Synthetic and Systems Biotechnology 9 (2024) 531–539

Contents lists available at ScienceDirect

Synthetic and Systems Biotechnology

journal homepage: www.keaipublishing.com/en/journals/synthetic-and-systems-biotechnology

Original Research Article

Biotransformation of ethylene glycol by engineered *Escherichia coli*

Wenlong Yan [a,b,1], Xinhua Qi [a,b,1], Zhibei Cao [a,b], Mingdong Yao [a,b], Mingzhu Ding [a,b,*], Yingjin Yuan [a,b]

[a] Frontiers Science Center for Synthetic Biology and Key Laboratory of Systems Bioengineering (Ministry of Education), School of Chemical Engineering and Technology, Tianjin University, Tianjin, 300072, China
[b] Frontiers Research Institute for Synthetic Biology, Tianjin University, Tianjin, 300072, China

ARTICLE INFO

Keywords:
PET biological recycling
Ethylene glycol utilization
Glycolic acid production
Escherichia coli
Synthetic biology
Metabolomics analysis

ABSTRACT

There has been extensive research on the biological recycling of PET waste to address the issue of plastic waste pollution, with ethylene glycol (EG) being one of the main components recovered from this process. Therefore, finding ways to convert PET monomer EG into high-value products is crucial for effective PET waste recycling. In this study, we successfully engineered *Escherichia coli* to utilize EG and produce glycolic acid (GA), expecting to facilitate the biological recycling of PET waste. The engineered *E. coli*, able to utilize 10 g/L EG to produce 1.38 g/L GA within 96 h, was initially constructed. Subsequently, strategies based on overexpression of key enzymes and knock-out of the competing pathways are employed to enhance EG utilization along with GA biosynthesis. An engineered *E. coli*, characterized by the highest GA production titer and substrate conversion rate, was obtained. The GA titer increased to 5.1 g/L with a yield of 0.75 g/g EG, which is the highest level in the shake flake experiments. Transcriptional level analysis and metabolomic analysis were then conducted, revealing that overexpression of key enzymes and knock-out of the competing pathways improved the metabolic flow in the EG utilization. The improved metabolic flow also leads to accelerated synthesis and metabolism of amino acids.

1. Introduction

Polyethylene terephthalate (PET) is a widely used synthetic plastic, obtained through the polymerization of terephthalic acid (TPA) and ethylene glycol (EG) [1]. In the early 20th century, PET was initially employed for producing disposable plastic bottles and rapidly popularized worldwide, which had become an indispensable part of people's life [2]. Nevertheless, owing to inadequate treatment strategies and the robust mechanical characteristics of PET plastic products, the widespread utilization of PET has given rise to grave environmental challenges, including soil pollution and disruptions in marine ecosystems [3–5]. Presently, conventional physical and chemical methods continue to be prevalent, resulting in significant environmental harm and substantial energy consumption [6–8]. Hence, it becomes imperative to explore a gentle and efficient approach for PET degradation. PET biodegradation has attracted more and more attentions as an environmentally friendly alternative, requiring mild temperature and low energy consumption [9–11]. Furthermore, PET degradation products are readily recyclable, offering the potential for the biological recycling of PET waste.

Certain wild or engineered strains have been proved to secrete PET-degrading enzymes to degrade PET [12–15]. PET degradation monomers can undergo further metabolism by other strains, ultimately transforming into high-value chemicals. Our laboratory has constructed an artificial microbial consortia system to degrade PET [16]. Our findings reveal that EG, one of the products of PET degradation, exerts a specific inhibitory effect on PET hydrolase. Related studies also reported that EG was identified as one of the competitive inhibitors of PET hydrolase [17]. Additionally, EG exhibits toxicity to strains, thus impeding microbial growth and adversely affecting degradation efficiency [18]. Consequently, developing engineered strains capable of utilizing EG can effectively enhance the enzymatic hydrolysis of PET. Furthermore, the conversion of EG into other high-value products via recombinant strains contributes to PET recycling.

Peer review under responsibility of KeAi Communications Co., Ltd.
* Corresponding author. Frontiers Science Center for Synthetic Biology and Key Laboratory of Systems Bioengineering (Ministry of Education), School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, China.
E-mail address: mzding@tju.edu.cn (M. Ding).
[1] The authors contributed equally to this work.

https://doi.org/10.1016/j.synbio.2024.04.006
Received 19 December 2023; Received in revised form 5 March 2024; Accepted 8 April 2024
Available online 11 April 2024

Glycolic acid (GA), an alpha-hydroxy acid, is widely used in the synthesis of plastics, polymers, cosmetics, and industrial detergents [19–21]. Traditional chemical methods of producing GA suffer from poor selectivity and excessive by-products, making the separation and purification process challenging. Therefore, producing glycolate through biological methods is a promising alternative that can address these issues [22]. Conventional bio-based production methods rely highly on sugar-based feedstocks, such as glucose and xylose. For instance, Pereira et al. [23] successfully engineered *E. coli* to produce GA using xylose as a carbon source, achieving a yield of 0.63 g/g xylose. Alkim et al. [24] constructed an *E. coli* strain that produces GA via the glyoxylate and synthetic xylulose-1 phosphate pathway from xylose-rich sugar mixtures, with the GA yield on the xylose fraction of the sugar mixture reaching 0.75 g/g. In another study, a synergetic system was designed to simultaneously utilize acetate and glucose to produce GA, glucose providing NADPH while acetate providing the main carbon backbone, achieving a final GA yield of 73.3 g/L with a productivity of 1.04 g/(L·h) [25]. Deng et al. [19] balanced the flux distributions between the tricarboxylic acid (TCA) cycle and glyoxylate shunt in *E. coli* to direct more carbon flux to GA, achieving a 0.504 g/g-glucose yield. In another research utilizing glucose as carbon backbone for GA production, a GA-responsive biosensor was established and agar plate- and 48-well deep-well plate-scale high-throughput screening methods was developed for the rapid screening of superior GA producers from a large library [26]. The optimum strain was obtained after rounds of screening, achieving a titer of 40.9 ± 3.7 g/L GA. Instead of using sugar-based feedstocks, we propose utilizing EG, a main PET degradation product, to produce GA. This approach is conducive to realize the conversion of PET waste to high-value products, achieving the up-cycling of PET waste. Another motivation in utilizing EG as a feedstock derives from that it can be obtained from CO_2, either through electrochemical reduction or other conversion technologies [27,28]. Therefore, considering EG as a substrate can potentially sequester carbon.

In order to realize the upgrading and utilization of PET monomer EG, engineered *E. coli* MGG capable of utilizing EG to produce GA was obtained. Furthermore, different strategies were employed to enhance GA biosynthesis. Among the engineered strains, the MGGA strain exhibited the fastest full utilization of EG. Moreover, the FYGA strain was characterized by the highest substrate conversion rate. To further understand the mechanisms underlying the increased EG utilization rate and GA production, transcriptional level analysis and metabolomic analysis of the engineered strains were conducted.

2. Materials and methods

2.1. Culture conditions

The strains used in this study are shown in Table 1. Strains were grown using lysogeny broth (LB) for all strain construction and fermentation pre-cultures. Pre-cultures were grown in LB media in 5 mL test tube cultures overnight at 37 °C, 220 rpm and transferred to fresh 250 mL shake-flaks containing 50 mL LB at the initial OD_{600} value of 0.2, culturing to logarithmic growth stage under the same condition. Then the cultures were transferred to fresh 250 mL shake-flaks containing 50 mL fermentation medium at the initial OD_{600} value of 0.2 under the same condition. We used modified M9 media containing 10 g/L EG, 1 g/L NH_4Cl, 3 g/L KH_2PO_4, 6.8 g/L Na_2HPO_4, 0.5 g/L NaCl, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.1% (V/V) trace metal solution and 2 g/L yeast extract. Trace metal solution was prepared by dissolving 1.6 g $FeCl_3$, 0.2 g $CoCl_2$·6 H_2O, 0.1 g $CuCl_2$, 0.2 g $ZnCl_2$·4H_2O, 0.2 g $NaMoO_4$ and 0.05 g H_3BO_3 in 1 L 0.1 M HCl solution. For all cultures, ampicillin was added as appropriate at 100 μg/mL. During the fermentation of engineered *E. coli*, when the OD_{600} value reached 0.6, 1 mM IPTG was added to induce the full expression of heterologous genes.

Table 1
Strains and plasmids used in this study.

Name	Relevant genotype	Reference
Strains		
MG1655 (DE3)	Wild-type (DE3)	Beijing Zoman
MG1655	Wild-type	Beijing Zoman
MGE	*E. coli* MG1655, pEc01001	This study
MGEQ	*E. coli* MG1655, pEc01002	This study
MGO	*E. coli* MG1655, pEc01003	This study
MGG	*E. coli* MG1655; pEc01004	This study
MGGA	*E. coli* MG1655; pEc01005	This study
FYG	*E. coli* MG1655, Δ*fucO*, Δ*yqhD*, pEc01004	This study
FYGA	*E. coli* MG1655, Δ*fucO*, Δ*yqhD*, pEc01005	This study
GLCG	*E. coli* MG1655, Δ*GlcDEF*, pEc01004	This study
GLCGA	*E. coli* MG1655, Δ*GlcDEF*, pEc01005	This study
FYGLCG	*E. coli* MG1655, Δ*fucO*, Δ*yqhD*, Δ*GlcDEF*, pEc01004	This study
Plasmids		
pET-28a (+)	Expression plasmid of *E. coli*, T7, Kan^R	This laboratory
pTrc99a	Expression plasmid of *E. coli*, trc, Amp^R	This laboratory
pKD46	araBp-gam-bet-exo, bla (Amp^R), repA101 (ts), oriR101	This laboratory
pEc01001	pET-28a (+): *PedE*	This study
pEc01002	pET-28a (+): *PQQ-PedE*	This study
pEc01003	pTrc99a: *fucO*	This study
pEc01004	pTrc99a: *Gox0313*	This study
pEc01005	pTrc99a: *Gox0313-aldA*	This study

2.2. Plasmids construction

All plasmids used in this study are shown in Table 1. The codon was optimized with *E. coli* MG1655 as the chassis, and the heterologous genes *PedE*, *PQQ* and *Gox0313* were synthesized. The RBS sequence was added before the synthesis of the heterologous genes, and the restriction sites were introduced at both ends of the synthetic fragment. The gene sequences are shown in Supplementary Table 1. The *PedE* gene and plasmid vector pET-28a (+) were assembled by *Xba*I and *Sal*I double digestion and T4 connection to obtain the engineered plasmid pEc01001. The *PQQ* gene and plasmid pEc01001 were digested by *Xba*I and *Hin*dIII and combined with T4 to obtain the engineered plasmid pEc01002. During the construction of the engineered plasmid pEc01003, *fucO (I7L/L8V)* fragment was obtained from the *E. coli* genome by designing the primer front-end sequence, as well as adding RBS sequence and restriction endonuclease site. The fragment and plasmid vector pTrc99a were assembled by *Bam*HI and *Eco*RI double restriction endonuclease and T4 connection to obtain the engineered plasmid pEc01003. The *Gox0313* gene and plasmid vector pTrc99a were digested by *Bam*HI and *Eco*RI and combined with T4 to obtain the engineered plasmid pEc01004. The *aldA* gene fragment was obtained from the *E. coli* genome by PCR. RBS sequence and restriction endonuclease sites *Xba*I and *Sal*I were added when designing the primer sequence. The engineered plasmid pEc01005 was obtained by combining the *aldA* gene fragment with the plasmid vector pEc01004 through *Bam*HI and *Eco*RI double restriction endonuclease and T4 connection.

2.3. Strains engineering

λ-Red recombination [29] was used for the deletion of *fucO*, *yqhD* and *GlcDEF* in *E. coli* MG1655. The *fucO*, *yqhD* and *GlcDEF* gene fragments were obtained from the *E. coli* genome by PCR. The plasmid pKD46 was transferred into *E. coli* MG1655 firstly. The upper and lower 500 bp homologous arms of target gene to be knocked out were obtained by PCR. The homologous arm was connected with the resistance label through overlap homologous recombination, obtaining the target fragment. Then, the target fragment was transferred into *E. coli* MG1655

(DE3) expressing the λ-Red recombinase from pKD46 induced by 10 mM arabinose. To obtain the colonies in which the target genes were replaced by the resistance label, colony PCR was performed using the verified primers. The resulting mutant strains were confirmed by colony PCR with the verified primers. As pKD46 has temperature sensitive origins of replication, it was propagated at low temperature (30 °C), and eliminated at high temperature (37 °C). All the transformations were performed by electroporation.

2.4. Analysis of EG and GA

In order to detect the concentration of metabolites, the strain culture was centrifuged for 5 min at 12,000 rpm, and the supernatant was then filtered through a 0.22 μm syringe filter. The concentrations of EG and GA were measured via high performance liquid chromatography (HPLC) with an HPX-87H ion exchange column (7.8 mm × 300 mm; Bio-Rad, Hercules, CA, USA) using a refractive index and UV detectors. The mobile phase was 5 mM sulfuric acid at a flow rate of 0.6 mL/min, and the HPLC column was operated at 60 °C.

2.5. Transcription level analysis

Engineered strains MGG, MGGA, FYG, FYGA, GLCG, GLCGA in the middle log-phase were harvested for mRNA isolation. The harvested strains were treated according to the manufacturer's manual using a Column Viral RNAOUT Kit (TianDZ, Beijing, China). cDNA was synthesized using a SPARKscript II RT Kit. Subsequently, qPCR was performed using 2 × SYBR qPCR Mix. Taking the endogenous gene *gapA* of *E. coli* MG1655 as the reference gene, the transcriptional level difference of genes involved in the pathway of utilizing EG to produce GA in the engineered strain was analyzed.

2.6. Untargeted metabolomics analysis

The fermentation broth of strains MGG, MGGA and FYGA was centrifuged at 5000 rpm, 4 °C for 10 min to obtain the strains, and the supernatant was discarded. PBS buffer was added to blow and suck for cleaning the strains, and the cleaned strains were obtained by centrifugation at 5000 rpm, 4 °C for 10 min, and the supernatant was discarded (repeat this operation). Quickly store the strains in a −80 °C refrigerator. The samples were thawed slowly at 4 °C 25 mg strains were weighed. 800 μL extract and 10 μL internal standard were added. The mixed samples were ground for 5 min. Then, the ultrasonication was performed at 4 °C for 10 min. And the obtained sample were refrigerated at −20 °C for 1 h. The samples were centrifuged at 25,000 rpm, 4 °C for 15 min 600 μL supernatant was taken and 200 μL complex solution (methanol: water = 1:9) was added. After ultrasound, the mixed solution was centrifuged at 25,000 rpm, 4 °C for 15 min. The obtained supernatant was transferred to the sample bottle.

BEH C18 column (2.1 × 100 mm) was used. Liquid A of mobile phase in positive ion mode is 0.1 % formic acid solution, and liquid B is methanol solution containing 0.1 % formic acid. Liquid A of mobile phase in negative ion mode is 10 mM ammonium formate solution, and liquid B is 95 % methanol solution containing 10 mM ammonium formate. The flow rate of mobile phase was set at 0.35 mL/min, the column temperature was set at 45 °C, and the single injection volume was set at 5 μL. The gradient program was as follows: 0–1 min, 2 % liquid B; 1–9 min, 2%–98 % liquid B; 9–12 min, 98 % liquid B; 12–12.1 min, 98%–2% liquid B; 12.1–15 min, 2 % liquid B.

3. Results and discussion

3.1. Construction of the primary strains for EG utilization

To construct a strain that could utilize EG, *E. coli* MG1655 was chosen as a suitable chassis strain, which possesses the metabolic pathway of endogenous utilization of glycolic aldehyde. The endogenous glyoxylic acid pathway in MG1655 is shown in Fig. 1a.

While MG1655 possesses an endogenous glyoxylic acid pathway that allows glycolic aldehyde to enter the tricarboxylic acid cycle, it lacks the oxidase that can catalyze the conversion of EG to glycolic aldehyde. It suggests that wild *E. coli* MG1655 may be unable to utilize EG. Subsequently, to verify whether MG1655 can utilize EG, MG1655 was cultivated in M9 medium with 10 g/L EG as the major carbon source. As depicted in Fig. 1b, after 104 h of cultivation, the concentration of EG in the medium exhibited minimal change, and the OD_{600} value of MG1655 remained below 2. These results indicate that wild *E. coli* MG1655 cannot directly use EG as a sole carbon source for growth, consistent with the research of Boronat et al. [30]. The slight initial increase in the OD_{600} value may be attributed to that *E. coli* utilized other nutrients in the culture medium during the initial growth stage.

Therefore, the key to construct engineered *E. coli* capable of utilizing EG and producing GA lies in achieving the conversion of EG to glycolic aldehyde. In this study, three different genes including the alcohol dehydrogenase gene *PedE* from *Pseudomonas putida* KT2440, along with the corresponding cofactor pyrroloquinoline quinone gene (*PQQ*), the *Gox0313* gene from *Gluconobacter oxydans*, and the *fucO* variant *fucO (I7L/L8V)* from the *E. coli* gene *fucO* were chosen as oxidases to catalyze the conversion of EG into glycolic aldehyde. *E. coli* MG1655 served as the chassis for constructing the EG utilization pathway, as illustrated in Fig. 2a. MGE, MGEQ, MGO and MGG strains were obtained by expressing *PedE*, *PedE* and cofactor *PQQ*, *fucO* variant *fucO (I7L/L8V)* and *Gox0313* genes in *E. coli* MG1655, respectively.

PedE enzyme is a pyrroloquinoline quinone dependent alcohol dehydrogenase (PQQ-ADH), whose function relies on the presence of PQQ cofactors. It has been proved that *E. coli* cannot produce PQQ by itself, but it can effectively express PQQ-ADH by adding exogenous PQQ or modifying *E. coli* to produce PQQ by genetic engineering. Therefore, the strain MGE needs to add 1 mM PQQ cofactor when fermenting to ensure the realization of enzyme function. The shaking flask fermentation experiment was conducted in M9 medium with 10 g/L EG as the major carbon source (Fig. 2b). Compared with the control strain MG1655, the strains MGE, MGEQ and MGO can utilize a small amount of EG, whereas MGG demonstrated complete utilization of 10 g/L EG for growth within 96 h. The OD_{600} value and GA production of strain MGG during fermentation were determined. The strain MGG could not only grow with 10 g/L EG as the major carbon source, but also convert it into GA.

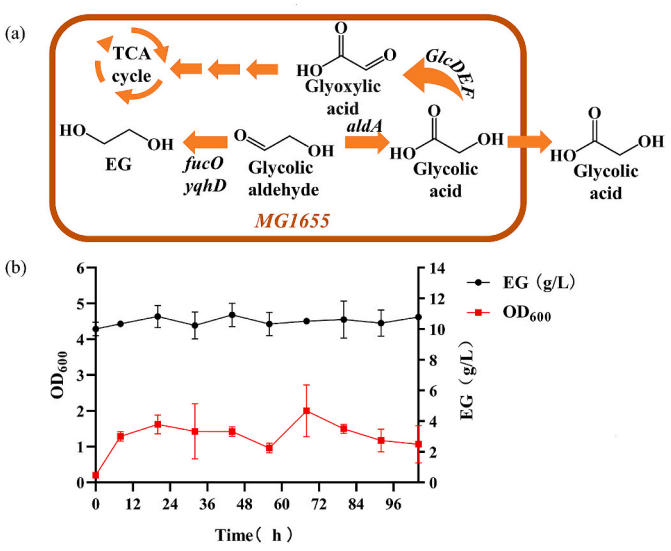

Fig. 1. The endogenous pathway and EG utilization of *E. coli* MG1655. (a) The endogenous glyoxylic acid pathway in *E. coli* MG1655 (b) Cell growth and EG utilization of *E. coli* MG1655.

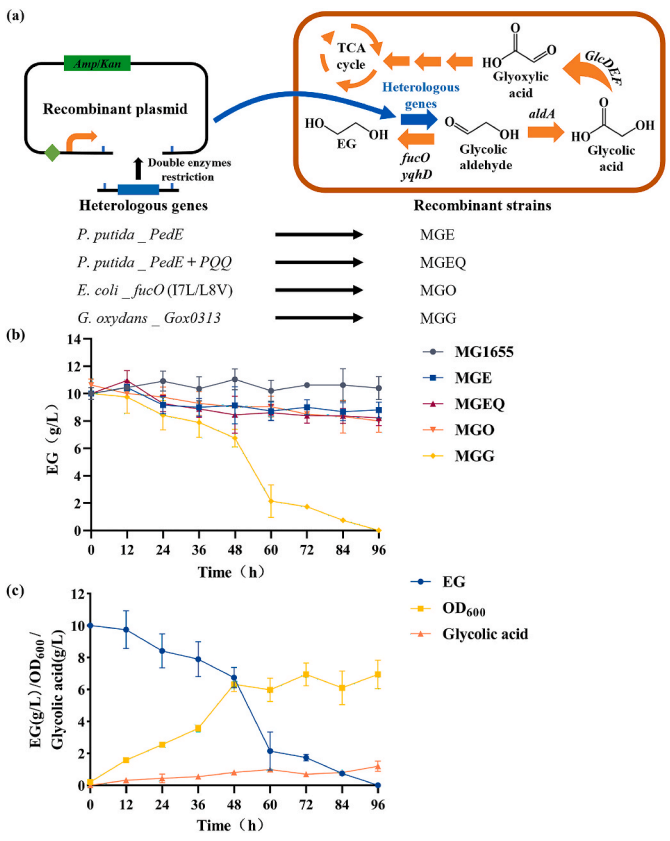

Fig. 2. The construction process and EG utilization of engineered strains. (a) The construction process of engineered strains (b) EG utilization of engineered strains (c) EG utilization, cell growth and GA production of strain MGG.

The engineered strain MGG can completely convert 10 g/L EG to 1.2 g/L GA within 96 h.

3.2. Enhancement of GA biosynthetic pathway

The engineered strain MGG successfully facilitated the conversion of EG to GA. The following step is to enhance GA biosynthetic pathway to increase the EG conversion efficiency and the production of GA.

In the process of constructing microbial cell factories, overexpression of key enzymes and knock-out of the competing pathways are commonly used to enhance the yield of products [31–34]. Accordingly, three strategies were employed to enhance GA biosynthetic pathway. The first strategy involved the overexpression of the glycolic aldehyde gene (*aldA*) to enhance the efficiency of EG conversion into GA. The second strategy focused on knocking out the endogenous glycolic aldehyde reductase genes, *fucO* and *yqhD*, to prevent the conversion of glycolic aldehyde into EG in *E. coli*, thereby improving its ability to utilize EG. The third strategy entailed knocking out the endogenous GA oxidase gene *GlcDEF*, which inhibits the GA utilization pathway, resulting in an increased accumulation of GA. These strategies were combined to obtain strains with varying levels of EG conversion efficiency and different levels of GA production (Fig. 3). In total, six strains were obtained. The FYG, MGGA and GlcG strains were constructed by knocking out the *fucO* and *yqhD* genes, overexpressing the *aldA* gene and knocking out the *GlcDEF* genes on the basis of MGG, respectively. The FYGlcG strain was developed by knocking out the *GlcDEF* genes on the basis of FYG. The FYGA strain was obtained by knocking out the *fucO* and *yqhD* genes on the basis of MGGA. The GlcGA strain was constructed by overexpressing the *aldA* gene on the basis of GlcG.

Strains were cultivated with 10 g/L EG as the major carbon source. The results are presented in Fig. 4. As shown in Fig. 4a, compared with the wild *E. coli* MG1655, strains MGG, MGGA and FYGA could effectively utilize EG for growth. Among them, strain MGG and MGGA fully utilized 10 g/L EG within 120 h, with strain MGGA exhibiting a faster rate of EG utilization than MGG. The strain FYGA utilized 6.8 g/L EG

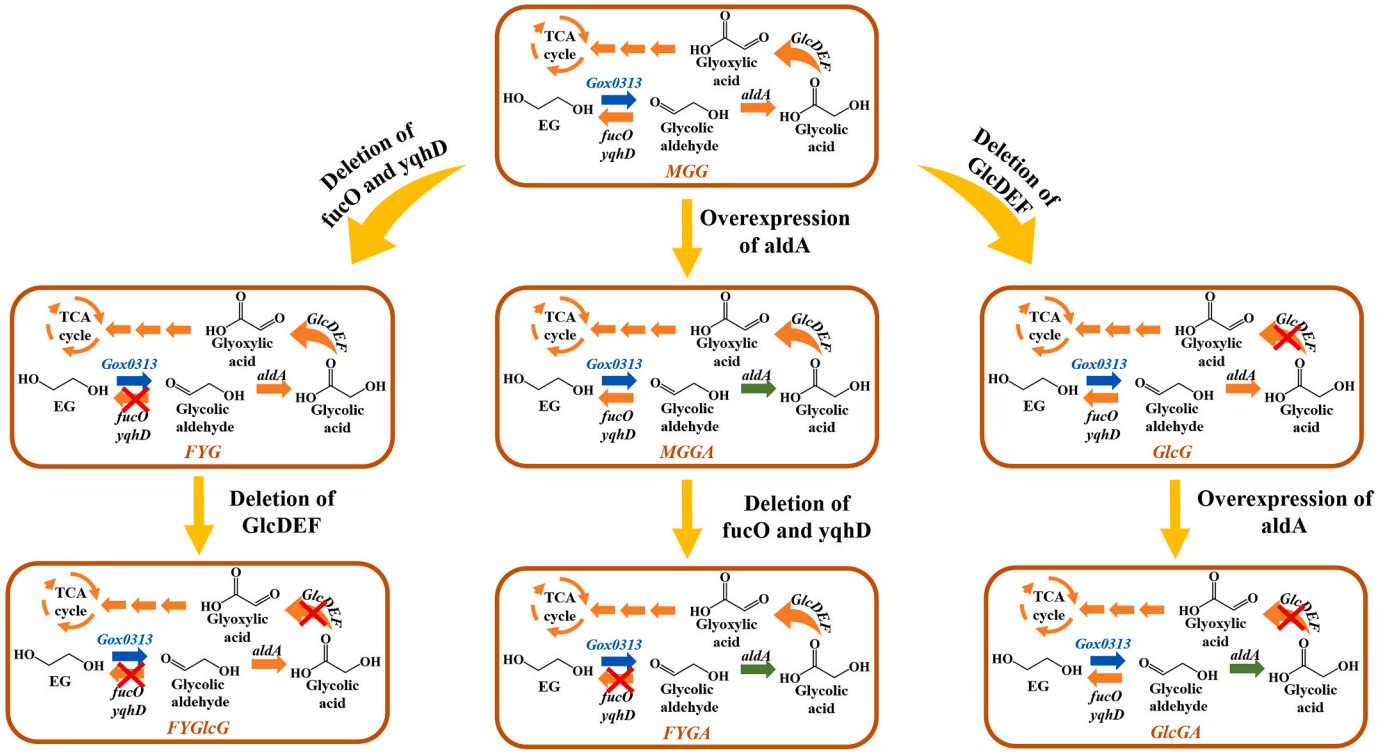

Fig. 3. Different strains obtained by combining three strategies. The blue arrow indicates the introduction of heterologous genes, the green arrow indicates the overexpression of genes, and the red cross indicates the knockout of genes.

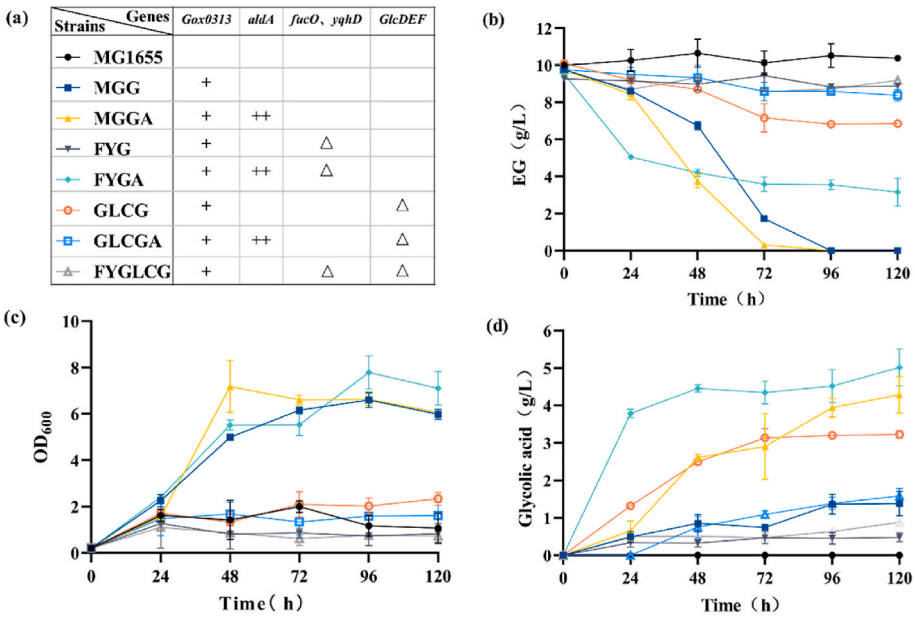

Fig. 4. Cell growth, EG utilization and GA production profiles of different *E. coli* (a) the genetic modifications of engineered strains ("+" represents introduction of exogenous gene; "++" represents overexpression of endogenous gene; "△" represents knockout of endogenous gene) (b) EG concentration (c) OD_{600} (d) GA concentration.

within 120 h. Compared with the strains MGG and MGGA, FYGA exhibited a faster EG utilization rate than MGG and MGGA in the first 48 h, but this rate decreased between 48 h and 120 h (Fig. 4b). Fig. 4c illustrates that when EG served as the major carbon source, strains MGG, MGGA, and FYGA exhibited robust growth, whereas other strains displayed minimal growth. Within the initial 48 h, the OD_{600} values of strains MGG, MGGA, and FYGA steadily increased, followed by a stabilization phase. During this stable period, the OD_{600} values consistently remained around 6–7, indicating that strains MGG, MGGA, and FYGA effectively utilized EG for growth (Fig. 4c). Comparing the GA production capabilities of different strains, it is evident that strain FYGA achieved the highest GA yield, reaching 5.1 g/L with a yield of 0.75 g/g EG. Notably, it's the current highest level in the shake flake experiments. Within the first 24 h of culture, the GA production increased rapidly. Its maximum productivity reached 0.158 g/L/h during the initial 24 h, which is higher than the latest research [35]. In the subsequent 24 h, the strain FYGA produced GA slower, and the concentration of GA changed little after 48 h (Fig. 4d). Compared with strain MGG and MGGA, strain FYGA exhibited the ability to rapidly utilize EG to produce GA. It could be attributed to the knock-out of endogenous genes *fucO* and *yqhD* in FYGA, which can promote the conversion of EG to GA. It is worth noting that the reaction catalyzed by *fucO* from EG to glycolic aldehyde is reversible. In previous studies, *fucO* or its mutants were overexpressed to realize the utilization of EG [35,36]. Differently, in this study, *fucO* and another reductase enzyme *yqhD* were deleted to ensure that glycolic aldehyde does not reverse back to EG, while a heterologous gene *Gox0313* replaced *fucO* for the utilization of EG. The results demonstrate that this system we developed can more effectively utilize EG and produce GA. However, as the fermentation process continued, strain FYGA ceased utilizing EG to produce GA, whereas strains MGG and MGGA could fully utilize all available EG in the culture medium. This change may be attributed to the high concentration of GA produced by strain FYGA during the initial stages of fermentation, which inhibited the conversion of EG to GA.

To verify whether GA has inhibitory effect on strain FYGA, subsequent experiments were conducted. Strain FYGA was cultured using 10 g/L EG as the carbon source, and 1 g/L, 2 g/L, 3 g/L, 4 g/L and 5 g/L GA were added, respectively. The OD_{600} value was measured every 12 h, and the results are depicted in Supplementary Fig. 1. Compared with the control group, the growth of *E. coli* could be inhibited by exogenous GA, and the higher the concentration of GA, the more obvious the inhibition effect. When the addition of GA reached 4 g/L, *E. coli* hardly grew. However, in the actual fermentation process of strain FYGA, the concentration of GA already reached 3.8 g/L after 24 h fermentation, while the OD_{600} value continued to increase. It may be due to the gradual increase of the concentration of GA during fermentation. When the growth limit concentration was reached, the strain had already grown, so the inhibitory effect was not as evident. The experiment of exogenous addition of GA indicates that high concentration of GA can inhibit the growth of strain FYGA, potentially explaining why the strain FYGA cannot fully use 10 g/L of EG in the culture medium. We also conducted fermentation experiments using FYGA strain at higher EG concentrations of 15 g/L and 20 g/L, respectively. There was no significant increase in the production of GA and the utilization of EG (Fig. S4). This indicated that the strain was sensitive to GA. When GA reached a certain high concentration, it inhibited the further utilization of EG, thereby limiting the further increase in acetic acid production. The results were consistent with the research of Wei et al. [37] that high concentration of GA will inhibit the biotransformation process. Another study also revealed that the accumulation of GA inhibits the activity of glyoxylate oxidase, thereby impeding the glyoxylate metabolism [38]. It may lead to insufficient energy supply and affect the growth of strains. Based on this, laboratory adaptive evolution [39,40], discovering tolerance-related genes via systems biology tools [41,42] or other methods [43] can be employed in subsequent experiments to alleviate the inhibitory effects of GA on strain FYGA, ultimately obtaining the mutant strain that can fully utilize EG to produce GA.

Besides, the strains GlcG, GlcGA and FYGlcG, which deleted the *GlcDEF* genes, all exhibited a low GA yield. It may be attributed to that the deletion of *GlcDEF* can affect the growth of strains. This finding aligns with the research conducted by Deng et al. [19] that the deletion of *GlcDEF* resulted in a lower yield of GA and weak cell growth. As shown in Fig. 6b, all strains that knock out *GlcDEF*, hardly grew on the culture medium with 10 g/L of EG as substrate.

3.3. Transcript levels analysis in the engineered strains

In order to further analyze the impact of knockout and

overexpression of endogenous genes on the conversion of EG to GA, the transcription level of MGG, MGGA, FYG, FYGA, GLCG and GLCGA strains were analyzed. At the fifth hour of shake flask fermentation, total RNA of six strains were extracted. Subsequently, the cDNA was obtained through reverse transcription of RNA. Then qPCR reaction was performed. Using the endogenous gene *gapA* of *E. coli* MG1655 as the reference gene, the transcription level difference between exogenous and endogenous genes involved in the pathway of converting EG to GA was analyzed (Fig. 5).

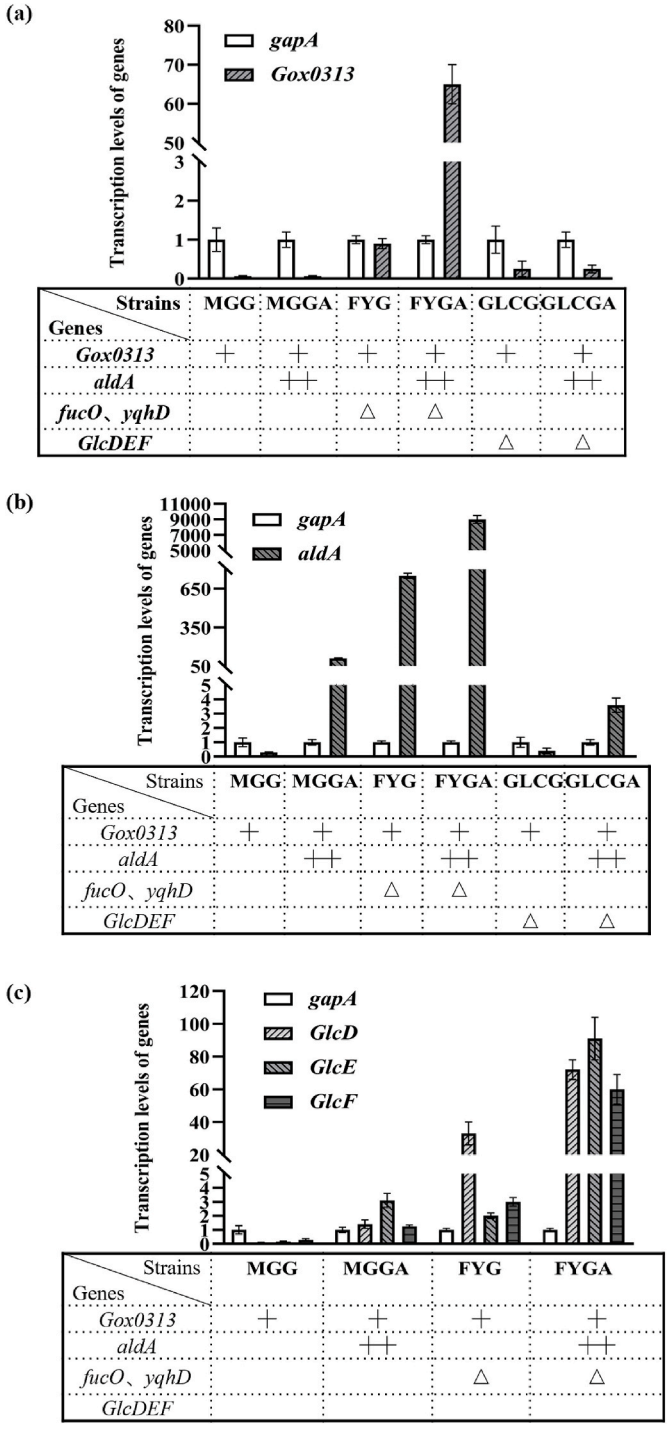

Fig. 5. Transcription level analysis of engineered *E. coli* (a) exogenous gene *Gox0313* (b) endogenous gene *aldA* (c) endogenous genes *GlcDEF* ("+" represents introduction of exogenous gene; "++" represents overexpression of endogenous gene; "△" represents knockout of endogenous gene).

As shown in Fig. 5a, the transcriptional level of the exogenous gene *Gox0313* in strain FYGA is significantly higher than that in strain MGGA, indicating that the deletion of the endogenous genes *fucO* and *yqhD* can promote the expression of the heterologous gene *Gox0313*. This conclusion can also be drawn by comparing the transcriptional level of *Gox0313* in strain FYG and strain MGG. The transcription level of the heterologous gene *Gox0313* is the highest in the strain FYGA, significantly exceeding that in the other six strains. Since the heterologous gene *Gox0313* encodes the alcohol oxidase, which catalyzes the conversion of EG to glycoldehyde, the strain FYGA is expected to utilize EG at the fastest rate at the 5th hour, consistent with the results shown in Fig. 4a. In addition, the transcription level of the gene *Gox0313* in the strain FYGA is about 72 times higher than that in the strain FYG. The result indicates that overexpression of the gene *aldA* can also enhance the expression of the gene *Gox0313*, thus promoting the conversion of EG to glycolic aldehyde. The transcription level of gene *Gox0313* in strain GLCG and GLCGA is higher than that in strain MGG and MGGA, respectively, revealing that the deletion of endogenous gene *GlcDEF* can facilitate the expression of gene *Gox0313*.

Besides, as a result of the overexpression of the *aldA* gene in strains MGGA, FYGA, and GLCGA, there was an enhancement in the transcription level of the *aldA* gene in these strains (Fig. 5b). The transcriptional level of gene *aldA* in strain FYGA was approximately 80 times higher than that in strain MGGA. It demonstrats that the deletion of genes *fucO* and *yqhD* can not only boost the expression of exogenous gene *Gox0313* but also promote the expression of endogenous gene *aldA*, thus accelerating the conversion of EG to GA. Consequently, strain FYGA exhibited improved GA production compared to strain MGGA. Additionally, the transcriptional level of the *aldA* gene in strain FYG was notably higher than that in strain MGG, showing an approximate 737-fold increase. This finding reinforces the idea that the deletion of endogenous genes *fucO* and *yqhD* can effectively enhance the expression of the endogenous *aldA* gene.

The transcription level of genes *GlcDEF* in FYGA and FYG strains was significantly higher than that in MGGA and MGG strains, respectively (Fig. 5c). This phenomenon suggests that the deletion of endogenous genes *fucO* and *yqhD* promotes the expression of *GlcDEF*. Additionally, the transcription level of genes *GlcDEF* in strain FYGA and MGGA was markedly higher than that in FYG and MGG strains, respectively. The result indicates that the overexpression of the *aldA* gene can also stimulate the expression of the endogenous *GlcDEF*. Furthermore, when combined with the differences in GA production, as illustrated in Fig. 4c, it can be concluded that the increase in GA production will further stimulate the expression of the downstream *GlcDEF*, thus enhancing the metabolism and utilization of GA.

3.4. Intracellular metabolomics analysis of the engineered strains

In this study, the strain MGG was obtained to efficiently utilize EG through introducing the gene *Gox0313*. Subsequently, the MGGA strain, exhibiting the fastest full utilization of EG, was obtained by overexpressing the *aldA* gene on the basis of MGG. Additionally, the FYGA strain, characterized by the highest substrate conversion rate, was obtained through the overexpression of the *aldA* gene and the deletion of the *fucO* and *yqhD* genes on the basis of MGG. To investigate the mechanisms underlying the increased EG utilization rate and GA production, intracellular metabolomics analysis of the strains MGG, MGGA, and FYGA strains were conducted. The results of metabolomic analysis were shown by volcano maps and cluster heat maps of differential metabolites among MGG, MGGA and FYGA strains (Fig. S2, Fig. S3). Compared with MGG strain, a total of 376 differential metabolites were detected in MGGA strain, with 180 metabolites upregulated and 196 metabolites downregulated. In comparison to MGGA strain, a total of 413 differential metabolites were detected in FYGA strain, with 191 metabolites upregulated and 222 metabolites downregulated. Furthermore, to elucidate the mechanisms contributing to the enhanced EG

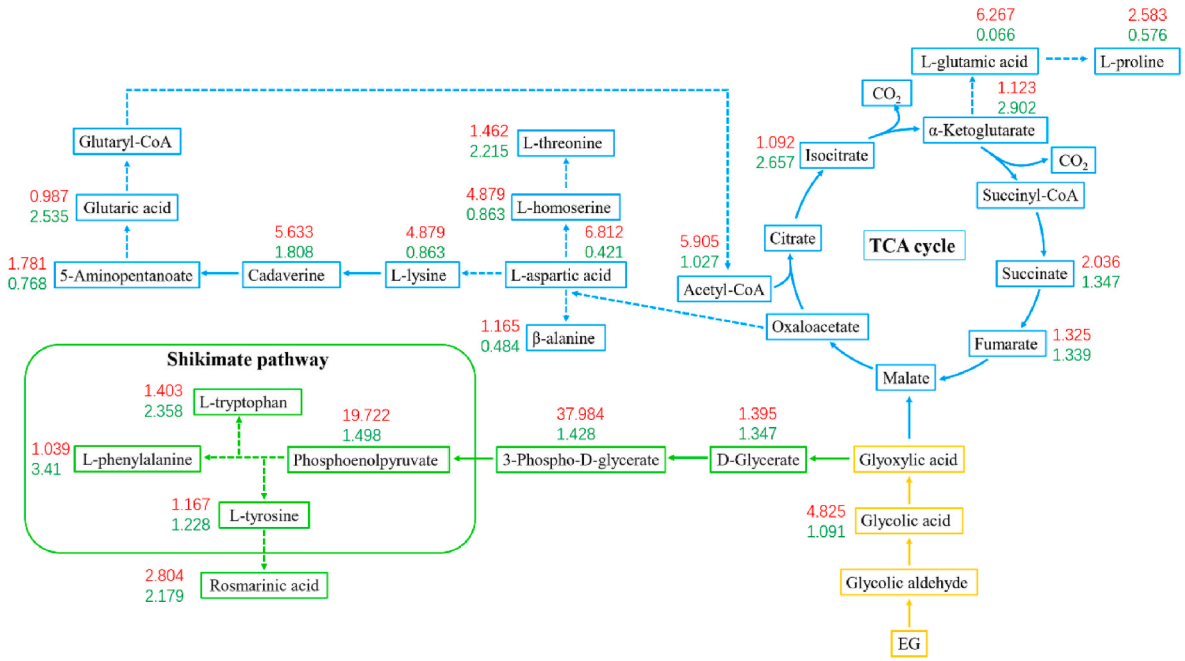

Fig. 6. Metabolite differences in EG utilization pathways of MGG, MGGA and FYGA. The red number represents the fold change of metabolites in FYGA compared to metabolites in MGGA and the green number represents the fold change of metabolites in MGGA compared to metabolites in MGG.

utilization rate and GA production, we conducted a detailed analysis of differential metabolites related to the EG utilization pathways (Fig. 6). In the figure, the red numbers represented the fold change of metabolites in FYGA compared to those in MGGA, while the green numbers represented the fold change of metabolites in MGGA compared to those in MGG.

Compared to MGG strain, EG metabolic pathway of strain MGGA were enhanced. There were two downstream metabolic pathways of EG utilization in *E. coli*, including the pathways from glyoxylic acid to shikimic acid and the TCA cycle, respectively. As shown in Fig. 6, both metabolic flows of EG utilization in MGGA strain were improved, indicating that the overexpression of *Gox0313* gene enhanced the EG utilization pathway. Additionally, the TCA cycle was one of the major biochemical hubs of the cell, primarily responsible for energy generation [44–46]. The enhanced TCA cycle accelerates energy generation, thus promoting the growth of strains, which may be one of the contributing factors to the improved EG utilization.

In comparison to MGGA strain, metabolic flows of EG utilization in FYGA strain were significantly improved. The two most substantially changed metabolites, 3-phospho-D-glycerate and phosphoenolpyruvate, increased to 37.984 times and 19.722 times than that in MGGA, respectively. The significant improvement in both metabolic flows of EG utilization may account for the rapid increase in EG utilization rate and GA production.

Furthermore, as depicted in Fig. 6, it was evident that the enhancement of metabolic fluxes into both the TCA cycle and the shikimic acid pathway in the MGGA and FYGA strains robustly reinforces the synthesis and metabolic pathways of amino acids. Notably, the content of various amino acids underwent substantial changes, including L-tryptophan, L-phenylalanine, L-tyrosine, L-aspartic acid, L-homoserine, L-threonine, L-lysine, β-alanine, L-glutamic acid, and L-proline. Additionally, metabolomic results also indicated the production of a new compound rosmarinic acid. Rosmarinic acid was also a high value-added product, exhibiting various biological activities, such as anti-inflammatory, antioxidant, antibacterial and antiviral properties [47–49]. And with the overexpression of *Gox0313* genes and the knockout of *fucO* and *yqhD* genes, the production of rosmarinic acid increased by 2.179-fold and 2.804-fold, respectively. The result underscored the potential of the engineered strains for efficient rosmarinic acid production. Moreover, these engineered strains were promised for modification to produce other high value-added products linked to the TCA cycle and the shikimic acid pathway, such as malate [50], shikimate [51], α-ketoglutaric acid [52], *p*-coumaric acid and other aromatic chemicals [53].

4. Conclusions

In this study, the PET monomer EG was utilized as the substrate for *E. coli* MG1655 to grow and produce GA. By screening genes from diverse sources to construct EG utilization pathway in *E. coli* MG1655, engineered *E. coli* to utilize EG and produce GA was successfully constructed. Subsequently, by reinforcing the GA production pathway and knocking-out of the competing pathways, the GA titer increased to 5.1 g/L with a yield of 0.75 g/g EG, which is the highest level in the shake flake experiments. Transcriptional level analysis and metabolomic analysis of the engineered strains were then conducted to further understand the mechanisms underlying the increased EG utilization rate and GA production. The results reveal that overexpression of key enzymes and knock-out of the competing pathways improved the metabolic flow in the EG utilization, ultimately enhancing the bioconversion of EG to GA. The improved metabolic flow also leads to accelerated synthesis and metabolism of amino acids. In conclusion, the research shows promise to use engineered *E. coli* for biological recycling of PET waste, and the engineered strains developed here can efficiently utilize PET monomer EG to produce GA. These engineered strains also hold promise as a platform for the production of other high-value-added products.

CRediT authorship contribution statement

Wenlong Yan: Conceptualization, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Xinhua Qi:** Conceptualization, Investigation, Visualization, Methodology. **Zhibei Cao:** Writing – review & editing. **Mingdong Yao:** Supervision. **Mingzhu Ding:** Conceptualization, Writing – review & editing, Supervision, Funding acquisition. **Yingjin Yuan:** Supervision.

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.

Acknowledgements

This work was supported by the National Key Research and Development Program of China (2019YFA0706900) and National Natural Science Foundation of China (22278310).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.synbio.2024.04.006.

References

[1] Mohanan N, Montazer Z, Sharma PK, Levin DB. Microbial and enzymatic degradation of synthetic plastics. Front Microbiol 2020;11:580709. https://doi.org/10.3389/fmicb.2020.580709.
[2] Koshti R, Mehta L, Samarth N. Biological recycling of polyethylene terephthalate: a mini-review. J Polym Environ 2018;26(8):3520–9. https://doi.org/10.1007/s10924-018-1214-7.
[3] Ivar do Sul JA, Costa MF. The present and future of microplastic pollution in the marine environment. Environ Pollut 2014;185:352–64. https://doi.org/10.1016/j.envpol.2013.10.036.
[4] Urbanek AK, Kosiorowska KE, Mironczuk AM. Current knowledge on polyethylene terephthalate degradation by genetically modified microorganisms. Front Bioeng Biotechnol 2021;9:771133. https://doi.org/10.3389/fbioe.2021.771133.
[5] Yang H, Chen G, Wang J. Microplastics in the marine environment: sources, fates, impacts and microbial degradation. Toxics 2021;9(2):41. https://doi.org/10.3390/toxics9020041.
[6] Anuar NFSK, Huyop F, Ur-Rehman G, Abdullah F, Normi YM, Sabullah MK, et al. An overview into polyethylene terephthalate (PET) hydrolases and efforts in tailoring enzymes for improved plastic degradation. Int J Mol Sci 2022;23(20):12644. https://doi.org/10.3390/ijms232012644.
[7] Kushwaha A, Goswami L, Singhvi M, Kim BS. Biodegradation of poly(ethylene terephthalate): mechanistic insights, advances, and future innovative strategies. Chem Eng J 2023;457:141230. https://doi.org/10.1016/j.cej.2022.141230.
[8] Qi X, Yan W, Cao Z, Ding M, Yuan Y. Current advances in the biodegradation and bioconversion of polyethylene terephthalate. Microorganisms 2021;10(1):39. https://doi.org/10.3390/microorganisms10010039.
[9] Wei R, Zimmermann W. Biocatalysis as a green route for recycling the recalcitrant plastic polyethylene terephthalate. Microb Biotechnol 2017;10(6):1302–7. https://doi.org/10.1111/1751-7915.12714.
[10] Lee S, Lee YR, Kim SJ, Lee JS, Min K. Recent advances and challenges in the biotechnological upcycling of plastic wastes for constructing a circular bioeconomy. Chem Eng J 2023;454:140470. https://doi.org/10.1016/j.cej.2022.140470.
[11] Kim N-K, Lee S-H, Park H-D. Current biotechnologies on depolymerization of polyethylene terephthalate (PET) and repolymerization of reclaimed monomers from PET for bio-upcycling: a critical review. Bioresour Technol 2022;363:127931. https://doi.org/10.1016/j.biortech.2022.127931.
[12] Yoshida S, Hiraga K, Takehana T, Taniguchi I, Yamaji H, Maeda Y, et al. A bacterium that degrades and assimilates poly(ethylene terephthalate). Science 2016;351(6278):1196–9. https://doi.org/10.1126/science.aad6359.
[13] Taniguchi I, Yoshida S, Hiraga K, Miyamoto K, Kimura Y, Oda K. Biodegradation of PET: current status and application aspects. ACS Catal 2019;9(5):4089–105. https://doi.org/10.1021/acscatal.8b05171.
[14] Roberts C, Edwards S, Vague M, Leon-Zayas R, Scheffer H, Chan G, et al. Environmental consortium containing Pseudomonas and Bacillus species synergistically degrades polyethylene terephthalate plastic. Msphere 2020;5(6):10–1128. https://doi.org/10.1128/mSphere.01151-20.
[15] Liu P, Zhang T, Zheng Y, Li Q, Su T, Qi Q. Potential one-step strategy for PET degradation and PHB biosynthesis through co-cultivation of two engineered microorganisms. Eng Microbiol 2021;1:100003. https://doi.org/10.1016/j.engmic.2021.100003.
[16] Qi X, Ma Y, Chang H, Li B, Ding M, Yuan Y. Evaluation of PET degradation using artificial microbial consortia. Front Microbiol 2021;12:778828. https://doi.org/10.3389/fmicb.2021.778828.
[17] Barth M, Oeser T, Wei R, Then J, Schmidt J, Zimmermann W. Effect of hydrolysis products on the enzymatic degradation of polyethylene terephthalate nanoparticles by a polyester hydrolase from Thermobifida fusca. Biochem Eng J 2015;93:222–8. https://doi.org/10.1016/j.bej.2014.10.012.
[18] Knott BC, Erickson E, Allen MD, Gado JE, Graham R, Kearns FL, et al. Characterization and engineering of a two-enzyme system for plastics depolymerization. Proc Natl Acad Sci USA 2020;117(41):25476–85. https://doi.org/10.1073/pnas.2006753117.
[19] Deng Y, Ma N, Zhu K, Mao Y, Wei X, Zhao Y. Balancing the carbon flux distributions between the TCA cycle and glyoxylate shunt to produce glycolate at high yield and titer in Escherichia coli. Metab Eng 2018;46:28–34. https://doi.org/10.1016/j.ymben.2018.02.008.
[20] Zhu T, Yao D, Li D, Xu H, Jia S, Bi C, et al. Multiple strategies for metabolic engineering of Escherichia coli for efficient production of glycolate. Biotechnol Bioeng 2021;118(12):4699–707. https://doi.org/10.1002/bit.27934.
[21] Liu M, Ding YM, Xian M, Zhao G. Metabolic engineering of a xylose pathway for biotechnological production of glycolate in Escherichia coli. Microb Cell Factories 2018;17:1–11. https://doi.org/10.1186/s12934-018-0900-4.
[22] Salusjarvi L, Havukainen S, Koivistoinen O, Toivari M. Biotechnological production of glycolic acid and ethylene glycol: current state and perspectives. Appl Microbiol Biotechnol 2019;103(6):2525–35. https://doi.org/10.1007/s00253-019-09640-2.
[23] Pereira B, Li Z-J, De Mey M, Lim CG, Zhang H, Hoeltgen C, et al. Efficient utilization of pentoses for bioproduction of the renewable two-carbon compounds ethylene glycol and glycolate. Metab Eng 2016;34:80–7. https://doi.org/10.1016/j.ymben.2015.12.004.
[24] Alkim C, Trichez D, Cam Y, Spina L, Francois JM, Walther T. The synthetic xylulose-1 phosphate pathway increases production of glycolic acid from xylose-rich sugar mixtures. Biotechnol Biofuels 2016;9. https://doi.org/10.1186/s13068-016-0610-2.
[25] Yu Y, Shao M, Li D, Fan F, Xu H, Lu F, et al. Construction of a carbon-conserving pathway for glycolate production by synergetic utilization of acetate and glucose in Escherichia coli. Metab Eng 2020;61:152–9. https://doi.org/10.1016/j.ymben.2020.06.001.
[26] Xu S, Zhang L, Zhou S, Deng Y. Biosensor-based multigene pathway optimization for enhancing the production of glycolate. Appl Environ Microbiol 2021;87(12). https://doi.org/10.1128/aem.00113-21.
[27] Tamura J, Ono A, Sugano Y, Huang C, Nishizawa H, Mikoshiba S. Electrochemical reduction of CO2 to ethylene glycol on imidazolium ion-terminated self-assembly monolayer-modified Au electrodes in an aqueous solution. Phys Chem Chem Phys 2015;17(39):26072–8. https://doi.org/10.1039/c5cp03028e.
[28] Kuhl KP, Cave ER, Abram DN, Jaramillo TF. New insights into the electrochemical reduction of carbon dioxide on metallic copper surfaces. Energy Environ Sci 2012;5(5):7050–9. https://doi.org/10.1039/c2ee21234j.
[29] Datsenko KA, Wanner BL. One-step inactivation of chromosomal genes in Escherichia coli K-12 using PCR products. Proc Natl Acad Sci USA 2000;97(12):6640–5. https://doi.org/10.1073/pnas.120163297.
[30] Boronat A, Caballero E, Aguilar J. Experimental evolution of a metabolic pathway for ethylene glycol utilization by Escherichia coli. J Bacteriol 1983;153(1):134–9. https://doi.org/10.1128/jb.153.1.134-139.1983.
[31] Chen XL, Gao C, Guo L, Hu GP, Luo QL, Liu J, et al. DCEO biotechnology: tools to design, construct, evaluate, and optimize the metabolic pathway for biosynthesis of chemicals. Chem Rev 2018;118(1):4–72. https://doi.org/10.1021/acs.chemrev.6b00804.
[32] Liu D, Mannan AA, Han Y, Oyarzun DA, Zhang F. Dynamic metabolic control: towards precision engineering of metabolism. J Ind Microbiol Biotechnol 2018;45(7):535–43. https://doi.org/10.1007/s10295-018-2013-9.
[33] Liu Z, Xu J, Feng Z, Wang Y. Multi-strategy engineering unusual sugar TDP-L-mycarose biosynthesis to improve the production of 3-O-α-mycarosylerythronolide B in Escherichia coli. Synth Syst Biotechnol 2022;7(2):756–64. https://doi.org/10.1016/j.synbio.2022.03.002.
[34] Deng C, Lv X, Liu Y, Li J, Lu W, Du G, et al. Metabolic engineering of Corynebacterium glutamicum S9114 based on whole-genome sequencing for efficient N-acetylglucosamine synthesis. Synth Syst Biotechnol 2019;4(3):120–9. https://doi.org/10.1016/j.synbio.2019.05.002.
[35] Pandit AV, Harrison E, Mahadevan R. Engineering Escherichia coli for the utilization of ethylene glycol. Microb Cell Factories 2021;20(1):1–17. https://doi.org/10.1186/s12934-021-01509-2.
[36] Panda S, Fung VYK, Zhou JFJ, Liang H, Zhou K. Improving ethylene glycol utilization in Escherichia coli fermentation. Biochem Eng J 2021;168. https://doi.org/10.1016/j.bej.2021.107957.
[37] Wei G, Yang X, Zhou W, Lin J, Wei D. Adsorptive bioconversion of ethylene glycol to glycolic acid by Gluconobacter oxydans DSM 2003. Biochem Eng J 2009;47(1–3):127–31. https://doi.org/10.1016/j.bej.2009.07.016.
[38] Franden MA, Jayakody LN, Li W-J, Wagner NJ, Cleveland NS, Michener WE, et al. Engineering Pseudomonas putida KT2440 for efficient ethylene glycol utilization. Metab Eng 2018;48:197–207. https://doi.org/10.1016/j.ymben.2018.06.003.
[39] Wu T, Liu J, Li M, Zhang G, Liu L, Li X, et al. Improvement of sabinene tolerance of Escherichia coli using adaptive laboratory evolution and omics technologies. Biotechnol Biofuels 2020;13(1):1–15. https://doi.org/10.1186/s13068-020-01715-x.
[40] Hirasawa T, Maeda T. Adaptive laboratory evolution of microorganisms: methodology and application for bioproduction. Microorganisms 2023;11(1):92. https://doi.org/10.3390/microorganisms11010092.
[41] Yan W, Cao Z, Ding M, Yuan Y. Design and construction of microbial cell factories based on systems biology. Synth Syst Biotechnol 2023;8(1):176–85. https://doi.org/10.1016/j.synbio.2022.11.001.
[42] Hasunuma T, Sanda T, Yamada R, Yoshimura K, Ishii J, Kondo A. Metabolic pathway engineering based on metabolomics confers acetic and formic acid tolerance to a recombinant xylose-fermenting strain of Saccharomyces cerevisiae. Microb Cell Factories 2011;10(1):1–13. https://doi.org/10.1186/1475-2859-10-2.
[43] Mohedano MT, Konzock O, Chen Y. Strategies to increase tolerance and robustness of industrial microorganisms. Synth Syst Biotechnol 2022;7(1):533–40. https://doi.org/10.1016/j.synbio.2021.12.009.

[44] Vuoristo KS, Mars AE, Sanders JPM, Eggink G, Weusthuis RA. Metabolic engineering of TCA cycle for production of chemicals. Trends Biotechnol 2016;34 (3):191–7. https://doi.org/10.1016/j.tibtech.2015.11.002.
[45] Kwong WK, Zheng H, Moran NA. Convergent evolution of a modified, acetate-driven TCA cycle in bacteria. Nat Microbiol 2017;2(7):1–3. https://doi.org/10.1038/nmicrobiol.2017.67.
[46] Ryan DG, Yang M, Prag HA, Blanco GR, Nikitopoulou E, Segarra-Mondejar M, et al. Disruption of the TCA cycle reveals an ATF4-dependent integration of redox and amino acid metabolism. bioRxiv 2021:e72593. https://doi.org/10.1101/2021.07.27.453996.
[47] Babaei M, Zamfir GMB, Chen X, Christensen HB, Kristensen M, Nielsen J, et al. Metabolic engineering of Saccharomyces cerevisiae for rosmarinic acid production. ACS Synth Biol 2020;9(8):1978–88. https://doi.org/10.1021/acssynbio.0c00048.
[48] Pattananandecha T, Apichai S, Julsrigival J, Ungsurungsie M, Samuhasaneetoo S, Chulasiri P, et al. Antioxidant activity and anti-photoaging effects on UVA-Irradiated human fibroblasts of rosmarinic acid enriched extract prepared from Thunbergia laurifolia leaves. Plants-Basel 2021;10(8):1648. https://doi.org/10.3390/plants10081648.
[49] Noor S, Mohammad T, Rub MA, Raza A, Azum N, Yadav DK, et al. Biomedical features and therapeutic potential of rosmarinic acid. Arch Pharm Res (Seoul) 2022;45(4):205–28. https://doi.org/10.1007/s12272-022-01378-2.
[50] Wu N, Zhang JH, Chen YR, Xu Q, Song P, Li YF, et al. Recent advances in microbial production of L-malic acid. Appl Microbiol Biotechnol 2022;106(24):7973–92. https://doi.org/10.1007/s00253-022-12260-y.
[51] Li ZD, Gao C, Ye C, Guo L, Liu J, Chen XL, et al. Systems engineering of Escherichia coli for high-level shikimate production. Metab Eng 2023;75:1–11. https://doi.org/10.1016/j.ymben.2022.10.010.
[52] Chen XL, Dong XX, Liu J, Luo QL, Liu LM. Pathway engineering of Escherichia coli for α-ketoglutaric acid production. Biotechnol Bioeng 2020;117(9):2791–801. https://doi.org/10.1002/bit.27456.
[53] Panda S, Zhou JFJ, Feigis M, Harrison E, Ma XQ, Yuen VFK, et al. Engineering Escherichia coli to produce aromatic chemicals from ethylene glycol. Metab Eng 2023;79:38–48. https://doi.org/10.1016/j.ymben.2023.06.012.