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Improving glutarate production in Escherichia coli by enhancing the CoA precursors

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May 21, 2020

Abstract

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Improvingglutarate production in*Escherichia coli* by enhancing the CoA precursors

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Abstract: Glutarate is an attractive C5 platform chemical having wide application in nylon and plasticizer. However, microbial glutarate is mainly accumulated in the degradation of lysine and other methods were rarely explored for producing the glutarate from glucose directly. Here, we utilized a reversed adipic acid degradation pathway (RADP) and improved the glutarate production by increasing the precursors (malonyl-CoA and acetyl-CoA) based on the previously studied strain Bgl4146. Specifically, the conversion system of intracellular acetyl-CoA to malonyl-CoA was firstly constructed and optimized to balance acetyl-CoA synthase and acetyl-CoA carboxylase under different dissolved oxygen, enhancing the glutarate production from 0.09 g/L to 0.49 g/L. Then, modulating CoA balance by monitoring the expression of acetate kinase

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and pyruvate dehydrogenase resulting in a rise in glutarate titer up to 0.70 g/L. Finally, the optimized strain Bgl51464 was able to produce 7.97 g/L glutarate in a 5-L bioreactor. This strategy was described here, which could lay a certain foundation for the development of effective CoA balance to produce industrially high value-added chemicals.

Key words: Glutarate; The reverse adipate degradation pathway; Malonate; Acetate; Malonyl-CoA; Acetyl-CoA

Introduction

Glutaric acid, a C5 chain dicarboxylic acid, is used as the building block for synthesizing nylon and plasticizer (J. Adkins, J. Jordan, & D. R. Nielsen, 2013; Bermúdez, León, Alemán, & Muñoz-Guerra, 2000; W. Li et al., 2019; Zhang, Gao, Guo, Guo, Kang, Xiao, Yan, Tao, Zhang, & Dong, 2018). Currently, glutaric acid is prepared using nitric acid oxidation and separated from a mixture of dicarboxylic acids, but its yield is not high (Castellan, Bart, & Cavallaro, 1991; Nishikido, Tamura, & Fukuoka, 1979; Niu, Draths, & Frost, 2002; Polen, Spelberg, & Bott, 2013; Sato, Aoki, & Noyori, 1998). The most notable natural route for glutarate production was discovered in*Pseudomonas putida* , which could utilize L-lysine degradation to produce glutarate by the 5-aminovalerate (AMV) pathway(JC & JR, 1977; Revelles, Espinosa-Urgel, Fuhrer, Sauer, & Ramos, 2005; Revelles, Wittich, & Ramos, 2007; Zhang, Gao, Guo, Guo, Kang, Xiao, Yan, Tao, Zhang, & Dong, 2018). In order to avoid the supply of precursor lysine and improve the glutarate production, the lysine overproducing*Corynebacterium glutamicum*overexpressed the AMV pathway from *P. putida* to produce glutaric acid in high titers and yields (J. Adkins, J. Jordan, & D. R. Nielsen, 2013; Kim et al., 2019; Maria, Gideon, Michael, Christoph, & Judith, 2016; Rohles et al., 2018; Shin et al., 2016). At the same time, in order to better study the AMV pathway, those genes were also heterologous expressed in *Escherichia coli*(J. Adkins, J. Jordan, & D. R. Nielsen, 2013; Park et al., 2013). The maximum titer of 0.82 g/L for glutarate was reached after 48 hours of fermentation (J. Adkins, J. Jordan, & D. R. Nielsen, 2013). In order to obtain higher output, lysine and α-KG were supplemented to the cell catalytic system resulting in 1.7 g/L glutarate in *E. coli* WL3110 strain(Park et al., 2013). Recently, glutarate titer was also greatly improved through whole cell bio-catalyst or whole-cell immobilized (Hong et al., 2018; S.-Y. Yang et al., 2019). And Li *et al* used the native lysine catabolic pathway in *E. coli* to synthesize glutarate and finally achieved both high titer and high yield(W. Li et al., 2019).

The above all results are based on AMV pathway or lysine carbolic pathway. In addition to the AMV pathway, a variety of attempts for the glutarate production and exploration were made in *E. coli* . Recently, the reverse adipic acid degradation pathway (RADP) was utilized to balance the production of adipate and glutarate and finally achieved 4.8 g/L glutarate (Zhao, Huang, et al., 2018; Zhao, Li, & Deng, 2018). Then the glutarate in RADP was accumulated to 6.3 g/L by malonate absorption(Sui et al., 2020). The RADP seems to be a potential pathway to produce glutarate from glucose in*E. coli* . The RADP contained the following enzymes: Tfu_0875 (β-ketothiolase), Tfu_2399 (3-hydroxyacyl-CoA dehydrogenase), Tfu_0067 (3-hydroxyadipyl-CoA dehydrogenase), Tfu_1647 (5-carboxy-2-pentenoyl-CoA reductase) and Tfu_2576-7 (adipyl-CoA synthetase) (Sui et al., 2020; Zhao, Huang, et al., 2018; Zhao, Li, & Deng, 2018) (Fig. 1). The RADP could convert acetyl-CoA and malonyl-CoA to glutaric acid and malonyl-CoA was crucial for the glutarate production. Therefore, cerulenin was used for increasing the amount of malonyl-CoA available by inhibiting fatty acid synthesis pathway(Heath & Rock, 1995; Rogers & Church, 2016; Zhao, Li, & Deng, 2018). And *matB*(malonic acid synthetase) and *matC* (malonic acid carrier protein) were overexpressed, absorb the malonate to improve the intracellular malonyl-CoA(Sui et al., 2020; Wu, Du, Zhou, & Chen, 2013). However, only increasing the content of intracellular malonyl-CoA was not sufficient for glutarate production. Both precursors of glutaric acid should be considered to increase production.

The acetyl-CoA could be transformed to malonyl-CoA by catalyzing acetyl-CoA carboxylase (ACC) (S. J. Li & Cronan, 1992; Lussier, Colatriano, Wiltshire, Page, & Martin, 2012; Xu, Li, Zhang, Stephanopoulos, & Koffas, 2014; Zhu, Wu, Du, Zhou, & Chen, 2014). But acetyl-CoA mainly flew to the tricarboxylic acid (TCA) cycle to maintain basic metabolism and growth, whereas only a minor proportion was used by ACC to synthesize malonyl-CoA(Tokuyama et al., 2019), and a diminutive proportion was engaged in fatty

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acid synthesis(Zha, Rubin-Pitel, Shao, & Zhao, 2009) in *E. coli.* Therefore, it was important to balance the supply and demand of acetyl-CoA and malonyl-CoA to flow to the target product. Here, our research is based on the previous RADP pathway to produce glutaric acid (Fig. 1). Firstly, we constructed a transformation system of acetyl-CoA to malonyl-CoA for enhancing the available intracellular malonyl-CoA in *E. coli* . Furthermore, the acetic acid uptake pathway was introduced into the metabolic pathway and the degradation pathway was knocked out to increase and balance intracellular acetyl-CoA and malonyl-CoA. Meanwhile, the dissolved oxygen was effectively controlled so that more carbon source flew to CoA. Finally, the optimal strains, Bgl51464 was cultured in a 5-L fermenter.

1. **Materials and methods**
2. **Strains and plasmids**

The strains, plasmids, and primers are shown in Supplementary Tables S1 and S2. In order to promote the transformation of acetyl-CoA to malonyl-CoA *in vivo* , the*accABCD* (Xu et al., 2014) from*E. coli* MG1655 was firstly linearized by PCR using accA-1 F and accA-1 R, accB-1 F and accB-1 R, accC-1 F and accC-1 R and accD-1 F and accD-1 R, respectively. Then the pACYCDuet-1 was digested using*Bam* H I and *Avr* II and all products were ligase by Gibson assembly (Gibson et al., 2009) and transformed into competent cells, forming strain pACC1. The *accBC*and *dtsR1* were amplified from *C. glutamicum* (Zhu et al., 2014) using accBC-2 F and accBC-2 R and dtsR1-2 F and dtsR1-2 R primers, respectively. The pACYCDuet-1 was linearized using *kpn* I and*Avr* II. The all products were assembled like pACC1, resulting in strain pACC2. To increase the CoA *in vivo* , the *acs* from*E. coli* MG1655 was amplified using acs-3 F and acs-3 R. Both the pACYCDuet-1 and the *acs* fragment were digested using *Bam* H I and *Not* I and ligated by T4 DNA ligase, naming pACC3. The*accBC* and *dtsR1* were amplified from pACC2 using accBC-2 F and dtsR1-2 R. The pACC3 plasmid was digested by *kpn* I and*Avr* II and all products were assembled, forming strain pACC4. All above constructs were screened by colony PCR and Sanger sequencing using veri-pACYC F and veri-pACYC R. All subsequent plasmid constructions (packA, and ppoxB) also used the Gibson assembly method as described above. The sgRNA target gene and knock gene template are shown in Supplementary Table S3.

2.2 Tolerance and degradation test in *E. coli*

All tolerance and degradation were studied for *E. coli* Bgl4146 at 37°C with 200 rpm. The seed solution was inoculated into Lysogeny broth (LB) and cultured overnight at 37°C. Then the seeds were inoculated (2% working volume) into 50 mL M9 minimal medium supplemented with 5 g/L glutarate as the only carbon source for glutarate degradation or different concentrations of acetate(0 g/L, 3 g/L, 6 g/L, 9 g/L, 12 g/L) as the single carbon source for acetate degradation. The glutarate tolerance test was performed in an optimized super optimal broth (SOB)(Zhao, Li, & Deng, 2018), which was also used in glutarate production by the engineered strains as described below. Briefly, SOB medium with different final concentrations (0, 5, 10, 15, 20, 30, 40, 50, 100, or 150 g/L glutarate and adjusted to pH 7.00) were inoculated and initial OD_{600} remained around 0.3.

2.3 Strain engineering

The *ackA* and *poxB* gene were knocked out on the basis of Bgl4(BL21(DE3)Δ*arcA* Δ*ldhA* Δ*atoB* Δ*pflB*) (Zhao, Li, & Deng, 2018) and performed according to CRISPR-Cas9 engineering in *E. coli*(Jiang et al., 2015). The related sgRNA was designed and shown in Supplementary 3.

2.4 Glutarate Fermentation

For shaken flask experiments for glutarate production, single colony was picked into LB and grown overnight at 37°C, 200 rpm. Lately, the cultures at an inoculation volume of 2% were added to the optimized SOB media (previously demonstrated as an effective media for glutarate over-production by*E. coli* (Zhao, Li, & Deng, 2018a)), grown at 37°C until OD_{600} ˜ 0.6 and then induced by 0.8 mM IPTG(Zhao, Li, & Deng, 2018). The induced cultures were incubated at 30°C, 200 rpm. 4 g/L glucose and 3 g/L or 6 g/L or 9 g/L or 12 g/L acetate were supplemented when necessary, respectively. After inoculation, the flasks were instantly

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moved to aerobic condition or microaerobic condition controlling by the fermentation bung (Nastasia, 2001) or anaerobic condition using an anaerobic workbench(J.-L. Yu, X.-X. Xia, J.-J. Zhong, & Z.-G. Qian, 2017).

For the fed-batch fermentation, the seed cultures were grown at a rate of 2% into the initial 8 g/L glucose and 6 g/L acetate. The Bgl51464 was cultured at 37°C until the OD_{600} ˜ 0.6. After induction with IPTG, the temperature was reduced to 30°C. And 0.5 vvm, 400 rpm and pH 7.0 were conducted during the whole process. When the glucose was used up, the 50% glycerol was supplemented by pH-stat feeding strategy(Lee, 1996). Samples were taken regularly to detect cell growth and analyze metabolites.

2.5 Metabolite analysis

After centrifuging the sample, the supernatant was obtained through a 0.22 μM filter and then analyzed. All metabolites were analyzed by HPLC equipment (Agilent, Waldbronn, Germany) with the HPX-87H column (Bio-Rad, CA, USA). The different detectors (refractive index and ultraviolet) were used at the same time and the ultraviolet wavelength was controlled at 210 nm. The 30 mM H_2SO_4 at 0.3 mL/min at 42 °C was used in the detect process(Babu et al., 2015; Zhao, Li, & Deng, 2018).

3. Results

3.1 Glutarate tolerance and degradation test

It has generally been believed that glutarate was toxic to any microorganism, and different strains had different tolerances to toxicity(J. Adkins, J. Jordan, & D. R. Nielsen, 2013; W. Li et al., 2019; Rohles et al., 2018). Although many studies have been done on its physiological roles(J. Adkins, J. Jordan, & D. R. Nielsen, 2013; W. Li et al., 2019; Rohles et al., 2018), glutarate tolerance remained unclear. Thus, glutarate tolerance was first performed before production. The tolerance examination was conducted in an optimized SOB medium(Zhao, Li, & Deng, 2018) with Bgl4146 at 37 °C, which was also used in glutarate production. The cells in exponential growth were placed into increasing glutarate concentrations (Fig. 2A). After cultivating to 30 g/L glutarate, cells grew well although the growth curve was lower than the control (no glutarate). When the concentration of glutaric acid reached 50 g/L, the cells still grew slowly. However, the cells hardly grew and even some cell lysis was discovered when the glutarate concentration increasing to 100 or 150 g/L. Therefore, at least 50 g/L glutarate was able to be tolerated by Bgl4146, this was verified in fermentation production of glutaric acid(W. Li et al., 2019).

Then we also tested the glutarate degradation in Bgl4146 because of the *CsiD* and *YgaF* in *E. coli* involving in the utilization of glutarate(Zhang, Gao, Guo, Guo, Kang, Xiao, Yan, Tao, Zhang, & Dong, 2018). The Bgl4146 was detected in M9 with 5 g/L glutarate as the single carbon source for glutarate degradation (Fig. 2B). However, the strain hardly grew and consumed only 0.1% glutarate in 96 h (Fig. 2B). Therefore, we would not consider the degradation of glutaric acid in the next study.

3.2 Improving glutarate production by increasing precursors

Due to the mutual cooperation of metabolism and regulatory network in the cell, the biosynthesis of essential metabolites (such as CoA derivative biosynthesis) was generally retained at a relatively stable level and the derivative was rarely overexpressed (Pitera, Paddon, Newman, & Keasling, 2007). Therefore, the metabolic fluxes resulting in the production of the CoA precursors in RADP were considered as the optimization objectives by metabolic engineering. Specifically, four strategies were adopted in order to increase the usability of acetyl-CoA and malonyl-CoA for glutarate production. These methods included 1) overexpression of acetyl-CoA carboxylase (ACC) for the conversion of acetyl-CoA and malonyl-CoA; 2) overexpression of *acs* (acetyl-CoA synthase) for acetate uptake pathway; 3) Blocking of the acetate competition pathway by deleting the *ackA* (acetate kinase) gene; 4) Blocking of the acetate synthesis pathway by deleting the *poxB* (pyruvate dehydrogenase) gene.

It was worth noting that the dissolved oxygen levels had a crucial effect on the glutarate production(J.-L. Yu et al., 2017; Zhao, Li, & Deng, 2018). Subsequently, the different recombinant strains were fermented under different dissolved oxygen conditions (aerobic condition, microaerobic condition, anaerobic condition)

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for glutarate production (Fig. 3). Overexpression of ACC (acetyl-CoA carboxylase, *accABCD*) gene from *E. coli* by Bgl41461 changed the glutarate production concentration by 0.93, 0.82, 0.99-fold than the control strain Bgl4146 under same aerobic condition, microaerobic condition and anaerobic condition, respectively. And the maximum glutarate titer by Bgl41461 was 0.34 g/L under microaerobic conditions. Overexpression of ACC (two subunits of acetyl-CoA carboxylase, *accBC* and *dtsR1*) from *C. glutamicum* (Gande et al., 2007; Miyahisa et al., 2005) alone Bgl41462 changed the glutarate production concentration by 1.27, 1.06, 0.78-fold than the control Bgl4146 under same aerobic condition, microaerobic condition and anaerobic condition, respectively. And the maximum glutarate titer by Bgl41462 was 0.44 g/L under microaerobic conditions. The glutarate titer was better improved on Bgl41462 by expressing *accBC* and *dtsR1* and microaerobic condition was useful for glutarate fermentation. But we found the production of acetic acid was affected by dissolved oxygen during the fermentation process and the by-product acetate titer was relatively high in microaerobic condition compare to other conditions in Bgl4146(Fig. S1)(Akesson, Hagander, & Axelsson, 2001). At the same time, the over-accumulation of the metabolic by-product acetate inhibited cell growth(Luli & Strohl, 1990; Nakano, Rischke, Sato, & Märkl, 1997) and production of recombinant proteins(Glazyrina et al., 2010; Lee, 1996), which was one of the barriers to achieving high yield and production of target compound.

In order to balance glutarate and acetate production, overexpressing the single *acs* (acetyl-CoA synthase) gene in *E. coli* was proven to be the best natural approach for acetate digestion (Lin, Castro, Bennett, & San, 2006; Zha et al., 2009). Therefore, we introduced the *acs* from *E. coli* to degrade acetate to increase the available acetyl-CoA in Bgl41463. As shown in Fig. 3, overexpression of *acs* from *E. coli* only to digest acetic acid into acetyl-CoA by Bgl41463 increasing the glutarate production by 0.94, 0.89, 1.05-fold than the control Bgl4146 under same aerobic condition, microaerobic condition and anaerobic condition, respectively. And the maximum glutarate titer by Bgl41463 was 0.37 g/L under microaerobic conditions. However, those results were not ideal for only increasing the content of intracellular acetyl-CoA or malonyl-CoA. Both precursors of glutaric acid should be considered together to increase glutarate production. Therefore, the well overexpressing *accBC* and *dtsR1* was tried to co-express with *acs* to enhance the glutarate production. Overexpression of *acs* , *accBC* and *dtsR1* together by Bgl414164 increased the glutarate production concentration by 1.38, 1.19, 1.54-fold than the control Bgl4146 under same aerobic, microaerobic and anaerobic condition, respectively. And the glutarate production had been greatly improved, reaching 0.49 g/L by Bgl41464 under microaerobic condition. This may be due to the increase in the content of acetyl-CoA and malonyl-CoA (Zhu et al., 2014). The better production strain Bgl41464 was applied for the further study under the microaerobic condition.

3.3 Improving glutarate by acetate uptake pathway

The strain Bgl41464 tried to uptake acetate directly from the medium for furthermore enhancing glutarate titer. Before that, the natural acetate degradation way was explored individually. We firstly tested the growth of Bgl4146 under different acetate concentrations (Fig. S2). The acetate growth and degradation were measured in M9 medium (pH 7.0) supplemented with acetate as the only carbon source. But 3 g/L and 6 g/L acetate could be consumed completely about 48h and 96h, respectively. In order to grow better and accelerate the consumption of acetate, we cultivated Bgl41464 in the fermentation medium with and without glucose (Fig. 4A and 4B). In order to improve the content of acetyl-CoA, the competition route of acetic acid was destroyed by eliminating the *ackA* gene and the acetate synthesis pathway was destroyed by knockout of the *poxB* gene for obtaining more acetyl-CoA. Because Zhu *et al* found that a combination of the three manipulations (deletion of *ackA* , overexpression of *acs* , *accBC* and *dtsR1*) caused a 16.3-fold increasing in the intracellular malonyl-CoA level than that in wild-type *E. coli* (Zhu et al., 2014). Therefore, we explored the impact of the deletions of acetate synthesis pathway (*ackA* or *poxB*) for glutarate production in *E. coli*(Fig. 4A and 4B). We found that the glutarate production with glucose fermentation was about three times than without glucose fermentation. And the glutarate production reached a maximum of 0.70 g/L under 6 g/L acetate with strain Bgl51464 (BL21(DE3) Δ*arcA* Δ*ldhA*Δ*atoB* Δ*pflB* Δ*ackA* carrying pAD1, pAD4, pAD6 and pACC4).

3.4 Glutarate production in 5-L bioreactor

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The best strain Bgl51464 was fed-batch fermentation to improve glutarate production using the glucose, acetic acid and glycerol as a mixture carbon source (Fig. 5). We finally obtained a 7.97 g/L maximal glutarate at 63 h, representing a 16.27-fold higher than that of strain Bgl41464 with microaerobic condition in shake flask (Fig. 5). And the OD_{600} reached a maximum of 24.3 at 113.5 h. After 30 h of fermentation, the acetate synthesis and consumption were kept in a dynamic balance (Fig. 5).

Discussion

Glutaric acid, an important C5 compound, is used in industrial production. Our team has previously used the reversed adipic acid degradation pathway (RADP) to produce adipate and glutarate in *E. coli* (Sui et al., 2020; Zhao, Huang, et al., 2018; Zhao, Li, & Deng, 2018). In this work we used the previous RADP pathway to enhance glutarate production based on*E. coli* Bgl4146(Zhao, Li, & Deng, 2018). The precursors of glutaric acid were acetyl-CoA and malonyl-CoA. In order to increase the precursors of glutarate production, these methods applied to enhance the precursors were described: 1) overexpression and optimization of ACC (acetyl-CoA carboxylase) from different strains to enhance the transformation of acetyl-CoA to malonyl-CoA; 2) overexpression of *acs* (acetyl-CoA synthase) to promote the decomposition of acetate into acetyl-CoA; 3) Combination of ACC and *acs* simultaneously to increase 2 CoA precursors for glutarate output; 4) Knockout of *ackA* or *poxB* to decrease acetate titer. The best strain Bgl51464 produced 7.97 g/L glutarate by fed-batch fermentation. This can be used as an effective method for the production of CoA-derived other high value-added chemicals.

The glutarate production was closely related to the dissolved oxygen(J. L. Yu, X. X. Xia, J. J. Zhong, & Z. G. Qian, 2017; Zhao, Li, & Deng, 2018). The glutarate titers varied under different dissolved oxygen levels and microaerobic condition could be suitable to produce glutarate (Fig. 3). The synthetic precursors of glutaric acid were malonyl-CoA and acetyl-CoA. Partially impairing the fatty acid pathway and tricarboxylic acid cycle by microaerobic condition could well drive acetyl-CoA and malonyl-CoA toward glutarate production while sustaining certain cell growth and metabolism requirements(Brose, Golovko, & Golovko, 2016; Zhao, Li, & Deng, 2018). At the same time, the production of acetic acid was also affected by dissolved oxygen during the fermentation process and relatively high in microaerobic condition (Fig. S1)(Akesson et al., 2001). And the over-accumulation of the metabolic by-product acetate inhibited cell growth(Luli & Strohl, 1990; Nakano et al., 1997) and production of recombinant proteins(Glazyrina et al., 2010; Lee, 1996), which was one of the barriers to achieving high yield and production of target compounds (Mazumdar, Clomburg, & Gonzalez, 2010; Wolfe, 2005). On the premise of glutarate production as the first principle, we need to balance the acetate production in the fermentation. Then, acetyl-CoA synthase (encoded by*acs*) was introduced to consume acetate. We finally found that Bgl41464 was better than other strains and glutarate production reached 0.49 g/L under microaerobic condition (Fig. 3).

The balance between acetyl-CoA and malonyl-CoA was crucial for glutarate production. Firstly, the acetyl-CoA was transformed to malonyl-CoA by overexpressing ACC (acetyl-CoA carboxylase). As shown in Fig. 3, overexpression of ACC (acetyl-CoA carboxylase,*accABCD*)(My et al., 2013; Xu et al., 2014) gene from *E. coli*by Bgl41461 had a certain negative effect on the glutarate production. And the maximum glutarate titer by Bgl41461 was 0.34 g/L under microaerobic conditions, which was 0.82-fold that of the control Bgl4146 (Fig. 3). However overexpression of the ACC gene from *C. glutamicum* consisting of two subunits, *accBC* and*dtsR1* (Gande et al., 2007; Miyahisa et al., 2005) alone by Bgl41462 had a great influence on the glutarate production. And the maximum glutarate titer by Bgl41462 was 0.44 g/L under microaerobic conditions, which was 1.06-fold that of the control Bgl4146 (Fig. 3). Though the glutarate titer was improved by overexpression of *accBC* and*dtsR1* in shake flask, it seemed that only increasing the content of malonyl-CoA was not sufficient for the synthesis of glutaric acid. Therefore, the acetyl-CoA synthase (encoded by *acs*) was introduced to decompose the metabolic by-product acetic acid to produce acetyl-CoA. Overexpression of *acs* , *accBC* and *dtsR1*by Bgl41464 increases the maximum titer of glutaric acid to 0.49 g/L under microaerobic conditions, which was 1.18-fold higher than the control Bgl4146 (Fig. 3). This combination balanced intracellular CoA and increased glutarate production.

Acetate can be used as a potential substrate for production of CoA-derived valuable compounds. However,

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the ability to digest acetate was restricted in *E. coli* due to slow growth and low recombinant protein expression(Wolfe, 2005). The*acs* pathway (overexpression of acetyl-CoA synthetase) and the reversible *pta -ackA* pathway (overexpression of phosphotransacetylase/acetate kinase) were two natural roads for digesting acetate in *E. coli*(Wolfe, 2005), the *acs* pathway was the best strategy for acetate decomposition(Lin et al., 2006; Zha et al., 2009). Therefore, the*acs* was overexpressed by Bgl41464, resulting in 0.49 g/L glutarate (Fig. 3). In order to improve the titer, we optimized the engineering strains and added acetate to the medium. The Bgl51464 finally was fermented and obtained 0.70 g/L glutarate with 6 g/L acetate in this study while the highest glutarate titer was 0.56 g/L when 10.4 g/L malonic acid was supplemented (Sui et al., 2020; Thuronyi, Privalsky, & Chang, 2017; Walker et al., 2013; Wu et al., 2013). Compared with the previous glutarate fermentation by the uptake of malonate, the uptake of acetate was cheaper, more efficient and obtained a higher titer in the shake flask. The acetate had many benefits as a potential fermentation substrate: 1) Acetate had a cheap source and a variety of routes(Xiao et al., 2013; J. Yang & Nie, 2016); 2) Acetate was easily soluble in water and easily transfers mass during fermentation; 3) Acetate was decomposed into acetyl-CoA, which can be used as an important precursor for many expensive chemicals. While malonic acid was a very valuable chemical for the synthesis of many flavors, fragrances, and pharmaceuticals and it mainly was produced from cyanoacetic acid or diethyl malonate(Pollak & Romeder, 2000). Importantly, the acetate uptake increased the all precursors of glutarate (acetyl-CoA and malonyl-CoA) while malonate uptake could only increase the glutarate precursor, malonyl-CoA. This may be the key reason for the difference in glutarate titer. Therefore, regardless of the availability of the added carbon source or the final effect on the glutarate production, acetate would be a good carbon source for the fermentation of chemicals.

Acknowledgements

This work was supported by the National Key R&D Program of China (2019YFA0905502), the National Natural Science Foundation of China (21877053), the Top-Notch Academic Programs Project of Jiangsu Higher Education Institutions (TAPP), the National First-class Discipline Program of Light Industry Technology and Engineering (LITE2018-24), the Fundamental Research Funds for the Central Universities (JUSRP51705A).

Competing financial interests

The authors declare no competing financial interests.

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Fig. 1 Glutarate biosynthetic pathway optimization results in *Escherichia coli*. *Tfu_0875* : α-ketothiolase, *Tfu_2399* : 3-hydroxyacyl-CoA dehydrogenase, *Tfu_0067* : 3-hydroxyadipyl-CoA dehydrogenase, *Tfu_1647* : 5-carboxy-2-pentenoyl-CoA reductase, *Tfu_2576-7* : adipyl-CoA synthetase, malonic acid synthetase: *matB* , malonic acid carrier protein: *matC* . The *ArcA* , *ldhA* , *atoB* ,*pflB* , *ackA* and *poxB* genes were deleted using the CRISPR/Cas9 system. A rough arrow represents genes or enzymes subject to overexpression, X represents those deleted. *ldhA* , L-lactate dehydrogenase; *atoB* , acetyl-CoA C-acetyltransferase; *arcA* , aerobic respiration control protein; *pflB* , formate C-acetyltransferase 1; *ackA* , acetate kinase; *poxB* , pyruvate dehydrogenase; *pta* , phosphate acetyltransferase;*acs* , acetyl-CoA synthetase.

Fig. 2 Glutarate tolerance and degradation test. A. Time profiles of Bgl4146 treated with various glutarate concentrations. Cells were grown at 37 °C in the SOB medium (pH 7.0) and exposed at exponential growth phase (OD_{600}˜0.3). B. Growth of Bgl4146 on glutarate. Growth (blue line) and the consumption of glutarate (orange line) was measured in M9 supplemented with 5 g/L glutarate as the single carbon source. Bgl4146: Bgl4 carrying pAD1, pAD4 and pAD6; Bgl4: BL21(DE3) Δ*arcA* Δ*ldhA* Δ*atoB*Δ*pflB* ; pAD1: pRSFDuet-1 carrying *Tfu_0875* and*Tfu_2399* ; pAD4: pTrc99a carrying *Tfu_0067* and*Tfu_1647* ; pAD6: pCDFDuet-1 carrying *Tfu_2576-7* . Data shown are mean ± s.d. (n = 3 independent experiments).

Fig. 3 Different recombinant strains for glutarate production. A. Aerobic Condition. B. Microaerobic Condition. C. Anaerobic Condition. The all strains were fermented in SOB with 4 g/L glucose initially. Error bars represent the s.d. from three independent assays. Bgl4146: Bgl4 carrying pAD1, pAD4 and pAD6; Bgl4: BL21(DE3) Δ*arcA* Δ*ldhA* Δ*atoB* Δ*pflB* ; pAD1: pRSFDuet-1 carrying *Tfu_0875* and *Tfu_2399* ; pAD4: pTrc99a carrying*Tfu_0067* and *Tfu_1647* ; pAD6: pCDFDuet-1 carrying*Tfu_2576-7* ; Bgl41461: Bgl4146 carrying pACC1 (pACYCDuet-1 carrying *accABCD* from *E. coli*); Bgl41462: Bgl4146 carrying pACC2 (pACYCDuet-1 carrying *accBC* , *dtsR1* from*Corynebacterium glutamicum*); Bgl41463: Bgl4146 carrying pACC3 (pACYCDuet-1 carrying *acs* from *E. coli*); Bgl41464: Bgl4146 carrying pACC4 (pACYCDuet-1 carrying *acs* , *accBC* ,*dtsR1*).

Fig. 4 Enhanced production of glutarate at different acetate concentrations in *E. coli* Bgl41464, Bgl51464, Bgl61464. A. The glutarate production at different acetate concentrations under 4 g/L glucose and microaerobic condition. B. The glutarate production at different acetate concentrations under microaerobic condition. Bgl4: BL21(DE3) Δ*arcA* Δ*ldhA* Δ*atoB* Δ*pflB* ; Bgl5: BL21(DE3) Δ*arcA* Δ*ldhA* Δ*atoB* Δ*pflB*Δ*ackA* ; Bgl6: BL21(DE3) Δ*arcA* Δ*ldhA* Δ*atoB*Δ*pflB* Δ*ackA* Δ*poxB* ; Bgl41464: Bgl4 carrying pAD1, pAD4, pAD6 and pACC4; Bgl51464: Bgl5 carrying pAD1, pAD4, pAD6 and pACC4; Bgl61464: Bgl6 carrying pAD1, pAD4, pAD6 and pACC4; Bgl4: BL21(DE3) Δ*arcA* Δ*ldhA* Δ*atoB* Δ*pflB* ; Bgl5: BL21(DE3) Δ*arcA* Δ*ldhA* Δ*atoB* Δ*pflB*Δ*ackA* ; Bgl6: BL21(DE3) Δ*arcA* Δ*ldhA* Δ*atoB*Δ*pflB* Δ*ackA* Δ*poxB* ; pAD1: pRSFDuet-1 carrying*Tfu_0875* and *Tfu_2399* ; pAD4: pTrc99a carrying*Tfu_0067* and *Tfu_1647* ; pAD6: pCDFDuet-1 carrying*Tfu_2576-7* ; pACC4: pACYCDuet-1 carrying *acs* ,*accBC* , *dtsR1* .

Fig. 5 Fed-batch production of glutarate in 5 L bioreactors. Fermentation process of Bgl51464. The medium initially contained 6 g/L acetate. Substrates consumptions, metabolites, and cell growth during fed-batch fermentation with 8 g/L glucose initially, 0.5 vvm aeration rate and 400 rpm agitation rate in SOB medium. Error bars represent the s.d. from three independent assays. Bgl51464: Bgl5 carrying pAD1, pAD4, pAD6 and pACC4; Bgl5: BL21(DE3) Δ*arcA* Δ*ldhA* Δ*atoB*Δ*pflB* Δ*ackA* ; pAD1: pRSFDuet-1 carrying *Tfu_0875*and *Tfu_2399* ; pAD4: pTrc99a carrying *Tfu_0067* and*Tfu_1647* ; pAD6: pCDFDuet-1 carrying *Tfu_2576-7* ; pACC4: pACYCDuet-1 carrying *acs* , *accBC* , *dtsR1* .

Supplementary material captions

Table S1 Strains and plasmids used in this study

Table S2 Primers used in this study

Table S3 DNA sequences

Fig. S1 The acetate production under different dissolved oxygen condition in Bgl4146

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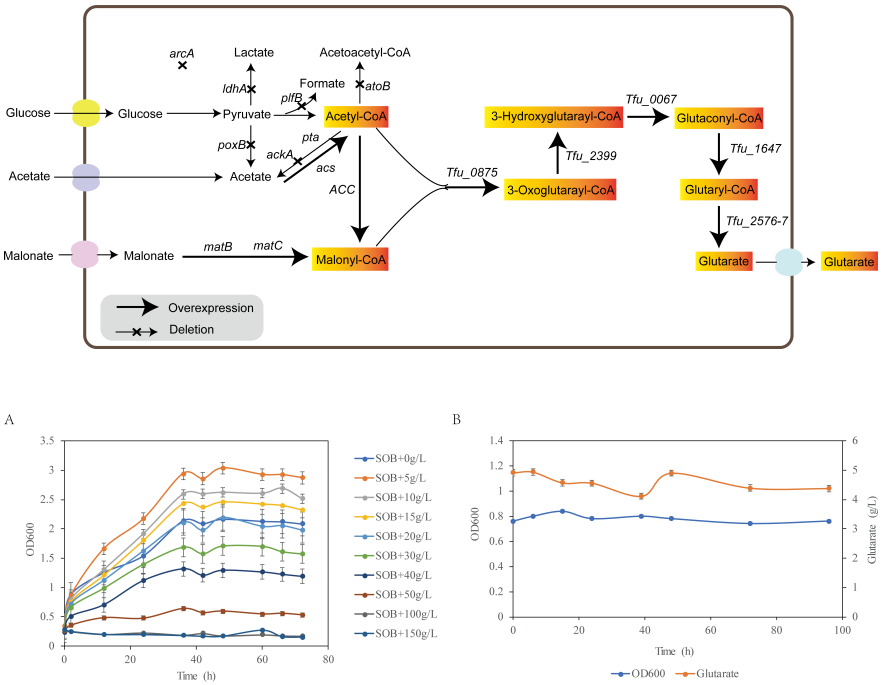
Fig. S2 Acetate growth and degradation test



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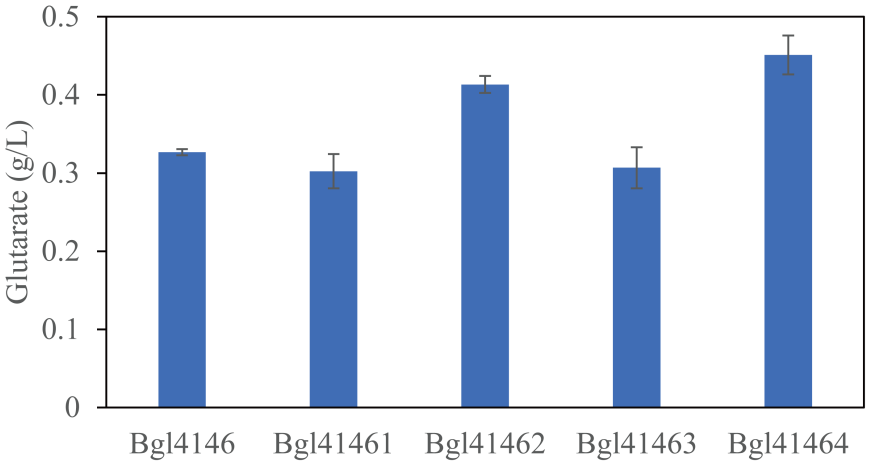
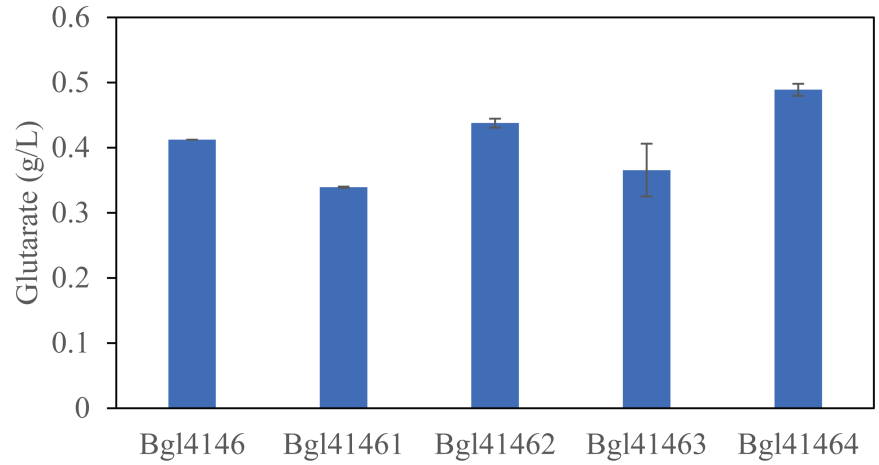
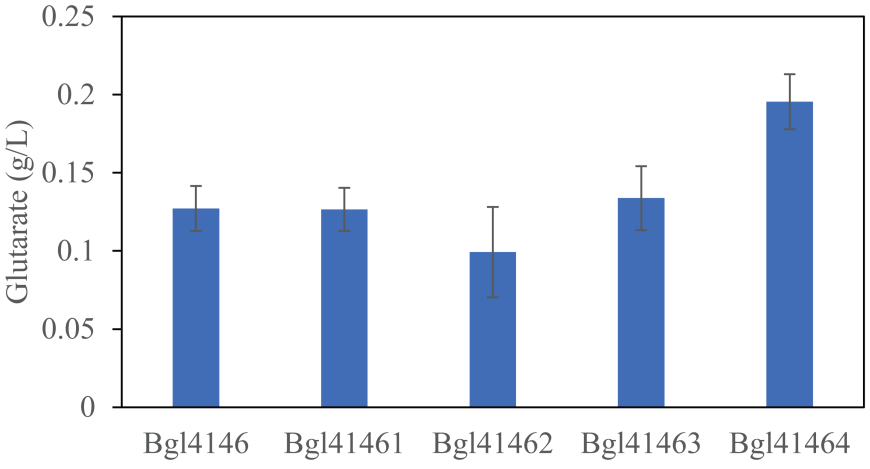
A



B



C



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