Bioproduction of eriodictyol by *Escherichia coli* engineered co-culture

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**Abstract**

Eriodictyol (ED) is a flavonoid in the flavanones subclass. It is abundantly present in a wide range of medicinal plants, citrus fruits, and vegetables. In addition, ED owns numerous importantly medicinal bioactivities such as inhibition of proliferation, metastasis and induction of apoptosis in glioma cells or inhibition of glioblastoma migration, and invasion. This study described the heterologous production of ED by *E. coli* based co-culture engineering system from the simple carbon substrate D-glucose. Two *E. coli* strains were engineered and functioned as constitutive components of biological system. Specifically, the first strain (upstream module) contained genes for synthesis of *p*-coumaric acid (pCA) from D-glucose. And, the second strain (downstream module) consisted of genes for the synthesis of ED from pCA. The highest yield in ED production was achieved 51.5 ± 0.4 mg/L using stepwise optimal culture conditions, while monoculture was achieved 21.3 ± 0.2 mg/L only. In conclusion, co-culture was the most efficient alternative approach for the synthesis of ED and other natural products.

**Graphical abstract**

**Keywords** Biosynthesis · Co-culture *E. coli* · Eriodictyol · Metabolic engineering

**Abbreviations**

| Abbreviation | Description          |
|--------------|----------------------|
| G6-P         | Glucose-6-phosphate  |
| PYR          | Pyruvate             |
| F6P          | Fructose-6-phosphate |
| G3P          | Glucose 3-phosphpate |
| PpsA         | Phosphoenolpyruvate synthase |
| TktA         | Transketolase A      |
| PEP          | 3-Deoxy-D-arabino-heptulosonate-7-phosphate |
| E4P          | Erythrose 4-phosphate |

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Introduction

Eriodictyol (ED) [IUPAC name (2S)-2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-2,3-dihydrochromen-4-one], is a flavonoid in the flavanones subclass abundantly found in various medicinal plants, citrus fruits, and vegetables (Islam et al. 2020). ED has multiple therapeutic effects such as anti-oxidant, anti-cancer, anti-inflammatory, and neuro-protective bioactivity (Clavin et al. 2007; Deng et al. 2020; Islam et al. 2020; Li et al. 2020; Lv et al. 2021). Such numerous pharmacological and biological roles of ED have attracted much research interest for increasing titer and quality (Marin et al. 2017).

Traditionally, ED is mainly extracted from Yerba santa (Eriodictyon californicum), a vascular plant locally distributed in North America (Marohn 2001). For example, ultrasound-assisted extraction method was used to extract ED from Anacardium occidentale L. leaves and content of ED resulted in 5.9 ± 0.5 (mg/kg dry extract) (Chotphruethipong et al. 2019). Additionally, this phytomedicine and its derivatives are also found in Eupatorium arnottianum (Clavin et al. 2007), Rosa canina (Hvattum 2002), and Millettia duchesnei (Ley et al. 2005).

In planta biochemistry, ED is a hydroxylated derivative of naringenin (NRN). In particular, NRN, a basic core for all flavonoids, is synthesized by the phenylpropanoid route in the presence of key enzymes including PAL or TAL to convert substrate L-phenylalanine or L-tyrosine, respectively, to p-coumaric acid (pCA). Next, various enzymes include 4CL, CHS and CHI will be catalyze the formation of p-coumaroyl-CoA, naringenin chalcone and NRN. NRN can be hydroxylated by different ways. For example, it is the substrate for flavonoid 3’-hydroxylase (F3’H) or flavone synthase (FNS) to generate ED and apigenin respectively (Tsao 2010; Wang et al. 2011).

With the exception of extraction from plants, ED was also produced via enzymatic synthesis using F3’H, a cytochrome P450. This enzyme could be used as purified form whole-cell biotransformation or microbial bioproduction by modular engineering pathway reconstruction (Bruglieri et al. 1999). For example, ED was synthesized by biotransformation in the recombinant S. cerevisiae containing Gerbera hybrid-derived F3’H gene. Particularly, hydroxylation-functionalized yeast was used for whole-cell bioconversion of NRN to ED. Moreover, the highest titer was 200 mg/L of ED in the selective media (Amor et al. 2010). In other studies, ED had been heterologously synthesized by various recombinant E. coli mono-cultures. Firstly, E. coli was used to produce ED by adding exogenous caffeic acid resulting in a titer of 11 mg/L (Leonard et al. 2007). Subsequently, the substrates as malonate and 2 mM caffeic acid were supplemented to the culture of recombinant E. coli to improve the production of ED up to 50 mg/L (Leonard et al. 2008).

However, researchers also showed several disadvantages of microbial mono-culture for producing complex natural products as a metabolic burden due to all genes accumulated in heterologous production in a host. In addition, the application of various molecular bio-techniques in a single host may inhibit its growth and formation of by-products. Recently, co-culture has been a crucial synthetic method and widely applied to the synthesis of different complex natural products. Different species hosts have been used as components in co-culture as E. coli – Saccharomyces cerevisiae (Yuan et al. 2020), Synechococcus elongate – E. coli (Liu et al. 2021) and Streptomyces sp. with Bacillus sp. (Shin et al. 2018) to produce of resveratrol, isoprene, and denticgerumycin E, respectively. Furthermore, Clostridium spp. was used with various microbial strains as Rhodobacter sphaeroides or Thermoanaerobacterium thermosaccharolyticum GD17 to produce bio-fuels and bio-solvents (Du et al. 2020). Based on those works, it is obviously clear that the co-culture microbial system positively affects chemicals and natural products production.

In this study, a co-culture system comprising two metabolically engineered E. coli strains was constructed to produce NRN from D-glucose (Fig. 1). In order to achieve the optimal strain contributed to this system, both E. coli strains were rationally engineered by deletion and overexpression of some relevant genes, which play essential roles in the process of tyrosine biosynthesis, as shown in Fig. 1. In the upstream strain (E. coli MCA), ppsA and tktA genes were overexpressed to enhance the production of PEP and E4P, respectively. Next, pheA gene was inactivated to inhibit the branching conversion of PPA to PPY. Furthermore, Rhodotorula glutinis—originated TAL gene was introduced to convert L-tyrosine to pCA. In the downstream strain (E. coli ED1), Rhizobium tropilii—originated matB (malonyl-CoA synthetase) and matC (dicarboxylate carrier protein) were
cloned to increase the intracellular pool of malonyl-CoA. Next, biosynthetic genes of NRN including 4CL, CHS and CHI from pCA was used. Finally, the pCW(Ori +)-mutant (M13), *Bacillus megaterium*– derived cytochrome P450 BM3, was selected to produce ED from NRN. In addition, several culture conditions such as inoculation size, temperature, and inoculation ratio of two microorganisms were optimized to increase the titer of product.

**Materials and methods**

**Culture media, reagents, and chemicals**

Luria–Bertani (LB) medium containing the appropriate antibiotics, ampicillin (100 µg/mL), chloramphenicol (30 µg/mL), streptomycin (100 µg/mL) or kanamycin (30 µg/mL) was utilized for cultivating the general strains for cloning, screening the recombinant plasmid or seed culture. Minimal M9 was used for the synthesis of pCA, NRN and ED. M9 mineral medium contained Na2HPO4·7H2O (12.8 g/L), KH2PO4 (3 g/L), NaCl (0.5 g/L), NH4Cl (1 g/L), MgSO4 (249 mg/L), CaCl2 (11.1 mg/L), and thiamine (10 µg/L). In addition, 15 g/L D-glucose was extra added for the culture of the upstream module to test pCA production. 2 g/L malonate was also supplemented for the culture of the downstream module.

Extraction chemicals were purchased from Merck (Germany) or Sigma (USA). Nanodrop 2000 UV–Vis Spectrophotometer (Thermo, USA) and HPLC–PDA (Agilent, USA) were used for chromatographic analysis.

**Plasmid and strain construction**

*Inactivation of pheA gene*. A pair of primer: 5′-GGC CTC CCA AAT CGG GGG GCC TTT ATT GAT AAC AAA AAG GCA ACA CTG TGT AGG TGT AGG CGG CTG CTT C-3′ (forward primer) and 5′-TGA AAA GCT GAA GGT GCC GGA TGA TGT GAA TCA TCC GGC ACT GGA TTA TTA CTG GCA TGG GAA TTA GCC ATG GTC C-3′ (reverse primer) was used to knock-out pheA using PCR-based efficient method (Datsenko and Wanner 2000). This work resulted in the mutant *E. coli* BL21(DE3)/ΔpheA.

Cloning and expression of tktA and ppsA. tktA and ppsA were cloned and expressed using pairs of primers for cloning

**Fig. 1** Design and application of co-culture engineering strategy for bioproduction of ED. G6-P glucose-6-phosphate, PYR pyruvate, F6P fructose-6-phosphate, G3P glucose 3-phosphate, PpsA phosphoenolpyruvate synthase, TktA transketolase A, PEP 3-deoxy-D-arabino-heptulosonate-7-phosphate, E4P erythrose 4-phosphate, AroG DAHP synthase, DAHP 3-deoxy-D-arabino-heptulosonate-7-phosphate, SHIK shikimate 5-phosphate, S3P 3-enolpyruvylshikimate-5-phosphate, PPA phenylpyruvate, PPY phenylpyruvate, L-Phe L-phenylalanine, TAL tyrosine ammonia lyase, 4CL 4-coumarate-CoA ligase, CHS chalcone synthase, CHI chalcone isomerase, F3′H flavonoid 3′-hydroxylase, CPR cytochrome P450 reductase, matC malonate transporter protein, matB malonyl-CoA synthetase.
Cloning and expression of matB and matC. PCR cloning of matB and matC was used pairs of primers as followed: For_matB (BglII): GGGATCC AGGGAGGCGCAGGATG TCTT ACG / Rev_matB (NcoI): CATATG GGGAGGGAATCATGGTATG / Rev_matB (EcoR I): CCG GAA TTC TTA TGC CAG CAT CTT CAG CAG AAC GTT GTT GAT. Consequently, those genes were introduced into pETDuet-1 containing ampicillin-resistant gene to obtain pETD-ppsA-tktA. Pair of primers for PCR of Rhodotorula glutinis—originated TAL gene included For_TAL (NcoI): CatG CCA TGG CGC CGC GCC CGA CTT CTC and Rev_TAL (EcoR I): CCG GAA TTC TTA TGC CAG CAT CTT CAG CAG AAC GTT GTT GAT. This strain was used to investigate the production of ED from pCA.

E. coli MCA was transferred various recombinant plasmids as pCDF-matB-matC (Str), pET28-CL (4-coumaryl CoA ligase) (Km), pAC-CHS (chalcone synthase)-CHI (chalcone isomerase) (Cm) and pCW(Ori +)-mutant 13 (M13) (Amp) to generate E. coli MED.

All strains and DNA plasmids were listed in Table 1. DNA manipulation such as DNA plasmid extraction, purification, digestion, and ligation will be followed standard protocols as described (Sambrook et al. 2001).

Verification of constructed plasmids

All the recombinant plasmids were digested by pair of restriction enzymes as described and then their molecular sizes were electrophoretically separated on 5 – 7% agarose gel. Those fragment genes were independently collected and purified by using GeneJET PCR Purification Kit (Thermo Scientific, USA). Finally, they were sent to DNA sequence by Sanger’s method to check any error.

Whole-cell biotransformation process for a single population of E. coli

E. coli MCA, E. coli ED1, and E. coli MED strains were used as mono-culture for the production of pCA from D-glucose, ED from pCA, and ED from D-glucose, respectively. M9 medium plus 15 g/L D-glucose was used for E. coli MCA while E. coli ED1 were cultured in the minimal M9 plus 2 g/L melonate (Wang et al., 2020). E. coli MED was cultured in minimal M9 plus 15 g/L D-glucose and 2 g/L melonate. Particularly, the E. coli strains were firstly cultured overnight at 37 °C in LB medium as the seed cultures. Subsequently, it was centrifuged and re-suspended in a fresh M9 medium. For mono-cultures, the initial net cell density of each of re-suspended culture was inoculated into the minimal M9 medium with the value of 5 × 10^6 cell per mL.

Co-culture techniques for the production of ED.

E. coli upstream and downstream cell ratios (100:1, 10:1, 1:1, 1:10 and 1:100 (v/v) with a constant initial net cells density of 5 × 10^6 cells per mL of culture), as well as temperatures (25, 30, 33.5 and 37 °C) were adopted for production testing in 15 mL test tubes containing 3 mL minimal M9 medium.

To investigate the effect of increasing initial net cells density on the consortia’s capacity for production of ED, initial net co-culture inoculum was increased from original 5 × 10^6 to 5 × 10^7 cells per mL of culture (the inoculation ratio = 1:1). 250 mL conical flask contained 50 mL
suspension culture and maintained at various temperatures (25, 30, 33 and 37 °C).

Time for culture in the range of 12–60 h. The formation of pCA, NRN and ED were intervals analyzed by HPLC.

**Extraction and chromatographical analysis of pCA, NRN and ED**

This procedure was followed Zhu et al., 2013 with several modifications using dimethyl sulfoxide (DMSO) to obtain ED and intermediate metabolites. The Mightysil RP-C18 column (4.6 × 250 mm) (Kanto Chemical Co. Inc., Japan) was maintained at 25 °C and used to separate ED and its intermediates. Acetonitrile (100%) (solvent B) and deionized distilled water containing 0.1% trifluoro acetic acid (TFA) (solvent A) were used as mobile phase on HPLC 1260, diode array detector (Agilent). The binary program using solvent B: 10% (0–8 min), 10–35% (8–15 min), 35–70% (15–20 min) and 70–100% (20–25 min) running at 1 mL/min. was adapted for HPLC analysis. The UV absorption at 330 and 280 nm was measured. Under these conditions, the retention time for pCA, NRN, and ED was 6.5, 13.3, and 12.5 min, respectively. For quantification of flavonoids, a calibration curve of authentic pCA, NRN, and ED were individually drawn using 10-, 20-, 30-, 40-, and 50 µg/ml concentrations, respectively. LC–ESI/MS analysis were carried out in the Center for Applied Spectroscopy, Institute of Chemistry, Vietnam Academy of Science and Technology (VAST).

**Statistical analysis**

Data are averages of results for three biological replicates. Error bars represent standard deviations from the means.

**Results**

**Synthesis of pCA from D-glucose using mono culture E. coli MCA**

To evaluate the ability of single E. coli strain to synthesize pCA, this strain was cultured in minimal M9 plus 15 g/L glucose. In another report, we tested the production of pCA from different carbon sources as glucose and glycerol (data not published). As a result, D-glucose was the choice to produce a better titer of pCA (25.3 ± 0.1 mg/L) after 48 h. Hence it was used for further study (Fig. 2A).

**Synthesis of ED from pCA using mono-culture E. coli**

Different E. coli host strains were constructed with the genes (4CL, CHS, CHI, and M13) of the biosynthetic pathway of ED to assess their ability for protein expression and
functional activity. Mainly, *E. coli* BL21(DE3) containing those genes were designated as *E. coli* ED1. Dependence of biosynthetic dynamic of ED using *E. coli* ED1 on incubation time was shown in Fig. 2B. ED concentration was gradually increased from 12–48 h of culture and reached the highest amount (21.3 ± 0.2 mg /L) after 48 h. Hence, the host *E. coli* ED1 was further used for the study.

Production of ED in the mono-culture *E. coli* MED strain

*E. coli* MED strain contained all genes required for ED biosynthesis from D-glucose (Table 1). This work aimed to demonstrate that the entire genetic complement of the ED biosynthetic pathway functions correctly in *E. coli*. Production of ED and its intermediates was shown in Fig. 2C. Moreover, the highest titer of ED obtained was reached 18.1 ± 0.5 mg/L after 48 h.

Co-culture for the synthesis of ED

Design of synthetic co-culture system

*E. coli*—based co-culture included two modules. Module 1 (*E. coli* MCA, upstream) contained genes for the biosynthetic pathway of L-tyrosine from D-glucose. Notably, gene doses of *ppsA* and *tktA* were increased to enhance the intracellular accumulation of main precursors, PEP and E4P. Furthermore, the inactivation of *pheA* was to restrict the biocorversion of L-tyrosine to L-phenylanaline (Fordjour et al. 2019; Patnaik et al. 2008). And, it resulted in the mutant *E. coli*/ΔpheA/ppsA/tktA. On the other hand, the TAL gene was introduced into this strain for conversion of L-tyrosine to pCA. Module 2 (*E. coli* ED1) contained genes (4CL, CHS, CHI) for the synthesis of NRN from pCA and flavonoid-3'-hydroxylase type M13 to convert NRN to ED (Fig. 1).

Effect of initial inoculum ratio

Growth kinetic profile of *E. coli* BL21(DE3), MCA and ED1 were analyzed and showed in Fig. S1 (supporting materials). In the M9 medium plus 15 g/L glucose, *E. coli* BL21(DE3) showed the specific rate µ = 0.133 ± 0.01/h (1A). *E. coli* MCA in M9 medium plus 15 g/L glucose gave µ = 0.438 ± 0.03/h. And, growth of *E. coli* ED1 in the M9 medium plus 2 g/L melonate and 150 mg/L pCA showed µ = 0.434 ± 0.01/h. To investigate effect of inoculum size, up and downstream strains were mixed at different ratio (100:1; 50:1; 1:1; 1:10; 1:50 and 1:100) and cultured under the same condition (minimal M9 medium + 15 g/L glucose and 33 °C) with supplementation of D-glucose. The initial inoculum ratio affected the formation of intermediates (pCA, NRN) and ED, as shown in Fig. 3. According to the results obtained, we saw that the most favorable ratio is 1:1 and ED titer of 25.5 ± 0.1 mg/L.

Effect of temperature

Various temperature ranges were used to set up culture conditions, including 25, 30, 33, and 37 °C. Production titer of ED was gradually increased at 25 °C and achieved...
38.4 ± 0.2 mg/L at 33 °C as the best result before decreasing (Fig. 4).

Effect of initial net inoculum

To investigate the impact of increasing initial net cell density of each module on the titer of ED production, the initial cell density of each up and downstream module strain was enhanced from 5 × 10^6 to 5 × 10^7 cells per mL and cultured at a temperature of 33 °C. Firstly, these culture conditions were tested on a test tube scale. As shown in Table 2, the highest concentration of ED achieved was 44.5 ± 0.3 mg/L at 33 °C after 48 h. Moreover, compared to the previous conditions, it resulted in a 1.5-fold increase in titer.

**Table 2** Effect of initial net inoculum on production of eriodictyol

| Culture time (hour) | pCA (mg/L) | NRN (mg/L) | ED (mg/L) |
|---------------------|------------|------------|-----------|
| 16                  | 13.8 ± 0.2 | 22.7 ± 0.1 | 37.6 ± 0.5 |
| 32                  | 15.2 ± 0.3 | 24.8 ± 0.3 | 39.8 ± 0.3 |
| **48**              | **16.5 ± 0.3** | **26.9 ± 0.5** | **44.5 ± 0.3** |
| 60                  | 13.4 ± 0.4 | 26.1 ± 0.4 | 42.1 ± 0.5 |

Bold letter in the Table 2 mentioned the highest amount of product after 48 hours of fermentation.

Finally, to evaluate the scalability of this synthetic consortium for the production of ED and its intermediates, shake flask culture was utilized under mentioned above conditions, including the ratio of MCA: ED1 = 1:1, 33 °C, and initial net inoculum density of 5 × 10^7 cells per mL. The titer of ED was measured and achieved of 51.5 ± 0.4 mg/L at 48 h as the highest amount (Fig. 5).

**Fig. 3** Comparison of bioproduction of ED in various co-culture systems. Each system contained different initial inoculum ratios of *E. coli* MCA and *E. coli* ED1. Amount of pCA, NRN and ED were marked by blue, orange and grey color, respectively. X-axis and Y-axis showed strain ratio (v/v) and concentration, respectively. Data are averages of results for three biological replicates. Error bars represent standard deviations from the means.

**Fig. 4** Effect of temperature on the production of ED by *E. coli* co-culture system. Amount of pCA, NRN and ED were marked by blue, orange and grey color, respectively. X-axis and Y-axis showed temperature and concentration, respectively. Data are averages of results for three biological replicates. Error bars represent standard deviations from the means.

**Fig. 5** ED productivity of the synthetic co-culture at a shake flask scale with inoculation ratio (1:1), initial net cells density (5 × 10^7 cells / mL of culture) and 33 °C. Amount of pCA, NRN and ED were marked by blue, orange and grey color, respectively. X-axis and Y-axis showed culture time and concentration, respectively. Data are averages of results for three biological replicates. Error bars represent standard deviations from the means.

Scale up the co-culture for production of ED using shake flask

Discussions

It has been shown that different strains in the same species have similar growth rates and sustainable coordination due to their similar physiological, biochemical, and genetic properties. As a result, co-culture of those strains will minimize the competition for nutritional resources and avoid growth inhibition caused by metabolite depletion. Furthermore, the co-culture system was also suitable for the production of valuable natural products with a long synthetic route, for instance, resveratrol glucosides, apigenin glucoside, acacetin, and 3-amino-benzoic acid (Thuan et al. 2018a, 2018b;
Table 3 Production titers of ED by different microbial hosts

| Hosts      | Externally fed substrates | Production titers (mg/L) | References                        |
|------------|---------------------------|--------------------------|-----------------------------------|
| Prokaryote |                           |                          |                                   |
| E. coli    | caffeic acid              | 0.03                     | Leonard et al. (2006)             |
| E. coli    | p-coumaric acid and malonate | 50                      | Leonard et al. (2008)             |
| E. coli    | L-Tyrosine                | 107                      | Zhu et al. (2014)                 |
| Streptomyces albus | D-glucose              | 0.002                    | Marin et al. (2017)               |
| E. coli    | D-glucose                 | 51.5 ± 0.4               | This study                        |
| Eukaryote  |                           |                          |                                   |
| S. cerevisiae | caffeic acid            | 6.5                      | Yan et al. (2005)                 |
| S. cerevisiae | naringenin              | 200                      | Amor et al. (2010)                |
| S. cerevisiae | naringenin              | 3.000                    | Gao et al. (2020)                 |

Wang et al. 2020; Zhang and Stephanopoulos 2016; Zhang et al. 2017. All those showed the improved titer of targeted compounds. It was the main reason that E. coli – based the up- and downstream modules were designed for the bioproduction of ED from D-glucose and investigated in this study.

Based on the co-culture engineering strategy (Fig. 1), we investigated the dynamic bioconversion of E. coli MCA, E. coli ED1 and E. coli MED, respectively. Firstly, as shown in Fig. 2A, MCA strain was checked for production of pCA from D-glucose and it resulted in a final product yield of 25.3 ± 0.1 mg/L. Next, ED1 strain was proven to produce ED at different amounts at 48 h of culture (Fig. 2B). Thereby, those strains could be used as up- and down-stream module of co-culture for ED production. Independently, MED strain was also constructed and checked for production of ED from D-glucose as mono-culture. And, as shown in Fig. 2C, it this strain a maximum ED titer of 18.1 ± 0.5 mg/L was achieved.

For optimization of co-culture system, the inoculum ratio of strains was proven significantly to affect the co-culture performance. As a result, various ratios were experimentally investigated and resulted in the combination of MCA:ED1 = 1:1 with a titer of 25.5 ± 0.1 mg/L (Fig. 2B). This ratio produced an ED titer higher than 1.2 fold compared to mono-culture (25.5 ± 0.1/21.3 ± 0.2). To further improve the production of the co-culture system, a range of temperatures from 25 – 37 °C was applied to test with the ratio of MCA: ED1 = 1:1. And, this resulted in 38.4 ± 0.2 mg/L of ED at 33 °C (Fig. 2C).

Previous studies demonstrated that a high density of initial net inoculum could increase growth rate and volumetric productivity (Goers et al. 2014). For example, higher density of S. cerevisiae and E. coli as components in co-culture system in an optimal ratio of those strains led to enhance production titer of NRN and RES production (Yuan et al. 2020; Zhang et al. 2017). In this work, the initial inoculum ratio of strain was increased from 5 × 10^6 to 5 × 10^7 cells per mL. As a result, a maximal ED titer of 44.5 ± 0.3 mg/L was achieved at 33 °C after 48 h. This result was higher than 2.45-fold (44.5 ± 0.3/21.3 ± 0.2) and 1.16-fold (44.5 ± 0.3/38.4 ± 0.2) compared to the monoculture and low initial inoculum density, respectively. Finally, the scalable possibility of the synthetic consortium was checked with a shake flask. As a result, an enhanced titer of ED was found and achieved 51.5 ± 0.4 mg/L or 1.16-fold (51.5 ± 0.4/44.5 ± 0.3) higher than the test tube scale. The improvement in the results obtained may be due to volumetric productivity, mass transfer, dissolved oxygen, and aeration speed (Klockner and Buchs 2012; Yuan et al. 2020).

Previously, Streptomyces albus was used as host for de-novo synthesis of ED from D-glucose. However, the titer of ED was very low (0.002 mg/L). And, it was shown that one of the reasons was due to the low concentration of intracellular malonyl-CoA (Marin et al. 2017). To circumvent this problem, we designed a co-culture E. coli system to synthesize ED from D-glucose, a simple, ready, and cheap substrate. Our co-culture setup and stepwise optimal culture conditions reached a maximum production of 51.5 ± 0.4 mg/L of ED. However, its production titer was still lower than several earlier reports. For example, Zhu et al. 2014 synthesized a fusion gene tF3'H-tCPR (tF3'H from G. hybrida and tCPR from C. roseus, respectively). Next, a recombinant host was successfully designed to synthesize 107 mg/L ED in titer from L-tyrosine as initial substrate. Furthermore, it was reported the maximal titer of ED (3 g/L) by biotransformation in S. cerevisiae using naringenin as substrate (Gao et al. 2020). Notably, using naringening as substrate, the biological activity of key enzymes, F3’H, CPR and their chimera have been considered as the most important factors affected on production titer. (Amor et al. 2010; Zhu et al. 2014; Gao et al. 2020). Thereby, combination of engineered key enzymes and co-culture engineering has great potential to improve its production. The comparison of ED production titer by different hosts was shown in Table 3.
Conclusion

An artificial E. coli – E. coli engineered co-culture system was constructed to synthesize ED from D-glucose. Using this system, the highest titer of ED was 51.5 ± 0.4 mg/L at 33 °C after 48 h. Consequently, co-culture is an alternat-
ively efficient approach to producing the natural product without much genetic or metabolic modification. Further
modification to improve titer of a targeted compound may concentrate on (i) increased pool of intracellular cofac-
tors (NADH, FAD, FMN) and precursors (malonate, pCA), (ii) proteins / genes of channel to import or export
natural product via cell membrane, (iii) plug and play biolog-
ical system performance using extracellular supplies as biosensors to control pH, temperature and substrate
concentration.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s11274-022-03294-5.

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