One stone two birds: Biosynthesis of 3-hydroxypropionic acid from CO₂ and syngas-derived acetic acid in *Escherichia coli*

Ningyu Lai a,1, Yuanchan Luo a,1, Peng Fei a, Peng Hu d, Hui Wu a,b,c,*

a State Key Laboratory of Bioreactor Engineering, School of Biotechnology, East China University of Science and Technology, 130 Meilong Road, Shanghai, 200237, China
b Shanghai Collaborative Innovation Center for Biomaterials, 130 Meilong Road, Shanghai, 200237, China
c Key Laboratory of Bio-based Material Engineering of China National Light Industry Council, 130 Meilong Road, Shanghai, 200237, China
d Shanghai GTL Biotech Co., Ltd., 1688 North Guoquan Road, Shanghai, 200438, China

ARTICLE INFO

**Keywords:**
Syngas-derived acetic acid  
3-Hydroxypropionic acid  
Metabolic engineering  
*Escherichia coli*  
Dynamic regulation

ABSTRACT

Syngas, which contains large amount of CO₂ as well as H₂ and CO, can be converted to acetic acid chemically or biologically. Nowadays, acetic acid becomes a cost-effective nonfood-based carbon source for value-added biochemical production. In this study, acetic acid and CO₂ were used as substrates for the biosynthesis of 3-hydroxypropionic acid (3-HP) in metabolically engineered *Escherichia coli* carrying heterogeneous acetyl-CoA carboxylase (Acc) from *Corynebacterium glutamicum* and codon-optimized malonyl-CoA reductase (MCR) from *Chloroflexus aurantiacus*. Strategies of metabolic engineering included promoting glyoxylate shunt pathway, inhibiting fatty acid synthesis, dynamic regulating of TCA cycle, and enhancing the assimilation of acetic acid. The engineered strain LNY07(M*DA*) accumulated 15.8 g/L of 3-HP with the yield of 0.71 g/g in 48 h by whole-cell biocatalysis. Then, syngas-derived acetic acid was used as substrate instead of pure acetic acid. The concentration of 3-HP reached 11.2 g/L with the yield of 0.55 g/g in LNY07(M*DA*). The results could potentially contribute to the future development of an industrial bioprocess of 3-HP production from syngas-derived acetic acid.

1. Introduction

The rising concentration of carbon dioxide (CO₂), mainly due to the accelerated consumption of fossil fuels and other human activities, has caused an increase in global temperatures [1–3]. With such environmental concerns, there is growing interest focusing on upgrading CO₂ to value-added products such as succinate [11], itaconic acid [12], fatty acid [13], isopropanol [14], and so on.

3-Hydroxypropionic acid (3-HP), which is composed of two functional groups (carboxylic and hydroxyl) and can be easily transformed into other compounds (e.g., acrylic acid, acrylamide, 1,3-propanediol (PDO), and methacrylic acid) [15], is a widely used agent for organic synthesis [16]. Various metabolic pathways and microorganisms have been explored for 3-HP production, including malonyl-CoA pathway in *Pseudomonas denitrificans* [17], CoA-independent pathway in *Klebsiella*...
pneumoniae [18], β-alanine pathway in Saccharomyces cerevisiae [19], malonyl-CoA pathway in Escherichia coli [20], and many other autotrophic or heterotrophic routes [21]. Among these processes, research on 3-HP production via malonyl-CoA route has been carried out in different chassis microorganisms model organisms, such as E. coli [22], S. cerevisiae [23], and Schizosaccharomyces pombe [24]. Production of 3-HP through malonyl-CoA route, is suitable for most carbon sources, including acetic acid, due to the malonyl-CoA is a universal intermediate in cell metabolism [20].

Although acetic acid can be utilized by many microorganisms, its consumption rate is much slower than those of sugar utilization, and reduced cell growth is also showed [25,26]. In E. coli, the acetic acid can be firstly converted to acetyl-CoA via two pathways, which were catalyzed by acetyl-CoA carboxylase (AckA-Pta) or acetyl-CoA synthetase (Acc) (Fig. 1) [27]. Acetyl-CoA is then converted to malonyl-CoA with CO₂ fixation catalyzed by acetyl-CoA carboxylase (Acc). Since the majority of cellular malonyl-CoA is usually consumed to produce fatty acids [28], leaving only a small amount available for 3-HP production. Then, malonyl-CoA reductase (Mcr) was applied to redirect the malonyl-CoA flux away from fatty acid to 3-HP formation [20]. Furthermore, the biosynthesis of 3-HP from malonyl-CoA, catalyzed by Mcr, requires 2 mol of NAPDH [17,29,30].

In this work, a metabolic engineered E. coli carrying codon-optimized Mcr (N940V, K1106W and S1114R) from Corynebacterium glutamicum was constructed to produce 3-HP via malonyl-CoA [20]. And to further increase 3-HP production, several engineering strategies were applied, including promoting glyoxylate shunt pathway by deletion of fabR, inhibiting fatty acid synthesis by overexpression of the native fabR, dynamic regulating of TCA cycle by controlling the expression of sdh. Finally, the utilization pathway of acetic acid was enhanced by replacing the promoter of ackA-pta.

Fig. 1. Simplified metabolic pathways of 3-HP biosynthesis by engineered E. coli strain using acetic acid as carbon source under aerobic condition. AcP, acetyl-phosphate; Ac-CoA, Acetyl-CoA; 3-HP, 3-hydroxypropionic acid; CIT, citrate; ICT, isocitrate; GOX, glyoxylate; α-KG, α-ketoglutarate; SucCoA, succinyl-CoA; Suc, succinate; FUM, fumarate; MAL, malate; OAA, oxaloacetate; PEP, phosphoenolpyruvate; PYR, pyruvate. ackA, acetate kinase; pta, phosphotransacetylase; acc, acetyl-CoA synthetase; mcr, malonyl-CoA reductase; gltA, glutamate synthase; aceA, α-ketoglutarate dehydrogenase; sucAB, succinyl-CoA synthetase; suCD, succinate dehydrogenase; fabR, fatty acid degradation repressor; fabA, beta-hydroxacyl-ACP acyl carrier protein dehydrogenase; fabB, beta-ketoacyl-ACP acyl carrier protein synthase; fabD, fabF, fatty acid degradation repressor; Ptc-mut, modified trc promoter.
2. Experimental section

2.1. Strains and plasmids construction

All the bacterial strains and plasmids used in the experiments are described in Table 1, and all the primers used for amplification of different genes are listed in Table 2. The temperature-sensitive Promoter A promoter including repressor protein CI857, amplifying by PCR using the plasmid pET28a-M*DA, as template, was substituted for the native promoter of sdh. The native promoter of ackA-pmA in strain BL27 was replaced by the modified trc promoter (Prc-mut, Table 2) [31]. Deletion of fadR gene and replacement of native promoter of sdh or ackA-pmA based on E. coli BL27 were created using the one-step inactivation method [32]. For the fadR gene deletion, the kanamycin resistance cassette flanked by FRT was amplified from pKD4 using primers with homologous arm for homologous recombination.

The mer gene from C. aurantius was codon-optimized and synthesized by Sangon Biotech Co. Ltd.. To obtain the mutated mer (N940V, K1106W, and S1114R) gene from C. aurantius, amplification by PCR from primers F-mcr-mut/R-mcr-mut, F-N940V/R-K1106W, and F-S1114R/R-S1114R respectively. These three fragments were combined by overlap PCR with primers F-mcr-mut/R-S1114R. Gene segments of dtsR and accBC from C. glutamicum were amplified by PCR and then overlapped together to form dtsR-accBC. The ribosome binding sequence (AAGGAGATGAC) was added before the start codons of accBC and dtsR respectively. The mutated mcr gene was ligated to linear vector pET28a which was digested by Bsal and Sfil, yielding plasmid pET28a-mcr-mut. Then, the DNA fragments dtsR-accBC was inserted into pET28a-mcr-mut to form the plasmid pET28a-mcr-mut-dtsR-accBC (named as pET28a-M*DA).

2.2. Culture medium and conditions

Luria-Bertani (LB) medium (per liter: 10 g tryptone, 5 g yeast extract, 10 g sodium chloride) was used for strains construction and plasmids amplification. During strain and plasmid construction, the strains with the temperature-sensitive plasmids pKD46 and pCP20 were incubated at 42 °C for 3 h, 10 g sodium chloride) was used for strains construction and plasmids amplification. During strain and plasmid construction, the strains with the temperature-sensitive plasmids pKD46 and pCP20 were incubated at 42 °C for 3 h, and then at 37 °C for 8 h. For shake flask fermentation, the secondary pre-culture was inoculated into 5 mL of LB media and cultured at 37 °C for 220 rpm for 8 h. For shake flask fermentation, the secondary pre-culture was inoculated into 5 mL of LB media and cultured at 37 °C for 220 rpm for 8 h. The fermentation medium was prepared by supplementing the minimal M9 medium with 10 g/L of ammonium acetate and 5 g/L of yeast extract. The minimal M9 medium contained (per liter): 40 mg biotin, 15.1 g Na2HPO4.12H2O, 3.0 g KH2PO4, 0.5 g NaCl, 1.0 g NH4Cl, 0.5 g MgSO4.7H2O, 0.011 g CaCl2, and 0.2 mL of 1% (w/v) vitamin B1. The appropriate antibiotics were included at the following concentrations:
50 µg/mL kanamycin, 34 µg/mL chloramphenicol, and 100 µg/mL ampicillin. When the OD$_{600}$ reached around 1.0 or 2.5 due to different experiments, Isopropyl-β-D-thiogalactopyranoside (IPTG) was provided at a final concentration of 0.1 mM for inducing the overexpression of MCR and Acc. Cultures subsequently were incubated at 25°C for 3-HP production. The pH was maintained at about 7.0 by the addition of an appropriate amount of 3 M H$_2$SO$_4$ solution. All experiments had 3 biological replicates.

### 2.3. Syngas-derived acetic acid from biological culture broth

The biological culture broth of syngas-derived acetic acid was obtained from _M. thermoacetica_ (ATCC 49707) strain, which converted a gas mixture of CO$_2$ and CO or H$_2$ into acetic acid in an anaerobic bioreactor [33].
The anaerobic acetogen *M. thermoacetica*, was cultivated at 60 °C under strict anaerobic conditions in an enhanced culture medium containing the following components (per liter): 10 g morpholino ethane sulfonic acid (MES), 10 g Yeast Extract, 1.4 g KH₂PO₄, 1.1 g K₂HPO₄, 2.0 g (NH₄)₂SO₄, 0.5 g MgSO₄·7H₂O, 20 mL ATCC 1754 PETC trace elements solution (http://www.atcc.org), 10 mL of 0.3% cysteine solution, and 0.5 mL of 0.2% resazurin (a color indicator for anaerobic conditions).

The 1-L glass bubble column reactor was used for cultivation of *M. thermoacetica*. Throughout the fermentation process, gas composition of CO₂/CO/H₂ (4/3/3) was maintained constant with a four-channel mass flow controller and pH was controlled at around 6 by addition of 5 N NaOH or HCl. Prepared media (excluding cysteine), sterilized and then added into the reactor, was purged with oxygen-free nitrogen overnight. The cells were grown on syngas mixture with a total gas flow rate of 100 sccm (standard cubic centimeters per minute). A cysteine solution was added to remove dissolved oxygen in the medium for anaerobic growth conditions, and the bioreactor was inoculated with 5% v/v.

2.4. Whole-cell bioconversion for 3-HP production

The whole-cell fermentation experiments were performed using the concentrated genetically engineered *E. coli* strain LNY07(M*DA). In the whole-cell bioconversion experiments (40 OD₆₀₀), the preculture and culture conditions were the same as that of the previous shake-flask fermentation. The cells were harvested after 25 h cultivation (mid-log phase of growth) by centrifugation at 5500 rpm and 4 °C for 10 min, washed once with M9 medium and resuspended in 50 mL of the same medium supplemented with 20 g/L ammonium acetate or biological culture broth containing syngas-derived acetic acid. The flasks were incubated at 25 °C and 220 rpm. The pH was maintained at 7.0 using 3 M H₂SO₄.

2.5. Analytical methods

Growth was monitored by using a UV–visible spectroscopy system (Xinmao, Shanghai, China) at OD₆₀₀. Acetic acid and 3-HP were determined by high-performance liquid chromatography (HPLC) at 50 °C on an Aminex HPX-87H column (300 × 7.8 mm, Bio-Rad, USA) as well as a refractive index detector (RID) (Agilent, USA). A mobile phase of 2.5 mM H₂SO₄ solution was used at a flow rate of 0.5 mL/min. All culture samples were centrifuged at 12,000 rpm for 10 min and then filtered through a 0.22-μm filter before analysis.

3. Results and discussion

3.1. Effect of push-and-pull strategy on 3-HP accumulation

The push-and-pull strategy for metabolic engineering was applied in this study. As mentioned previously, there are generally two pathways to deplete acetic acid in *E. coli*, AckA-Pta pathway and Acs pathway [31, 34]. Both pathways can convert acetic acid to acetyl-CoA, but there are obvious differences between these two pathways. The AckA-Pta pathway was discovered to be central for mutual conversion between acetic acid and acetyl-CoA [27]. The direction of reaction in this pathway can be rapidly transformed from acetic acid production to acetic acid consumption [35], which is a major pathway for acetic acid assimilation. In contrast, Acs pathway is responsible only for conversion acetic acid to acetyl-CoA. For acetic acid uptake, the former pathway consumes less energy than the latter because AcS in *E. coli* consumes ATP and produces AMP instead of ADP [36]. Mutant cells lacking AckA-Pta pathway grew poorly in high concentrations of acetic acid [37]. Previous studies have shown that when high concentrations of acetic acid is used as carbon source, the AckA-Pta pathway is the main route for acetic acid assimilation [31,34]. In previous study, the plasmid pET28a-MDA has been proven enough to produce 3-HP at high concentration from glucose [38]. We constructed the pET28a-M*DA (MCR with 3 mutations) (N940V, K1106W, and S114R) on the basis of pET28a-MDA, since the MCR with 3 mutations (N940V, K1106W, and S114R) performed much better than MCR in the production of 3-HP from glucose [20]. Compared with BL27 (MDA), BL27 (M*DA) achieved a higher 3-HP production (~1.75-fold more) and yield on acetic acid (0.44 g/g vs. 0.23 g/g) (Figs. 2C and 5). It indicated that the mutant of MCR can still maintain the higher activity in the cultivation of acetic acid as well as glucose.

Fig. 4. Profiles of cell density (A), acetic acid concentration (B), 3-HP concentration (C) and yield of 3-HP (D) in cultivation of different strains: LNY05 (M*DA), LNY06 (M*DA), and LNY07 (M*DA).
yeast extract is mostly used for the growth of *E. coli*, and acetic acid is the main carbon source for the production of 3-HP. In order to enhance acetic acid utilization and improve 3-HP production in our engineering strains, the native promoter shared by *ackA* and *pta* genes in the strain *E. coli* BL27 was replaced to a modified *trc* promoter (*Ptrc-mut, Table 2*), yielding strain LNY01. The strain LNY01(M*DA*) produced 3.22 g/L of 3-HP in 48 h, about 13.0% higher than that of BL27 (M*DA*) (Fig. 2A and B). And the yield of 3-HP in LNY01 (M*DA*) reached 0.472 g/g, which is about 8.0% higher than that of the control strain. The results indicated that promoting the acetic acid up-take rate was beneficial for the 3-HP production.

When *E. coli* grows on acetic acid as a sole carbon source, the glyoxylate cycle is a critical and up-regulated, which can replenish dicarboxylic acid intermediates from the TCA cycle for cell metabolism and increase the utilization rate of exogenous acetic acid [39]. In the pathway, isocitrate is cleaved by isocitrate lyase (encoded by *aceB*) to succinate and glyoxylate (Fig. 1) [40]. IclR (isocitrate lyase repressor, encoded by *iclR*) is known as a repressor protein binding to a site which overlaps the aceB promoter [41]. Thus, the most common approach to enhance the glyoxylate cycle is deletion of *iclR*. FadR (fatty acid degradation repressor) is recognized as a fatty acid metabolism regulator, which not only represses fatty acid degradation pathway [42,43], but also activates expression of genes essential for the unsaturated fatty acid synthesis [44]. Additionally, it has been reported that FadR activates the expression of *iclR* by binding to a part of the upstream site of the *iclR* promoter [41]. In this work, we investigated the effect of deletion of *fadR* on 3-HP production. The *fadR* gene was deleted in *E. coli* BL27 strain, resulting in LNY02 strain. And the plasmid pET28a-M*DA* was inserted into LNY02 to obtain the LNY02(M*DA*) strain. It was found that the cell biomass of strain LNY02(M*DA*) were enhanced (Fig. 2A), and the 3-HP production and acetic acid assimilation rate were also higher than that of strain BL27 (M*DA*) (Fig. 2B and C). LNY02 (M*DA*) produced 3.16 g/L 3-HP, which was 10.9% higher than that of BL27 (M*DA*). In addition, the yield of 3-HP on acetic acid (0.46 g/g) was increased slightly due to the increased substrate consumption and product accumulation. The consumption rate of acetic acid was significantly increased by combining with “push” (enhancing the pathway of acetic acid uptake by overexpression of *ackA-pta*) and “pull” (enhancing the pathway of acetic acid utilization by deletion of *fadR*). Between 16 and 32 h, the acetic acid consumption rate of strain LNY04(M*DA*) reached 0.3 g/L/h, which was a 68% increase compared to BL27 (M*DA*). The results demonstrated that the deletion of *fadR* shown a positive effect on the 3-HP production and yield.

Although the cell growth, 3-HP production, and acetic acid uptake rate were successfully enhanced, the yield was still low. The native promoter of *ackA* and *pta* in LNY02 was further replaced, yielding the LNY04 strain. Compared to BL27 (M*DA*), LNY04(M*DA*) showed improved 3-HP titer by 9.1% (from 2.85 to 3.11 g/L) in 48 h cultivation (Fig. 2A and C). In addition, all acetic acid had been consumed by the strain LNY04(M*DA*) in 32 h (Fig. 2B), and the 3-HP production rate of LNY04(M*DA*) was also enhanced significantly (Fig. 2C). It indicated the strain has great potential to convert acetic acid into 3-HP.

### 3.2. Effect of temperature-controlled TCA cycle on 3-HP accumulation

To further regulate the carbon flux and improve 3-HP accumulation, we decided to dynamically regulate TCA cycle by controlling the expression of *sdh*. The bacteriophage λ promoters (P*E*, P*P*) enable a simple temperature change to switch on-off the expression of genes efficiently and rapidly by using the temperature-sensitive repressor CI857 [45,46]. In a previous study, the native lactate dehydrogenase gene (*ldhA*) in *E. coli* were controlled by the promoters, which repressed *ldhA* when growth at 33 °C and was able to increase the biomass yield by 10% (compared with the static strategy), while switching to 42 °C induced the expression of *ldhA* and increased the production of lactate [45]. In our case, *sdh* is active under the control of the P*P* promoter during cell growth (favored by an inactive CI857 at 39 °C) and inactive due to CI857 is active at low temperatures and binds to the P*P* promoter in the phase of 3-HP production (favored by an active CI857 at 25 °C). Through the dynamic regulation of *sdh*, we expect to decouple the growth and 3-HP production. We obtained LNY03, LNY05, LNY06 and LNY07 strains by changing the native promoter of *sdh* into the temperature-sensitive promoter P*E* in BL27, LNY01, LNY02 and LNY04 strain, respectively. As the native promoter is replaced, different OD_{600} of induction were studied in the strain LNY03(M*DA*). When the OD_{600} of induction was 1, only 2.46 g/L of acetic acid was consumed, about 28% of the initial acetic acid with least 3-HP accumulation (Fig. 3). And when the OD_{600} of induction was 2.5, LNY03(M*DA*) produced the highest concentration of 3-HP. The experimental results showed that the expression level of SDH affects carbon flux distribution and it was found...
that the optimum OD_{600} value for induction of the 3-HP biosynthesis pathway was 2.5 (Fig. 3). The strains LNY03(M*DA), LNY05(M*DA), LNY06(M*DA) and LNY07(M*DA) generated 3.48, 3.45, 3.46 and 3.92 g/L of 3-HP in 48 h (Figs. 3B and 4B), which were 22.1, 7.2, 9.5 and 26.0% higher than those of the control strains without promoter exchanging. The yield of 3-HP in LNY07 (M*DA) reached 0.57 g/g, which is about 76% of theoretical maximum (0.75 g/g) (Fig. 5). The result showed that replacing the promoter of sdh with the temperature-sensitive promoter enhanced the rate of 3-HP production significantly.

3.3. The 3-HP production in whole-cell bioconversion of engineered E. coli strain

Whole-cell catalysis has the advantages of higher cell density, higher product yield and productivity, lower energy requirements, etc. In the experiment we used concentrated E. coli strain LNY07(M*DA), which had both a high titer and high yield of 3-HP as whole-cell biocatalyst, acetic acid as the sole carbon source. The initial OD_{600} of LNY07 (M*DA) was around 40. After 44 h of bioconversion, almost all the acetic acid was consumed and 15.8 g/L 3-HP was obtained (Fig. 6A), with the yield increasing to 0.71 g/g, about 94% of the maximum theoretical pathway. The titer of 3-HP in the biotransformation was significantly higher than the concentration obtained using fed-batch cultures. The titer of 3-HP increased significantly due to the high cell density, and it indicated that the carbon metabolic flux were redirected into the 3-HP production pathway by whole-cell bioconversion. The cell density decreased during the process, which may occur due to the repeatedly addition of acetic acid (at 17 and 34 h) (Fig. 6A), which was to prolong the production of 3-HP and better test the life span of 3-HP production activity. And the production rate of 3-HP was maintained constantly during the cultivation, about 0.35 g/L/h.

3.4. Utilization of syngas-derived acetic acid for 3-HP

Here, the syngas-derived acetic acid, which was produced by M. thermoacetica, was used as a sole carbon source for 3-HP biosynthesis by LNY07(M*DA). The results of acetic acid consumption, 3-HP production and cell density during the cultivation are shown in Fig. 6B. The initial concentration of acetic acid in the syngas fermentation broth was 20.5 g/L, and the initial OD_{600} of LNY07(M*DA) was around 39. As a result, the strain consumed almost all the acetic acid in the biologically produced culture medium and accumulated 11.2 g/L 3-HP from the syngas-derived acetic acid. The titer of 3-HP dropped due to the initial concentration of acetic acid in the biologically produced culture medium. No additional acetic acid was added during the cultivation. In addition, the cell density did not show any significant decrease compared to the condition of using chemically synthesized acetic acid. The complex composition in the culture broth of M. thermoacetica may help the cell density. Since the yield of 3-HP dropped a little (0.55 g/g), it indicated that more acetic acid maybe used to the cell maintenance than that of using chemically synthesized acetic acid. In this study, the current titer of 3-HP from syngas-derived acetic acid was the highest concentration ever reported. This result indicates a great potential for the metabolically engineered E. coli strain to generate 3-HP from syngas-derived acetic acid.

The current results can be compared with other studies using E. coli as host to produce 3-HP from acetic acid. The engineered E. coli converted 8.98 g/L of acetic acid into 3.00 g/L of 3-HP in 48 h cultivation with overexpression of mcr and acs and deletion of iclR, when 50 μM cerulenin was added to repress fatty acid synthesis pathway [29]. In two-stage bioreactor (glucose is used for cell growth and acetic acid for 3-HP formation), the engineered E. coli strain with upregulated glyoxylate shunt produced 7.3 g/L of 3-HP with yield of 0.26 mol/mol (0.39 g/g) [30]. In comparison, the engineered E. coli LNY07(M*DA) obtained 15.8 g/L of 3-HP with the yield of 0.71 g/g from chemically synthesized acetic acid and 11.2 g/L of 3-HP with the yield of 0.55 g/g from syngas-derived acetic acid. The study demonstrates an effective route to produce 3-HP from acetic acid. Despite the encouraging results in the whole-cell bioconversion experiment, there are still challenges. Compared with the use of glucose as the substrate, when acetic acid is used as the substrate, the cell growth is slower and the final titer of 3-HP is lower. Studies have been conducted to balance the activities of key enzymes and use glucose to synthesize 40.6 g/L of 3-HP in E. coli [28]. In order to increase the production of 3-HP from acetic acid, further strain modification or bioprocess optimization should be considered.

4. Conclusions

In this study, the engineered E. coli strains can produce 3-HP using acetic acid efficiently. Several strategies were applied to enhance 3-HP production from acetic acid, including exchanging promoter of ackApta, deletion of fadR, and temperature-controlling the expression of sdh. The engineered strain LNY07(M*DA) produced 15.8 g/L of 3-HP with the yield of 0.71 g/g from chemically synthesized acetic acid and 11.2 g/L of 3-HP with the yield of 0.55 g/g from syngas-derived acetic acid. The results demonstrate an effective route to use syngas-derived acetic acid as raw materials to produce 3-HP and other important chemicals, especially malonyl-CoA derived compounds.
Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Ningyu Lai: Formal analysis, Writing – original draft. Yuanchan Luo: Formal analysis. Peng Fei: Formal analysis. Peng Hu: Formal analysis. Hui Wu: Conceptualization, Supervision.

Acknowledgements

We thank Professor Shu Quan for kindly providing us with the strain, E. coli BL27. This study was supported by the Natural Science Foundation of Shanghai (19ZR1472700), the Fok Ying-Tong Education Foundation, China (Grant No. 161017), the National Natural Science Foundation of China (Grant No. 21776083), the Fundamental Research Funds for the Central Universities (Grant No. 22221818014). Partially supported by Open Funding Project of the CAS Key Laboratory of Synthetic Biology.

References

[1] Dibenedetto A, Nocito F. The future of carbon dioxide chemistry. ChemSusChem 2020;13:11–11. https://doi.org/10.1002/cssc.202000205.
[2] Shen S, Wang G, Zhang M, Tang Y, Gu Y, Jiang W, Wang Y, Zhan Z. Effect of temperature and surfactant on biomass growth and higher-alcohol production during syngas fermentation by Clostridium carboxydrivorum P7. Bioresearches and Bioprocessing 2020;2:56. https://doi.org/10.18403/2020-00344-4.
[3] Burkart MD, Hazari N, Tway CL, Zeitzer EL. Opportunities and challenges for catalysis in carbon dioxide utilization. ACS Catal 2019;9:7397–56. https://doi.org/10.1021/acscatal.9b02116.
[4] Liu H, Song T, Fei K, Wang H, Xie J. Microbial electrocatalysis of organic chemicals from CO₂ by Clostridium scatologenes ATCC 25775. Bioresearches and Bioprocessing 2018;5:7. https://doi.org/10.18403/2018-01819-5.
[5] Unrean P, Tee KL, Wong TS. Metabolic pathway analysis for in silico design of chemicals from CO₂ by metabolic engineered Pyrococcus furiosus. Bioresources and Bioprocessing 2019;4:69. https://doi.org/10.18403/2019-01928-4.
[6] Xrpt E. DOE invests $17 million to advance carbon utilization projects [accessed October]. https://www.energy.xrpt.com/news/doe-invests-17-million-to-advance-carbon-utilization-projects-990539.
[7] Fei Q, Chang HN, Shiang L, Choi J-D.R. Exploring low-cost carbon sources for microbial lipids production by fed-batch cultivation of Cryptococcus albidos. Biotechnol Bioproc Eng 2011;16:462–7. https://doi.org/10.1007/s13205-010-0070-y.
[8] Hu P, Rismansi-Yazdi H, Stephanopoulos G, Aneron, CO₂ fixation by the acetogenic bacterium Morethella microbiola. AIChE J 2013;59:3176–83. https://doi.org/10.1002/aic.14127.
[9] Ge X, Yang L, Sheets JP, Yu Z, Li Y. Biological conversion of methane to liquid fuels: status and opportunities. Biotechnol Bioeng 2011;108:686–705. https://doi.org/10.1002/bit.23799.
[10] Song H, Choi O, Pandey A, Kim YG, Joo JS, Sang B-I. Simultaneous production of methane and acetate by thermophilic mixed culture from carbon dioxide in a bioreactor. J Biotechnol Bioeng 2019;6:49. https://10.1186/s40643-019-0282-4.
[11] Li Y, Huang B, Yang H, Fang G, Zhang X, Wu H. Efficient isopropanol biosynthesis by engineered Escherichia coli. Biotechnol Bioeng 2020;21. https://10.1002/bit.26508.
[12] Brown TD, Jones-Mortimer MC, Kornberg HL. The enzymic interconversion of acetate and acetyl-coenzyme A in Escherichia coli W. Biotechnol Bioeng 2018;113:2652–60. https://doi.org/10.1002/bit.26303.
[13] Liu R, Li, Yang Z, Liu H, Liu X, Miao A, Zhao G. Malonyl-CoA pathway: a promising route for 3-hydroxypropionate biosynthesis. Curr Rev Biotechnol 2017;9:923–41. https://doi.org/10.1016/j.jbior.2017.07.009.
[14] Maloy SR, Nunn WD. Role of gene fadR in the regulation of fatty acid degradation in Escherichia coli. J Bacteriol 1981;5:1038–45. https://doi.org/10.1128/jb.141.8.1038-1045.1981.
[15] Wang B, Wang P, Zheng E, Chen X, Zhao H, Song P, Su R, Li X, Zhu G. Biochemical properties and physiological roles of NADP-dependent malic enzyme in Escherichia coli. J Microbiol 2011;49:797–802. https://10.1007/s13205-010-0457-5.
[16] Qi L, Sunnarborg A, Laporte DC. Regulated expression of a repression reporter: FadR activates iclR. J Bacteriol 1996;178:4704–9. https://10.1128/jb.178.15.4704-4709.1996.
[17] Clark D. Regulation of fatty acid degradation in Escherichia coli: analysis by operon fusion. J Bacteriol 1981;148:485–91. https://10.1128/jb.148.1.485-491.1981.
[18] Hughes KT, Simons RW, Nunn WD. Regulation of fatty acid degradation in Escherichia coli: fadR superrepressor mutants are unable to utilize fatty acids as the sole carbon source. J Bacteriol 1988;170:1666–71. https://10.1128/jb.170.4.1666-1671.1988.
[44] Nunn WD, Giffin K, Clark D, Cronan Jr JE. Role for fadR in unsaturated fatty acid biosynthesis in Escherichia coli. J Bacteriol 1983;154:554-60. https://10.1128/JB.154.2.554-560.1983.

[45] Zhou L, Niu DD, Tian KM, Chen XZ, Prior BA, Shen W, Shi GY, Singh S, Wang ZX. Genetically switched D-lactate production in Escherichia coli. Metab Eng 2012;14:560-8. https://10.1016/j.mib.2012.05.004.

[46] Venayak N, Anesiadis N, Cluett WR, Mahadevan R. Engineering metabolism through dynamic control. Curr Opin Biotechnol 2015;34:142–52. https://10.1016/j.copbio.2014.12.022.