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Republic of China.Enzymatic production of  
5-aminovalerate from L-lysine using  
L-lysine monooxygenase and  
5-aminovaleramide amidohydrolase

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5-Aminovalerate is a potential C5 platform chemical for synthesis of valerolactam, 5-hydroxyvalerate, glutarate, and 1,5-pentanediol. It is a metabolite of L-lysine catabolism through the aminovalerate pathway in *Pseudomonas putida*. L-Lysine monooxygenase (DavB) and 5-aminovaleramide amidohydrolase (DavA) play key roles in the biotransformation of L-lysine into 5-aminovalerate. Here, DavB and DavA of *P. putida* KT2440 were expressed, purified, and coupled for the production of 5-aminovalerate from L-lysine. Under optimal conditions, 20.8 g/L 5-aminovalerate was produced from 30 g/L L-lysine in 12 h. Because L-lysine is an industrial fermentation product, the two-enzyme coupled system presents a promising alternative for the production of 5-aminovalerate.

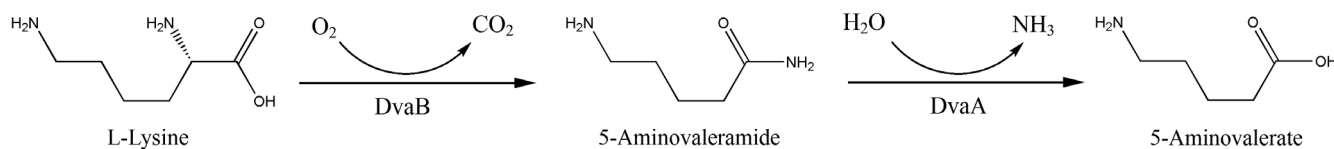
Polyamides (also known as nylons) are a class of plastics with a variety of uses as textiles and mechanical parts<sup>1</sup>. Polyamides are formed either as co-polymers via condensation of diacids with diamines, or as homopolymers of amino acids<sup>1-4</sup>. Nylon-5 is a polyamide homopolymer produced from amino acid monomers of 5-aminovalerate<sup>5</sup>. Although nylon 5 has not achieved commercial realization, it possesses properties similar to those of nylon-4,6, and serves as a suitable substitute<sup>5</sup>. Thus, it would be desirable to find an effective method for the biotechnological production of 5-aminovalerate.

5-Aminovalerate is naturally produced from L-lysine through the 5-aminovalerate pathway<sup>6,7</sup>. L-Lysine monooxygenase (DavB) and 5-aminovaleramide amidohydrolase (DavA) play key roles in the 5-aminovalerate pathway of various microorganisms<sup>6,7</sup>. DavB catalyzes the oxidation of L-lysine to produce 5-aminovaleramide. DavA then catalyzes 5-aminovaleramide into 5-aminovalerate<sup>6,7</sup>.

Fermentation of high concentrations of L-lysine is easily carried out<sup>8-14</sup>. Currently, L-lysine has an annual production of more than 2 million tons<sup>15</sup>. Considering current preparation processes, L-lysine might be a suitable starting material for 5-aminovalerate production. In a previous study, recombinant *Escherichia coli* strains expressing the *davB* and *davA* genes of *Pseudomonas putida* KT2440 were constructed for the production of 5-aminovalerate from L-lysine<sup>16</sup>. When the recombinant *E. coli* was cultured in a medium containing both glucose and L-lysine, 3.6 g/L (30.7 mM) 5-aminovalerate was produced from 7 g/L (47.9 mM) L-lysine<sup>16</sup>.

Production of 5-aminovalerate from L-lysine by enzymatic methods is another area of emphasis. The advantages of this method are the simple composition of the reaction mixture, high conversion rate of substrate, and convenience of recovery<sup>17</sup>. For example, L-lysine  $\alpha$ -oxidase can oxidize L-lysine into 6-amino-2-ketocaproic acid with a high conversion rate<sup>18-20</sup>. Hydrogen peroxide, which is produced during the process of L-lysine oxidation, will further oxidize 6-amino-2-ketocaproic acid to form 5-aminovalerate as the final product<sup>21</sup>. Under optimum conditions for enzyme activity, 17.5 g/L (120 mM) L-lysine was transformed into 13.4 g/L (114 mM) 5-aminovalerate after aerobic incubation for 5 days at 37°C<sup>21</sup>.

As shown in Fig. 1, DavB and DavA formed a coupled system for the production of 5-aminovalerate from L-lysine. In this work, DavB and DavA from *P. putida* KT2440 were expressed and purified. After optimization of the biocatalysis conditions, production of 5-aminovalerate from L-lysine with high yield and high concentration was accomplished through the DavB and DavA coupled system.



**Figure 1** | Scheme for the production of 5-aminovalerate from L-lysine by the DavB and DavA coupled system.

## Results

**Expression and purification of DavB and DavA.** The genes *davB* and *davA* were amplified by polymerase chain reaction (PCR) from the genome of *P. putida* KT2440. The fragments were sequenced. The deduced amino acid sequences had no mutational changes in the DavB and DavA proteins of *P. putida* KT2440<sup>6</sup>. DavB and DavA were expressed and purified to homogeneity through Ni-affinity chromatography. The purified DavB and DavA proteins were both detectable as single bands of 62.4 kDa and 29.3 kDa on SDS-PAGE, respectively (Fig. S1). DavB is a FAD-dependent monooxygenase that catalyzes oxidative decarboxylation of L-lysine with a specific activity of 6.1 U/mg. DavA is a hydrolase that catalyzes the production of 5-aminovalerate from 5-aminovaleramide. The specific activity of DavA was not determined because of the lack of commercial 5-aminovaleramide.

**Feasibility of 5-aminovalerate production using the coupled system.** To identify the L-lysine oxidation activity of DavB, 5 g/L L-lysine was used as a substrate in 100 mM phosphate buffer (pH 7.4). DavB was added to the reaction broth at a concentration of 0.5 g/L. The reaction was conducted at 30°C for 3 h. Catalytic conversion of 4.5 g/L L-lysine resulted in the accumulation of 5-aminovaleramide in the reaction mixture (Fig. S2A and Table S1). Although 5-aminovaleramide hydrolyzed spontaneously into 5-aminovalerate, only 1.1 g/L 5-aminovalerate was obtained without the addition of DavA (Fig. S2A). Alternatively, when 0.5 g/L DavA was also added to the reaction mixture, 3.4 g/L 5-aminovalerate was obtained from 5 g/L L-lysine (Fig. S2B and Table S1). The yield of 5-aminovalerate was 0.85 mol/mol. Thus, it is desirable to use the DavB and DavA coupled system for 5-aminovalerate production from L-lysine with high yield.

**Optimization of biocatalysis conditions.** To achieve higher product concentration, the biocatalytic conditions of 5-aminovalerate production from L-lysine were optimized. The influence of reaction pH and temperature on 5-aminovalerate production in 100 mM phosphate buffer containing 10 g/L L-lysine, 0.5 g/L DavB, and 0.5 g/L DavA were determined. The pH was adjusted ranging from 5.0 to 9.0. The reaction was initiated by adding the DavB and DavA at 30°C followed by incubation for 3 h. As shown in Fig. 2A, 5-aminovalerate production was the highest at pH 7.0. The transformation of L-lysine to 5-aminovalerate decreased under acidic or alkaline conditions.

To determine the effect of reaction temperature, the same reaction mixture was used as described above (pH at 7.0). After bioconversion at 16, 25, 30, 37, 45, or 55°C for 3 h, the highest 5-aminovalerate production was obtained at 37°C, as shown in Fig. 2B. To determine the effect of L-lysine concentration, reactions with six different L-lysine concentrations (5, 10, 30, 50, 70, or 90 g/L) were conducted at pH 7.0 and 37°C. Samples were taken after 3 h. Conversion rates were determined under each reaction condition. The optimal L-lysine concentration was found to be 30 g/L as shown in Fig. 2C. 5-Aminovalerate production increased as the L-lysine concentration increased up to 30 g/L, and decreased thereafter. The ratio of DavA to DavB (0.5 g/L) was also optimized using 30 g/L L-lysine as the substrate at a pH of 7.0 and a temperature of 37°C. As shown in Fig. 2D, 5-aminovalerate production was highest when the ratio of

DavA to DavB was 1 : 1. A ratio of 1 : 1 (DavA/DavB) was used in subsequent experiments.

**Production of 5-aminovalerate from L-lysine under optimal conditions.** After optimizing the reaction conditions, the biocatalytic production of 5-aminovalerate from L-lysine was conducted as follows: the reaction mixture contained 30 g/L L-lysine as substrate and 0.5 g/L each of DavB and DavA as the biocatalysts. The reaction was performed at 37°C and a pH of 7.0. As shown in Fig. 3, under the optimal conditions, 20.8 g/L (178 mM) 5-aminovalerate was obtained from 30 g/L (205 mM) L-lysine after 12 h. The yield of 5-aminovalerate from L-lysine was 0.87 mol/mol.

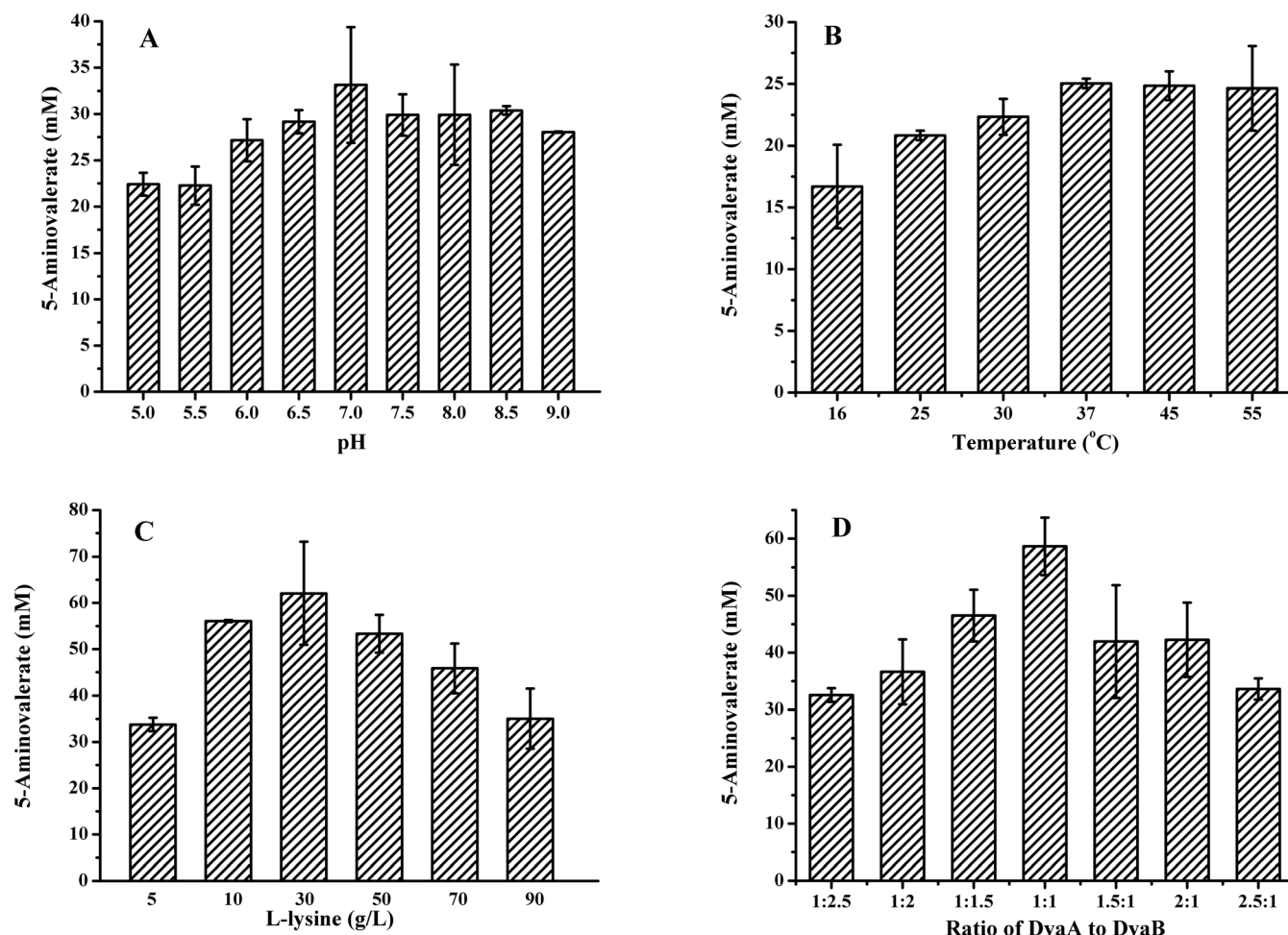
## Discussion

With concerns about environmental problems and fossil fuel resource availability, there has been much interest in replacement of petro-chemical derived materials with biologically based, degradable plastic polymers<sup>22–26</sup>. The production of biodegradable plastics requires the availability of high amounts of monomeric units, such as 3-hydroxypropionate<sup>27,28</sup>, lactic acid<sup>29,30</sup>, succinic acid<sup>31,32</sup>, and 1,3-propanediol<sup>33,34</sup>. 5-Aminovalerate, another monomeric unit produced from renewable sources, can be used to produce the biologically based, degradable plastic polymer, nylon-5. This compound can also be used as a C5 platform chemical for synthesizing 1,5-pentanediol, glutarate, and 5-hydroxyvalerate<sup>16,35</sup>. For the utilization of this important intermediate, an efficient 5-aminovalerate producing process is desirable.

5-Aminovalerate synthesis from L-lysine is mediated by DavB and DavA in *P. putida* KT2440. Previous studies have tried to produce 5-aminovalerate from L-lysine using whole-cell cultures of *E. coli* containing the *davB* and *davA* genes. Since 5-aminovalerate is further converted to glutarate via glutarate semialdehyde in these microorganisms, it proved difficult to produce 5-aminovalerate with high yield. In a previous work, Park et al. constructed a recombinant *E. coli* strain for the production of 5-aminovalerate from L-lysine<sup>16</sup>. The yield of 5-aminovalerate from L-lysine in this study was 0.64 mol/mol.

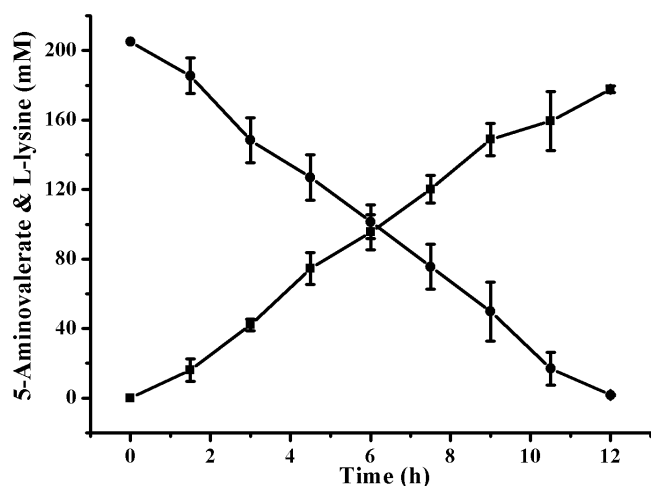
Alternatively, enzymatic synthesis of 5-aminovalerate using immobilized L-lysine  $\alpha$ -oxidase has been developed<sup>21</sup>. Production of 13.4 g/L 5-aminovalerate from 17.5 g/L L-lysine was accomplished using this method, which is the highest reported yield of 5-aminovalerate to date. In the present work, coupled reactions catalyzed by purified DavB and DavA were used to produce 5-aminovalerate from L-lysine. Although the yield of 5-aminovalerate (0.87 mol/mol) was lower than the process using L-lysine  $\alpha$ -oxidase (0.95 mol/mol), the highest reported product concentration (20.8 g/L) for 5-aminovalerate production from L-lysine was accomplished using the DavB and DavA coupled system. Because of the simple composition of the biocatalytic system, 5-aminovalerate can easily be separated from the reaction broth. Although most of the L-lysine was biotransformed, 5-aminovaleramide accumulation in the reaction mixture was detected indicating the presence of inactive DavA at the end of the reaction (Fig. S3). Increasing the stability and reusability of biocatalysts, especially DavA, through co-immobilization of DavB and DavA might make the coupled process more practical in future research.

In conclusion, the potential C5 platform chemical 5-aminovalerate was produced from L-lysine through a DavB and DavA coupled



**Figure 2 | Optimization of biocatalysis conditions.** (A) pH. (B) Temperature. (C) Concentration of L-lysine. (D) Ratio of DvaA to DvaB. Data points represent means  $\pm$  SD of three parallel replicates.

system. The concentration and yield of 5-aminovalerate were 20.8 g/L and 0.87 mol/mol, respectively. Because L-lysine can be easily produced by fermentation, the biocatalysis process introduced in this study provides a technically and economically valuable method for 5-aminovalerate production.



**Figure 3 | Production of 5-aminovalerate from L-lysine by the DavB and DavaA coupled system under optimal conditions.** (■) L-lysine, (●) 5-aminovalerate. Data points represent means  $\pm$  SD of three parallel replicates.

## Methods

**Chemicals.** 5-Aminovalerate, L-lysine, isopropyl- $\beta$ -D-1-thiogalactopyranoside (IPTG), phenylisothiocyanate (PITC), phenylmethanesulfonyl fluoride (PMSF), and dithiothreitol (DTT) were all purchased from Sigma. PCR primers were obtained from Sangon (Shanghai, China). Ampicillin was purchased from Amresco (USA). Restriction enzymes were obtained from Fermentas (Lithuania). T<sub>4</sub> DNA ligase and FastPfu DNA polymerase were purchased from MBI (USA) and Transgen Biotech (China), respectively. All other chemicals were of analytical grade and commercially available.

**Bacterial strains, primers, and plasmids.** Bacterial strains, primers, and plasmids used in this study are listed in Table 1. *E. coli* BL21(DE3) was used for protein expression. *E. coli* DH5a was used for general cloning. Luria-Bertani (LB) medium was used for *E. coli* cultivations. *P. putida* KT2440 was cultured in minimal salt medium (MSM) supplemented with 10.0 g/L DL-lactate as the carbon source<sup>36,37</sup>. Ampicillin was used at a concentration of 100  $\mu$ g/mL.

**Cloning and expression of *davB* and *dava*.** Genomic DNA of *P. putida* KT2440 was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). The *davB* gene was amplified from genomic DNA of *P. putida* KT2440 using primer B1 with a *Bam*HI restriction site insertion and primer B2 with an *Eco*RI restriction site insertion. The PCR product was ligated to the pEasy-Blunt vector. The resulting plasmid was designated pEasy-Blunt-*davB*. pEasy-Blunt-*davB* was then digested with *Bam*HI and *Eco*RI. The gel purified *davB* fragment was ligated to pETDuet-1 vector that had been digested with the same restriction enzymes. The *dava* gene fragment was obtained from the genome of *P. putida* KT2440 using primers A1 (with a *Bam*HI restriction site) and A2 (with an *Eco*RI restriction site), ligated to the pEasy-Blunt vector, and then ligated to the pETDuet-1 vector using the process described above.

**Purification of DavB and Dava.** *E. coli* BL21(DE3) carrying plasmid pETDuet-*davB* or pETDuet-*dava* was grown at 30°C in LB medium with 100  $\mu$ g/mL ampicillin in an optical density of 0.5 at 620 nm. IPTG was added at a concentration of 0.5 mM to induce the expression of DavB or Dava. After cultivation for another 3 h at 30°C,





Table 1 | Strains, plasmids, and primers used in this study

Strain, plasmid and primer	Genotype, properties and sequence	Source or reference
<b>Strain</b>		
<i>P. putida</i> KT2440	Wild type	DSM6125
<i>E. coli</i> DH5 $\alpha$	<i>supE44</i> $\Delta$ <i>lacU169</i> ( $\phi$ 80 <i>lacZ</i> $\Delta$ M15) <i>hsdR17</i> <i>recA1</i> <i>endA1</i> <i>gyrA96</i> <i>thi-1</i> <i>relA1</i>	Novagen
<i>E. coli</i> BL21 (DE3)	F <sup>-</sup> <i>ompT</i> <i>hsdSB</i> ( <i>rB</i> <sup>-</sup> <i>mB</i> <sup>-</sup> ) <i>gal</i> ( $\lambda$ <i>cI</i> 857 <i>ind1</i> <i>Sam7</i> <i>nin5</i> <i>lacUV5</i> T7gene1) <i>dcm</i> (DE3)	Novagen
<b>Plasmid</b>		
pEasy-Blunt	Cloning vector; Ap <sup>r</sup>	Transgene
pETDuet-1	Overexpression vector; Ap <sup>r</sup>	Novagen
pETDuet- <i>dvaB</i>	pETDuet-1 with <i>dvaB</i> gene of <i>P. putida</i> KT2440	This study
pETDuet- <i>dvaA</i>	pETDuet-1 with <i>dvaA</i> gene of <i>P. putida</i> KT2440	This study
pEasy-Blunt- <i>dvaB</i>	pEasy-Blunt with <i>dvaB</i> gene of <i>P. putida</i> KT2440	This study
pEasy-Blunt- <i>dvaA</i>	pEasy-Blunt with <i>dvaA</i> gene of <i>P. putida</i> KT2440	This study
<b>Primer<sup>a</sup></b>		
A1	5'-GGATCCGATGCGCATCGCTCTGTACCAG-3' (BamHI)	This study
A2	5'-GAATTCCTCAGCCTTTACGCAGGTGCAGC-3' (EcoRI)	This study
B1	5'-GGATCCGATGAACAAGAAGAACCGCCAC-3' (BamHI)	This study
B2	5'-GAATTCCTCAATCCGCCAGGGCGATCGGG-3' (EcoRI)	This study

<sup>a</sup>Restriction sites are underlined, and the restriction enzymes are indicated in parentheses.

cells were harvested and washed with 0.85% (wt/vol) sodium chloride solution by centrifugation at 8,000 rpm for 5 min. The cell pellet was subsequently suspended in binding buffer (pH 7.4; 20 mM sodium phosphate, 20 mM imidazole, and 500 mM sodium chloride) containing 0.5 mM PMSF, 1 mM DTT, and 10% glycerol. Cells were disrupted by sonication (Sonic 500 W; 20 KHz) for 10 min in an ice bath. The cell lysate was centrifuged at 8,000 rpm for 30 min at 4°C. The supernatant was loaded onto a HisTrap HP column (5 mL) and eluted with 40% binding buffer and 60% elution buffer (pH 7.4; 20 mM sodium phosphate, 500 mM imidazole, and 500 mM sodium chloride) at a flow rate of 5 mL/min. The fractions containing DavB or DavA were concentrated by ultrafiltration, desalted with gel G-25, and detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

**Production of 5-aminovalerate from L-lysine.** DavB and DavA were coupled for 5-aminovalerate production from L-lysine. A typical coupled reaction was carried out in a 50-mL Erlenmeyer flask with 10 mL reaction broth containing 100 mM phosphate buffer (pH 7.4), 10 g/L L-lysine, 0.5 mg/mL DavB, and 0.5 mg/mL DavA. The reaction was conducted at 30°C 200-rpm shaking. Samples (0.2 mL) were taken periodically and centrifuged at 12,000 rpm.

**Analytical methods.** Cell growth was measured via optical density measured at 620 nm (OD<sub>620</sub>) using an Ultrospec™ 2100 pro UV/visible spectrophotometer. The oxidase activity of DavB for L-lysine was assayed by measuring the rate of oxygen consumption as follows: the reaction mixture contained 10 mM L-lysine, 100 mM phosphate buffer (pH 7.4), and an appropriate amount of enzyme in a final volume of 0.5 mL. The enzyme activity assay was started with the addition of enzyme solution. Consumption of oxygen was measured using a Clark-type oxygen electrode (Oxytherm System; Hansatech Instruments Ltd). One unit of enzyme activity was defined as the amount of enzyme catalyzing the consumption of one micromole of O<sub>2</sub> per min. The protein concentration was determined by the Lowry procedure using bovine serum albumin as the standard<sup>38</sup>.

Aqueous concentrations of L-lysine and 5-aminovalerate were measured using a high-performance liquid chromatography (HPLC) system (Agilent 1100 series, Hewlett-Packard) equipped with a UV-Vis detector. Derivatization of 5-aminovalerate and L-lysine by PITC was performed before injection and separation on a ZORBAX SB-C18 (5  $\mu$ m, 4.6 mm  $\times$  250 mm) maintained at 38°C<sup>39,40</sup>. The mobile phase (supplied at 1 mL/min) consisted of a solution of (A) pH 6.5, 100 mM sodium acetate-acetonitrile (97 : 3) and (B) acetonitrile-deionized (4 : 1) ramping (A : B) from 0 : 100 to 100 : 0 over 1 h.

- Adkins, J., Pugh, S., McKenna, R. & Nielsen, D. R. Engineering microbial chemical factories to produce renewable "biomonomers" *Front. Microbiol.* **3**, 313 (2012).
- Qian, Z. G., Xia, X. X. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of putrescine: a four carbon diamine. *Biotechnol. Bioeng.* **104**, 651–662 (2009).
- Qian, Z. G., Xia, X. X. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of cadaverine: a five carbon diamine. *Biotechnol. Bioeng.* **108**, 93–103 (2011).
- Le Vo, T., Kim, T. & Hong, S. Effects of glutamate decarboxylase and gamma-aminobutyric acid (GABA) transporter on the bioconversion of GABA in engineered *Escherichia coli*. *Bioprocess Biosyst. Eng.* **35**, 645–650 (2012).
- Bermudez, M., Leon, S., Aleman, C. & Munoz-Guerra, S. Comparison of lamellar crystal structure and morphology of nylon 46 and nylon 5. *Polymer* **41**, 8961–8973 (2000).

- Revelles, O., Espinosa-Urgel, M., Fuhrer, T., Sauer, U. & Ramos, J. L. Multiple and interconnected pathways for L-lysine catabolism in *Pseudomonas putida* KT2440. *J. Bacteriol.* **187**, 7500–7510 (2005).
- Revelles, O., Espinosa-Urgel, M., Molin, S. & Ramos, J. L. The davDT operon of *Pseudomonas putida*, involved in lysine catabolism, is induced in response to the pathway intermediate delta-aminovaleric acid. *J. Bacteriol.* **186**, 3439–3446 (2004).
- Adachi, N. *et al.* Direct L-lysine production from cellobiose by *Corynebacterium glutamicum* displaying beta-glucosidase on its cell surface. *Appl. Microbiol. Biotechnol.* **97**, 7165–7172 (2013).
- Chen, Z., Meyer, W., Rappert, S., Sun, J. & Zeng, A. P. Coevolutionary analysis enabled rational deregulation of allosteric enzyme inhibition in *Corynebacterium glutamicum* for lysine production. *Appl. Environ. Microbiol.* **77**, 4352–4360 (2011).
- Coello, N., Montiel, E., Concepcion, M. & Christen, P. Optimisation of a culture medium containing fish silage for L-lysine production by *Corynebacterium glutamicum*. *Bioresour. Technol.* **85**, 207–211 (2002).
- Geng, F., Chen, Z., Zheng, P., Sun, J. & Zeng, A. P. Exploring the allosteric mechanism of dihydrodipicolinate synthase by reverse engineering of the allosteric inhibitor binding sites and its application for lysine production. *Appl. Microbiol. Biotechnol.* **97**, 1963–1971 (2013).
- Meiswinkel, T. M., Rittmann, D., Lindner, S. N. & Wendisch, V. F. Crude glycerol-based production of amino acids and putrescine by *Corynebacterium glutamicum*. *Bioresour. Technol.* **145**, 254–258 (2013).
- Takeo, S., Murata, R., Kobayashi, R., Mitsuhashi, S. & Ikeda, M. Engineering of *Corynebacterium glutamicum* with an NADPH-generating glycolytic pathway for L-lysine production. *Appl. Environ. Microbiol.* **76**, 7154–7160 (2010).
- van Ooyen, J., Noack, S., Bott, M., Reth, A. & Eggeling, L. Improved L-lysine production with *Corynebacterium glutamicum* and systemic insight into citrate synthase flux and activity. *Biotechnol. Bioeng.* **109**, 2070–2081 (2012).
- Anastasiadis, S. L-lysine fermentation. *Recent Pat. Biotechnol.* **1**, 11–24 (2007).
- Park, S. J. *et al.* Metabolic engineering of *Escherichia coli* for the production of 5-aminovalerate and glutarate as C5 platform chemicals. *Metab. Eng.* **16**, 42–47 (2013).
- Li, Y., Chen, J. & Lun, S. Y. Biotechnological production of pyruvic acid. *Appl. Microbiol. Biotechnol.* **57**, 451–459 (2001).
- Chauhan, N., Narang, J., Sunny & Pundir, C. S. Immobilization of lysine oxidase on a gold-platinum nanoparticles modified Au electrode for detection of lysine. *Enzyme Microb. Technol.* **52**, 265–271 (2013).
- Kusakabe, H. *et al.* A new antitumor enzyme, L-lysine alpha-oxidase from *Trichoderma viride*. Purification and enzymological properties. *J. Biol. Chem.* **255**, 976–981 (1980).
- Lukasheva, E. V. & Berezov, T. T. L-Lysine alpha-oxidase: physicochemical and biological properties. *Biochemistry (Mosc.)* **67**, 1152–1158 (2002).
- Pukin, A. V., Boeriu, C. G., Scott, E. L., Sanders, J. P. M. & Franssen, M. C. R. An efficient enzymatic synthesis of 5-aminovaleric acid. *J. Mol. Catal. B Enzym.* **65**, 58–62 (2010).
- Asada, Y., Miyake, M., Miyake, J., Kurane, R. & Tokiwa, Y. Photosynthetic accumulation of poly-(hydroxybutyrate) by cyanobacteria—the metabolism and potential for CO<sub>2</sub> recycling. *Int. J. Biol. Macromol.* **25**, 37–42 (1999).
- Jung, Y. K., Kim, T. Y., Park, S. J. & Lee, S. Y. Metabolic engineering of *Escherichia coli* for the production of polylactic acid and its copolymers. *Biotechnol. Bioeng.* **105**, 161–171 (2010).
- Yang, T. H. *et al.* Biosynthesis of polylactic acid and its copolymers using evolved propionate CoA transferase and PHA synthase. *Biotechnol. Bioeng.* **105**, 150–160 (2010).



25. Reinecke, F. & Steinbuchel, A. *Ralstonia eutropha* strain H16 as model organism for PHA metabolism and for biotechnological production of technically interesting biopolymers. *J. Mol. Microbiol. Biotechnol.* **16**, 91–108 (2009).
26. Rojas-Rosas, O., Villafana-Rojas, J., Lopez-Dellamary, F. A., Nungaray-Arellano, J. & Gonzalez-Reynoso, O. Production and characterization of polyhydroxyalkanoates in *Pseudomonas aeruginosa* ATCC (9027) from glucose, an unrelated carbon source. *Can. J. Microbiol.* **53**, 840–851 (2007).
27. Rathnasingh, C., Raj, S. M., Jo, J. E. & Park, S. Development and evaluation of efficient recombinant *Escherichia coli* strains for the production of 3-hydroxypropionic acid from glycerol. *Biotechnol. Bioeng.* **104**, 729–739 (2009).
28. Rathnasingh, C. *et al.* Production of 3-hydroxypropionic acid via malonyl-CoA pathway using recombinant *Escherichia coli* strains. *J. Biotechnol.* **157**, 633–640 (2012).
29. Zhou, S., Causey, T. B., Hasona, A., Shanmugam, K. T. & Ingram, L. O. Production of optically pure D-lactic acid in mineral salts medium by metabolically engineered *Escherichia coli* W3110. *Appl. Environ. Microbiol.* **69**, 399–407 (2003).
30. Mazumdar, S., Clomburg, J. M. & Gonzalez, R. *Escherichia coli* strains engineered for homofermentative production of D-lactic acid from glycerol. *Appl. Environ. Microbiol.* **76**, 4327–4336 (2010).
31. Bechthold, I., Bretz, K., Kabasci, S., Kopitzky, R. & Springer, A. Succinic acid: a new platform chemical for biobased polymers from renewable resources. *Chem. Eng. Technol.* **31**, 647–654 (2008).
32. Jiang, M. *et al.* Succinic acid production from corn stalk hydrolysate in an *E. coli* mutant generated by atmospheric and room-temperature plasmas and metabolic evolution strategies. *J. Ind. Microbiol. Biotechnol.* **41**, 115–123 (2014).
33. Saxena, R. K., Anand, P., Saran, S. & Isar, J. Microbial production of 1, 3-propanediol: recent developments and emerging opportunities. *Biotechnol. Adv.* **27**, 895–913 (2009).
34. Pflügl, S., Marx, H., Mattanovich, D. & Sauer, M. Heading for an economic industrial upgrading of crude glycerol from biodiesel production to 1,3-propanediol by *Lactobacillus diolivorans*. *Bioresour. Technol.* **152**, 499–504 (2014).
35. Adkins, J., Jordan, J. & Nielsen, D. R. Engineering *Escherichia coli* for renewable production of the 5-carbon polyamide building-blocks 5-aminovalerate and glutarate. *Biotechnol. Bioeng.* **110**, 1726–1734 (2013).
36. Gao, C., Wang, X., Ma, C. & Xu, P. Production of hydroxypyruvate from glycerate by a novel biotechnological route. *Bioresour. Technol.* **131**, 552–554 (2013).
37. Gao, C. *et al.* Efficient production of 2-oxobutyrate from 2-hydroxybutyrate using whole cells of *Pseudomonas stutzeri* strain SDM. *Appl. Environ. Microbiol.* **76**, 1679–1682 (2010).
38. Markwell, M. A. K., Haas, S. M., Bieber, L. L. & Tolbert, N. E. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87**, 206–210 (1978).
39. Chen, S. The facile HPLC enantioresolution of amino acids, peptides on naphthylethylcarbamate-beta-cyclodextrin bonded phases using the acetonitrile-based mobile phase after their pre-column derivatization with phenylisothiocyanate: factors that affect the resolution. *Amino Acids.* **26**, 291–298 (2004).
40. Hariharan, M., Naga, S. & VanNoord, T. Systematic approach to the development of plasma amino acid analysis by high-performance liquid chromatography with ultraviolet detection with precolumn derivatization using phenyl isothiocyanate. *J. Chromatogr.* **621**, 15–22 (1993).

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## Author contributions

C.M., C.G. and P.X. conceived and designed the experiments. P.L., H.Z., M.L., M.H. and Z.L. performed the experiments. C.M., C.G. and P.X. contributed reagents and materials. P.L., H.Z. and C.G. analyzed the data. C.G., C.M. and P.X. wrote the paper. All authors reviewed the manuscript.

## Additional information

**Supplementary information** accompanies this paper at <http://www.nature.com/scientificreports>

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