCATAGGGGTTCCTAAAGAGATAAAA
41	aladh hindlll re	ATTCGATATCAAGCTTttaAGCACCCGCCACAGATGATTCATCCTG
42	NcoI_aladh_Bs_for	aggagatataccatgggcATCATAGGGGTTCCTAAAGAGATAAAAAAC
43	Notl_aladh_Bs_re	aagcattatgcggccgcTTAAGCACCCGCCACAGATGATTCATCCTG
44	NdeI_tacv_for	aaggagatatacatatgatgCAGAAACAGCGTACCACCTCTCAGTGG
45	AvrII_tacv_re	aagcattatgcggccgcTTAAGCACCCGCCACAGATGATTCATCCTG
46	p22 IF ADH-Bs for 2	AAGGAGATATACATATGAAAGCAGCAGTTGTGGAACAGTTT
47	p22 IF ADH-Bs re 2	TGCTCGAGTGCGGCCGCTTTATCTTCCAGGGTCAGAACAAC
49	sgRNA ppc Rv	caggtatcgagcacttcgattatacctaggactgagct
50	sgRNA ppc Fw	agtgctcgatacctgccgttttagagctagaaatagc
51	HomSeqUp ppc Fw	gctttttttgaattcGTGCCGCAATAATGTCGGATGCGAT
52	HomSeq Up ppc Rv	TGCAGAAGAGGAAGAATTACCCCAGACACCCCATCTTATC
53	HomSeq Dw ppc Fw	TCTTCCTCTTCTGCAAACCCTCGTG
54	HomSeq Dw ppc Rv	cagggtaatagatctGCCCATAGCACCACGCCGATTACTG
55	sgRNA glcB Rv	TTTCAGAGCCATCGGCGGGGTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC
56	sgRNA glcB Fw	GCCGATGGCTCTGAAATTGCTAGCATTATACCTAGGACTGAGCTAGCT
57	HomSeqUp g;cB Fw	TGCTTTTTTGAATTCGGTGGAAACCACGGGCATTGACAG
58	HomSeq Up glcB Rv	CACAATATAGACGCTGCAGCTTCGACGATAACATCGTTGATGTGC
59	HomSeq Dw glcB Fw	CGATGTTATCGTCGAAGCTGCAGCGTCTATATTGTGAAACCGAAAATGCAC
60	HomSeq Dw glcB Rv	GCTTCTGCAGGTCGACGTGACGGAACCCAGGCTGTGTTTGC