Improving cellular malonyl-CoA level in \textit{Escherichia coli} via metabolic engineering

Wenjuan Zha$^a$, Sheryl B. Rubin-Pitel$^a$, Zengyi Shao$^a$, Huimin Zhao$^{a,b,*}$

$^a$ Department of Chemical and Biomolecular Engineering, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA
$^b$ Departments of Chemistry, Biochemistry, and Bioengineering, Institute for Genomic Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

\textbf{ARTICLE INFO}

Article history:
Received 16 November 2008
Received in revised form 28 January 2009
Accepted 28 January 2009
Available online 5 February 2009

\textbf{Keywords:}
Malonyl-CoA
Metabolic engineering
Polyketides
Flavonoids
Type III polyketide syntheses

\textbf{ABSTRACT}

\textit{Escherichia coli} only maintains a small amount of cellular malonyl-CoA, impeding its utility for overproducing natural products such as polyketides and flavonoids. Here, we report the use of various metabolic engineering strategies to redirect the carbon flux inside \textit{E. coli} to pathways responsible for the generation of malonyl-CoA. Overexpression of acetyl-CoA carboxylase (Acc) resulted in 3-fold increase in cellular malonyl-CoA concentration. More importantly, overexpression of Acc showed a synergistic effect with increased acetyl-CoA availability, which was achieved by deletion of competing pathways leading to the byproducts acetate and ethanol as well as overexpression of an acetate assimilation enzyme. These engineering efforts led to the creation of an \textit{E. coli} strain with 15-fold elevated cellular malonyl-CoA level. To demonstrate its utility, this engineered \textit{E. coli} strain was used to produce an important polyketide, phloroglucinol, and showed near 4-fold higher titer compared with wild-type \textit{E. coli}, despite the toxicity of phloroglucinol to cell growth. This engineered \textit{E. coli} strain with elevated cellular malonyl-CoA level should be highly useful for improved production of important natural products where the cellular malonyl-CoA level is rate-limiting.

© 2009 Elsevier Inc. All rights reserved.

1. Introduction

Malonyl-CoA is the major building block for natural products such as fatty acids, polyketides, and flavonoids, which have significant applications in medicine (antibacterials, antifungals, anticancers, and immunosuppressants), veterinary medicine (antibiotics), and agriculture (insecticides), and potential as anticancers, and immunosuppressants), veterinary medicine

Escherichia coli possesses a very low level of cellular malonyl-CoA (Takamura and Nomura, 1988), which can be a potential barrier to the wide utilization of this host for large-scale manufacturing of important polyketides. Therefore, it is desirable to create an \textit{E. coli} strain with improved cellular malonyl-CoA level.

In the central metabolism of \textit{E. coli} (Fig. 1), a carbon source, such as glucose, is consumed through a series of enzymatic reactions to form pyruvate. Subsequently, pyruvate is oxidatively decarboxylated by the pyruvate dehydrogenase complex to produce CO$_2$ and acetyl-CoA. While the majority of acetyl-CoA enters the tricarboxylic acid (TCA) cycle, a small portion participates in fatty acid biosynthesis, where acetyl-CoA is converted to malonyl-CoA by acetyl-CoA carboxylase (Acc). In \textit{E. coli}, intracellular malonyl-CoA concentration is tightly regulated to be very low, so as to coordinate the rate of fatty acid biosynthesis with phospholipid production, macromolecule synthesis, and cell growth (for review, see Magnuson et al., 1993).

Metabolic engineering has emerged as a powerful tool to improve productivity by genetic manipulation of multistep catalytic systems involved in cell metabolism. In recent years, application of metabolic engineering has blossomed in both academia and industry (Aldor and Keasling, 2003; Stephanopoulos, 1999). Most metabolic engineering studies have focused on manipulating enzyme levels through the amplification, addition, or deletion of particular pathways. For example, these strategies were successfully applied to increase the conversion of glucose to...
pyruvate in *E. coli* (Causey et al., 2004). The concentration of acetyl-CoA inside *E. coli* was increased by engineering pathways that consume or produce acetyl-CoA (Lin et al., 2004; Vadali et al., 2004a, b), and this strategy has also been applied to *Saccharomyces cerevisiae* (Shiba et al., 2007). In particular, the *E. coli* intracellular malonyl-CoA concentration was enhanced by overexpression of a key enzyme, acetyl-CoA carboxylase, leading to improved production of the important polyketides (2S)-flavanones (Miyahisa et al., 2005) and flavonoid (Leonard et al., 2007) as well as fatty acids (Lu et al., 2008). Similarly, overexpression of the endogenous acetyl-CoA carboxylase in *S. cerevisiae* enhanced production of 6-MSA (Wattanachaisaereeekul et al., 2008). Nevertheless, there has been no effort to evaluate the combination of various distinct metabolic engineering strategies to achieve greater improvement in cellular malonyl-CoA level.

In this study, we applied various rational modification strategies to cellular pathways to redirect carbon flux toward malonyl-CoA, and investigated their synergism on improving intracellular malonyl-CoA level in *E. coli*. These strategies include overexpression of *acs*, inactivation of malonyl-CoA-consuming fatty acid synthesis, overexpression of acetyl-CoA synthase (*acs*), an enzyme involved in the acetate assimilation pathway, and knockout of the competing pathways for acetate and ethanol synthesis. Finally, we applied some of our engineered *E. coli* strains for the production of an important polyketide, phloroglucinol, which is a core precursor to the synthesis of various high-value bioactive compounds and energetic compounds. Previously, we have shown that phloroglucinol is produced by the type III polyketide synthase PhID from *Pseudomonas fluorescens* via condensation of three molecules of malonyl-CoA (Achkar et al., 2005b; Zha et al., 2006). Here, we overexpressed PhID in selected metabolically engineered *E. coli* strains and observed an increase in phloroglucinol production.

2. Materials and methods

2.1. Materials

Luria Broth (LB) supplemented with 2% glucose was used throughout as the medium for malonyl-CoA overproduction. Various combinations of ampicillin, kanamycin, and chloramphenicol were added in cultures of plasmid-bearing *E. coli*. The pACYC-Duet-1 and pRSF-Duet-1 expression vectors and *E. coli* BL21 (DE3) strain were obtained from Novagen (Madison, WI, USA). *Phusion* DNA polymerase, T4 DNA ligase, and restriction endonucleases were purchased from New England Biolabs (Beverly, MA, USA). QIAprep Spin Plasmid Miniprep Kit, QIAquick Gel Extraction Kit, and QIAquick PCR Purification Kit were obtained from Qiagen (Valencia, CA, USA). Oligonucleotide primers were obtained from Integrated DNA Technologies (Coralville, IA, USA). CoASH, acetyl-, butyryl-, and malonyl-CoAs were purchased from Sigma (St. Louis, MO, USA). All the other reagents unless specified were obtained from Sigma–Aldrich.

2.2. Construction of plasmids

The plasmids constructed and used in this study are listed in Table 1. The fabF gene was PCR-amplified from the *E. coli* K-12 genome, and cloned into pACYC-Duet-1 between the EcoRV and KpnI sites, resulting in plasmids pACYC-FabF. Likewise, the *E. coli* *acs* gene was PCR-amplified and cloned into pACYC-Duet-1 and pACYC-FabF between the NcoI and HindIII sites, resulting in plasmids pACYC-Acs and pACYC-FabF/Acs, respectively. The *P. fluorescens* Pf-5 phID gene was cloned into pACYC between NcoI and KpnI to create plasmid PACYC-PhID. The phID gene was also cloned into pACYC-Acs between the Ndel and KpnI sites, creating plasmid pACYC-Acs/PhID.

2.3. Construction of knockout strains

Strains used in this work are listed in Table 1. Deletion of *E. coli* chromosomal genes, *ackA-pta* or *adhE*, was carried out by the Red recombinase method (Datsenko and Wanner, 2000). Briefly, the *ackA-pta* or *adhE* gene was PCR-amplified from the *E. coli* genome and cloned into pKD46. Integration of linear DNA was facilitated by an arabinose-inducible Red recombinase carried in pKD46 (temperature conditional). Integrants were selected for kanamycin resistance (10 mg/L). FRT-flanked antibiotic resistance genes used for selection were deleted by using a temperature-conditional plasmid pCP20 expressing FLP recombinase from a thermo-inducible promoter. The double knockout strain (*ackA-pta+AdhE*) was created by the standard P1 transduction method (Miller, 1992). At each step, mutations were verified by analyses of PCR products.

2.4. Culture conditions

For intracellular CoA-ester pool analysis, inoculants of wild type or engineered *E. coli* strains were grown in LB at 37 °C.
overnight. A small portion of this culture was then diluted 1:100 in fresh LB medium containing 2% glucose, and continued growing at 37 °C until OD600 = 1.0–1.2. Then, IPTG was added to a final concentration of 1 mM, and protein expression was induced in a final volume of 2 mL. The instrument was tuned by the direct infusion of a solution of CoA (100 µM) in the ion source to optimize the parameters of the ESI–MS/MS system, first based on the maximum generation of protonated molecular ions (parents) and then on those of the corresponding fragment ions (daughters) for the CoA-esters of interest. Quantification was done in the multiple reaction monitoring (MRM) mode in MS/MS using the mass ions set to detect transitions of the parent ion to the daughter ion specific to the selected analytes. The transitions (m/z parent → m/z daughter) for the four CoA-esters of our interest were as follows: (1) malonyl-CoA, 852.5 → 808.5; (2) CoA, 766.5 → 419.2; (3) acetyl-CoA, 808.6 → 4611; and (4) butyryl-CoA, 836.5 → 489.2.

2.5. Extraction of intracellular CoA-esters

An aliquot of 0.5 mL cell culture was removed, chilled immediately on ice, and centrifuged at 5000 rpm, 4 °C for 10 min. The cell pellet was resuspended in 1 mL of 6% perchloric acid to facilitate cell lysis. Then, 0.3 mL of 3 M potassium carbonate was added while vortexing to neutralize the acid. The solution was centrifuged to pellet the cell debris. The supernatant was collected and stored chilled until analysis of CoA compounds using LC–ESI–MS/MS (described below). To determine the dry cell weight, 2 mL of the same culture was filtered through a 0.45 µm cellulose membrane (Millipore, Billerica, MA, USA), followed by washing with distilled water. The membranes with cell residue were dried in a conventional oven. The weight difference between empty membranes and those with cell residues represented dry cell weight.

2.6. LC–ESI–MS/MS analysis of CoA-esters

The analysis of intracellular CoA-esters extracted from cultures of E. coli strains was performed using an Agilent Quattro micro MS interface consisting of a separation module connected directly to a Micromass Quattro micro MS. A 150 × 4.6 mm² C18 reverse-phase HPLC column (Agilent Technologies, Santa Clara, CA, USA) was used to perform HPLC separation. The analytes were eluted at a flow rate of 300 µL/min with a gradient of 15 mM ammonium formate (A) and 10% 10 mM ammonium acetate in methanol (v/v) (B) at 25% B for the first 5 min, to 100% B in 10 min. The column effluent was directed to an MS instrument, equipped with an ESI ion source, which was operated in the negative ion mode. The instrument was tuned by the direct infusion of a solution of CoA (100 µM) in the ion source to optimize the parameters of the ESI–MS/MS system, first based on the maximum generation of protonated molecular ions (parents) and then on those of the corresponding fragment ions (daughters) for the CoA-esters of interest. Quantification was done in the multiple reaction monitoring (MRM) mode in MS/MS using the mass ions set to detect transitions of the parent ion to the daughter ion specific to the selected analytes. The transitions (m/z parent → m/z daughter) for the four CoA-esters of our interest were as follows: (1) malonyl-CoA, 852.5 → 808.5; (2) CoA, 766.5 → 419.2; (3) acetyl-CoA, 808.6 → 4611; and (4) butyryl-CoA, 836.5 → 489.2.
concentrations in an extremely intricate sample matrix. Recently, a combined usage of LC with electrospray ionization tandem mass spectrometry (ESI–MS/MS) was reported for analysis of intracellular short organic CoA-esters from Streptomyces venezuelae (Park et al., 2007). Using HPLC to separate analytes and further relying on tandem MS/MS data to distinguish analytes based on their molecular weight as well as the fragmented mass profiles that are characteristic or typical of each analyte, the LC–ESI–MS/MS method provides easy separation and detection of individual CoA-ester from the complex matrix of cellular components. In addition, the amount of each CoA-ester can be readily quantified according to the peak area of their characteristic daughter ion peak on MS/MS spectra. Herein, we applied this method to quantify the three intracellular CoA-esters of interest (CoASH, acetyl-CoA, and malonyl-CoA) in our genetically engineered E. coli strains.

Direct injection analysis of pure CoA-esters by ESI revealed that negative ion mode gave higher signal intensity than positive ion mode, and thus, the negative ion mass spectrum [M-H]⁻ was acquired for CoA-ester analysis throughout this study. Direct injection of pure compounds onto LC–MS/MS showed that malonyl-CoA, CoASH, acetyl-CoA, and butyryl-CoA (used as internal standard for quantification) were eluted at 4.3, 6.1, 7.9, and 11.8 min, respectively. Since the daughter fragment ions generated from a specific CoA-ester in tandem MS/MS vary depending on the structure of its acyl-side chain, for malonyl-CoA, acetyl-CoA, butyryl-CoA, and CoASH, each had a unique fragmentation pattern, and could be unambiguously identified in the sample matrix (data not shown). For quantification, the characteristic daughter ions specific to the four individual CoA-esters were analyzed with regard to their peak area in MRM mode (Fig. 2). Calibration curves were established for each CoA-ester, and there was a good linear correlation (R² > 0.98) between the concentration of CoA-ester and the peak area. The limit of quantification (LOQ), based on a signal-to-noise ratio greater than 5, was determined to be at nM scale for each of the CoA-ester standards in the MRM mode. These data demonstrate that the LC–ESI–MS/MS method developed in this work is selective and sensitive enough for analysis of CoA-esters derived from E. coli cells.

In order to determine the intracellular CoA-ester concentrations in real time, it is critical to rapidly quench cellular metabolism during sample preparation, since the turnover rates of most metabolic reactions are in the order of sub-seconds. To process E. coli samples, we used a modified protocol from Boynton et al. (1994), where perchloric acid was used to facilitate the disruption of cell membrane and simultaneously inactivate the cellular enzymes.

3.2. Overexpression of acetyl-CoA carboxylase

Using the LC–ESI–MS/MS analytical method described above, intracellular malonyl-CoA concentration of the wild-type E. coli was determined to be around 0.07 nmol/mg dry cell weight, consistent with the reported value that was determined by an enzymatic method (0.01–0.23 nmol/mg dry cell weight) (Takamura and Nomura, 1988). Our previous effort to produce an important polyketide, phloroglucinol, in E. coli by heterologous expression of its synthase, PhlD from P. fluorescens, led to low yield of only 0.7 g/l (Achkar et al., 2005a). Since PhlD uses malonyl-CoA as the sole substrate to synthesize phloroglucinol, we hypothesized that the low concentration of cellular malonyl-CoA might limit phloroglucinol production in E. coli. It was suggested in literature that acetyl-CoA carboxylase, which catalyzes the conversion of acetyl-CoA to malonyl-CoA, is the rate-controlling enzyme in fatty acid synthesis (Davis et al., 2000). Therefore, an obvious strategy to improve cellular malonyl-CoA concentration would be to increase Acc enzyme level through episomal overexpression (Fig. 1). The most intensively studied E. coli Acc enzyme consists of four subunits (Davis et al., 2000) and is known to be feedback inhibited by acyl-acyl carrier proteins (ACPs) from the E. coli fatty acid biosynthetic pathway (Davis and Cronan, 2001). Although studies have shown that overexpression of the E. coli Acc enzyme led to increased cellular level of malonyl-CoA (Davis et al., 2000), we found that overexpression of this native Acc affected cell viability, and the phloroglucinol production was not improved at all (data not shown). On the other hand, Acc from Corynebacterium glutamicum consists of only two subunits, and it has been reported that overexpression of this Acc enzyme in E. coli led to increased heterologous production of flavanone, one of whose precursors is also malonyl-CoA (Miyahisa et al., 2005). We chose to introduce the same C. glutamicum Acc into E. coli for this metabolic engineering work. We found that functional expression of C. glutamicum Acc from the strong T7 promoter required low induction temperature, for induction at 37 °C gave no change to cellular malonyl-CoA concentration (data not shown), whereas induction at 25 °C resulted in significant increase in malonyl-CoA concentration. Therefore, 25 °C was used as the induction temperature throughout our studies. An E. coli BL21 (DE3) strain overexpressing C. glutamicum Acc and the wild-type strain were grown under the same conditions, and their intracellular CoA pool was analyzed by LC–ESI–MS/MS as described in Section 2. Overexpression of Acc alone increased the cellular malonyl-CoA concentration by 3-fold, while acetyl-CoA concentration remained almost unchanged (Table 2).

3.3. Inhibition of fatty acid synthesis

In E. coli, malonyl-CoA is only consumed during biosynthesis of fatty acids. In addition, multiple components in the fatty acid synthase complex regulate the cellular concentration of malonyl-CoA to be at a very low level (Magnuson et al., 1993). Thus, inhibition of fatty acid synthesis in E. coli would not only abolish a competing pathway consuming malonyl-CoA, but also alleviate the inherent negative regulation. It has been shown that blocking fatty acid synthesis by the antibiotic cerulenin triggered a large accumulation of malonyl-CoA (Davis et al., 2000). This inhibitor specifically inactivates the β-ketoacyl-acyl carrier protein synthases (KAS) I and II (FabB and FabF). However, the cost of cerulenin (more than $20 mg⁻¹) makes it impractical to use in our metabolic engineering work. Subrahmanyam and coworkers reported that overexpression of β-ketoacyl-ACP synthase II (FabF) also caused cessation of fatty acid synthesis, and subsequently a temporary increase in malonyl-CoA concentration within a few
minutes of inducing FabF overexpression (Subrahmanyan and Cronan, 1998). A possible model for such inhibition was proposed that malonyl-CoA:ACP transacylase (FabF), with whom the association is essential for ketosynthases’ condensation activity, was exhausted by large excess expression of FabF, thus leaving little FabD for other KAS isozymes (such as FabB) to carry out the complete fatty acid synthesis. However, blocking fatty acid synthesis by means would dreadfully affect cell viability, as demonstrated by many inhibition studies. Here, we hypothesized that if overexpression of FabF is induced right before the E. coli culture enters its stationary growth phase, a continuous and long-term increase in the malonyl-CoA concentration would occur without significantly compromising the cell density. To test this hypothesis, the E. coli fabF gene was cloned under the control of a T7 promoter. The gene was overexpressed through the regulation of a T7 promoter for overexpression. Analysis of the intracellular CoA pool from BL21(DE3) cells overexpressing FabF showed that blocking fatty acid synthesis resulted in a 4-fold higher concentration of intracellular malonyl-CoA (Table 2). However, when Acc and FabF were coexpressed in E. coli, we did not observe an additive effect on improving the malonyl-CoA level. This antagonism may arise due to non-obvious and complex interactions between FabF and Acc, which are involved in large metabolic and regulatory networks. Therefore, the combination of Acc and FabF was not investigated further in our metabolic engineering efforts.

3.4. Increase of acetyl-CoA availability

The immediate precursor to malonyl-CoA is acetyl-CoA. In E. coli metabolism, acetyl-CoA serves as a key metabolic intermediate to the tricarboxylic acid cycle, where the acetyl group is broken down to CO2 and H2O, accompanied with energy generation. Besides this major function, acetyl-CoA is also involved in several other pathways, such as those leading to the formation of acetate and ethanol (Fig. 1) (Causey et al., 2004).

Acetate is a toxic byproduct that inhibits the growth of E. coli cells (Luli and Strohl, 1990). Two enzymes, phosphotransacetylase (Pta) and acetate kinase (AckA), are responsible for conversion of acetyl-CoA to acetate. In addition, an assimilation pathway recycles acetate back to acetyl-CoA through the action of acetyl-CoA synthetase (Acs). Both pathways have been manipulated in previous studies. Deletion of the AckA-Pta pathway was shown to give decreased extracellular acetate production while increasing the cellular level of acetyl-CoA (Yang et al., 1999), but at the expense of cell survival (Chang et al., 1999). Recently, Leonard and coworkers reported an improved heterologous production of flavonone in E. coli upon overexpression of Acs, which was thought to be a result of increased availability of the precursor, acetyl-CoA by Acs overexpression (Leonard et al., 2007). Ethanol is another byproduct from acetyl-CoA, and the conversion is catalyzed by the bifunctional alcohol/alkdehyde dehydrogenase (AdhE) in E. coli. Therefore, eliminating this enzyme activity would also be expected to improve the availability of acetyl-CoA.

Here, we sought to investigate the effect of manipulating the above-mentioned pathways on cellular levels of malonyl-CoA. For this purpose, the E. coli ackA gene was overexpressed through the regulation of a T7 promoter. The ackA-pta genes were deleted from E. coli BL21(DE3) chromosome to generate the ΔackA-pta strain (BWZ1). Likewise, the adhE gene was deleted to generate the ΔadhE strain (BWZ2). To create the double knockout BL21(DE3, ΔackA-pta ΔadhE) strain (BWZ3), P1 transduction was used to transduce the ΔadhE deletion to BWZ1. We then evaluated the improvement of cellular malonyl-CoA level by these strategies, which would provide more acetyl-CoA, together with overexpression of Ack or FabF. Overexpression of the acetate assimilation enzyme, Acs, that recycles acetate back into acetyl-CoA, did not significantly affect the cellular malonyl-CoA concentration in the Acc and/or FabF overexpression strains (Tables 3 and 4). However, deletion of the ackA-pta pathway consuming acetyl-CoA for the byproduct acetate in conjunction with Acc overexpression
resulted in a 2-fold increase in the malonyl-CoA level compared with that from Acc overexpression alone (Tables 2 and 3). More importantly, the dual manipulation of acetate production from acetyl-CoA, involving assimilation (Acs overexpression) and pathway knockout (ΔackA-pta), showed synergistic effect together with Acc overexpression, giving a total of 15-fold improved cellular level of malonyl-CoA compared with that in the wild-type E. coli (Table 3). Moreover, deletion of a second competing pathway (ΔadhE) that produces byproduct ethanol from acetyl-CoA led to a slightly further increase of cellular malonyl-CoA concentration (Table 3). On the other hand, the effect of FabF overexpression on cellular malonyl-CoA level seemed to be impaired by the increased availability of acetyl-CoA (Table 4). We speculated that there might be a certain degree of inhibition activity of FabF. Throughout the concentration of acetyl-CoA and activity of FabF. The increased flux toward malonyl-CoA production results from the increased availability of acetyl-CoA (due to loss of the acetate-acetyl-CoA recycling pathway) and simultaneous overexpression of C. glutamicum Acc, which is likely not effectively inhibited by E. coli acyl-ACP (Table 3). Finally, the cellular CoASH concentration was always below the detection limit of ESI–MS/MS, even in the wild-type E. coli strain. This phenomenon might be caused by extra glucose in the culture medium, as there have been reports that the CoASH pool dramatically decreased when cells were grown in sufficient glucose medium (Chohnan et al., 1997).

3.5. Production of phloroglucinol in the engineered E. coli strains

To demonstrate the utility of engineered E. coli strains with elevated malonyl-CoA concentration, we used the biosynthesis of phloroglucinol as a model system. The phloroglucinol synthetase PhlD was expressed in wild-type BL21(DE3) and two engineered E. coli strains, i.e. (1) B-Acc, BL21(DE3) overexpressing Acc, and (2) BWZ3-Acc/Acs, BL21(DE3) with ackA-pta and adhE double deletion, and coexpression of Acc and Acs. After inducing protein expression by IPTG, phloroglucinol production was monitored at 24 and 48 h. As shown in Table 5, at 48 h culture time the two engineered strains improved phloroglucinol concentration approximately 1.5-fold compared with previously published data, and approximately 2-fold compared with wild-type production in this study. When phloroglucinol yield is normalized against cell density to account for the slower growth rate of the engineered strains, improvements of strains B-Acc and BWZ3-Acc/Acs relative to BL21(DE3) expressing PhlD are 2.7- and 3.7-fold, respectively (Table 5). Given the fact that malonyl-CoA in the sole substrate for phloroglucinol synthesis, the 2.7-fold improved phloroglucinol production in the B-Acc strain correlates well with the 3-fold elevated malonyl-CoA concentration from Acc overexpression. However, the 3.7-fold improvement is lower than expected for BWZ3-Acc/Acs expressing phlD on plasmid pACYC-PhlD.

### Table 4

<table>
<thead>
<tr>
<th>Malonyl-CoA</th>
<th>Acetyl-CoA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration (nmol/mg DCW)</td>
<td>Fold change relative to WT</td>
</tr>
<tr>
<td>Wild-type (WT)</td>
<td>0.07 ± 0.01</td>
</tr>
<tr>
<td>4FabF expression</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>4Acs expression</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>4ΔackA-pta</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>4Acs expression and ΔackA-pta</td>
<td>0.19 ± 0.02</td>
</tr>
<tr>
<td>4Acs expression, ΔackA-pta and ΔadhE</td>
<td>0.45 ± 0.07</td>
</tr>
</tbody>
</table>

Strains WT and B-FabF are included as a reference for comparison. DCW: dry cell weight.

Engineered strains are 4FabF, 4Acs/FabF, 4BWZ1-FabF, 4BWZ1-Acs/FabF, and 4BWZ3-Acs/FabF.

### Table 5

<table>
<thead>
<tr>
<th>Strain</th>
<th>Phloroglucinol concentration (mg L⁻¹)</th>
<th>Fold change relative to WT</th>
<th>Normalized concentration (mg L⁻¹ OD₆₀₀)</th>
<th>Fold change relative to WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>4Wild-type (WT)</td>
<td>720</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4WT</td>
<td>510</td>
<td>1</td>
<td>58</td>
<td>1</td>
</tr>
<tr>
<td>4Acc expression</td>
<td>1110</td>
<td>2.2</td>
<td>160</td>
<td>2.7</td>
</tr>
<tr>
<td>4Acs expression, Acc expression, ΔackA-pta and ΔadhE</td>
<td>1280</td>
<td>2.5</td>
<td>220</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Results from this study represent the average of triplicate experiments.

Engineered strains are 4BL21(DE3) expressing PhlD on plasmid pJA2.042, 4BL21(DE3) expressing PhlD on plasmid pACYC-PhlD, 4B-Acc expressing phlD on plasmid pACYC-PhlD, and 4BWZ3-Acc/Acs expressing phlD on plasmid pACYC-PhlD.
minimal medium, phloroglucinol significantly inhibits cell growth at a concentration of 500 mg/L, whereas the observed phloroglucinol concentrations in the B-Acc and BWZ3-Acc/ACS strains exceeded 500 mg/L by 24 h (data not shown) and were slightly above 1000 mg/L by 48 h, shown in Table 5. It is possible that the toxicity of phloroglucinol at this concentration might have prevented further increase in phloroglucinol production. For compounds exhibiting no or less toxicity to E. coli, a much larger improvement in productivity should be reasonably expected from the BWZ3-Acc/ACS strain.

In conclusion, by applying various metabolic engineering strategies individually and in combination to redirect the carbon flux inside E. coli to pathways responsible for the generation of malonyl-CoA, we engineered several E. coli strains with highly elevated cellular malonyl-CoA concentration. The usefulness of theses strains was demonstrated by the improved heterologous production of an important polyketide, phloroglucinol. We believe that these engineered E. coli strain will be useful for biosynthesis of natural products where the cellular malonyl-CoA level is rate-limiting.

Acknowledgments

This research was supported by a grant from the Office of Naval Research (N00014-02-1-0725). S. B. R.-P. acknowledges support from the National Institutes of Health Cell and Molecular Biology Training Grant Program and the National Science Foundation Graduate Research Fellowship Program.

References


