Engineering *Escherichia coli* Co-Cultures for Production of Curcuminoids From Glucose

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Curcuminoids (cus) have attracted increasing attention because of the antioxidant, anticancer, and antitumor activities while their production is limited because of its main source, turmeric plant, demonstrates extensive seasonal variation. In this study, we constructed *Escherichia coli* co-culture system for the rapid production of curcuminoids from glucose. Firstly, the overexpression of curcuminoid synthase and four different strategies related to increasing the intracellular malonyl-CoA pool were conducted in engineered *E. coli*. We found that bisdemethoxycurcumin (BDMC) is the main product and that high level of malonyl-CoA pool is essential for BDMC production. We also obtained the maximum titer (13.8 mg L⁻¹) of BDMC within 4 h by fast preparation directly from p-coumaric acid. Secondly, we developed a process for BDMC synthesis from glucose using a co-culture system where an *E. coli* strain is used to produce p-coumaric acid from glucose and another *E. coli* strain converted p-coumaric acid into the final product. Compared to the mono-culture system, the co-culture is more potent and resulted in 6.28 mg L⁻¹ of BDMC from glucose within 22 h of fermentation in a 3-L bioreactor. This is the first time a co-culture method is employed for the production of curcuminoids from glucose in a lab scale bioreactor. This system provides a new method transforming inexpensive substrate into value-added products.

1. Introduction

The rhizome of turmeric (*Curcuma longa*) is an important medicinal and flavoring agent in Asian countries. The pharmacological properties of this medicinal plant, specifically its main active chemical constituents, curcuminoids, have attracted increased attention in the recent literature.¹⁻⁴ Curcuminoids (specifically bisdemethoxycurcumin, demethoxycurcumin, and curcumin) were first studied because of their antibiotic activity, however, more therapeutic properties of curcuminoids, such as antioxidant, anticancer, and antitumor activities, have since been discovered.⁵⁻⁷

Traditionally, curcuminoids have been produced through plant extraction. However, extraction from plant sources results in a complex mixture of product compounds.⁸ Another drawback in the production of curcuminoids from plant sources is its dependence on seasonal variations. Metabolic engineering and the use of recombinant microbial hosts such as *Escherichia coli* can overcome the aforementioned production roadblocks as it has been demonstrated in the recent past for the production of several other curcuminoid compounds.⁹

Since the first report demonstrating the synthesis of BDMC using the curcuminoid synthase (CUS) form *Oryza sativa*, a number of articles have been published using engineered *E. coli* to produce curcuminoids from a variety of synthetic pathways.¹⁰⁻¹³ In a recent work, p-coumaric acid was supplemented to the *E. coli* fermentation broth resulting in the production of 91 ± 23 mg L⁻¹ of BDMC after 60 h.¹¹ Several other phenylpropanoic acids (such as cinnamic, caffeic, and ferulic acid,) have also been used as substrates for curcuminoid production.¹¹ However, feeding phenylpropanoic acid substrates, at the commercial scale, would result in an uneconomical process due to their high value relative to curcuminoids and low solubility in aqueous solutions. Some articles have reported the overexpression of tyrosine ammonia lyase (TAL) to produce the phenylpropanoic acids, p-coumaric, and cinnamic acid using the native amino acid substrates, tyrosine, or phenylalanine, respectively.¹²,¹³ This method is also problematic due to low solubility of aromatic amino acids at neutral pH. Introduction of a curcuminoid metabolic pathway in a phenylalanine or tyrosine overproducing strain of *E. coli*, makes it possible to enable the de

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DOI: 10.1002/biot.201700576
nov o production of curcuminoids from simple sugars.[9,14,15] It is also possible to use glucose as a cheaper and initial substrate to synthesize phenylpropanoic acids.[14]

In addition to the phenylpropanoyl-CoA starter unit, the extender unit, malonyl-CoA, is also necessary for curcuminoid biosynthesis.[10,16] Although only one mole of malonyl-CoA is required for the production of one mole of curcuminoids, the competition with fatty acid production results in low availability of malonyl-CoA for recombinant pathways.[14] Overexpressing acetyl-CoA carboxylase (ACC) is the most common and effective method to enhance malonyl-CoA pool in *E. coli*.[17–19] Katsuyama et al. showed that overexpressing the ACC enzymes resulted in a more than 10-fold increase in BDMC titers.[31] These results warrant further investigation into proper management of the malonyl-CoA metabolite pool to optimize curcuminoid production.

Another important issue to be considered when building recombinant pathways is metabolic burden,[29] especially for pathway requiring energy in the form of ATP and reducing equivalents in the form of NAD(P)H.[19] Fortunately, recent progresses in using co-cultures for the production of chemicals has helped address the issue of metabolic burden.[23,31]

In this work, we constructed a recombinant pathway for curcuminoid production in *E. coli*. We also developed a co-culture approach in order to achieve rapid de novo BDMC production for the first time. Using glucose and a synthetic co-culture fermentation strategy, we were able to achieve 6.3 mg L\(^{-1}\) BDMC production in a 3-L bioreactor within 22 h.

2. Experimental Section

2.1. Strains, Media, and Culture Conditions

*E. coli* DH5α was used to propagate plasmids and *E. coli* BL21 Star™ (DE3) or BL21 Star™ (DE3) ΔuscCΔfumC was used for protein expression and curcuminoid biosynthesis.[17] *E. coli* rpoA14 (DE3) with plasmid pETM6-RgTAL\(^{\text{syn}}\) was used to biosynthesize p-coumaric acid.[20] LB agar plates or liquid medium with antibiotics (80 μg ml\(^{-1}\) ampicillin, 50 μg ml\(^{-1}\) kanamycin, or 50 μg ml\(^{-1}\) streptomycin, 25 μg ml\(^{-1}\) chloramphenicol) were used for cell growth at 37 °C. AMM media was used for curcuminoid production.[21] When IPTG (isopropyl-β-d-thiogalactoside) was added for induction, culture temperature was reduced to 30 °C.

2.2. Plasmid Construction

Plasmids pETM6, pCDM4, and pACYCDuet-1 were used to construct different expression modules.[22] Based on previous studies, we selected the curcuminoid synthase (CUS) from *Oryza sativa* for curcuminoids biosynthesis and 4-coumarate-CoA ligase (4CL) from *Arabidopsis thaliana* to convert p-coumaric acid to 4-coumaroyl-CoA.[10,23] The 4CL and CUS templates were synthesized and codon-optimized, and cloned into the pETM6 vector using NdeI/XhoI and NdeI/SpeI, respectively. The resulting vectors were then used to subclone a single vector expressing both 4CL and CUS in monocistronic form using standard ePathBrick protocols.[22]

Plasmid pACYC-accABCD encoding the four-subunit *E. coli* acetyl-CoA carboxylase (ACC) and plasmid pACYC-matBC carrying *matB* and *matC* genes have been described previously.[24,25] The tal gene from *Rhodotorula glutinis* encoding phenylalanine/tyrosine ammonia lyase was cloned into pCDM4 via EcoRV/XhoI. The primers used in this work can be found in Table S2, Supporting Information.

2.3. Curcuminoid Production

To optimize the level of the intracellular malonyl-CoA pool and the concentration of p-coumaric acid, several recombinant *E. coli* strains were designed, as shown in Table S1, Supporting Information. The strains from LB agar plates were precultured in AMM medium at 37 °C. After overnight incubation, we used a 2% inoculation to culture the strains in 2 ml of fresh AMM medium (20 g L\(^{-1}\) glucose added) in a 48-well plate fixed in a shaking incubator. After 2 h, the temperature was shifted to 30 °C and IPTG was then added at a final concentration of 1 mM. Different concentrations (25, 50, 75, and 100 mg L\(^{-1}\)) of p-coumaric acid were fed into media after 2 h of fermentation. Disodium malonate at various final concentrations (0.5, 1, 2, 3, and 4 g L\(^{-1}\)) was also fed to the *matBC* containing strains after induction. Curculin was added at a final concentration of 2 mM to inhibit fatty acids synthesis 1 h post induction.

For curcuminoid production in monoculture, 10 ml AMM medium (20 g L\(^{-1}\) glucose added) in 125 ml shake-flask was inoculated with 2% overnight *E. coli* culture and cultivated at 37 °C with 200 rpm shaking. When OD\(_{600}\) reached 0.6, the temperature was shifted to 30 °C and IPTG was added for induction of enzyme expression. The disodium malonate was fed after 4 h of fermentation and curcuminoid titer was analyzed 22 h post inoculation via HPLC.

For curcuminoid production in a synthetic co-culture system, the first strain rpoA14(DE3) containing plasmid pETM6-RgTAL\(^{\text{syn}}\) and the second strain BL21Star™(DE3) containing plasmid pETM6-A4CL-OSCUS were separately cultivated in LB medium overnight and were inoculated into AMM medium (20 g L\(^{-1}\) glucose added) with different inoculation ratios (2% total inoculation with various ratios 1:2, 1:1, 2:1, 5:1, and 10:1). After 2 h, the OD\(_{600}\) of these co-cultures reached 0.6. Fermentation temperature was shifted to 30 °C and IPTG was added. The disodium malonate was fed 4 h post inoculation and curcuminoid titer was analyzed 22 h post inoculation via HPLC.

For bioreactor cultivation using the co-culture system, 1.5 L AMM medium (10 g L\(^{-1}\) glucose) was added to a 3-L Bioflo115 fermenter (New Brunswick Scientific) and a 30 ml overnight inoculation of LB cultures of the rpoA14 and BL21 production strains were added at a 1:10 inoculation ratio. Air flow rate was maintained at a constant 1.5 L min\(^{-1}\). The pH was maintained at 7.2 by automated addition of concentrated NaOH or HCl. Before IPTG induction, 37 °C was set for cell growth while agitation was set at 500 rpm. After 4 h, we decreased fermentation temperature to 30 °C and added IPTG for induction. We also supplemented glucose (10 g h\(^{-1}\), 100 g glucose total) to increase cell density and p-coumaric acid synthesis. Additionally, 1 g L\(^{-1}\) disodium malonate was added to enhance the malonyl-CoA pool. During the curcuminoid production stage, the dissolved oxygen (DO)

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**Biotechnol. J. 2018, 13, 1700576**

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value was maintained around 12% by automatically adjusting agitation rate.

2.4. Curcuminoid Extraction and Analysis

Fermentation broth was mixed with an equal volume ethanol and vortexed for 10 s prior to centrifugation (5 min, 20 000 × g, 4 °C). The curcuminoids were extracted from the supernatant, which showed a characteristic yellow color. We injected 25 μl for analysis into an Agilent 1200 series HPLC equipped with a ZORBAX SB-18 column (5 μm, 4.6 × 150 mm) and absorbance at 425 nm was monitored using a diode array detector. The mobile phase was acetonitrile (solvent A) and water (solvent B) (both contain 0.1% formic acid) at a flow rate of 1 ml min⁻¹. HPLC program was as follows: 10–100% A (0–10 min), 100% A (10–15 min), and 10% A (15–20 min). The HPLC retention time of standard BDMC was 8.4 min. All experiments were conducted in duplicate and data were determined to be statistically significant (p-value < 0.05) using a two-tailed unpaired t-test.

3. Results and Discussion

3.1. Production of Bisdemethoxycurcumin From p-Coumaric Acid in Monoculture

BDMC is biosynthesized from two moles of the starter unit 4-coumaryl-CoA and one mole of the extender unit malonyl-CoA by curcumin synthase (CUS). Since E. coli cannot produce 4-coumaryl-CoA naturally, we introduced the 4-coumarate-CoA ligase (At4CL) from A. thaliana and fed p-coumaric acid to enable the production of 4-coumaryl-CoA (Figure 1A). In the initial part of our work, we used the pETM6-4CL-CUS plasmid to introduce the required enzymes in E. coli. The IPTG-inducible T7 promoter controlled transcription of all genes. HPLC analysis revealed low conversion of p-coumaric acid to BDMC (Figure 1B), leading us to hypothesize that the endogenously supplied malonyl-CoA substrate was limiting production. After 9 h of fermentation we achieved a maximal production of only 0.385 ± 0.013 mg L⁻¹ BDMC by monoculture fermentation, though its titer decreased (Figure 1B). In addition to low productivity, we also observed a reduced growth rate of the BDMC-producing cells. After 20 h of shake flask fermentation, we observed a final OD₆₀₀ of only 2.1 ± 0.12, which represents a significant decrease from typical E. coli growth that can reach a final OD₆₀₀ of about 10. Even though the use of a codon-optimized CUS gene resulted in significant CUS expression as shown by SDS–PAGE analysis of the whole cell lysate supernatant (Figure S1, Supporting Information), the production of BDMC remained low. Therefore, we hypothesized that enhancing the malonyl-CoA pool and/or the cell density may in duplicate and data were determined to be statistically significant (p-value < 0.05) using a two-tailed unpaired t-test.

3.2. Enhancement of Malonyl-CoA Pool

In order to further improve BDMC production, we used several methods to enhance the malonyl-CoA pool, including overexpression of acetyl-CoA carboxylase (AccABCD) enzyme cluster, improvement of acetyl-CoA level through targeted gene deletions in the tricarboxylic acid (TCA) cycle, directed synthesis of malonyl-CoA from malonate, and inhibition of fatty acid biosynthesis using the antibiotic cerulenin. In the second method, we knocked out fumC and sucC genes to decrease the TCA-cycle flux and covert more phosphoenolpyruvate (PEP) into acetyl-CoA, which is the direct precursor of malonyl-CoA (Figure 2B). These two genes have previously been shown to increase production of malonyl-CoA derived flavonoids when deleted. In the third method, we overexpressed MatB and MatC enzymes to convert externally supplied sodium malonate to malonyl-CoA (Figure 2C). Malonate is transported into the cytoplasm through the action of the membrane protein MatC and directly activated to malonyl-CoA by the MatB enzyme. Finally, through the addition of cerulenin to the culture media we were able to downregulate the major sink for malonyl-CoA (fatty acid biosynthesis) to subsequently...
enhance the malonyl-CoA pool (Figure 2D). Cerulenin acts through the inactivation of the FabB and FabF enzymes in the fatty acid biosynthesis pathway of *E. coli* and has been shown to increase the production of a variety of malonyl-CoA derived products.[19,26] However, this increase results in substantial decrease in cell growth (0.8–1.0 OD_{600}) compared to other strategies (≈2 OD_{600}).

As shown in Figure 2E, we observed that the third strategy, which is based on feeding malonate, resulted in the highest production of BDMC (5 mg L\(^{-1}\)). This represents a 25-fold improvement over the first strategy, ACC pathway overexpression. We also showed that the addition of cerulenin resulted in 3.5–5-folds improvement in curcuminooid titer when compared to the strategies of enhanced flux of acetyl-CoA into malonyl-CoA synthesis. When cerulenin was added, the BDMC productivity on a per-cell basis was 0.75 mg/L/OD_{600} which was significantly lower than the 2.5 mg/L/OD_{600} achieved with malonate added to the medium. Overall, these data clearly demonstrate that enhancing the malonyl-CoA pool is critical to improving the conversion of 4-coumaroyl-CoA into BDMC.

### 3.3. Optimization of BDMC Production From \(\alpha\)-Coumaric Acid and Malonate

Since our previous studies showed that malonate feeding is the most efficient way to improve curcuminooid production titers, we then decided to optimize the malonate feeding concentration and feeding time in order to further improve production. In order to investigate utilization efficiency of substrate, we used various concentrations of \(\alpha\)-coumaric acid precursor and fed 1 mg L\(^{-1}\) malonate in the growth media. A concentration of 100 mg L\(^{-1}\) of \(\alpha\)-coumaric acid did not lead to a higher...
production of the final product. Instead, the lowest concentration (25 mg L\(^{-1}\)) of \(p\)-coumaric acid resulted in the highest curcuminoid production (Figure 3A). Based on a previous report that showed that \(p\)-coumaric acid caused a reduction in capsular size of cells, we deduced that high concentration of \(p\)-coumaric acid may have similar effects on \(E.\) coli and inhibit BDMC biosynthesis.[34] Among the different strains tested, the recombinant \(E.\) coli strain 3# overexpressing matBC showed the highest conversion efficiency of \(p\)-coumaric acid substrate into curcuminoids.

In addition, we also investigated the time period of malonate feeding (Figure 3B). We found that early malonate feeding, together with IPTG addition, resulted in the highest curcuminoid yield. We also identified the optimum concentration of disodium malonate for enhanced production of BDMC by feeding with different concentrations (0–4 g L\(^{-1}\)) of malonate in the fermentation broth as shown in Figure 3C. Specifically, the highest production (1.92 ± 0.01 mg L\(^{-1}\)) of BDMC at 12 h was achieved when 1 g L\(^{-1}\) of disodium malonate was fed into the medium. In Figure 3D, we also found that, compared to the recombinant strain overexpressing acetyl-CoA carboxylase, the strain overexpressing matB-matC produced BDMC the fastest, reaching the highest titers in the first 4 h of fermentation and a production rate of 0.3 mg/L/h. Higher concentrations of disodium malonate resulted in decreased production, possibly due to metabolic pathway imbalance resulting in decreased concentrations of coenzyme A.[10,27] Finally, we obtained the highest titer (4.8 mg L\(^{-1}\)) of BDMC after 24 h fermentation using 25 mg L\(^{-1}\) of \(p\)-coumaric acid, with 1 g L\(^{-1}\) of disodium malonate added 2 h after beginning the fermentation. As shown in Figure 3, the strains 1# and 2# not only showed very low yield of BDMC, but also showed a rapid decrease of BDMC which had completely disappeared after 32 h of fermentation.

3.4. Fast Preparation of Bisdemethoxycurcumin

In the previous sections, we summarized the work that allowed the production of BDMC from precursor metabolites, such as \(p\)-coumaric acid. We found that BDMC yields were relatively low and long fermentation times can result in degradation of BDMC. We therefore decided to further improve production rates by testing different fermentation conditions. After inducing the \(E.\) coli recombinant strain carrying plasmids pEMT6-4CL-CUS and pACYC-MatBC for protein expression at 25 °C for 12 h, we harvested the cells by centrifugation and suspended them in 2 ml fresh AMM medium supplemented with 20 g L\(^{-1}\) glucose, 25 mg L\(^{-1}\) \(p\)-coumaric acid, and 1 g L\(^{-1}\) disodium malonate. The second part of the fermentation, involving the biosynthesis of curcuminoids, was conducted at two different temperatures, 30 and 37 °C (Figure S2A, Supporting Information). We found that 37 °C was more beneficial for BDMC production, most likely reflecting the higher catalytic activity of CUS.[10] Under these

![Figure 3. Optimization of BDMC production with \(p\)-coumaric acid and malonate. A) Effect of different concentrations of \(p\)-coumaric acid and 1 mg L\(^{-1}\) malonate on three strains for BDMC production; (B) Effect of different malonate feeding points (same to IPTG inducing time) on strain 3# for BDMC production; (C) Effect of different concentrations of disodium malonate and 25 mg L\(^{-1}\) \(p\)-coumaric acid on strain 3# for BDMC production; (D) BDMC fermentation time course of three engineered strains. Strain 1#, \(E.\) coli BL21 Star\textsuperscript{TM} (DE3) w/pEMT6-4CL-CUS, strain 2#, \(E.\) coli BL21 Star\textsuperscript{TM} (DE3) w/pEMT6-4CL-CUS, pACYC-ACC, strain 3#, \(E.\) coli BL21 Star\textsuperscript{TM} (DE3) w/pEMT6-4CL-CUS, pACYC-MatBC. Malonate was added only in the case of strain 3#.](image)
conditions, maximum titer of 13.8 mg L\(^{-1}\) of BDMC was achieved after 4 h. We also observed that high titers of BDMC gave the cell culture an obvious yellow color (Figure S2B, Supporting Information).

### 3.5. De Novo Production of Curcuminoids From Glucose

Though we can easily obtain curcuminoids by feeding p-coumaric acid and malonate to engineered E. coli, it is still uneconomical to use p-coumaric acid as a precursor. It has been suggested that tyrosine could be added as an “inexpensive” substrate to produce curcuminoids. However, similar to p-coumaric acid, feeding tyrosine is uneconomical and is coupled with additional problems such as low solubility and limited uptake rate when added in the medium.

In order to achieve the production of curcuminoids from glucose, we therefore proposed a co-culture approach of two different recombinant E. coli strains. The first strain was a tyrosine producer, E. coli rpoA14 (DE3), carrying plasmid pEMT6-TAL\(^{\text{SYN}}\) that allowed the conversion of intracellular tyrosine to p-coumaric acid. Since E. coli can easily absorb and secrete p-coumaric acid, we designed another strain, E. coli BL21 Star\(^{\text{TM}}\) (DE3) (w/pEMT6-4CL-CUS) to produce BDMC with the precursor substrate p-coumaric acid produced from the first strain (Figure 4A). We also designed a recombinant E. coli strain carrying genes tal, 4cl, and cus that was used as a control strain. From the fermentation time course, we found that the co-culture system could achieve higher titer of BDMC with a maximum titer of 0.48 ± 0.03 mg L\(^{-1}\) reached at 8 h after the start of the fermentation (Figure 4B).

We found that co-culture system caused more loss of BDMC in late fermentation stage (Figure 4C). One possible reason for this is that the co-culture results in higher BDMC titer that may activate its degradation metabolism in E. coli.\(^{[33]}\) Though the final yield of BDMC was relatively modest and culture optimization was needed, the co-culture system demonstrated the potential of using this method to produce high value-added curcuminoids from glucose feedstock.\(^{[28]}\)

### 3.6. Optimization of Curcuminoids Production in Co-Culture System

While glucose is an inexpensive substrate when producing higher value chemicals such as curcuminoids, the canonical metabolic pathway leading to its conversion to curcuminoids is long and requires a number of cofactors, such as coenzyme A and ATP.\(^{[29,30]}\) The simultaneous optimization of all parameters necessary to improve titers and yields can therefore impose significant metabolic burden on the cell and as such, the use of a co-culture approach can offer a competitive alternative.\(^{[31]}\) However, several parameters need to be optimized when co-cultures are used. In this study, we considered that inoculation ratio and induction point might be the most important parameters to affect curcuminoid production. First, we used different inoculation ratios (1:2, 1:1, 2:1, 5:1, and 10:1) of E. coli BL21 Star\(^{\text{TM}}\) (DE3) and rpoA14 (DE3) production hosts. We found that high content of rpoA14 (DE3) was beneficial to the accumulation of p-coumaric acid but inhibited the production of BDMC. Using an inoculation ratio of 10:1 as shown in Figure 5A, we could obtain 3 mg L\(^{-1}\) BDMC. In addition, we also observed that changing the induction point time to 1 h resulted in a final titer of 3.18 mg L\(^{-1}\) of BDMC (Figure 5B), which is about a sixfold increase compared to the initial co-culture system.

Next, we attempted the co-culture system in a 3-L bioreactor. In order to maintain high cell density, glucose carbon source was fed as specified in the methods above and the induction point was delayed to 4 h. As shown in Figure 5C, the maximum cell density (OD\(_{600}\)) reached 15. The maximum titer of BDMC in cell broth was 6.28 mg L\(^{-1}\) after 22 h of fermentation (Figure 5C). The final fermentation broth displayed a bright yellow pigment in the 3-L bioreactor, with some E. coli cells sticking to the bioreactor and giving its wall a bright yellow color (Figure 5D). In the future, we intend to develop accurate methods that will allow us to accurately determine the dynamics of our co-culture systems, possibly by tracking recombinant gene abundance using qPCR.\(^{[32]}\)
4. Conclusion

In the present work, we constructed an artificial pathway for curcuminoid production in *E. coli*. Overexpression of curcuminoid synthase from *Oryza sativa* in *E. coli* resulted in the production of the major curcuminoid, BDMC from *p*-coumaric acid. We also demonstrated that enhancement of the intracellular malonyl-CoA pool is essential for increasing the final production titer of BDMC. Expression of a recombinant pathway that allows the conversion of malonate to malonyl-CoA encoded by genes *matB* and *matC* resulted in a 25-fold improvement of final BDMC titer. It also enabled the maximum titer (13.8 mg L$^{-1}$) of BDMC within only 4 h after resuspension of the induced cell culture (16 h after initial induction) using a two-stage fermentation approach. In addition, we compared curcuminoid production from glucose when a consolidated bioprocess was used where the three-step conversion of tyrosine to BDMC was inserted in a single *E. coli* strain versus a co-culture process. In the co-culture process, an *E. coli* strain was used to produce *p*-coumaric acid and another *E. coli* strain converted *p*-coumaric acid into the final curcuminoid product. The comparison of the two methods demonstrated that the co-culture system resulted in better yield of conversion of the inexpensive substrate glucose to curcuminoid. After optimizing the inoculation ratio and induction point of the *E. coli* co-culture, 6.28 mg L$^{-1}$ of BDMC was obtained from glucose within 22 h of fermentation in a 3-L bioreactor. This is the first example of a co-culture method that has been employed for the production of curcuminoids from glucose in a lab scale bioreactor. This system provides a new method for transforming inexpensive substrate into value-added products. Future improvement of curcuminoids production will possibly result from fermentation optimization and a highly selective CUS enzyme, including the use of a new medium and rational protein design.[35,36]

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

Acknowledgement

This work was supported by Award CBET-1403815 by the National Science Foundation (U.S.).

Conflict of Interest

The authors declare no financial or commercial conflict of interest.

Keywords

bisdemethoxycurcumin, co-culture, curcuminoid synthase (CUS), malonyl-CoA

Received: September 13, 2017
Revised: November 9, 2017
Published online: December 5, 2017