Investigation of the key factors on 3-hydroxypropionic acid production with different recombinant strains

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Abstract 3-Hydroxypropionic acid (3-HP) is an important compound and precursor for a series of chemicals and polymeric materials. In this study, the 3-HP producing bacteria were constructed and studied for efficient synthesis of 3-HP. The results indicated that the instability of glycerol dehydratase (GDHt) affected the 3-HP production significantly, which was successfully solved by the expression of glycerol dehydratase reactivase (GdrB), with fivefold increase in 3-HP yield. Meanwhile, NAD⁺-regenerating enzymes GPD1 (glycerol-3-phosphate dehydrogenase) was expressed; however, the results showed 3-HP was significantly decreased from 56.73–4 mM, and malic acid was obviously increased. Analysis of the C flux distribution showed that the main reason for the results was the lack of NAD⁺. The addition of NAD⁺ further increased the 3-HP production to 23.87 mM, demonstrating that the “regeneration of NAD⁺” was the major factor for enhancing 3-HP production.

Keywords 3-hydroxypropionic acid · Glycerol dehydratase · Acetaldehyde dehydrogenase · Glycerol · NAD⁺ regeneration

Introduction

With the increasing concerns on environment and energy, biodiesel was regarded as a viable fuel and has attracted much attention due to its wide utilization as a fossil diesel substitution (Leung et al. 2010). Glycerol is an inevitable byproduct generated during the biodiesel production processes, and the tremendous growth of biodiesel industry has led to a dramatic decrease in price of crude glycerol over the past few years, resulting in a research hotspot on the utilization of glycerol to produce high-valued chemicals (Cho et al. 2015; Clomburg and Gonzalez 2013; Diaz-Alvarez et al. 2011; Viana et al. 2012). 3-Hydroxypropionic acid (3-HP) is an important building block for a series of chemicals and polymeric materials, which shows great potential applications in commodity, environmental treatment, and chemical industries (Chen et al. 2014; Honjo et al. 2015; Kim et al. 2014; Tokuyama et al. 2014). With the development of biocatalysts, the bio-production of 3-HP becomes available under mild and environment-friendly reaction conditions (Kumar et al. 2013). Enzymes including glycerol dehydratase (GDHt) and aldehyde dehydrogenase (AldH) are mainly involved in the biosynthesis of 3-HP, by which the glycerol is first catalyzed to 3-hydroxypropionaldehyde (3-HPA), followed by the conversion to 3-HP. As a rate-limiting enzyme for the conversion of glycerol to 3-HPA, GDHt is consisted of three subunits, i.e., α , β , and γ , and exists as the form of $\alpha_2\beta_2\gamma_2$ heterohexamers. It is reported that GDHt is a vitamin B₁₂-dependent enzyme ubiquitous in different microorganisms, such as *Citrobacter butyricum*, *Klebsiella pneumoniae*, and *Citrobacter freundii* (Jiang et al. 2015, 2016). AldH is responsible for the catalysis of oxidation of aldehydes to carboxylic acids, and has been found mostly in the *Escherichia coli* and *Saccharomyces cerevisiae* (Ko et al. 2012).

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Most of AldHs are NAD⁺-dependent and generate NADH during the catalysis process under aerobic condition (Luo et al. 2012; Sabet-Azad et al. 2013). To consume the excessive NADH, 3-HP producing strains produce a large quantity of byproducts including organic acid and alcohols, which significantly reduces the 3-HP yield from glycerol (Ashok et al. 2013b). To generate NAD⁺ efficiently, 3-HP production which should be conducted under aerobic condition with oxygen serves as an electron acceptor. However, under aerobic conditions, the enzyme activity of GDHt is inhibited, since the Co–C bond of coenzyme vitamin B₁₂ could be destroyed by oxygen (Xu et al. 2009). Zhu et al. (2009) reported that the GDHt activity under aerobic condition was 46% lower than that under the anaerobic condition. Thus, to solve this problem, NAD⁺ regeneration strategies should be considered during 3-HP preparation process.

In this study, the recombinant strains of *E. coli* were constructed. The GDHt and α -ketoglutaric semialdehyde dehydrogenase (KGSADH) with site-directed mutagenesis were co-expressed and a glycerol-3-phosphate dehydrogenase (GPD1) responsible for NAD⁺ regeneration was introduced into the genetically engineered strains to increase the 3-HP yield. The results obtained in this study demonstrated the efficiency of the constructed strains for the biosynthesis of 3-HP.

Materials and methods

Materials

Strains and plasmids used in this study are listed in Table 1. *E. coli* BL21 (DE3) (Invitrogen Co., Carlsbad,

CA, USA) was used for plasmid construction and 3-HP production. T4 DNA ligase and restriction endonuclease were obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA). 3-HP was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan).

Construction of recombinant *E. coli* strains

The genomic DNA of *Klebsiella pneumoniae* DSM 2026 was isolated and used as template for the amplification of *dhaB123* and *gdrAB* genes, and the genomic DNA of *A. brasiliense* was isolated and used as template for the amplification of KGSADH genes. The primers used in this study were listed in Table 2. The *dhaB123* and *gdrA* fragments (GenBank: U30903) with *Eco*RI and *Hind*III restriction sites, and the *gdrB* fragment (Gene ID: 95102055) with the *Hind*III restriction sites were digested by the corresponding endonucleases and ligated into the pre-digested pGEM-T vectors separately, resulting in recombinant plasmids denoted as pGBG1 and pGBG2, respectively.

The plasmids, pACYCDuet-tac, pET28a, pETDuet-1, and pTrc-99a, were used for expressing the *dhaB123* and *gdrA* fragments, resulting in plasmids referred to as pABG1, pEBG1, pDBG1, and pTBG1. The pGBG2 was digested with *Hind*III and the fragment of *gdrB* with 0.36 kb was cloned into pDBG1. The resulting plasmid containing *dhaB123* and *gdrAB* in the pETDuet-1 vector is referred to as pDBG2.

The pEBG1, pDBG1, pABG1, and pTBG1 were transformed individually into *E. coli* BL21 (DE3) competent cells to yield R-EBG1, R-DBG1, R-ABG1, and R-TBG1, respectively. Details about the construction of plasmids are

Table 1 Bacterial strains and plasmids used in this study

Strain and plasmid	Description	Source
Strain		
<i>E. coli</i> BL21(DE3)	Expression host	Novagen
Plasmid		
pGEM-T Easy	Lac Z α ; cloning vector; Amp ^r	Promega
pACYCDuet-tac	Expression vector with the tac promoter	Lab collection
pCDFDuet-tac	Expression vector with the tac promoter	Lab collection
pCBG1	Template for <i>dhaB</i> and <i>gdrA</i> genes	This study
pCBG2	Template for <i>gdrB</i> genes	This study
pCKS	KGSADH in pCDFDuet-tac, Sm ^r	This study
pEBG1	<i>dhaB</i> and <i>gdrA</i> in pET-28a, Kan ^r	This study
pDBG1	<i>dhaB</i> and <i>gdrA</i> in pETDuet-1, Amp ^r	This study
pABG1	<i>dhaB</i> and <i>gdrA</i> in pACYCDuet-tac, Cm ^r	This study
pTBG1	<i>dhaB</i> and <i>gdrA</i> in pTrc-99a, Amp ^r	This study
pDBG2	<i>dhaB</i> and <i>gdrAB</i> in pETDuet-1, Amp ^r	This study
pCKSG	<i>gpd1</i> in pCDFDuet-tac-KGSADH, Sm ^r	This study

Table 2 Primers used in this study

Primers	Sequence (5'–3') and restriction enzyme ^a
dhaB1-4-F	CCGGAATTCATGAAAAGATCAAAACGATTTGCAGTACT (<i>EcoR</i> I)
dhaB1-4-R	GTTAAGCTTGATCTCCCACTGACCAAAGCTGG (<i>Hind</i> III)
KGSADH-F	GGAATTCATATGGCTAACGTGACTTATAC (<i>Nde</i> I)
KGSADH-R	CCGCTCGAGTTACACTGCCATAACAG (<i>Xho</i> I)
gpd1-F	CATGCCATGGATGTCTGCTGCTGCTGACCGT (<i>Nco</i> I)
gpd1-R	CCGGAATTCCTAGCAGCCGGATCTCAGTG (<i>EcoR</i> I)
gdrB-F	GAAAAGCTTGAGGGGACCGTCATGTGCT (<i>Hind</i> III)
gdrB-R	GAAAAGCTTTCAGTTTCTCTCACTTAACGGCAGG (<i>Hind</i> III)

^aRestriction sites are underlined **Fig. 1** Construction of the plasmids pEBG1, pDBG1, pDBG2, pABG1, pTBG1, pCKS, and pCKSG

shown in Fig. 1. To develop the final recombinant strains producing 3-HP, the plasmids pEBG1, pDBG1, pABG1, pTBG1, and pDBG2 were transformed together with pCKS into *E. coli* BL21 (DE3), respectively. The resulting strains were marked as R-EBGK1, R-DBGK1, R-ABGK1, R-TBGK1, and R-DBGK2. The pCKSG were transformed together with pDBG2 into *E. coli* BL21 (DE3) and the resulting strain was named as R-DBGK2.

SDS-PAGE analysis

The recombinant *E. coli* strains were grown in LB medium (0.5% yeast extract, 1% tryptone, and 1% NaCl) with appropriate antibiotics at 37 °C. The cultures were induced with 0.1 mM IPTG at 0.6 optical density of 600 nm (OD₆₀₀). The cells were harvested at 8 h after induction and centrifuged at 10,000g for 5 min. The cell pellets were washed twice with 100 mM potassium phosphate buffer

(pH 8.0) and resuspended in the same buffer. The cells were lysed using an Ultrasonic Cell Disruptor (VCX 130 PB, SONICS & MATERIALS INC, Newtown, CT, USA) at 1250 psi. The cell lysate was centrifuged at 8000g for 10 min, and the supernatants were used for SDS-PAGE and enzyme activity measurement. The supernatants were examined on SDS-PAGE under denaturing conditions (Laemmli 1970). Coomassie brilliant blue R-250 was used to stain the proteins.

Enzyme activity assay

The GDHt activity was measured by the method reported by Toraya et al. (1977) based on the reaction of aldehydes with MBTH (3-methyl-2-benzothiazolinone), resulting in the formation of azine derivatives, which can be determined spectrophotometrically. The reaction was performed in reaction mixture containing 100 µL crude cell extract,

0.2 M 1,2-propanediol, 0.05 M KCl, 0.035 M potassium phosphate buffer (pH 8.0), and 15 μ M vitamin B₁₂, in a total volume of 1 mL. After 10 min reaction at 37 °C, 1 mL ddH₂O was added and the amount of propionaldehyde was determined according to the absorbance at 305 nm. One unit of GDHt activity was defined as the amount of enzyme required to form 1 μ mol of propionaldehyde per min.

The KGSADH activity was determined at pH 8.0 using the method reported by Ashok et al. (2011) with slight modifications. Briefly, the enzyme lysate was incubated in 100 mM potassium phosphate buffer (pH 8.0), containing 1 mM DL-dithiothreitol at 37 °C for 5 min. The reaction was initiated by adding 2 mM acetaldehyde and 2 mM NAD⁺. The amount of NADH formed was determined using a molar extinction coefficient ($\Delta_{\epsilon 340}$) of 6.22×10^3 M/cm. One unit of aldehyde dehydrogenase activity was defined as the amount of enzyme required to reduce 1 μ mol of NAD⁺ to NADH in 1 min.

Cultivation of recombinant *E. coli* for 3-HP production

The recombinant *E. coli* was cultured in M9 medium for 3-HP production. M9 medium contained the following components per liter of deionized water: MgSO₄·7H₂O, 0.25 g; NaCl, 1 g; Na₂HPO₄·12H₂O, 22.7 g; KH₂PO₄, 3 g; NH₄Cl, 1 g; yeast extract, 4 g; and glycerol, 30 g. The cells were grown aerobically in 250 mL Erlenmeyer flasks containing 100 mL of the medium at 37 °C and 200 rpm in an orbital incubator shaker. The cells were induced with 0.1 mM IPTG and 15 μM of filter-sterilized vitamin B₁₂ was added when the OD₆₀₀ reached about 0.6.

Analytical methods

Cell growth was monitored by measurement of OD₆₀₀ of appropriately diluted sample with a UV-visible spectroscopy system (INESA Instrument, Shanghai, China). Dry cell weight (DCW) was determined as follows: 1 mL of cell suspension (10,000g, 5 min) was centrifuged, washed with deionized water, and centrifuged again, followed by resuspension in a small volume of deionized water and drying at 80 °C until constant. One OD₆₀₀ unit corresponded to 0.37 g/L dry cell weight.

The concentration of 3-HP, glycerol, and other metabolites in culture broth were determined by HPLC (Waters, Milford, MA, USA) equipped with a UV-Vis and RI detector and an ion exchange column (300 × 78 mm; Aminex HPX-87H; Bio-Rad, Hercules, CA, USA). The mobile phase was 5 mM H₂SO₄ and the flow rate was 0.6 mL/min. The column and flow cell temperatures were 65 °C and 45 °C, respectively.

Results and discussion

Site-directed mutagenesis of *kgsadh*

In this study, the site-directed mutagenesis of KGSADH was performed to improve its activity toward 3-HPA. The 3D structure of wild-type KGSADH was constructed using structure of succinic semialdehyde dehydrogenase (PDB accession NO. 3JZ4) by SWISS-MODEL homology modeling (<https://swissmodel.expasy.org/>) (Fig. 2) (Biasini et al. 2014). The mutant sites of 120E, 163K, 177K, 219P, 225K, 252E, and 278K residues were determined by energy

Table 3 Relative activity of mutant KGSADH

Aldehyde dehydrogenase	$V_{\max}$ (U/mg protein)	Relative activity(%)
KGSADH	1.43	100
Mutant KGSADH-E120D	2.63	184
Mutant KGSADH-K177R	2.30	161
Mutant KGSADH-K225R	1.86	130
Mutant KGSADH-E252D	–	–
Mutant KGSADH-E120Q	1.72	120
Mutant KGSADH-P219A	2.19	154
Mutant KGSADH-K278E	–	–
Mutant KGSADH-K163E	–	–
Mutant KGSADH-E120Q/P219A	4.75	333
Mutant KGSADH-E120D/P219A	6.03	422
Mutant KGSADH-K177R/P219A	1.92	135
Mutant KGSADH-K177R/E120D	1.20	84
Mutant KGSADH-K177R/E120Q	–	–

– No activity

analysis software Swiss-PDB Viewer 4.1 (<http://spdbv.vital-it.ch/>). The analysis of active site of aldehyde dehydrogenase demonstrated that it contains conserved residues, which directly interact with the aldehyde moiety of the substrate. Among them, 120E, 163K, and 219P residues are conserved sites, 177K residue is the coenzyme binding site, and the sites of 225K, 252E, and 278K residues are around active centre.

The site-directed mutagenesis at 120E, 163K, 177K, 219P, 225K, 252E, and 278K residues were carried out according to previous report (Liu et al. 2014, 2017). As shown in Table 3, the relative activities of mutants E120D, K177R, and P219A increased more than 50% than that of the wild-type enzyme. Moreover, double mutation was carried out based on E120D and K177R, and the mutants of KGSADH-E120Q/P219A, E120D/P219A, K177R/P219A, K177R/E120D, and K177R/E120Q were obtained. The double mutant E120D/P219A showed the highest relative activity of 422%. These results show that mutations at E120D and P219, located near the conserved domain residues, and have great effects on the catalytic activity of KGSADH.

Balanced expression of GDHt and KGSADH

In this study, four different vectors were used for the expression of GDHt, to balance the expression between GDHt and KGSADH; corresponding recombinant strains named R-EBG1, R-DBG1, R-ABG1, and R-TBG1 were engineered. As shown in Fig. 3, the SDS-PAGE analysis showed obvious bands of α -, β -, and γ -subunits of glycerol dehydratase (*dhaB*) with a molecular weight of 61, 21, and 16 kDa, respectively, and the corresponding band of

Fig. 3 SDS-PAGE analysis of GDHt and GdrA in recombinant *E. coli* strains. *M* Protein marker, lane 1 empty plasmid pTrc-99a, lane 2 pTBG1, lane 3 empty plasmid of pET-28a, lane 4 pEBG1, lane 5 empty plasmid of pACYCDuet, lane 6 pABG1, lane 7 empty plasmid of pETDuet, lane 8 pDBG1

glycerol dehydratase reactivase (*gdrA*) with a molecular weight of 63 kDa, which were consistent with the theoretical values, demonstrating the successful expression of these two enzymes. To further investigate the expression of GDHt gene carried by different plasmids, the GDHt activity was assayed.

The specific activities of GDHt in the recombinant strains harboring R-EBG1, R-DBG1, R-ABG1, and R-TBG1 were shown in Fig. 4. The GDHt activities in R-EBG1 and R-TBG1 were 11.56 and 8.94 U/mg, respectively, which indicated that lower copy plasmids (pET28a) were conducive to the activity of GDHt, as the expression of lower copy plasmids was much increased. Furthermore, fermentation of glycerol by these strains was carried out and the results showed that 3-HP was more efficiently produced by the strains of R-ABGK1 and R-DBGK1 with pACYC-tac and pETDuet plasmids, with 3-HP yields of 1.09 and

Fig. 4 Effects of different plasmids on the glycerol dehydratase activities and 3-HP production

1.33 g/L, respectively. Rathnasingh et al. (2009) reported that the imbalance between the GDHt activity and AldH activity was the main reason limiting 3-HP production. In this study, to solve the imbalance between the two key enzymes, the site-directed mutagenesis of *kgsadh* was done and proper plasmid for GDHt expression was chosen, and the results showed that these ways can enhance 3-HP yield to some extent.

Effect of GDHt reactivase gene *gdrB* on the production of 3-HP

It was reported that glycerol dehydratase could be irreversibly inactivated by glycerol because of the cleavage of the Co–C bond (Toraya et al. 1976). Glycerol dehydratase reactivase (GDR) was identified to be conducive for stabilize the GDHt activity through renovating the inactive B₁₂ (Rathnasingh et al. 2009; Toraya 2000). In this study, the reactivation capability of *gdrB* on GDHt was determined by the recombinant strains harboring *dhaB1-3*, *gdrAB*, and *kgsadh*. Figure 5 suggests that there is no significant difference in activities between R-DBGK1 and R-DBGK2. However, although the GDHt activity of R-DBGK2 showed only a slightly higher than that of R-DBGK1, the production level of 3-HP in the R-DBGK2 expressing *gdrB* was almost fivefold to that without the expression of *gdrB*, with a yield of 5.12 g/L during flask cultivation (Fig. 5). Moreover, the conversion of glycerol to 3-HP was improved from 6.87 to 41.4% (Table 4), indicating that the GDHt was more tolerant to glycerol after the expression of *gdrB*, and, therefore, efficiently enhanced the 3-HP yield.

Effect of NAD⁺ regeneration on 3-HP production

KGSADH is an NAD⁺-dependent aldehyde dehydrogenase and the regeneration of NAD⁺ in the biocatalysis is

Fig. 5 Effect of GdrB expression on the glycerol dehydratase activities and 3-HP production

beneficial to its activity. The NAD⁺ regeneration could be realized either by the addition of nitrate or by the expression of NADH oxidase (Ashok et al. 2013a; Lopez de Felipe et al. 1998), as well as the introduction of cytosolic glycerol-3-phosphate dehydrogenase (GPD1 or GPDH-C) carried out in this study, which reduces dihydroxyacetone phosphate to glycerol-3-phosphate, accompanied by the oxidation of NADH to NAD⁺ (Stryer et al. 2002).

The specific activities of KGSADH in the recombinant strain R-DBGK1 harboring *dhaB*, *gdrA*, and *kgsadh*, and R-DBGK2 harboring *dhaB*, *gdrA*, *kgsadh*, and *gpd1* were shown in Fig. 6. The co-expression of GPD1 resulted in KGSADH activity increased from 3.11 to 6.35 U/mg. However, 3-HP production between the two strains has no significant improvement (1.33–1.43 g/L), while it was much less than the strain of R-DBGK2 harboring *dhaB*, *gdrA*, *gdrB*, and *kgsadh* (5.12 g/L). When the GPD1 was expressed in R-DBGK2, the 3-HP production was reduced to 0.36 g/L (Table 4), with a conversion of glycerol decreased from 35.61 to 2.52%. Acetic acid and malic acid were found to be the main byproducts and malic acid accumulated to 49.37 mM. Figure 7 showed that after NAD⁺ regeneration by GPD1, C flux was inclined to generate dihydroxyacetone (DHA) and then went into the EMP pathway and tricarboxylic acid cycle (TCA). During the tricarboxylic acid cycle, malic acid was largely produced, while 3-HP production was inhibited due to the lack of NAD⁺. Subsequently, NAD⁺ was added during the fermentation of R-DBGK2 and R-DBGK2, and 3-HP production enhanced from 4 to 23.87 mM, increased by 496.75%, which indisputably indicated that NAD⁺ was important for 3-HP production. Therefore, in the following works, the EMP pathway should be metabolically engineered, to make more C flux to 3-HP synthetic pathway to further increase the catalytic efficiency of the engineered strain for the production of 3-HP.

Table 4 Results of different 3-HP producing strains

Strains/product	R-DBGK1	R-DBGK2	R-DBGK2 + NAD ⁺	R-DBGKG2	R-DBGKG2 + NAD ⁺
Consumed glycerol (mM)	165.76 ± 2.01	159.31 ± 3.91	151.16 ± 0.97	158.87 ± 2.2	150.83 ± 7.16
Biomass (C-mol%)	29.74 ± 1.12 (3.9 ± 0.1)	31.10 ± 0.02 (4.0 ± 0.1)	32.59 ± 0.09 (3.9 ± 0.1)	26.24 ± 3.92 (3.3 ± 0.2)	29.32 ± 0.53 (3.5 ± 0.1)
3-HP (C-mol%)	6.87 ± 0.12 (11.38 ± 0.11)	35.60 ± 0.82 (56.73 ± 0.11)	41.8 ± 1.2 (63.19 ± 0.05)	2.51 ± 0.84 (4.0 ± 1.5)	15.85 ± 0.39 (23.87 ± 0.56)
1, 3-PDO (C-mol%)	12.11 ± 0.75 (20.01 ± 1.01)	10.38 ± 0.16 (16.56 ± 0.66)	9.73 ± 0.92 (14.72 ± 1.84)	11.74 ± 2.38 (18.66 ± 2.76)	13.52 ± 1.46 (19.05 ± 1.31)
Acetic acid (C-mol%)	13.77 ± 0.4 (34.22 ± 1.27)	3.17 ± 0.10 (7.58 ± 0.33)	3.44 ± 0.83 (7.81 ± 2.15)	23.55 ± 2.86 (56.12 ± 6.83)	6.91 ± 0.16 (15.65 ± 0.33)
Succinate (C-mol%)	1.19 ± 0.16 (1.48 ± 0.22)	1.28 ± 0.13 (1.53 ± 0.12)	1.42 ± 0.11 (1.61 ± 0.18)	0 (0)	0 (0)
Formic (C-mol %)	1.05 ± 0.03 (5.22 ± 0.2)	1.10 ± 0.02 (5.27 ± 0.46)	0.96 ± 0.08 (5.03 ± 0.48)	1.01 ± 0.31 (4.79 ± 1.67)	0 (0)
Malic acid (C-mol%)	0 (0)	0 (0)	0 (0)	38.22 ± 3.22 (49.37 ± 3.28)	39.75 ± 2.68 (45.72 ± 1.79)
C circulate (%)	64.73	82.63	89.94	103.27	105.35
3-HP/Consumed glycerol (%)	6.87	35.61	41.80	2.52	15.83

The values in the parentheses indicate the final dry cell weight (g/L) and products (mM). The biomass was calculated on $\text{CH}_{1.93}\text{O}_{0.55}\text{N}_{0.25}\text{P}_{0.021}$ (Stephanopoulos Gregory et al. 1998)

Fig. 6 Effects of GPD1 expression on the activity of KGSADH and 3-HP production

Conclusion

In conclusion, the KGSADH was mutated in this study for efficient synthesis of 3-HP. The results showed that mutants of E120D/P219A increased the enzyme activity by 4.22-fold. The balance expression of *dhaB* and *kgsadh* also enhanced the production of 3-HP. The *gdrB* was found to exhibit obvious effect on 3-HP production. Co-expression of GPD1 together with *gdrB* dramatically changed the glycerol metabolic flux and decreased the 3-HP production.

Fig. 7 Part of the metabolic flux distributions for glycerol metabolism in recombinant *E. coli*

The C metabolic analysis indicates that large amount of malic acid produced due to the lack of NAD⁺, which further inhibited 3-HP production. The addition of extra NAD⁺ increased 3-HP production by 496.75%. This study paves a way for the efficient production of 3-HP.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

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