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RESEARCH

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Engineering *Escherichia coli* for utilization of PET degraded ethylene glycol as sole feedstock

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Abstract

From both economic and environmental perspectives, ethylene glycol, the principal constituent in the degradation of PET, emerges as an optimal feedstock for microbial cell factories. Traditional methods for constructing *Escherichia coli* chassis cells capable of utilizing ethylene glycol as a non-sugar feedstock typically involve overexpressing the genes *fucO* and *aldA*. However, these approaches have not succeeded in enabling the exclusive use of ethylene glycol as the sole source of carbon and energy for growth. Through ultraviolet radiation-induced mutagenesis and subsequent laboratory adaptive evolution, an EG02 strain emerged from *E. coli* MG1655 capable of utilizing ethylene glycol as its sole carbon and energy source, demonstrating an uptake rate of 8.1 ± 1.3 mmol/gDW h. Comparative transcriptome analysis guided reverse metabolic engineering, successfully enabling four wild-type *E. coli* strains to metabolize ethylene glycol exclusively. This was achieved through overexpression of the *gcl*, *hyi*, *glxR*, and *glxK* genes. Notably, the engineered *E. coli* chassis cells efficiently metabolized the 87 mM ethylene glycol found in PET enzymatic degradation products following 72 h of fermentation. This work presents a practical solution for recycling ethylene glycol from PET waste degradation products, demonstrating that simply adding M9 salts can effectively convert them into viable raw materials for *E. coli* cell factories. Our findings also emphasize the significant roles of genes associated with the glycolate and glyoxylate degradation I pathway in the metabolic utilization of ethylene glycol, an aspect frequently overlooked in previous research.

Keywords Ethylene glycol, Non-sugar feedstock, *Escherichia coli*, Metabolic engineering, Transcriptome analysis, Polyethylene terephthalate (PET), Laboratory adaptive evolution

Introduction

Traditional industrial chassis cells heavily rely on sugar-based feedstocks, such as glucose and xylose, for the production of high-value products. However, significant challenges arise primarily due to the higher cost of common sugar feedstocks compared to oil prices, posing a barrier for traditional industrial chassis cells to compete with conventional petrochemical counterparts [1, 2]. In response to this challenge, non-sugar feedstocks have emerged as viable alternatives. When evaluating these alternatives, it is crucial to acknowledge that many cannot be naturally catabolized by

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traditional industrial chassis cells. Therefore, it is necessary to consider feedstock toxicity, biocompatibility, preparation techniques, and costs, along with the development of appropriate metabolic pathways for feedstock utilization. Indeed, refactoring large metabolic pathways in traditional industrial chassis cells has proven challenging in the past [3, 4].

Ethylene glycol stands out as a highly promising non-sugar feedstock candidate for traditional industrial chassis cells, driven by several key factors. Firstly, Pandit and colleagues demonstrated that ethylene glycol exhibits the highest orthogonality scores compared to formate, glucose, and xylose in the biosynthesis of succinate, glycolate, and 2,3-butanediol [4, 5]. Secondly, ethylene glycol yields more reducing equivalents compared to traditional glucose under equimolar conditions (Fig. 3). Moreover, ethylene glycol is economically feasible, primarily synthesized by the petrochemical industry from ethylene. Notably, ethylene glycol can also be produced through the electrochemical conversion of CO_2, showcasing its potential to address climate change via carbon sequestration [6, 7]. Additionally, ethylene glycol can be sourced sustainably from glycerol, a renewable bio-resource and common waste product of the biodiesel industry and soap production. This presents a valuable opportunity for the upcycling of glycerol waste [8, 9]. Furthermore, ethylene glycol is a major degradation product of polyethylene terephthalate (PET) plastic, one of the most important pollutions on earth and in the seas [10]. Utilizing ethylene glycol derived from PET waste degradation as a non-sugar substrate provides an economically and environmentally friendly solution for recycling PET, representing a significant stride towards transforming waste into a valuable resource.

Escherichia coli (*E. coli*) has been engineered as an industrial chassis cell due to its rapid growth and ease of genetic manipulation. However, the wild-type *E. coli* is inherently unable to utilize ethylene glycol as a feedstock, despite possessing proposed pathways for ethylene glycol metabolism. Albert Boronat et al. identified spontaneous mutants of *E. coli* capable of exclusive growth on ethylene glycol as the sole source of carbon and energy, derived from mutants that could grow on propylene glycol. Subsequent analysis revealed a significant increase in the levels of propanediol oxidoreductase (fucO) and glycolaldehyde dehydrogenase (aldA) in this spontaneously mutated strain [11]. Building on these findings, prior research has predominantly focused on engineering ethylene glycol-utilizing *E. coli* by overexpressing *fucO* and *aldA*. More recently, an engineered *E. coli* MG1655 strain was developed, demonstrating efficient ethylene glycol consumption and glycolate production through the overexpression of these endogenous genes. Notably, the growth medium requires supplementation with glycerol in addition to ethylene glycol [12].

Simultaneously, Smaranika Panda et al. enhanced ethylene glycol utilization in *E. coli* by finely tuning the expression levels of the *fucO* and *aldA* genes, while also adjusting the composition of the growth medium [13]. However, achieving exclusive growth on ethylene glycol as the sole source of carbon and energy remains challenging. Importantly, Panda et al.'s research highlights the significance of incorporating a low concentration of glycerol (0.1 g/L) or supplementing amino acids to significantly improve the efficient utilization of ethylene glycol [14]. Therefore, the development of an *E. coli* chassis cell capable of thriving solely on ethylene glycol as the primary source of carbon and energy, coupled with a comprehensive understanding of ethylene glycol's metabolic utilization, is crucial for advancing its use as a non-sugar feedstock in industrial applications.

In the proposed pathways for ethylene glycol metabolism in *E. coli*, ethylene glycol undergoes sequential oxidation, resulting in the production of glyoxylate. Glyoxylate can serve as a sole source of carbon and energy for growth through the glycolate and glyoxylate degradation I pathway, where two molecules of glyoxylate condense to form tartronate semialdehyde [13]. Another hypothesis suggests that glyoxylate can undergo metabolic processes through the glyoxylate shunt, wherein it can condense with acetyl-CoA to produce malate as part of the glycolate and glyoxylate degradation II pathway [14]. Research has indicated that when *E. coli* utilizes glycolate or glyoxylate as its sole carbon and energy source, the key enzyme gcl in pathway I is indispensable and cannot be disrupted, whereas the key enzyme glcB in pathway II is dispensable. This suggests that *E. coli* primarily utilizes pathway I and employs pathway II as a secondary route when utilizing ethylene glycol as the sole carbon and energy source, and it is highly likely that the glycolate and glyoxylate degradation pathways I and II operate in parallel [15]. Further investigations are required to validate these pathways and mechanisms.

Besides *Escherichia coli*, significant progress has been made in engineering ethylene glycol-utilizing *Pseudomonas putida*. Mückschel et al. found that *P. putida* JM37 exhibited robust growth using ethylene glycol as its sole carbon and energy source, whereas strain *P. putida* KT2440 failed to grow under identical conditions despite possessing potential genes for ethylene glycol metabolism [16]. Expanding on these findings, Franden et al. demonstrated that overexpressing only gcl and glxR enabled *P. putida* KT2440 to grow on ethylene glycol as the sole carbon source [17]. In comparison to *P. putida*, *Escherichia coli* offers rapid growth and facile genetic manipulation capabilities. Therefore, the development of *E. coli* chassis

cells capable of utilizing ethylene glycol as the sole carbon and energy source could potentially yield significant outcomes.

In this study, we initially applied UV radiation mutagenesis to *E. coli* MG1655, enabling its growth on ethylene glycol as the sole source of carbon and energy. Subsequently, laboratory adaptive evolution was employed to further refine *E. coli*'s exclusive ability to thrive on ethylene glycol. Transcriptomic analysis provided insights into the capability of EG02 to utilize ethylene glycol as the sole source of carbon and energy for growth. Building upon this, we engineered four wild-type *E. coli* strains to utilize ethylene glycol exclusively as their carbon and energy source through reverse metabolic engineering. These research findings not only deepened our understanding of *E. coli*'s metabolic utilization of ethylene glycol, but also positioned ethylene glycol as a highly promising non-sugar feedstock candidate for an *E. coli* chassis cell. Even more excitingly, the simple addition of M9 salts to PET degradation products not only could make it a feasible non-sugar feedstock for *E. coli* chassis cells, but also offered an economically and environmentally friendly solution for recycling PET degradation products, primarily composed of ethylene glycol.

Materials and methods

Strains and culture conditions

The *E. coli* strains MG1655, ATCC 8739, DH5α, and BL21(DE3) underwent genetic modification to enable ethylene glycol metabolism. MG1655 served as the initial strain for utilizing ethylene glycol as the exclusive non-sugar feedstock, while DH5α acted as the cloning host. Strains were cultured at 37 °C in Luria–Bertani medium (LB, 1% w/v tryptone, 0.5% w/v yeast extract, and 1% w/v NaCl). M9 base medium with ethylene glycol (EG) as the sole non-sugar carbon source (X g/L ethylene glycol; X varied between 0.1 and 50 as specified in the text) was used. The M9 medium salts composition included 1 g/L ammonium chloride (NH_4Cl), 6.78 g/L disodium phosphate (Na_2HPO_4), 3 g/L monopotassium phosphate (KH_2PO_4), 0.5 g/L sodium chloride (NaCl), 0.241 g/L $MgSO_4$ solution, 0.011 g/L $CaCl_2$ solution, and 1 mL of a 1000 × trace elements stock solution. The trace elements stock solution contained 1.6 g/L $MnCl_2{\bullet}4H_2O$, 0.38 g/L $CuCl_2{\bullet}2H_2O$, 0.5 g/L $CoCl_2{\bullet}6H_2O$, 0.94 g/L $ZnCl_2$, 0.03 g/L H_3BO_3, 0.4 g/L $Na_2EDTA{\bullet}2H_2O$. The pH of the medium was adjusted to 7, and cultures were maintained at 37℃. Kanamycin (50 mg/L), chloramphenicol (30 mg/L), ampicillin (100 mg/L), streptomycin (100 mg/L), were added to the medium when appropriate.

1‰ of the activated seed culture was inoculated into 100 ml triangle flask, and wild-type *E. coli* MG1655 was cultured at 37 ℃, 250 rpm. The first generation of EG01 and reverse metabolism strains was cultured at 37 ℃, 250 rpm using M9 simple medium with ethylene glycol as the sole non-sugar carbon source. The second generation of acclimated EG02 strains needed additional chloramphenicol resistance during culture. Similarly, the strains cultured in the PET degradation environment were cultured at 37 ℃ and 250 rpm.

Plasmid construction

The plasmids involved in this experiment were assembled using type IIS restriction enzymes-based assembly method, Golden Gate [18], for which DNA primers were designed using J5 Device Editor [19]. The recombinant plasmids and specific primers are shown in Tables S1 and S2. The plasmids pET-32a (+), pACYCDuet-1, pRSFDuet-1, were used for plasmid construction. All of plasmids digesting with appropriate restriction enzymes then ligating vector and inserts to produce plasmids were used in validation the expression of related enzymes in the ethylene glycol metabolic pathways based on transcriptome analysis.

Inducing ethylene glycol-utilizing *E. coli* by ultraviolet irradiation

A scheme of the UV tolerance test for characterizing the survival of ethylene glycol-tolerant *E. coli* is illustrated in Figure S1 [20]. The strains cells in the logarithmic phase were centrifuged for 5 min (5000 rpm) and the solid cells were obtained after removing the supernatant. The solid cells were washed and suspended in sterile water and the cells density was adjusted to $OD_{600}=1$. Wild-type *E. coli* MG1655 cells in the exponential phase were diluted and spread on LB plates. The reference without UV exposure was diluted 10^6-fold and the cells were diluted by 10^3-fold for exposing to UV radiation in a sterile cabinet, irradiated with UV light at a distance of 30 cm for 0, 2, 4, 6 min, respectively. The power of UV lamp was 15 W and the UV wavelength was 254 nm. The number of mutant cells in the petri-dish was counted and the survival rate of cells can be calculated. The mutants with different radiation time were cultured in the M9 simple medium with ethylene glycol as the sole non-sugar carbon source medium at optimal growth condition and observed the growth of the cells. Then the strains with ethylene glycol metabolism ability were screened preliminarily.

The survival rates (SRs) of the strains were calculated using Eq. (1):

$$SR = N1/N2 \times 10^{-3}\%, \tag{1}$$

where $N1$ and $N2$ represented the number of clones with and without UV exposure, respectively. The experiments were done in triplicate.

Quantitative analysis of cell biomass and analytical methods

OD_{600} at specified time points was measured using a microplate reader (BioTek Epoch2). The raw data were converted into standard OD_{600} units through a standard curve.

Quantitative analysis of ethylene glycol (EG): The solution from strains at different culture periods was collected, centrifuged at 12,000 *g* for 5 min, and the supernatant was filtered using a nylon syringe filter with a pore size of 0.22 μm and a diameter of 13 mm. Subsequently, 10 μL of the filtered supernatant was analyzed using high-performance liquid chromatography (HPLC, Agilent 1260) with a Bio-Rad Aminex HPX-87H column (300 mm × 7.8 mm). The mobile phase used was 5 mM H_2SO_4 with a flow rate of 0.8 ml/min (isocratic flow). The column temperature was maintained at 60 ℃, and a UV detector set at a wavelength of 210 nm was employed for quantifying ethylene glycol. The observed retention time for ethylene glycol was 19.3 min.

Quantitative analysis of terephthalic acid (TPA), Mono(2-hydroxyethyl) terephthalic acid (MHET) and bis(2-hydroxyethyl) terephthalate (BHET): Different fermentation time of the strain supernatant, centrifuged at 12000*g* for 5 min, and the supernatant was filtered using a nylon syringe filter with a pore size of 0.22 μm and a diameter of 13 mm. 10 μL of the obtained filtered supernatant was analyzed using a high-performance liquid chromatography (HPLC, Agilent 1260). The column was the XTerra MS C18 Column, (5 μm, 4.6 mm × 250 mm). An isocratic flow was used with a flow rate of 0.6 mL/min. The mobile phase consists of carbinol and 0.1% (v/v) formic acid. The column temperature was 30 ℃. The detector was a UV detector with the wavelength set at 240 nm for quantifying terephthalic acid. The observed retention time for TPA was 12.8 min; the observed retention time for MHET was 22.3 min; the observed retention time for BHET was 27.4 min. Detailed standard product curves and HPLC detection methods are shown in Figure S2.

Adaptive laboratory evolution

EG01, equipped with ptrcDnaB-AID, a novel tool for random genomic base editing, was cultured in M9 (10 g/L EG) medium with an initial OD_{600} of 0.1. The culture was maintained at 37 °C with agitation at 250 rpm for 48 h, and the OD_{600} value was measured using a microplate reader (Tecan Infinite M200). It was transferred every two days, and the growth rate and proliferation of the bacteria were observed after each 12 successive generations.

De novo transcriptome sequencing

Cells of EG02 cultured with LB and M9 (10 g/L EG) medium were harvested. Total RNA of EG02 was extracted from the two media in the exponential phase (three independent biological replicates for each case). Then strand-specific RNA-seq library were prepared. Sequencing was performed on an Illumina NovaSeq 6000 platform and 150 bp paired-end reads were generated (GENEWIZ, Inc., Tianjin, China). Finally, clean reads were obtained after removing reads containing adapter, reads containing N base, and reads with low quality. To estimate gene expression, reads were aligned to the genome assembled using Bowtie2 (2.3.4.3). HTSeq (v0.9.1) was then used to count the reads numbers mapped to each gene. Differential expression analysis of EG02 in LB and M9 (10 g/L EG) medium was performed using the DESeq2R package (1.20.0). padj < 0.05 and $|\log_2(\text{fold change})| > 0$ were set as the threshold for significantly differential expression.

Preparation of PET degradation products

Untreated PET wastes (below 10% crystallinity) were introduced into a 3-L bioreactor (New Brunswick Bio-Flo 115, Eppendorf, Germany) containing 2.5 L of glycine–NaOH buffer (pH 9.0, 100 mM), along with purified DepoPETase, at a ratio of 0.4% W_{enzyme}/W_{PET}. The temperature was maintained at 50 °C using a water bath, with constant agitation at 500 rpm using a single marine impeller. The reaction pH was controlled at 8.7 ± 0.2 by adding 0.5 M NaOH through a peristaltic pump. After 120 h, M9 base salts were introduced to prepare the PET enzymatic degradation product feedstock.

Results

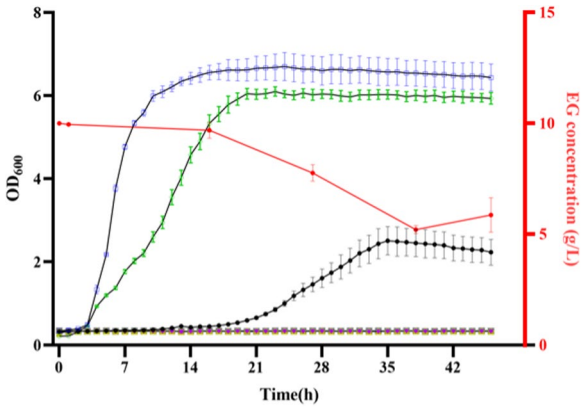
UV-induced mutagenesis enables *E. coli* to thrive exclusively on ethylene glycol as the sole source of carbon and energy

As previously mentioned, only adaptively evolved *E. coli* strains derived from mutants with the capability to grow on propylene glycol exhibit a remarkable ability to thrive on ethylene glycol as the exclusive source of carbon and energy [11]. Conversely, engineered ethylene glycol-utilizing *E. coli* strains, achieved through the overexpression of *fucO* and *aldA*, fall short of this achievement [12, 21]. To unravel the precise mechanism underlying *E. coli*'s metabolism of ethylene glycol and position ethylene glycol as a non-sugar feedstock alternative for *E. coli* chassis cells, we opted to employ UV radiation mutagenesis on *E. coli* to facilitate its growth on ethylene glycol as the sole source of carbon and energy. Given the lack of reports of spontaneous mutants of *E. coli* capable of exclusively utilizing ethylene glycol as a non-sugar feedstock, we semi-quantified the intensity of UV radiation by adjusting

bacterial concentration and exposure time on solid LB medium. The results, illustrated in figure S1, demonstrate that when 1 OD of *E. coli* was diluted 10^6 times without UV radiation or diluted 10^3 times and exposed to UV radiation for 2, 4 and 6 min, the corresponding survival rates were 33.9×10^{-4}%, 4.1×10^{-4}%, and 2.7×10^{-4}% [20], respectively. Subsequently, *E. coli* was diluted 10^3 times and exposed to UV radiation for 4 and 6 min on solid M9 medium containing 10 g/L ethylene glycol as the sole source of carbon and energy. Fortunately, after a 3-day incubation, a single colony emerged on one of the plates subjected to UV radiation for 6 min, as indicated by the yellow circle in figure S1. In contrast, no single colonies were observed on plates subjected to UV radiation for 4 min or on other plates, even after two weeks of cultivation.

To confirm the exclusive growth capacity of this mutant on ethylene glycol as the sole source of carbon and energy, additional assessments were conducted. The mutant was cultured in both LB and M9 liquid media containing 10 g/L ethylene glycol, alongside its parental strain, *E. coli* MG1655. Results showed that in LB liquid medium, the mutant exhibited a slightly superior doubling time of 1.3 h compared to *E. coli* MG1655 with a doubling time of 1.58 h. Similar to previous studies [11, 12, 14], *E. coli* MG1655, MG1655 (*fucO*) overexpressing *fucO*, and MG1655 (*fucO*/*aldA*) overexpressing both *fucO* and *aldA* displayed no growth in liquid M9 medium containing 10 g/L ethylene glycol, even after 84 h of cultivation. In contrast, the mutant exhibited significant growth in M9 liquid medium containing 10 g/L ethylene glycol, albeit with an extended doubling time of 5.2 h. Ethylene glycol consumption was also observed, confirmed by HPLC analysis with a 50% reduction (figure S2). Thus, this mutant indeed possessed the ability to exclusively grow on ethylene glycol as the sole source of carbon and energy and had been designated as EG01 (Fig. 1).

Additionally, we further elucidated the ethylene glycol metabolism capability of EG01. Increasing the feedstock ethylene glycol concentration from 5 to 10 g/L resulted in enhanced growth of EG01. However, growth inhibition occurred at concentrations of 20 g/L and 30 g/L. At 40 g/L, the growth of EG01 was nearly completely suppressed (Figure S3A). We also assessed EG01's growth performance at temperatures of 30 ℃, 37 ℃, and 42 ℃, with the optimal growth temperature aligning with typical *E. coli* strains at 37 ℃ (Figure S3B).

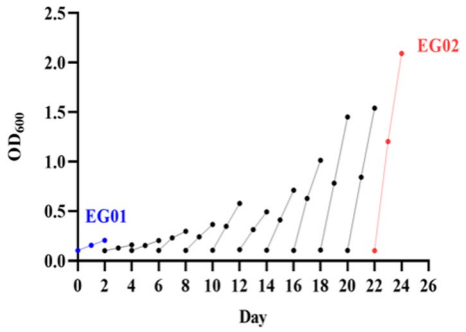


Symbol	Strain-medium	Doubling time (h^{-1})
	MG1655-LB	1.58
	MG1655-M9(10g/L EG)	—
	MG1655(fucO)-M9(10g/L EG)	—
	MG1655(fucO/aldA)-M9(10g/L EG)	—
	EG01-LB	1.26
	EG01-M9(10g/L EG)	5.22

Fig. 1 Characterization of ethylene glycol metabolism in *E. coli* MG1655 and UV-induced mutant *E. coli*. In LB medium, both EG01 (green) and *E. coli* MG1655 (blue) exhibited doubling times of 1.58 h^{-1} and 1.26 h^{-1}, respectively. In M9 medium with ethylene glycol as the sole carbon source, EG01 (black) had a doubling time of 5.22 $h.^{-1}$, whereas the growth of *E. coli* MG1655, MG1655 (*fucO*) overexpressing *fucO*, and MG1655 (*fucO*/*aldA*) overexpressing both *fucO* and *aldA* was undetectable. The red line illustrates the change in ethylene glycol consumption by EG01 in M9 (10 g/L EG) medium. Error bars indicated standard error ($n=3$)

Enhancing *E. coli* chassis cells for ethylene glycol as a non-sugar feedstock

Adaptive laboratory evolution produces evolved microbial strains with desired traits through the application of natural selection principles in a lab setting [22]. To address the limited growth of EG01 in liquid M9 (10 g/L EG) medium, we implemented laboratory adaptive evolution to enhance *E. coli*'s exclusive ability to grow on ethylene glycol as the sole source of carbon and energy. Additionally, to increase the mutation rate and expedite the acquisition of strains with enhanced ethylene glycol metabolism, we introduced DNAB-AID, a novel tool for random genomic base editing. Consequently, EG01, equipped with ptrcDnaB-AID, underwent continuous cultivation for 24 days, with transfers to fresh medium every two days, covering a span of 12 generations [23]. As depicted in Fig. 2, strain EG02 exhibited substantial growth improvement, achieving an OD value of 2.1 ± 0.6 compared to EG01's 0.2 ± 0.1 after 48 h of cultivation. Moreover, EG02 demonstrated an ethylene glycol uptake rate of 8.1 ± 1.3 mmol/gDW·h, surpassing EG01's 4.8 ± 0.8 mmol/gDW h in shake-flasks. Therefore, the growth performance of *E. coli* chassis cells relying solely on ethylene glycol as a non-sugar feedstock showed remarkable enhancement.



Strain	OD_{600}(48h)	Uptake rate of EG (mmol/gDW·h)
EG01	0.2 ± 0.1	4.8 ± 0.8
EG02	2.1 ± 0.6	8.1 ± 1.3

Fig. 2 Enhancing *E. coli* chassis cells for ethylene glycol as a non-sugar feedstock. EG01 underwent continuous cultivation for 24 days, with transfers to fresh medium every two days, covering a span of 12 generations, resulting in the strain EG02. Compared to EG01, after 48 h of cultivation in M9 (10 g/L EG) medium, the final biomass increased from 0.2 ± 0.1 OD to 2.1 ± 0.6 OD, and the uptake rate of ethylene glycol (uptake rate of EG) also rose from 4.8 ± 0.8 mmol/gDW.h to 8.1 ± 1.3 mmol/gDW.h. Error bars indicated standard error ($n = 3$)

Comparative transcriptome analysis of *E. coli* chassis cells for metabolic utilization of ethylene glycol

Traditional approaches to engineering ethylene glycol-utilizing *E. coli* typically focus on overexpressing genes such as *fucO* and *aldA*. However, these methods often do not achieve exclusive reliance on ethylene glycol as the sole carbon and energy source for growth [12–14]. In our pursuit of *E. coli* strains capable of such exclusive utilization, we selected EG02 for experimentation due to its enhanced ethylene glycol metabolism compared to EG01.

Whole transcriptome sequencing (RNA-seq) was performed to quantify gene expression in EG02 under two distinct conditions: LB and M9 (10 g/L EG) liquid medium. Three independent biological replicates were chosen for each condition, and strand-specific RNA-seq libraries were sequenced using an Illumina NovaSeq 6000 system following standard sample preparation protocols (figure S4).

In the proposed pathways for ethylene glycol metabolism in *E. coli*, ethylene glycol is sequentially oxidized by lactaldehyde reductase (fucO), aldehyde dehydrogenase A (aldA), and glycolate dehydrogenase (glcDEF) to glycolaldehyde, glycolic acid, and glyoxylic acid, respectively. It is noteworthy that in M9 medium (10 g/L EG), compared to LB medium, the gene expression levels of these five enzymes increased by fold changes of 4.02, 5.19, 9.40, 9.46, and 7.73, respectively. Subsequently, glyoxylate may be converted to 2-phosphoglycerate through the glycolate and glyoxylate degradation pathway I, involving consecutive four-step catalysis by gcl, hyi, glxR, and glxK. Their fold changes are 8.48, 9.38, 9.76, and 4.26, respectively. Alternatively, glyoxylate may be transformed into malate via the glyoxylate shunt as part of the glycolate and glyoxylate degradation pathway II, catalyzed by glcB, with a fold change of 7.34.

Comparative transcriptome analysis revealed that, compared to the initial genes *fucO* and *aldA*, the subsequent genes (*glcDEF*, *gcl*, *hyi*, and *glxR*), except for *glxK*, exhibited approximately twice the fold changes. Additionally, their expression levels, measured by FPKM (Fig. 3), showed a notable increase of at least tenfold. This disparity provides insights into EG02's capability to utilize ethylene glycol as the sole carbon and energy source for growth. This finding contrasts with conventional approaches for constructing ethylene glycol-utilizing strains, which primarily rely on the overexpression of *fucO* and *aldA* [11, 14].

Rational engineering of *E. coli* chassis cells utilizing ethylene glycol as the sole source

Expanding on the findings from our comparative transcriptome analysis, we have gained deeper insights into the expression levels of each enzyme involved in the proposed pathways for ethylene glycol metabolism in *E. coli*. Our analysis revealed that, in addition to the well-studied fucO and aldA, several other enzymes (glcDEF, glcB, hyi, gcl, glxR, and glxK) play crucial roles in the metabolism of ethylene glycol. This critical aspect has often been overlooked in previous research efforts, underscoring the need for a broader investigation into the enzymatic contributions to ethylene glycol utilization.

To verify this hypothesis, we conducted a systematic study by dividing the proposed pathways for ethylene glycol metabolism into four modules. The first module involves the sequential oxidation of ethylene glycol to glycolate, catalyzed by fucO and aldA, which has garnered significant attention. The second module focuses on the subsequent oxidation of glycolate to glyoxylate, mediated by glcDEF, all regulated by a single operon, facilitating their collective study for overexpression research. The third module encompasses the glycolate and glyoxylate degradation pathway I, where specific reactions are catalyzed by hyi, gcl, glxR, and glxK, all under the regulation of a single operon, facilitating their collective study for overexpression research. The fourth module involves the glyoxylate shunt, where glyoxylate condenses with acetyl-CoA to produce malate, facilitated by glcB catalysis, as a component of the glycolate and glyoxylate degradation pathway II. Despite glcB and glcDEF sharing a common operon, they have not been co-studied for overexpression

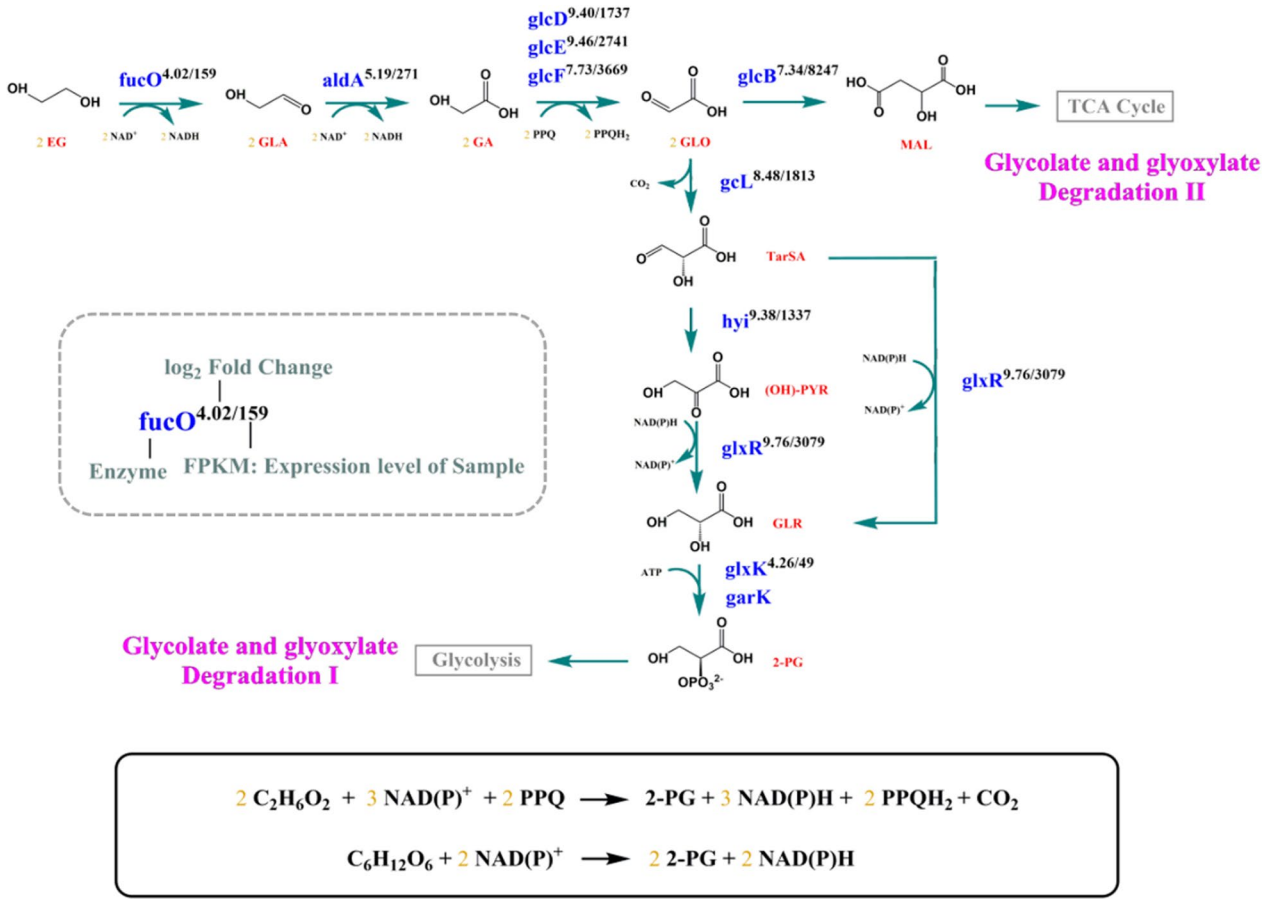


Fig. 3 Comparative transcriptome analysis of *E. coli* chassis cells for metabolic utilization of ethylene glycol. Whole transcriptome sequencing (RNA-seq) was performed to quantify gene expression in EG02 under two distinct conditions: LB liquid medium and M9 (10 g/L EG) medium. The analysis results are presented in the proposed pathways for ethylene glycol metabolism in *E. coli*. The gray dotted box provides an explanation of the data presentation style legend. FPKM (fragments per kilobase of exon model per million reads) indicates the expression level of each gene. The $\log_2$ fold change represents the fold increase in gene expression of EG02 in M9 (10 g/L EG) medium compared to LB medium. The solid black box compares the production of reducing equivalents when using glucose and ethylene glycol as sole carbon sources. fucO, lactaldehyde reductase; aldA, aldehyde dehydrogenase A; glcDEF, glycolate dehydrogenase; glcB, malate synthase G; gcl, glyoxylate carboligase; hyi, hydroxypyruvate isomerase; glxK, glycerate 2-kinase 2; glxR, tartronate semialdehyde reductase 2. EG, Ethylene glycol; GLA, glycolaldehyde; GA, Glycolate; GLO, Glyoxylate; TSA, (2R)-tartronate semialdehyde; (OH)-PYR, hydroxypyruvate; GLR, D-glycerate; 2PG, 2-phospho-D-glycerate; MAL, malate

due to their involvement in distinctly different catalytic reactions. In our investigation, we systematically overexpressed genes associated with these modules in *E. coli* MG1655 to elucidate their respective contributions to ethylene glycol metabolism.

Surprisingly, as depicted in Fig. 4B, overexpressing only the genes from the third module proved sufficient to enable *E. coli* MG1655 to thrive on ethylene glycol as the sole carbon and energy source. In contrast, separate overexpression of genes from the third module and the fourth module did not confer the capability for *E. coli* MG1655 to grow on ethylene glycol alone. Consistent with previous findings, overexpressing only the genes from the first module did not empower *E. coli* MG1655 to utilize ethylene glycol as the sole carbon and energy source. Furthermore, attempts to overexpress *fucO* alone failed to enable *E. coli* MG1655 to grow on ethylene glycol as the sole carbon source (Fig. 1). It is also possible that the growth rate was too slow to observe significant proliferation on ethylene glycol alone.

Subsequently, we individually co-overexpressed the third module genes with either the first module genes or the second module genes, as illustrated in Fig. 4B. Interestingly, co-overexpression of the second module genes enhanced *E. coli* MG1655's capability to metabolize ethylene glycol, whereas co-overexpression of the first module genes did not result in a similar improvement. These findings were consistent across three

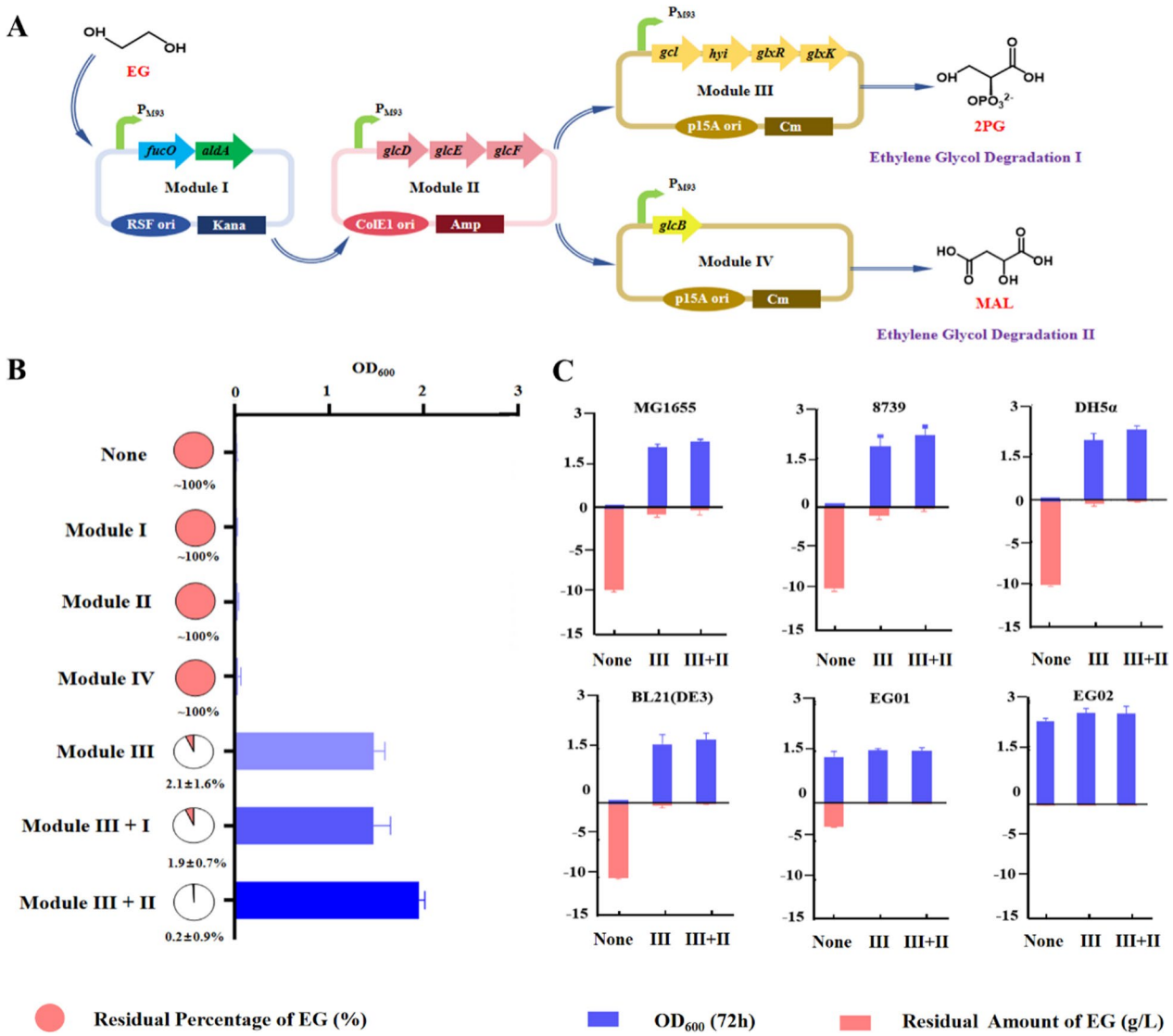


Fig. 4 Rational engineering of *E. coli* chassis cells utilizing ethylene glycol as the sole carbon source. **A** Schematic diagram illustrating plasmid construction for the pathways enabling ethylene glycol metabolism. Module I: PM93-*fucO/aldA*; Module II: PM93-*glcD/glcE/glcF*; Module III: PM93-*gcl/hyi/glxK/glxR*; Module IV: PM93-*glcB*. **B** Reverse metabolic engineering of *E. coli* MG1655 for ethylene glycol utilization. Individual or co-transformation of Module I, II, III, and IV plasmids into *E. coli* MG1655 strains. The OD_{600} value of the strain after 72 h is shown in the blue bar graph, while the residual percentage of ethylene glycol (%) is depicted in the red pie chart. **C** Rational engineering of six *E. coli* chassis cells (*E. coli* MG1655, ATCC8739, *E. coli* DH5α, *E. coli* BL21(DE3), EG01, and EG02) utilizing ethylene glycol as the sole carbon source. The OD_{600} value of the strains after 72 h is represented in the blue bar graph, while the residual amount of ethylene glycol (g/L) is shown in the red bar graph. M9 (10 g/L EG) medium was employed for culturing all *E. coli* strains mentioned above. Error bars indicate standard error ($n = 3$)

other commonly used strains in our laboratory (DH5α, BL21(DE3), ATCC 8739). Among these strains, engineered BL21(DE3) exhibited the strongest ethylene glycol metabolism capacity, designated as EG-BL21(DE3), albeit slightly weaker than EG02. Furthermore, we attempted to further enhance ethylene glycol metabolism by overexpressing these genes in both EG01 and EG02 strains. The results indicated no significant improvement in EG02's ethylene glycol metabolism, while EG01 showed noticeable enhancement, although it did not reach the level observed in EG02 (Fig. 4C). This suggests the involvement of additional unknown factors influencing ethylene glycol metabolism capability.

Complete metabolic utilization of ethylene glycol in PET degradation product

The current effective approach to tackling PET plastic pollution involves the enzymatic or chemical

depolymerization of the polymer into ethylene glycol and terephthalic acid (TPA) monomers [23–26]. However, the newly generated ethylene glycol poses environmental pollution concerns. From an environmental perspective, transforming ethylene glycol within PET degradation products into a non-sugar feedstock for an *E. coli* chassis cell emerges as the most efficient strategy to holistically address PET plastic pollution. Given that the complete enzymatic or chemical depolymerization of PET plastic results in equimolar proportions of ethylene glycol and TPA, it is crucial to explore the impact of TPA on the growth of ethylene glycol-utilizing *E. coli* chassis cells.

The ethylene glycol-utilizing *E. coli* strains, EG-BL21(DE3) and EG02 were cultured separately in M9 medium supplemented with varying concentrations of ethylene glycol (16 mM, 80 mM, 161 mM, 484 mM, 806 mM), along with equimolar levels of TPA-Na_2. Ethylene glycol was nearly completely metabolized after 72 h at initial concentrations of 16 mM, 80 mM, and 161 mM. However, at an initial concentration of 30 g/L (484 mM), a significant amount of ethylene glycol remained post-fermentation. The TPA content in the culture media remained unchanged before and after fermentation across all groups. At an initial concentration of 50 g/L (806 mM) of ethylene glycol and TPA-Na_2, ethylene glycol metabolism was notably hindered after 72 h, resulting in reduced growth of the ethylene glycol-utilizing *E. coli* strains (Fig. 5).

To investigate the inhibitory effects of high concentrations of ethylene glycol or TPA-Na_2 on EG-BL21(DE3) and EG02 (Fig. 5), both strains were cultured using a combination of 10 g/L (161 mM) ethylene glycol and 484 mM TPA-Na_2. Their biomass reached 3.01 OD and 3.27 OD after 72 h of culture at 37 °C. These findings, combined with those shown in Figure S3A, confirm that high concentrations of ethylene glycol primarily inhibit the growth of these *E. coli* chassis cells, rather than TPA-Na_2. Therefore, ethylene glycol in PET degradation products can be developed as a non-sugar feedstock for ethylene glycol-utilizing *E. coli* chassis cells.

Building on the insights gained from the aforementioned study, the subsequent focus is completely utilizing ethylene glycol in the enzymatic PET degradation product. In our earlier research, we optimized PET depolymerization using DepoPETase through directed evolution. This enhancement facilitated the complete depolymerization of untreated PET wastes (below 10% crystallinity) on a liter scale with an enzyme loading of 0.4% W_{enzyme}/W_{PET} at 50 °C in 120 h. According to HPLC analysis, the enzymatic degradation products of PET include 87 mM of

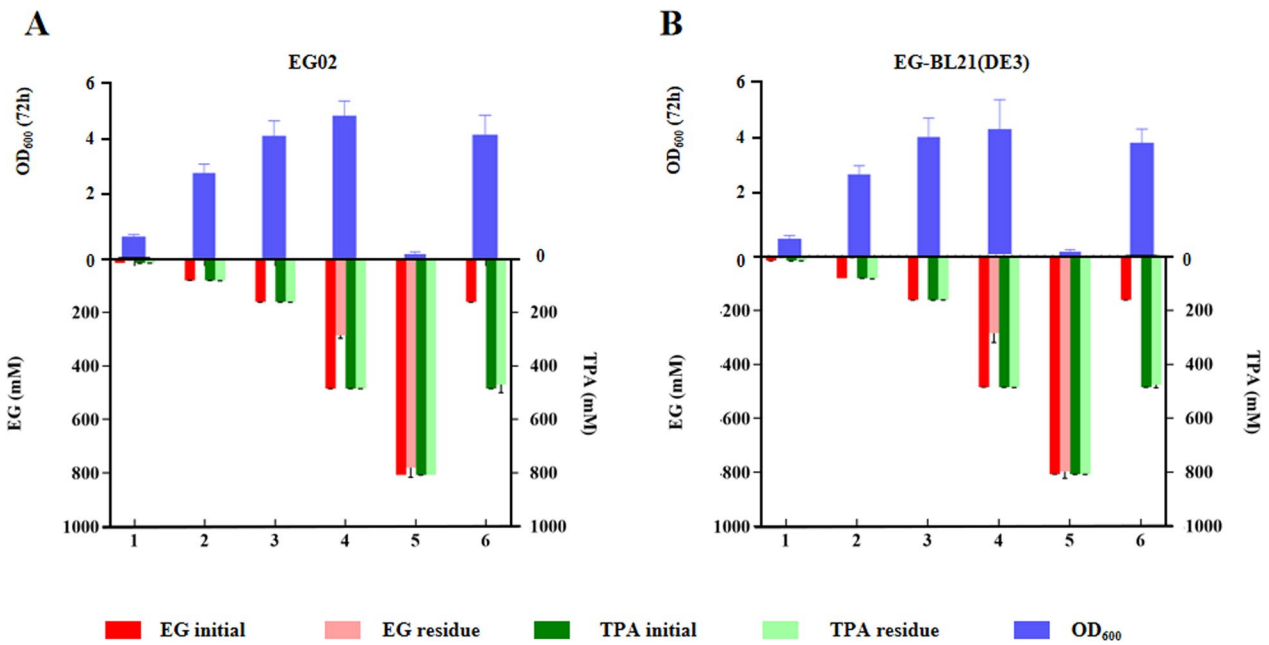


Fig. 5 The influence of TPA on the growth of ethylene glycol-utilizing *E. coli* chassis cells. **A** EG02; (**B**) EG-BL21(DE3). The *E. coli* strains, EG02 and EG-BL21(DE3), capable of utilizing ethylene glycol, were separately introduced into M9 medium containing varying concentrations of ethylene glycol, supplemented with equimolar doses of TPA-Na_2 (TPA). 1: 16 mM EG, 16 mM TPA; 2: 80 mM EG, 80 mM TPA; 3: 161 mM EG, 161 mM TPA; 4: 484 mM EG, 484 mM TPA; 5: 806 mM EG, 806 mM TPA; 6: 161 mM EG, 484 mM TPA. The red bars show the initial addition of ethylene glycol in M9 medium, with light red indicating the residual amounts after 72 h of fermentation. The green bars show the initial addition of TPA, with light green indicating the residual amounts after 72 h. The blue bars represent the OD_{600} values of the strain after 72 h of fermentation. Error bars indicated standard error ($n = 3$)

ethylene glycol, 87 mM of TPA, 32 mM of MHET (a PET enzymatic hydrolysis intermediate), 100 mM glycine–NaOH buffer for PETase enzymatic reaction, and trace amounts of residual DepoPETase [24]. The addition of M9 salts to PET enzymatic degradation products provided a straightforward method for their utilization as a non-sugar feedstock in cultivating ethylene glycol-utilizing *E. coli* chassis cells EG02 and EG-BL21 (DE3). To mitigate the impact of other components in the PET enzymatic degradation product, such as MHET, on the growth of engineered *E. coli* chassis cells, we performed a gradient dilution of the PET degradation product with M9 medium containing 87 mM ethylene glycol. Four sets of media were prepared in ratios of 0:5, 1:4, 4:1, and 5:0, EG02 and EG-BL21 (DE3) were inoculated into each. The results revealed that ethylene glycol-utilizing *E. coli* chassis cells EG02 and EG-BL21 (DE3) exhibited efficient metabolic utilization of ethylene glycol in all four media compositions, even in 100% PET enzymatic degradation product (Fig. 6BC). These findings suggest that the addition of M9 salts to PET degradation products could render them a viable non-sugar raw material for *E. coli* cell factories.

Discussion

In this study, we successfully endowed *E. coli* chassis cells with the unique ability to efficiently utilize ethylene glycol as an alternative, non-sugar feedstock. This capability was achieved through a combination of ultraviolet radiation-induced mutagenesis and subsequent adaptive

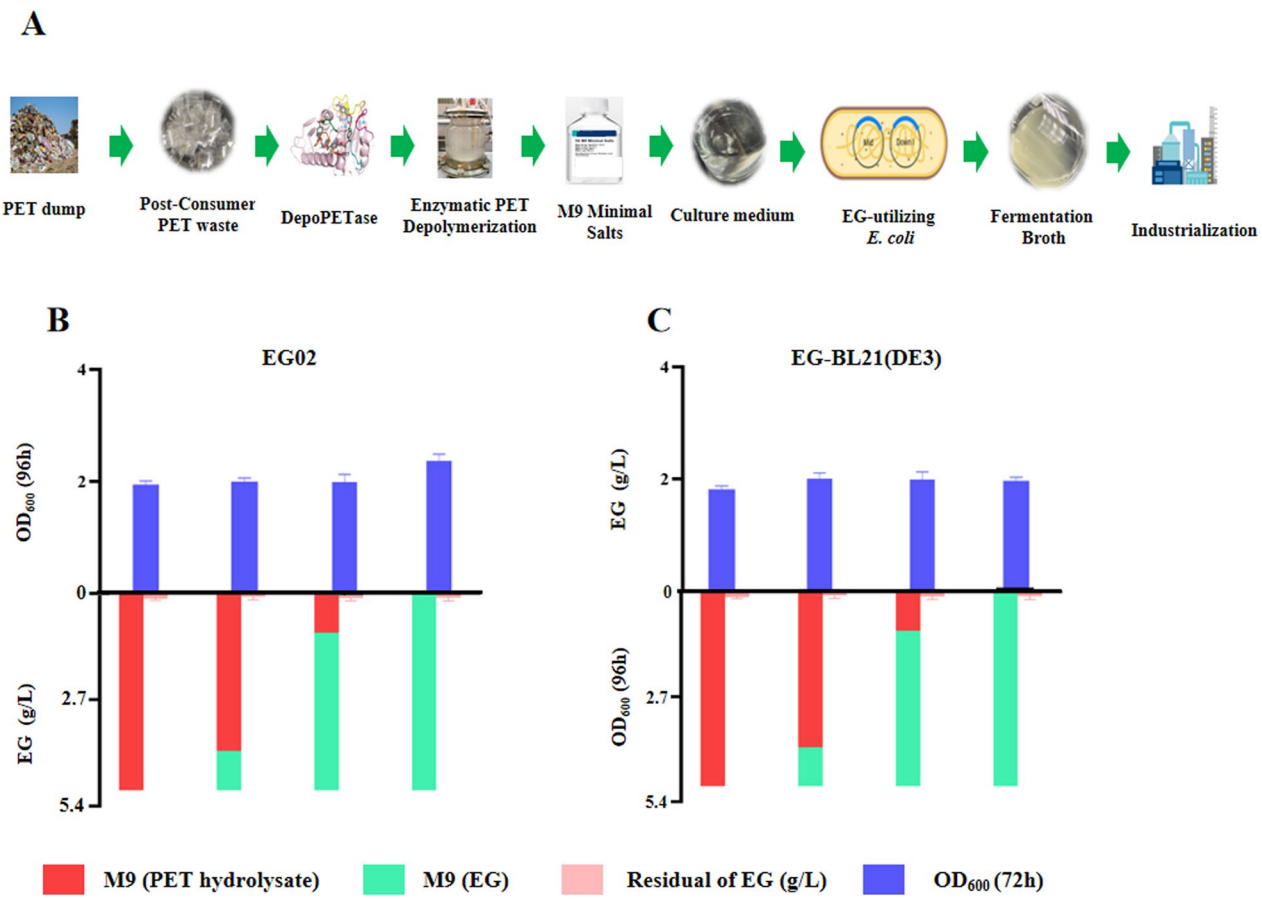


Fig. 6 Complete metabolic utilization of ethylene glycol in PET degradation product. **A** Schematic illustration of developing PET enzymatic degradation products as a non-sugar feedstock. **B** EG02; **C** EG-BL21(DE3). The Impact of other components, such as MHET, in PET enzymatic degradation products on the growth of EG02 and EG-BL21(DE3). A gradient dilution of the PET degradation product was conducted with M9 medium containing 87 mM equimolar ethylene glycol. Four sets of media were prepared in ratios of 0:5, 1:4, 4:1, and 5:0, and equal amounts of EG02 and EG-BL21(DE3) were inoculated into each. Red bars indicated the addition of PET enzymatic degradation product components in each culture medium. Green bars indicated the addition of M9 components in each culture medium. Light red bars indicated the residual ethylene glycol after 72 h of fermentation. The blue bars represented the OD_{600} value of the strain after 72 h of fermentation. Error bars indicated standard error ($n = 3$)

evolution experiments conducted in a laboratory setting. Among the various strains developed, strain EG02 emerged as the most proficient in metabolizing ethylene glycol, demonstrating a remarkable uptake rate of 8.1 ± 1.3 mmol/gDW.h.

We further validated the unique growth capability of ethylene glycol-utilizing *E. coli* chassis cells, confirming their ability to thrive on ethylene glycol as the sole source of carbon and energy. Additionally, we characterized their metabolic performance in utilizing ethylene glycol. These findings demonstrate the cells' efficiency and potential for biotechnological applications, emphasizing their robustness and adaptability in alternative feedstock utilization. Building upon the results of our comparative transcriptome analysis, we proposed that genes associated with the glycolate and glyoxylate degradation I pathway, rather than the *fucO* and *aldA* genes, play a crucial and determinant role in the metabolic utilization of ethylene glycol. This critical aspect has often been overlooked in previous research. However, Frazao and colleagues developed a growth-based assay using ethylene glycol as the sole carbon source and tested different NAD^+-dependent enzymes, including Ec.fucO, Ec.fucOI6L, and Go.adh. After 72 h of cultivation, the observed growth rates were very slow, potentially too low to yield reliable measurements [25]. In contrast, the ethylene glycol-utilizing *E. coli* chassis cells we engineered exhibited growth rates 10 to 20 times faster under identical conditions. This significant improvement indicates that our chassis cells are highly efficient in utilizing ethylene glycol, especially from PET waste degradation products.

Furthermore, both ethylene glycol-utilizing *E. coli* chassis cells, EG-BL21(DE3) and EG02, demonstrated efficient metabolism of the 87 mM ethylene glycol found in PET enzymatic degradation products after 72 h of fermentation. Notably, the addition of M9 salts to PET degradation products rendered them viable non-sugar raw materials for *E. coli* cell factories.

Additionally, we tested the ethylene glycol metabolism capabilities of both EG-BL21(DE3) and EG02 by directly adding higher concentrations of equimolar ethylene glycol and TPA-Na_2 to the M9 medium. When the initial concentration of ethylene glycol was 30 g/L (484 mM) or higher, the growth of both strains was significantly inhibited. To further investigate the inhibitory effects of high concentrations of ethylene glycol or TPA-Na_2 on EG-BL21(DE3) and EG02 (Fig. 5), both strains were cultured using a combination of 10 g/L (161 mM) ethylene glycol and 484 mM TPA-Na_2. These findings, combined with those shown in Figure S3A, confirm that high concentrations of ethylene glycol primarily inhibit the growth of these *E. coli* chassis cells, rather than TPA-Na_2.

In our study, the enzymatic degradation of post-consumer PET waste was conducted in a 100 mM glycine–NaOH buffer [24]. We also reviewed two other studies that used a 100 mM potassium phosphate buffer for the same purpose [26, 27]. The salt concentrations in the buffers across all three studies showed no significant differences. Therefore, ethylene glycol in PET degradation products can be developed as a non-sugar feedstock for ethylene glycol-utilizing *E. coli* chassis cells.

Biological processes are crucial for sustainable development and resource utilization by harnessing various raw materials. Traditionally, glucose and xylose have been the primary sugar feedstocks in industrial biological manufacturing, but they pose challenges such as high costs and potential threats to food security [1, 2]. Consequently, there is growing interest in non-sugar alternatives, with ethylene glycol emerging as a particularly promising candidate for industrial chassis cells. Pandit and colleagues demonstrated that ethylene glycol exhibits the highest orthogonality scores compared to formate, glucose, and xylose in the biosynthesis of succinate, glycolate, and 2,3-butanediol [4, 28]. Additionally, ethylene glycol yields more reducing equivalents than traditional glucose under equimolar conditions (Fig. 3). This characteristic is advantageous for using ethylene glycol as a substrate to synthesize chemicals that require higher energy and reducing power, such as carotenoids and lycopene [29]. However, during the metabolism of ethylene glycol, glyoxylate carboligase (gcl) condenses two molecules of glyoxylate to form tartronate semialdehyde, simultaneously producing one molecule of CO_2. Consequently, the carbon efficiency for converting ethylene glycol to pyruvate is significantly lower than that for glucose [29].

Ethylene glycol, derived cost-effectively from ethylene or sources like PET plastic degradation products, enhances the sustainability of biological industrial manufacturing by reducing reliance on finite natural resources [6–9, 30–33]. Developing ethylene glycol as a non-sugar feedstock improves industry adaptability to regional resource variations and changing demands. Moreover, substituting ethylene glycol with inexpensive waste and biomass resources reduces costs, enhances competitiveness in biological industrial products, and promotes sustainable manufacturing practices. In conclusion, research and development of ethylene glycol as a non-sugar feedstock hold significant implications for sustainable development. This innovation boosts raw material diversity, enhances production adaptability, and lowers costs in biological industrial manufacturing, paving the way for a more sustainable and innovative future in industrial biotechnology.

Conclusion

This study achieved the acquisition of *E. coli* chassis cells capable of exclusively utilizing ethylene glycol for growth via UV mutagenesis and laboratory adaptive evolution. Furthermore, through reverse metabolic engineering, four *E. coli* strains were successfully engineered to grow solely on ethylene glycol. Additionally, the supplementation of PET degradation products with M9 salts transformed them into a non-sugar feedstock for *E. coli* chassis cells. These findings present a practical solution for recycling ethylene glycol from PET waste degradation products. Moreover, the study also emphasizes the significant roles of genes associated with the glycolate and glyoxylate degradation I pathway in the metabolic utilization of ethylene glycol, an aspect frequently overlooked in previous research.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13068-024-02568-4.

Supplementarye Materials 1: Fig. S1. EG-utilizing E. coli EG01 was obtained by UV mutagenesis. *E. coli* MG1655 cells in the exponential stage were diluted and spread on LB plates and M9plates. The cells were diluted 10^3 times, exposed to ultraviolet radiation in a sterile cabinet, and the unirradiated reference was diluted 10^6 times. Because part of the UV radiation is blocked by the side wall of the plastic plate, the number of colonies was counted in the oval area of the plate. A single colonyin the M9plate was found after 6 min of irradiation. The red/green bar on the right showed the UV survival rate of *E. coli* exposed to ultraviolet radiation. Fig. S2. High-Performance Liquid ChromatographyMethod for PET Monomer Detection. On the left was the HPLC peak diagram of different concentrations of substances, and on the right was the element peak area and standard curve. A. Ethylene glycol; B. Terephthalate; C. Terephthalate monoester; D. terephthalateester. Error bars indicated standard error. Fig. S3. Characterization of Metabolic Performance in Ethylene Glycol Utilization by E. coli Chassis Cell EG01. A. Effect of ethylene glycol concentration on EG01 growth. B. The optimal growth temperature of EG01 was tested. Error bars indicated standard error. Fig. S4. Comparative Transcriptome Analysis of E. coli's Metabolic Utilization of Ethylene Glycol as the Sole Carbon Source. A. Pearson correlation between EG02 samples cultured with M9and LB. B. PCA analysis of the transcription of EG02 cultured with M9and LB. C. Differential expression of genes screened by RNA-seq, red representing 579 up-regulated genes; Green represents down-regulated 408 genes. D. Heatmap plot for DEGs of EG02 cultured with M9and LB. E. GO enrichment analysis of different expression genes. F. KEGG pathway analysis of differentially expressed genes compared with M9group and LB control group. The bubble diagram shows 20 signaling pathways associated with EG metabolism. Table S1 Plasmids used in this study. Table S2 Primers used in this study for plasmids construction

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Not applicable.

Author contributions
X.Z., C.B., M.C. and L.Z designed the research, analyzed data, and wrote the manuscript. J.C. and P.W. designed the research, performed experiments, analyzed data, and wrote the manuscript. Y.M. performed experiments, analyzed data. X.Z. wrote the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
A provisional patent has been submitted in part entailing the reported findings. The authors declare no conflict of interest.

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References

1. Zeng AP. New bioproduction systems for chemicals and fuels: needs and new development. Biotechnol Adv. 2019;37(4):508–18.
2. Mahadevan R, Burgard AP, Famili I, Van Dien S, Schilling CH. Applications of metabolic modeling to drive bioprocess development for the production of value-added chemicals. Biotechnol Bioprocess Eng. 2005;10(5):408–17.
3. Smanski MJ, Bhatia S, Zhao D, Park Y, BA Woodruff L, Giannoukos G, Ciulla D, Busby M, Calderon J, Nicol R, et al. Functional optimization of gene clusters by combinatorial design and assembly. Nat Biotechnol. 2014;32(12):1241–9.
4. Pandit AV, Srinivasan S, Mahadevan R. Redesigning metabolism based on orthogonality principles. Nat Commun. 2017;8:15188.
5. Ignea C, Raadam MH, Motawia MS, Makris AM, Vickers CE, Kampranis SC. Orthogonal monoterpenoid biosynthesis in yeast constructed on an isomeric substrate. Nat Commun. 2019;10(1):3799.
6. Bushuyev OS, De Luna P, Dinh CT, Tao L, Saur G, van de Lagemaat J, Kelley SO, Sargent EH. What should we make with CO_2 and how can we make it? Joule. 2018;2(5):825–32.
7. Na J, Seo B, Kim J, Lee CW, Lee H, Hwang YJ, Min BK, Lee DK, Oh HS, Lee U. General technoeconomic analysis for electrochemical coproduction coupling carbon dioxide reduction with organic oxidation. Nat Commun. 2019;10(1):5193.
8. Kandasamy S, Samudrala SP, Bhattacharya S. The route towards sustainable production of ethylene glycol from a renewable resource, biodiesel waste: a review. Catal Sci Technol. 2019;9(3):567–77.
9. Marchesan AN, Oncken MP, Maciel Filho R, Wolf Maciel MR. A roadmap for renewable C2–C3 glycols production: a process engineering approach. Green Chem. 2019;21(19):5168–94.
10. Tiso T, Winter B, Wei R, Hee J, de Witt J, Wierckx N, Quicker P, Bornscheuer UT, Bardow A, Nogales J, et al. The metabolic potential of plastics as biotechnological carbon sources–review and targets for the future. Metab Eng. 2022;71:77–98.
11. 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.
12. Pandit AV, Harrison E, Mahadevan R. Engineering *Escherichia coli* for the utilization of ethylene glycol. Microb Cell Fact. 2021;20(1):1–7.
13. Hansen RW, Hayashi JA. Glycolate metabolism in *Escherichia coli*. J Bacteriol. 1962;83(3):679–87.

14. Bock A, Sawers G. *Escherichia coli* and Salmonella cellular and molecular biology, 2nd edition. Fermentation. 1996: 262–282.
15. Karp PD, Billington R, Caspi R, Fulcher CA, Latendresse M, Kothari A, Keseler IM, Krummenacker M, Midford PE, Ong Q, et al. The BioCyc collection of microbial genomes and metabolic pathways. Brief Bioinform. 2019;20(4):1085–93.
16. Mückschel B, Simon O, Klebensberger J, Graf N, Rosche B, Altenbuchner J, Pfannstiel J, Huber A, Hauer B. Ethylene glycol metabolism by *Pseudomonas putida*. Appl Environ Microbiol. 2012;78(24):8531–9.
17. Franden MA, Jayakody LN, Li WJ, Wagner NJ, Cleveland NS, Michener WE, Hauer B, Blank LM, Wierckx N, Klebensberger J, et al. Engineering *Pseudomonas putida* KT2440 for efficient ethylene glycol utilization. Metab Eng. 2018;48:197–207.
18. Engler C, Kandzia R, Marillonnet S. A one pot, one step, precision cloning method with high throughput capability. PLoS ONE. 2008;3(11): e3647.
19. Hillson NJ, Rosengarten RD, Keasling JD. j5 DNA assembly design automation software. ACS Synth Biol. 2012;1(1):14–21.
20. Wang P, Zhao D, Li J, Su J, Zhang C, Li S, Fan F, Dai Z, Liao X, Mao Z, et al. Artificial diploid *Escherichia coli* by a CRISPR chromosome-doubling technique. Adv Sci. 2023;10(7): e2205855.
21. Panda S, Fung VYK, Zhou JFJ, Liang H, Zhou K. Improving ethylene glycol utilization in *Escherichia coli* fermentation. Biochem Eng J. 2021;168: 107957.
22. Dragosits M, Mattanovich D. Adaptive laboratory evolution–principles and applications for biotechnology. Microb Cell Fact. 2013;12(1):64.
23. Wang J, Zhao D, Li J, Hu M, Xin X, Price MA, Li Q, Liu L, Li S, Rosser SJ, et al. Helicase-AID: a novel molecular device for base editing at random genomic loci. Metab Eng. 2021;67:396–402.
24. Shi L, Liu P, Tan Z, Zhao W, Gao J, Gu Q, Ma H, Liu H, Zhu L. Complete depolymerization of PET wastes by an evolved PET hydrolase from directed evolution. Angewandte Chemie Int Edition. 2023;62(14):e202218390.
25. Frazão CJR, Wagner N, Rabe K, Walther T. Construction of a synthetic metabolic pathway for biosynthesis of 2,4-dihydroxybutyric acid from ethylene glycol. Nat Commun. 2023;14(1):1931.
26. Tournier V, Topham CM, Gilles A, David B, Folgoas C, Moya-Leclair E, Kamionka E, Desrousseaux ML, Texier H, Gavalda S, et al. An engineered PET depolymerase to break down and recycle plastic bottles. Nature. 2020;580(7802):216–9.
27. Lu H, Diaz DJ, Czarnecki NJ, Zhu C, Kim W, Shroff R, Acosta DJ, Alexander BR, Cole HO, Zhang Y, et al. Machine learning-aided engineering of hydrolases for PET depolymerization. Nature. 2022;604(7907):662–7.
28. Pandit AV, Harrison E, Mahadevan R. Engineering *Escherichia coli* for the utilization of ethylene glycol. Microb Cell Fact. 2021;20(1):22.
29. Wagner N, Wen L, Frazão CJR, Walther T. Next-generation feedstocks methanol and ethylene glycol and their potential in industrial biotechnology. Biotechnol Adv. 2023;69: 108276.
30. Wei R, Tiso T, Bertling J, O'Connor K, Blank LM, Bornscheuer UT. Possibilities and limitations of biotechnological plastic degradation and recycling. Nat Catal. 2020;3(11):867–71.
31. Wierckx N, Prieto MA, Pomposiello P, de Lorenzo V, O'Connor K, Blank LM. Plastic waste as a novel substrate for industrial biotechnology. Microb Biotechnol. 2015;8(6):900–3.
32. Li WJ, Jayakody LN, Franden MA, Wehrmann M, Daun T, Hauer B, Blank LM, Beckham GT, Klebensberger J, Wierckx N. Laboratory evolution reveals the metabolic and regulatory basis of ethylene glycol metabolism by Pseudomonas putida KT2440. Environ Microbiol. 2019;21(10):3669–82.
33. Ügdüler S, Van Geem KM, Denolf R, Roosen M, Mys N, Ragaert K, De Meester S. Towards closed-loop recycling of multilayer and colored PET plastic waste by alkaline hydrolysis. Green Chem. 2020;22(16):5376–94.

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