One-step fermentative production of aromatic polyesters from glucose by metabolically engineered Escherichia coli strains

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Aromatic polyesters are widely used plastics currently produced from petroleum. Here we engineer Escherichia coli strains for the production of aromatic polyesters from glucose by one-step fermentation. When the Clostridium difficile isocaprenoyl-CoA:2-hydroxyisocaproate CoA-transferase (HadA) and evolved polyhydroxyalkanoate (PHA) synthase genes are overexpressed in a d-phenyllactate-producing strain, poly(52.3 mol% 3-hydroxybutyrate (3HB)-co-47.7 mol% d-phenyllactate) can be produced from glucose and sodium 3HB. Also, various poly(3HB-co-d-phenyllactate) polymers having 11.0, 15.8, 20.0, 70.8, and 84.5 mol% of d-phenyllactate are produced from glucose as a sole carbon source by additional expression of Ralstonia eutropha β-ketothiolase (phaA) and reductase (phaB) genes. Fed-batch culture of this engineered strain produces 13.9 g l\(^{-1}\) of poly(61.9 mol% 3HB-co-38.1 mol% d-phenyllactate). Furthermore, different aromatic polyesters containing d-mandelate and d-3-hydroxy-3-phenylpropionate are produced from glucose when feeding the corresponding monomers. The engineered bacterial system will be useful for one-step fermentative production of aromatic polyesters from renewable resources.
Aromatic polyesters are widely used indispensable plastics currently produced from petroleum. Microbial fermentative production of polymers from renewable resources has received much attention to substitute petroleum-based polymers and help solving environmental problems. Thus, there has been much interest in fermentative production of aromatic polyesters from renewable non-food biomass, but without any success. Polyhydroxyalkanoates (PHAs) comprising various hydroxyalkanoic acids are natural biodegradable microbial polymers, which are accumulated by numerous microorganisms under nutrient limited conditions in the presence of excess carbon sources. The material properties of PHAs can be modulated by changing the monomer types and compositions. Over the last three decades, numerous metabolic engineering studies have been performed to produce PHAs containing specific monomers having different monomer compositions. Various kinds of monomers such as 3-hydroxypropionate, 3-hydroxybutyrate (3HB), 3-hydroxyvalerate, 4-hydroxybutyrate, 5-hydroxyvalerate, and 6-hydroxyhexanoate, and medium-chain length 3-hydroxyalkanoates have been shown to be incorporated into PHAs, either as homo- or co-polymers, giving diverse material properties.

More recently, one-step fermentative production of non-natural polymers, poly(δ-lactate), poly(lactate-co-glycolate), and other δ-lactate-containing PHAs by metabolically engineered bacteria have been reported. In these studies, an evolved propionate CoA-transferase (Pct) and an evolved PHA synthase (PhaC) were expressed in Escherichia coli; engineered Clostridium propionicum Pct converts 2-hydroxyacids to corresponding 2-hydroxyacyl-CoAs using acetyl-CoA as a CoA-donor and then engineered Pseudomonas sp. MBEL 6–19 PHA synthase (PhaC_{p6–19}) polymerizes these 2-hydroxyacyl-CoAs into polymers containing corresponding monomers. It has been previously reported that some bacteria such as Pseudomonas oleovorans and Pseudomonas putida strains can synthesize aromatic polyesters when grown in the culture medium containing n-phenylalkanoic acid as a direct precursor of aromatic monomer. These aromatic polyesters have only been produced by feeding the cells with corresponding aromatic monomers as substrates and have not been produced by direct fermentation from renewable feedstock carbohydrates such as glucose. Furthermore, those mentioned above contain aromatic groups far away from the main polymer carbon chain and thus polymer properties were found to be much different from petroleum-derived aromatic polymers, which often contain aromatic rings close to the main polymer chain, such as poly(ethylene terephthalate) (PET) and polystyrene. Such observations led us to develop metabolically engineered E. coli strains for one-step fermentative production of aromatic polyesters from glucose.

The following strategies were employed in this study. First, CoA-transferases that can efficiently activate phenyllactanoates into their corresponding CoA derivatives were discovered. Second, cells were metabolically engineered to produce phenyllactanoates from glucose. Third, the engineered phenyllactanoates overproducing E. coli strains were employed for in vivo production of aromatic polyesters by expressing engineered PHA synthase and CoA-transferases. Fourth, strains were further engineered to produce aromatic polyesters directly from glucose. Fifth, the enzyme expression levels were modulated to produce various aromatic polyesters having different monomer fractions. Finally, as proof-of-concept examples of expanding the range of aromatic polyesters that can be produced by fermentation, aromatic polyesters containing δ-mandelate and δ-3-hydroxy-3-phenylpropionate were produced by feeding the corresponding precursors.

Results

Validation of the CoA-transferase activity. First, we examined whether Pct can activate phenyllactate and mandelate into phenyllactyl-CoA and mandelyl-CoA, respectively. As the mutant of Pct (Pct540) has successfully been employed for in vivo production of polyesters containing 2-hydroxyacids such as glycolate, lactate, 2-hydroxybutyrate, and 2-hydroxyisovalerate, and various hydroxyacids, the substrate spectrum of Pct540 seems to be broad enough with respect to the number of carbon atoms and position of hydroxyl group. Unfortunately, however, Pct540 was found to have no catalytic activities toward phenyllactate and mandelate (Supplementary Fig. 1). Thus, a CoA-transferase capable of activating aromatic compounds into corresponding CoA derivatives had to be identified for aromatic polymer production.

There have been reports describing that Clostridium sporogenes cinnamoyl-CoA-phenyllactyl-CoA transferase (Flda) can convert phenyllactate into phenyllactyl-CoA using cinnamoyl-CoA as a CoA-donor. As cinnamoyl-CoA is a non-natural metabolite in E. coli, the possibility of using acetyl-CoA, which is abundant metabolite in the cell, as a CoA-donor was examined for the synthesis of phenyllactyl-CoA by Clostridium botulinum A strain ATCC 3502 Flda (using the synthesized gene), which shared 99.0% of amino acid sequence identity with that of C. sporogenes. However, Flda was found to have no catalytic activity on generating phenyllactyl-CoA using acetyl-CoA as a CoA-donor. A previous study reported that Streptomyces coelicolor 4-coumarate-CoA ligase (4CL) has an important role in phenylpropanoid metabolism via generating cinnamoyl-CoA from cinnamate. Thus, a biosynthetic route was designed and tested in vitro for the synthesis of cinnamoyl-CoA from cinnamate by 4CL (Supplementary Fig. 2 and Supplementary Note 1). A modified 4CL was applied to make cinnamoyl-CoA, which can be used as CoA-donor for Flda mediated phenyllactyl-CoA formation. As expected, phenyllactyl-CoA was successfully synthesized by in vitro sequential reaction of 4CL and Flda; 4CL converts cinnamate to cinnamoyl-CoA and then Flda converts phenyllactate into phenyllactyl-CoA (Supplementary Fig. 3). These results suggest that 4CL and Flda can potentially be used for phenyllactyl-CoA generation and consequently aromatic polyester production. Similarly, 4-hydroxyphenyllactate, another promising aromatic monomer, was also converted to 4-hydroxyphenyllactyl-CoA by in vitro sequential reaction of mutant 4CL and Flda (Supplementary Fig. 3).

In vivo polymerization of aromatic PHA using Flda. In the biosynthesis of non-natural polyesters, selection of a monomer-specific PhaC variant is crucial for the production of desired polyesters. To examine the performance of various PhaCs on aromatic PHAs production, engineered Pseudomonas sp. MBEL 6–19 PHA synthase (PhaC_{p6–19}) Variants (PhaC1202, PhaC1301, PhaC1310, PhaC1437, PhaC1439) were expressed in E. coli XL1-Blue strain together with 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase (aroE_{BR}), phenylalanine ammonia-lyase (PAL), 4CL, Flda, and Pct540 in MR medium (see Methods) containing 20 g l^{-1} (111 mM) of glucose, 1 g l^{-1} of D-phenyllactate, and 1 g l^{-1} of sodium 3HB. Here, 3HB was supplied to enhance the production of polymers, because it is converted by Pct540 into 3HB-CoA, a more favorable substrate of PhaC, thus allowing production of PHAs to sufficient amounts for further analysis. The E. coli XL1-Blue strains expressing different PHA synthase variants together with AroG_{BR}, PAL, 4CL, Flda, and Pct540 were able to produce varying amounts of random copolymers, poly(δ-lactate-co-3HB-co-D-phenyllactate), having different monomer compositions (Supplementary Fig. 4a).
Among the PhaC variants, PhaC1437 containing four amino acids substitutions (E130D, S325T, S477G, and Q481K) was found to be the best, which resulted in the production of poly(16.8 mol% D-lactate-76.9 mol% 3HB-co-4.8 mol% D-phenyllactate) to the polymer content of 12.8 wt% of dry cell weight in MR medium containing 20 g l\(^{-1}\) of glucose and 1 g l\(^{-1}\) of sodium 3HB (Supplementary Fig. 5 and Supplementary Table 1). It should be noted that a small amount of d-4-hydroxyphenyllactate was also incorporated into the polymer, which is predictable from in vitro FldA assay (Supplementary Fig. 3); d-4-hydroxyphenyllactyl-CoA was also generated by FldA and polymerized with d-phenyllactyl-CoA.

Identification of 2-hydroxyisocaproate CoA-transferase.
Although aromatic PHAs containing d-phenyllactate could be successfully produced, critical problems still exist in using the FldA system, such as rather low aromatic monomer content and narrow monomer spectrum. In vitro enzyme assay results suggested that FldA was able to transfer CoA to phenyllactate and 4-hydroxyphenyllactate, but not to others of our interest, such as manelate, 2-hydroxy-4-phenylbutyrate, 3-hydroxy-3-phenylpropionate, and 4-hydroxybenzoate (Supplementary Fig. 3). Thus, the Clostridium difficile isocaprenyl-CoA2-hydroxyisocaproate CoA-transferase (HadA), which has more than 48% of amino acid sequence identity to FldA was newly identified based on aromatic acid sequence similarity analysis (Supplementary Fig. 6).

Interestingly, despite isocaprenyl-CoA-based 2-hydroxyisocaproate-specific CoA transferring activity of HadA, we newly discovered that phenyllactate could be converted to phenyllactyl-CoA using acetyl-CoA derived CoA moiety (Fig. 2 and Supplementary Fig. 7). Moreover, HadA showed catalytic activity on converting mandelate, 4-hydroxymandelate, 4-hydroxymandelate, 4-hydroxybenzoate, 3-hydroxy-3-phenylpropionate, and 4-hydroxybenzoate (Supplementary Fig. 3). Thus, the Clostridium difficile isocaprenyl-CoA2-hydroxyisocaproate CoA-transferase (HadA), which has more than 48% of amino acid sequence identity to FldA was newly identified based on aromatic acid sequence similarity analysis (Supplementary Fig. 6).
hydroxypheylactate, 2-hydroxy-4-phenylbutyrate, 3-hydroxy-3-phenylpropionate, and 4-hydroxybenzoate to their corresponding CoA derivatives (Fig. 2 and Supplementary Fig. 7c–k). Thus, HadA has a potential to more efficiently produce diverse aromatic polyesters using acetyl-CoA as a CoA donor.

Enhanced carbon flux for D-phenyllactic acid production. For the overproduction of D-phenyllactate, the tyrR gene was deleted to make E. coli XBT strain due to the negative regulation of TyrR on aromatic amino acid biosynthesis. E. coli XBT strain expressing AroGβr, PheAfbr, and FldH could produce 0.5 g l⁻¹ of D-
phenyllactic acid from 16.4 g l\(^{-1}\) (91 mM) of glucose, which is 34.4\% higher than that obtained with \(E.\) coli XL1-Blue strain expressing the same genes. In order to remove competing pathways of D-phenyllactate biosynthesis, the \(poxB\) (encoding pyruvate oxidase), \(pflB\) (encoding pyruvate formate lyase), \(adhE\) (encoding acetaldehyde dehydrogenase/alcohol dehydrogenase), and \(frdB\) (encoding fumarate reductase) genes were deleted in \(E.\) coli XBT, to make \(E.\) coli XB201T. The \(E.\) coli XB201T strain expressing AroGfbr, PheAfbr, and FldH produced 0.55 g l\(^{-1}\) of D-phenyllactic acid from 15.7 g l\(^{-1}\) (87 mM) of glucose, which is 10\% higher than that obtained with the XBT strain (Supplementary Fig. 8). The \(tyrB\) gene encoding tyrosine aminotransferase and \(aspC\) gene encoding aspartate aminotransferase were also deleted, to increase carbon flux toward D-phenyllactic acid based on in silico genome-scale metabolic flux analyses (Supplementary Note 2). The resulting \(E.\) coli XB201T strain expressing AroGfbr, PheAfbr, and FldH allowed significantly enhanced production of D-phenyllactic acid up to 1.62 g l\(^{-1}\) from 18.5 g l\(^{-1}\) (102 mM) of glucose, which is 4.35-fold higher than that obtained with the original XL1-Blue strain expressing the same genes. The \(ldhA\) gene was further deleted in XB201TBA to prevent D-lactate formation, to make XB201TBAL strain. The engineered \(E.\) coli XB201TBAL strain expressing AroGfbr, PheAfbr, FldH, HadA, and PhaC1437 was able to produce poly (52.1 mol\% 3HB-co-47.9 mol\% D-phenyllactate) to the polymer content of 15.8 wt\% of dry cell weight in MR medium containing 20 g l\(^{-1}\) of glucose and 1 g l\(^{-1}\) of sodium 3HB (Fig. 3). It should be noted that D-4-hydroxyphenyllactate was not incorporated into the polymer anymore through reinforcing the flux toward D-phenyllactate. Moreover, poly(52.3 mol\% 3HB-co-47.7 mol\% D-phenyllactate) could be produced to a higher polymer content of 24.3 wt\% by fed-batch fermentation (Supplementary Fig. 9).

The importance of HadA in the production of PHAs containing diverse aromatic monomers was also evaluated by supplementation of monomers of interest such as D-mandelate and D-3-hydroxy-3-phenylpropionate. The final engineered \(E.\) coli XB201TBAL expressing AroGfbr, PheAfbr, FldH, HadA, and PhaC1437 was able to produce poly(55.2 mol\% 3HB-co-43.0 mol\% D-phenyllactate-co-1.8 mol\% D-mandelate) to the polymer content of 11.6 wt\% of dry cell weight and poly(33.3 mol%...
3HB-co-18.0 mol% D-phenyllactate-co-48.7 mol% D-3-hydroxy-3-phenylpropionate) to the polymer content of 14.7 wt% of dry cell weight in MR medium containing 20 g l⁻¹ of glucose and 1 g l⁻¹ of sodium 3HB together with 0.5 g l⁻¹ of their corresponding monomers, respectively (Supplementary Fig. 10). These results strongly suggest that the engineered E. coli system expressing HAdA and evolved PHA synthase can be broadly employed for the production of diverse aromatic polyesters.

**Synthetic promoter based flux modulation.** In the above studies, 3HB was supplemented to boost polymer accumulation as described earlier. Next, production of aromatic PHAs from glucose without 3HB supplementation was pursued in XB201TBAL strain expressing AroGfbr, PheAfbr, FldH, HadA, and PhaC1437. Expected, XB201TBAL strain expressing AroGfbr, PheAfbr, FldH, HadA, and PhaC1437 was able to produce poly(86.2 mol% 3HB-co-13.8 mol% D-phenyllactate) to the polymer content of 18.0 wt% of dry cell weight in MR medium containing glucose without 3HB feeding. In 96 h, 2.5 g l⁻¹ of poly (67.6 mol% 3HB-co-32.4 mol% D-phenyllactate) was produced with a polymer content of 43.8 wt%, which demonstrated that aromatic polyester can be produced by one-step fermentation of metabolic fluxes of PhaAB using synthetic Anderson promoters (http://parts.igem.org/). Five different plasmids expressing PhaAB under five promoters of different strength were constructed and introduced into the XB201TBAL strain expressing AroGfbr, PheAfbr, FldH, HadA, and PhaC1437. The D-phenyllactate monomer fraction could be modulated, showing increased fraction with increased PhaAB expression; polymers having 11.0, 15.8, 20.0, 70.8, and 84.5 mol% of D-phenyllactate could be produced (Fig. 4a, b and Supplementary Table 2); it should be noted that by expressing PhaAB under BBA_J23103 promoter, a polymer having a very high D-phenyllactate fraction (poly(15.5 mol% 3HB-co-84.5 mol% D-phenyllactate)) could be produced to the polymer content of 4.3 wt% of dry cell weight (Fig. 4b). These results suggest that aromatic polyesters having different aromatic monomer fractions can be produced by modulating metabolic fluxes. Next, the pH-stat fed-batch culture of E. coli XB201TBAL strain expressing AroGfbr, PheAfbr, FldH, HadA, PhaC1437, and PhaAB under BBA_J23114 promoter was performed in a medium containing glucose without 3HB feeding. In 96 h, 2.5 g l⁻¹ of poly (67.6 mol% 3HB-co-32.4 mol% D-phenyllactate) was produced with a polymer content of 43.8 wt%, which demonstrated that aromatic polyester can be produced by one-step fermentation of

![Diagram](https://example.com/diagram.png)
engineered *E. coli* from glucose (Supplementary Fig. 9). To further enhance production of aromatic PHAs, gene expression system was optimized by replacing the *ldhA* gene with the *fldH* gene in the chromosome of *E. coli* XB201TBA. In addition, the native promoter of the *ldhA* gene was replaced with the strong trc promoter to increase *fldH* expression. Furthermore, we employed pulsed-feeding method (see Methods). The resulting *E. coli* XB201TBAF strain expressing AroG*, PheA*, HadA, PhaC1437, and PhaAB under BBA_J23141 promoter allowed production of 13.9 g l\(^{-1}\) of poly(61.9 mol\% 3HB-co-38.1 mol\% \(\beta\)-phenyllactate) with a polymer content of 55.0 wt\% from glucose by fed-batch fermentation (Supplementary Fig. 9). This titer (13.9 g l\(^{-1}\)) is 5.6-fold higher than that (2.5 g l\(^{-1}\)) obtained with the *E. coli* XB201TBA strain expressing AroG*, PheA*, FlhD, HadA, PhaC1437, and PhaAB under BBA_J23141 promoter and is also much higher than that (< 1 g l\(^{-1}\)) obtained by fed-batch culture of the *E. coli* XB201TBA strain expressing AroG*, PheA*, FlhD, HadA, and PhaC1437 in a medium supplemented with glucose and sodium 3HB. These results demonstrate that aromatic PHAs could be successfully produced to a reasonably high concentration by fed-batch culture of the engineered strain (XB201TBAF strain expressing AroG*, PheA*, HadA, PhaC1437, and PhaAB under BBA_J23141 promoter). Although we provided proof-of-concept fermentation results here, it is expected that further optimization of cultivation condition will allow more efficient production of aromatic polyesters by one-step fermentation from glucose.

Next, material properties of aromatic polyesters produced in this study were examined to see whether they are suitable for industrial applications. In the case of PET, the most widely used petroleum-derived aromatic polyester, fiber-grade, and bottle-grade PET have a number-average molecular weight (\(M_n\); molecular mass) of 15–20 kg and 24–36 kg, respectively. The \(M_n\)'s of aromatic polyesters produced in this study are also in the range of 3.6–24.9 kg (Supplementary Table 2), showing a good potential to replace petroleum-based aromatic polyesters. To examine the material properties of the aromatic polyesters produced by metabolically engineered *E. coli* strains (Fig. 4c–i and Supplementary Note 3), films of five different aromatic polyesters produced above and poly(3HB) as a control were manufactured. The critical cohesive fracture energy (\(G_c\)) of poly(3HB) film was measured to be 0.28 ± 0.11 (SD) J m\(^{-2}\), whereas the \(G_c\) increased as the aromatic monomer fraction increased from 11.0 to 84.5 mol\% . The polymer film made of poly(15.5 mol\% 3HB-co-84.5 mol\% \(\beta\)-phenyllactate), the polymer having the highest \(\beta\)-phenyllactate fraction, showed increased \(G_c\) of 0.90 ± 0.12 (SD) J m\(^{-2}\), which is mainly attributed to the reduced crystallinity and high glass transition temperature (\(T_g\)) of the polymer. These results suggest that aromatic polyesters produced by one-step direct fermentation of engineered *E. coli* strains can be used to replace those aromatic polyesters currently produced from petroleum.

**Discussion**

In this study, we report the development of a bacterial platform system that allows production of various aromatic polyesters by one-step fermentation. The key strategies for the successful fermentative production of aromatic polyesters from glucose include identification and use of a novel broad substrate range CoA-transferase for activating aromatic compounds to their CoA derivatives and a mutant PHA synthase capable of polymerizing these aromatic CoA derivatives, and design and optimization of metabolic pathways to overproduce corresponding aromatic monomers in vivo. As demonstrated for several aromatic monomers, this system has a potential to be used for the production of even more diverse aromatic polyesters. For example, HadA (or related enzymes) and PHA synthase can be further engineered to accept desired aromatic monomers of interest. Recently, the crystal structure of *R. eutropha* PHA synthase has been determined\(^1\). This structure can serve as a model for rational protein engineering of various PHA synthases to broaden the substrate utilization range. It is expected that the bacterial platform system developed here will help establish sustainable bioprocesses for the production of aromatic polyesters from renewable non-food biomass to substitute petroleum-based counterparts.

**Methods**

**Plasmids and construction of bacterial strains.** All chemicals used in this study were purchased from Sigma-Aldrich unless noted otherwise. All bacterial strains and plasmids used in this study were listed in Supplementary Table 3. *E. coli* XL1-Blue (Strategene Cloning Systems, La Jolla, CA) was used for general gene cloning and all DNA manipulations were performed following the standard procedures\(^26\). PCR was performed with the Clonero Thermal Cycler (Bio-Rad, Hercules, CA). Primers used in this study (Supplementary Table 4) were synthesized by Genotech (Daejeon, Korea).

The pPr619C1437Ptrc540 was used for the expression of the *Pseudomonas* sp. 6–19 PHA synthase gene (*phaC, ACM68707.1*) containing quadruple mutations of E130D, S325T, S477G, and Q481K (*PhaC1437*), and the *C. propionicum* Pct gene containing methionine addition of V193A and four silent nucleotide mutations of T78C, T97A, A1125G, and T1158C\(^10\). The primers Phct540 and Phct540d were used for the amplification of the *C. propionicum* Pct gene. The PCR product was digested with *Nde*I and *Bam*HI and ligated with *Nde*I-Bam*HI-digested pET22b(+) plasmid.

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plasmid pET22b-his4CL(A294G) as a template. The PCR product was digested with XhoI and SacI, and ligated with XhoI–SacI-digested pACYC184KS to construct pACYC-4CL(A294G). pACYC-4CL(A294G)FdrA was constructed by cloning the fd ΔA gene amplified by PCR using the primers FdrA-F and FdrA-R containing C-terminal (His)₆-tag from pET22b-fdΔA as a template into pACYC-4CL(A294G)FdrA site. To make pACYC-4CL(A294G)FdrA, the fd ΔA gene was amplified by PCR with the primers FdrA-F and FdrA-R using pACYC-FdΔA as a template. The PCR product was digested with SfiI and HindIII sites, and ligated with SfiI–HindIII-digested pACYC-4CL(A294G)FdrA to construct pACYC-4CL(A294G)FdrA.

To construct pET22b-hisHadA, the C. difficile HadA gene (hadA; AA40822.1) fused with C-terminal (His)₆-tag was amplified by PCR using the primers HadA-hs and HadA-hsA using the genomic DNA of C. difficile as a template. The PCR product was digested with NdeI and NotI, and ligated with NdeI–NotI-digested pET22b(-) plasmid. p6S196C1437-HadA was constructed by replacing the pET54Δ0 gene in p6S146C1437pCT540 with the C. difficile hadA gene that was amplified by PCR with the primers HadA-hs and HadA-hsA using the genomic DNA of C. difficile as a template. The PCR product was digested with SfiI and NdeI, and ligated with SfiI–NdeI-digested DNA fragment of pS6196C1437pCT540.

For the construction of pKM212-ZE_PhaAB, the K. estorophra PHA biosynthesis operon promoter, the β-ketothiolase (phaC), and acetoacetyl-CoA reductase (phaB) coding regions were amplified from the chromosomal DNA of K. estorophra using PhaB-Bam-R using the genomic DNA of BBa_J23100 was amplified to include the 10 bp overhangs that add additional HindIII and KpnI recognition sites. The PCR product was digested with HindIII and KpnI and ligated with HindIII–KpnI-digested pET22b(-) plasmid. The PCR product was digested with SfiI and SpeI, and ligated with SfiI–SpeI-digested pET22b(-) plasmid. pET22b-his4CL(A294G)FdrA, pACYC-4CL(A294G)FdrA, and pET22b-hisHadA were used as templates for the in vitro expression of the genes fused with C-terminal (His)₆-tag was ampliﬁed by PCR with the primers FldH-sbF and FldH-hiR using the plasmid pACYC-FldH as a template. The PCR product was digested with Hinfl and NotI, and ligated with Hinfl–NotI-digested pET22b(-) plasmid. The PCR product was digested with SfiI and NdeI, and ligated with SfiI–NdeI-digested DNA fragment of pS6196C1437pCT540.

In silico ﬂux response analysis. The genome-scale metabolic model of E. coli J013662⁵², comprising 2,251 metabolic reactions and 1,135 metabolites, was used for in silico ﬂux response analysis to examine the effects of central and aromatic amino acid biosynthesis reactions on the ω-phenylacetic acid production. In order to enhance the ω-phenylacetic acid production, the reactions were categorized into six types based on the flux patterns obtained from the in silico ﬂux response analysis. Reactions that were predicted to be negatively correlated with ω-phenylacetic acid production rate were identiﬁed as potential knockout candidates. During the simulation, the glucose uptake rate was set at 10 mmol per gram of dry cell weight per hour. All simulations were performed in Python environment with Gurobi Optimizer 6.0 and GurobiPy package (Gurobi Optimization, Inc., Houston, TX). Reading, writing, and manipulation of the COBRA-compliant SBML ﬁles were implemented using COBRAPP⁵¹.

Culture condition. Luria–Bertani (LB) medium (containing per liter: 10 g tryptone, 5 g yeast extract and 10 g NaCl) was used for cultivation of E. coli for DNA manipulations. Recombinant E. coli XL1-Blue cultures were grown in a chemically deﬁned MR medium at 30 °C in a rotary shaker at 200 r.p.m. for the production of PHAs. MR medium (pH 7.0) contains (per liter): 6.67 g KH₂PO₄, 4 g (NH₄)₂HPO₄, 0.8 g MgSO₄·7H₂O, 0.8 g citric acid, and 5 ml trace metal solution. The trace metal solution contains (per liter of 0.5 M HCl): 10 g FeSO₄·7H₂O, 2 g CaCl₂, 2.2 g ZnSO₄·7H₂O, 0.5 g MnSO₄·4H₂O, 1 g CuSO₄·5H₂O, 0.1 g (NH₄)₂MoO₄·4H₂O, and 0.02 g NaBO₃·10H₂O. Glucose, MgSO₄·7H₂O, sodium 3HB (Acros Organics, USA), β-phenylacetic acid, and d-(−)-hydroxyphenyllactic acid, L-tyrosine, and L-phenylalanine were analysed by HPLC (1100 series HPLC, Agilent Technologies, Palo Alto, CA) equipped with a refractive index detector and a C18 column (5 μm, 4.6 × 150 mm, Agilent).

Fed-batch cultures were carried out in a 6.6L Bioreator (Bioflow 3000, New Brunswick Scientific Co., Edison, NJ) containing 21 L of MR medium supplemented with 20 g L⁻¹ of glucose and 1 g L⁻¹ of sodium 3HB. The dissolved oxygen concentration was maintained above 40% of air saturation by automatically supplying air with the rate of 21 min⁻¹ and by automatically controlling the agitation speed from 200 to 1,000 r.p.m. The culture pH was controlled at 7.0 by 28% NaOH solution with a pH-stat strategy and also by pulsed-feeding strategy. For the pH-stat fed-batch cultures, the pH of fermentation broth was maintained at pH 7.0 and the feeding solution was automatically fed when the pH rose above 7.02 as a set point. For the pulsed-feeding strategy, the feeding solution containing 700 g glucose, 15 g MgSO₄·7H₂O, and 250 mg thiamine. The feed-batch fermentation was performed by pH-stat strategy and also by pulsed-feeding strategy. For the pH-stat fed-batch cultures, the pH of fermentation broth was maintained at pH 7.0 and the feeding solution was automatically fed when the pH rose above 7.02 as a set point.

4CL enzyme assay. The activity of 4CL was determined by the spectrophotometric assay as follows: the changes in absorbance during cinnamoyl-CoA formation were monitored at wavelengths of 311 nm. The reaction mixture contained 400 mM Tris-HCl (pH 7.8), 5 mM ATP, 5 mM MgCl₂, 0.3 mM CoA, 0.5 mM hydroxylamine, and 50 μg of citrate synthase. The reaction was started by the adding 4CL enzyme at 30 °C for 5 min. The change in absorbance was measured by successive scanning of the wavelength with a spectrophotometer.

Cinnamoyl-CoA preparation and FldA enzyme assay. The cinnamoyl-CoA for FldA reaction was prepared as follows: the OASIS HLB SPE cartridge (Waters, Milford, MA) was first conditioned with 3 ml of methanol followed by addition of 3 ml of distilled water. The PK1 disruption strain was grown in Luria–Bertani medium at 30 °C and each enzyme was puriﬁed using Tacon talon metal affinity resin (3 ml of HisBlue TALON, Thermo Scientiﬁc, Waltham, MA). The crude enzyme mixture was applied to the cartridge followed by addition of 2 ml of 0.15% TCA solution. The attached CoA-thioesters were eluted with 1 ml of methanol and 10 mM NaOH solution containing 10 mM EDTA. The solution was concentrated to 1 ml of 5 mM Tris-HCl (pH 7.5) containing 5 mM DTT and 0.5 mM EDTA. For the pH-stat fed-batch cultures, the pH of fermentation broth was maintained at pH 7.0 and the feeding solution was automatically fed when the pH rose above 7.02 as a set point.

Analysis of aliphatic and aromatic acyl-CoAs. The formation of CoA-thioesters was conﬁrmed by using high-performance liquid chromatography (HPLC)-mass spectrometry (MS). For the analysis of aliphatic and aromatic acyl-CoAs, samples were prepared by method described above in cinnamoyl-CoA concentration. The crude enzyme mixture was applied to the cartridge followed by addition of 2 ml of 0.15% TCA solution. The attached CoA-thioesters were eluted with 1 ml of methanol–ammonium hydroxide (99: 1, v/v) and then the sample was further evaporated to dryness at room temperature by vacuum centrifugation at 15,493 g. The sample was dissolved with 50 mM phosphate buffer (pH 7.5). The reaction was carried out for 1 h at 30 °C with 0.5 mM phosphatase (E. coli). The glucose was removed by 10 mM NaOH solution containing 10 mg of purified FldA. After reaction, the concentration of cinnamoyl-CoA was measured by the spectrophotometric assay by measuring the absorbance at 311 nm.

Cell growth and metabolite analysis. Glucose concentration was measured using glucose analyser (model 2700 STAT, Yellow Spring Instrument, OH). Cell growth was monitored by measuring absorbance at 600 nm (OD₆₀₀) using an Ultraspec 3000 spectrophotometer (Amersham Biosciences, Upplands, Sweden). The metabolites (acetate, formate, lactate, succinate, and pyruvate) were analysed by HPLC (1515 isocratic HPLC pump, Waters) equipped with refractive index detector (2414, Waters) and MetaCarb 87 H column (Agilent). Elution was performed with 0.01 H₂SO₄, at a flow rate of 0.5 ml min⁻¹ at 25 °C. t−Phenylacetic acid, t−4-hydroxyphenylacetic acid, t−lysine, and t−phenylalanine were analysed by HPLC (1100 series HPLC, Agilent Technologies) equipped with U/U/SIS (G1315B, Agilent), refractive index (Shodex RI-71; Tokyo, Japan) detector and a Zorbax SB-C18 column (150 mm × 4.6 mm, 5 μm, Agilent). Elution was performed with mobile phase A (Water–0.1% trifluoroacetic acid) and mobile phase B (Acetonitrile). A linear gradient scheme started from 10% of mobile phase A and the fraction of mobile phase A was increased up to 50%. The flow rate was 1 ml min⁻¹ and analytes were detected at 220 nm.
Polymer analysis. The polymer contents and monomer compositions of synthesized PHAs were determined by gas chromatography (GC) or GC-MS (Supplementary Methods). The collected cells were washed three times with distilled water and then lyophilized for 24 h. The PHAs in lyophilized cells were converted to corresponding hydroxymethyl esters by acid-catalyzed methanolysis. The resulting methyl esters were analysed by GC (Agilent 6890N, Agilent) equipped with Agilent 7683 automatic injector, flame ionization detector, and a fused silica capillary column (Agilent DB-Wax, 30 m × 0.25 mm × 0.5 μm). Polymers were extracted from the cells by chloroform extraction method. Polymers content is defined as the weight percentage of polymer concentration to dry cell concentration (w/w of dry cell weight). The structure, molecular weights, and thermal properties of the polymers were determined by nuclear magnetic resonance spectroscopy, gel permeation chromatography, and differential scanning calorimetry, respectively (Supplementary Methods).

Transmission electron microscopy analysis. To confirm intracellular aromatic PHA synthesis in recombinant E. coli, transmission electron microscopy (TEM) analysis was performed. After 72 h of cultivation, 5 ml of cells were collected and washed twice with phosphate-buffered saline (pH 7.0). Cells were fixed by adding 2.5% (v/v) glutaraldehyde in phosphate-buffered saline (pH 7.0) and 1% (v/v) osmium tetroxide. Then, the samples were dehydrated and embedded in Epon 812. The ultra-thin section was carried out by Ultra-Microtome (Leica Ultracut UCT, Leica, Austria). The sample was stained with 2% uranyl acetate and lead citrate. TEM analysis was performed using Bio-TEM (Tecnai G2 Spirit, FEI, Hilborn, OR) at the Korea Basic Science Institute.

Grazing incidence X-ray scattering measurements. The samples for grazing incidence X-ray scattering (GIXS) measurements were prepared by spin coating of copolymer solutions in chloroform (2 wt%) on silicon substrate. GIXS measurements were performed on beamline 3 C in the Pohang Accelerator Laboratory (South Korea). X-rays with energy of 11.075 keV were used and the off-specular scattering was recorded using a MAR-CCD area detector and sample to detector distance was maintained to be 30 cm. The incidence angle of the X-ray beam (= 0.12°) was used to allow for complete penetration of X-ray beam into the polymer film.

Dual cantilever beam test. The dual cantilever beam (DCB) specimens were prepared with the geometry of glass/aromatic PHAs/Au/ePOxy/glass. A series of PHAs was dissolved in chloroform at the concentration of 2 wt% and then spin-coated on a bare glass substrate. After sufficient drying of the residual chloroform in a vacuum oven, a 50 nm-thick Au layer is deposited on the top of the polymer films by thermal evaporation. And then, the specimen was cut into 8 mm × 25.4 mm-long glass beam using Dicing Machine (DAD3350, DISCO Co., Japan) in the common pathway of aromatic biosynthesis in Escherichia coli K12: regulation of enzyme synthesis at different growth rates. J. Bacteriol. 127, 1085–1097 (1976).

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**Author contributions**

S.Y.L. conceived the project. S.Y.L., J.E.Y., and S.J.P. generated ideas and designed research. J.E.Y. and S.J.P. performed research and analytical experiments. W.J.K. performed in silico metabolic simulation. J.E.Y., S.J.P., S.Y.L., H.J.K., B.J.K., J.S., and H.L. analysed data. J.E.Y., S.J.P., and S.Y.L. wrote the paper and all authors approved the final manuscript.

**Additional information**

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**Competing interests:** S.Y.L., J.E.Y., and S.J.P. declare that they have competing financial interest as strains, enzymes, and genes described in this paper are patented and are of commercial interest. The remaining authors declare no competing financial interests.

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