Physiological investigations of the influences of byproduct pathways on 3-hydroxypropionic acid production in *Klebsiella pneumoniae*

**Xiaohan Li**¹* | **Liuni Chen**¹* | **Xiuling Wang**² | **Pingfang Tian**¹

¹Beijing Key Laboratory of Bioprocess, College of Life Science and Technology, Beijing University of Chemical Technology, Beijing, China
²College of Life Sciences, Hebei Agricultural University, Baoding, China

**Correspondence**
Pingfang Tian, Beijing Key Laboratory of Bioprocess, College of Life Science and Technology, Beijing University of Chemical Technology, 15 Beisanhuan East Road, Chaoyang, 100029 Beijing, China.
Email: tianpf@mail.buct.edu.cn

**Funding information**
National Key Research and Development Program of China, Grant/Award Number: 2018YFA0901800; National Natural Science Foundation of China, Grant/Award Number: 21476011; National High Technology Research and Development Program, Grant/Award Number: 2015AA021003; National Basic Research Program of China, Grant/Award Number: 2012CB725200; Fundamental Research Funds for the Central Universities, Grant/Award Number: YS1407

**Abstract**
*Klebsiella pneumoniae* can naturally synthesize 3-hydroxypropionic acid (3-HP), 1,3-propanediol (1,3-PD), and 2,3-butanediol (2,3-BD) from glycerol. However, biosynthesis of these industrially important chemicals is constrained by troublesome byproducts. To clarify the influences of byproducts on 3-HP production, in this study, a total of eight byproduct-producing enzyme genes including *pmd*, *poxB*, *frdB*, *fumC*, *dhaT*, *ilvH*, *adhP*, and *pflB* were individually deleted from the *K. pneumoniae* genome. The resultant eight mutants presented different levels of metabolites. In 24-h shake-flask cultivation, the *adhP* and *pflB* deletion mutants produced 0.41 and 0.44 g/L 3-HP, respectively. Notably, the *adhP* and *pflB* double deletion mutant *K. pneumoniae*Δ*adhP*Δ*pflB* produced 1.58 g/L 3-HP in 24-h shake-flask cultivation. When *K. pneumoniae*Δ*adhP*Δ*pflB* was harnessed as a host strain to overexpress PuuC, a native aldehyde dehydrogenase (ALDH) catalyzing 3-hydroxypropionaldehyde (3-HPA) to 3-HP, the resulting recombinant strain *K. pneumoniae*Δ*adhP*Δ*pflB* (pTAC-puuC) (pTAC-puuC is PuuC expression vector) generated 66.91 g/L 3-HP with a cumulative yield of 70.84% on glycerol in 60-h bioreactor cultivation. Additionally, this strain showed 2.3-, 5.1-, and 0.67-fold decrease in the concentrations of 1,3-PD, 2,3-BD, and acetic acid compared with the reference strain *K. pneumoniae* (pTAC-puuC). These results indicated that the byproducts exerted differential impacts on the production of 3-HP, 1,3-PD, and 2,3-BD. Although combinatorial elimination of byproduct pathways could reprogram glycerol flux, the enzyme 1,3-propanediol oxidoreductase (DhaT) that catalyzes 3-HPA to 1,3-PD and the enzymes ALDHs, especially, PuuC are most pivotal for 3-HP production. This study provides a deep understanding of how byproducts affect the production of 3-HP, 1,3-PD, and 2,3-BD in *K. pneumoniae*.

**KEYWORDS**
1,3-propanediol, 3-hydroxypropionic acid, gene knockout, glycerol, *Klebsiella pneumoniae*

---

*Xiaohan Li and Liuni Chen contributed equally to this work.*

© 2019 The Authors. *Journal of Basic Microbiology* published by Wiley-VCH Verlag GmbH & Co. KGaA

*J Basic Microbiol*. 2019;59:1195–1207. www.jbm-journal.com | 1195
1 | INTRODUCTION

Bioproduction of chemicals has emerged as an alternative to conventional chemical synthesis due to depletion of oil reserves and deterioration of the environment. 3-Hydroxypropionic acid (3-HP) is one of 12 value-added chemicals proposed by the United States Department of Energy (DOE) in 2004 [1]. As a versatile platform chemical, 3-HP can be easily converted into a series of bulk chemicals including acrylic acid, acrylonitrile [2], 1,3-propanediol (1,3-PD), 3-hydroxypropionaldehyde (3-HPA), and malonic acid [3]. In addition, 3-HP as a monomer can be polymerized to form poly(3-hydroxypropionate) (P3HP), which is an unnatural polyhydrox alkanoate exhibiting biocompatibility and biodegradation attributes [4]. To date, diverse microbes have been exploited in the synthesis of 3-HP, including Escherichia coli [5,6], Corynebacterium glutamicum [7], Saccharomyces cerevisiae [8], Methylobacterium extorquens [9], and Klebsiella pneumoniae [10]. K. pneumoniae is a promising species for production of 3-HP because of its noticeable biochemical properties such as active cell growth, high ability to metabolize glycerol as well as innate capability to synthesize 3-HP. In particular, K. pneumoniae can naturally produce vitamin B12, which is the cofactor of glycerol dehydrogenase, a key enzyme for biosynthesis of 3-HP and 1,3-PD [11,12]. That is, production of 3-HP and 1,3-PD by K. pneumoniae fermentation does not need additional vitamin B12 and thus, reduces production cost.

In K. pneumoniae, glycerol-based biosynthesis of 3-HP and 1,3-PD is governed by the dha regulon, which involves glycerol reduction and oxidation pathways (Figure 1) [11–14]. In the glycerol reduction pathways, glycerol is converted into 3-HPA by glycerol dehydratase (GDH; EC: 4.2.1.30, encoded by dhaB gene cluster, GenBank U30903). 3-HPA is then catalyzed into 1,3-PD by 1,3-propanediol oxidoreductase (DhaT; EC: 1.1.1.202) and 3-HP by aldehyde dehydrogenase (ALDH; NAD+-dependent, EC:1.2.1.1) [15]. In the glycerol oxidation pathways, glycerol is converted to dihydroxyacetone phosphate (DHAP), which subsequently enters the glycolic pathway and tricarboxylic acid (TCA) cycle (Figure 1). Both glycerol reduction and oxidation pathways generate byproducts, which not only compete with 3-HP for carbon flux but also entangle downstream separation [10,16].

Previous efforts focused on knocking out or knocking down of the competing pathways that generate byproducts including 1,3-PD, 2,3-butanediol (2,3-BD), lactic acid, acetic acid, formic acid, and ethanol [12,17,18]. For 3-HP production in K. pneumoniae, major byproducts include lactic acid, 1,3-PD and 2,3-BD [19]. Separation of 3-HP from lactic acid is extremely challenging because 3-HP (3-hydroxypropionic acid) and lactic acid (2-hydroxypropionic acid) are isomers [16]. So far, nearly no techniques can effectively separate 3-HP from lactic acid. Apart from lactic acid, 1,3-PD and 2,3-BD also substantially affect 3-HP production. To minimize 1, 3-PD, its synthesis gene dhaT can be deleted [17,18]. In fact, 1,3-PD is another value-added bulk chemical, which can be polymerized to form polytrimethylene terephthalate, a thermoplastic that can be spun into both fibers and yarns [1]. In K. pneumoniae, biosynthesis of 1,3-PD consumes NADH and simultaneously generates NAD+, and NAD+ is required for 3-HP production (Figure 1). Clearly, there exists a synergy between 3-HP and 1,3-PD production. Presumably, coproduction of 3-HP and 1,3-PD can coordinate 3-HP production and NAD+ regeneration [20,21]. Unlike 3-HP and 1,3-PD, 2,3-BD is derived from the glycerol oxidation pathway and also is an economically valuable chemical. So far, industrial production of 2,3-BD has made impressive progress [22,23]. In addition to the aforementioned lactic acid, 1,3-PD, and 2,3-BD, other byproducts such as acetic acid, formic acid, and ethanol also hinder or benefit 3-HP production.

Although massive efforts have been focused on deciphering the dha regulon, the relationship between byproducts and 3-HP remains unclear [24]. To clarify this, we individually deleted eight byproduct-synthesizing genes, including pmd (GenBank No. KPN_01632, 1-lactate dehydrogenase), poxB (KPN_00904, pyruvate oxidase), frdB (KPN_04552, fumarate reductase iron sulfur subunit), fumC (KPN_01517, fumarate hydratase, class II), dhaT (KPN_03491, 1,3-propanediol dehydrogenase), ilvH (KPN_00083, acetalactate synthase I/III small subunit), adhP (KPN_01853, alcohol dehydrogenase, propanol-preferring), and pfb (KPN_00931, formate C-acetyltransferase). Metabolic analysis of the mutants was done to systematically evaluate the impacts of each byproduct-producing gene on glycerol consumption, cell growth, and 3-HP production. The objective of double deletion of genes was to determine whether combinatorial knockout of byproduct-producing genes can reallocate metabolic flux. Overexpression of PuuC, an aldehyde dehydrogenase (ALDH) native to K. pneumoniae [25], was done to assess 3-HP production in this chassis strain. Overall, this study aims to offer a deeper understanding of byproduct pathways in K. pneumoniae, which may shed light on basic research and manufacturing of chemicals.

2 | MATERIALS AND METHODS

2.1 | Strains, vectors, and chemicals

The strain of E. coli Top10 was purchased from the China General Microbiological Culture Collection Center
(CGMCCC). The strain of *K. pneumoniae* DSM 2026 was purchased from DSMZ GmbH, Germany. Vector pET-28a is a product of Novagen. The original T7 promoter in pET-28a was replaced by the tac promoter, leading to the vector pTAC. Vector pTAC-puuC was previously constructed, where puuC is an ALDH native to *K. pneumoniae*, and its expression is controlled by the tac promoter [10]. The *E. coli* strain was cultivated in...
Luria-Bertani (LB) medium containing the following components per liter: NaCl, 10 g; peptone, 10 g; yeast extract, 5 g; and additional 15 g agar for solid LB medium. The *K. pneumoniae* strain was grown in medium for producing 3-HP, which contained the following ingredients per liter: glycerol, 40 g; K₂HPO₄·3H₂O, 3.4 g; KH₂PO₄, 1.3 g; MgSO₄·7H₂O, 0.5 g; CaCO₃, 0.1 g; (NH₄)₂SO₄, 4 g; yeast extract, 3 g; and 1.25 ml of trace element solution, which contained (per liter): FeSO₄, 32 g; ZnCl₂·6H₂O, 2.72 g; CuCl₂·2H₂O, 1.88 g; CoCl₂·6H₂O, 1.88 g; MnCl₂·4H₂O, 0.68 g; H₃BO₃, 0.24 g; Na₂MoO₄, 0.02 g and 40 ml concentrated HCl. Chloramphenicol (Cm) at a final concentration of 170 μg/ml was used for screening positive strains. Taq DNA polymerase, T4 DNA ligase, and restriction enzymes were purchased from TaKaRa or New English Biolabs. Primer synthesis was completed by Biomed (China). DNA sequencing were performed by the Beijing Genomics Institute (China). The chemicals for high-performance liquid chromatography (HPLC) analysis and enzymatic activity assay were products of Sigma. All primers and oligos used in this study are listed in Table 1.

### Table 1: Strains, vectors, and primers used in this study

| Strains, vectors, and primers | Description | Source |
|-------------------------------|-------------|--------|
| **Strains**                   |             |        |
| *Escherichia coli* Top10      | Cloning host and *puuC* gene donor | Biomed, China |
| *Klebsiella pneumoniae* DSM 2026 | Recombinant *K. pneumoniae* strain-harboring vector | DSMZ, Germany |
| *K. pneumoniae*(pTAC-*puuC*)  | *pTAC-*puuC*, where *puuC* is controlled by *tac* promoter | Li et al. [10] |
| *K. pneumoniae*Δ*pmd*         | *pmd* gene was deleted from *K. pneumoniae* | This study |
| *K. pneumoniae*Δ*poxB*        | *poxB* gene was deleted from *K. pneumoniae* | This study |
| *K. pneumoniae*Δ*frdB*        | *frdB* gene was deleted from *K. pneumoniae* | This study |
| *K. pneumoniae*Δ*fumC*        | *fumC* gene was deleted from *K. pneumoniae* | This study |
| *K. pneumoniae*Δ*daHA*        | *daHA* gene was deleted from *K. pneumoniae* | This study |
| *K. pneumoniae*Δ*ilvH*        | *ilvH* gene was deleted from *K. pneumoniae* | This study |
| *K. pneumoniae*Δ*adhP*        | *adhP* gene was deleted from *K. pneumoniae* | This study |
| *K. pneumoniae*Δ*pflB*        | *pflB* gene was deleted from *K. pneumoniae* | This study |
| *K. pneumoniae*Δ*adhP*Δ*pflB* | Both *adhP* and *pflB* genes were deleted from *K. pneumoniae* | This study |

| Vectors                      |            |        |
|-----------------------------|------------|--------|
| pET-28a                     | Expression vector; KanR | Novagen |
| pTAC                        | The original T7 promoter in pET-28a was replaced by *tac* promoter, leading to vector pTAC; KanR | Li et al. [10] |
| pTAC-*puuC*                 | Expression vector; *tac* promoter; KanR | Li et al. [10] |
| pET-*pmdUD                  | For knocking out *pmd* gene, pBR322 ori, KanR, CmR | This study |
| pET-*poxBUD                 | For knocking out *poxB* gene, pBR322 ori, KanR, CmR | This study |
| pET-*frdBUD                 | For knocking out *frdB* gene, pBR322 ori, KanR, CmR | This study |
| pET-*fumCUD                 | For knocking out *fumC* gene, pBR322 ori, KanR, CmR | This study |
| pET-*daHAUD                 | For knocking out *daHA* gene, pBR322 ori, KanR, CmR | This study |
| pET-*ilvHUD                 | For knocking out *ilvH* gene, pBR322 ori, KanR, CmR | This study |
| pET-*adhPUD                 | For knocking out *adhP* gene, pBR322 ori, KanR, CmR | This study |

| Primer name | Sequence (5’ to 3’) | Restriction enzyme |
|-------------|---------------------|-------------------|
| *pmd*       |                     |                   |
| Up-F        | 5’-GGAAGATCTTTGCGGCTGAGGGGCCTG-3’ | Bgl II |
| Up-R        | 5’-TCCGAGCTCCAAGTCAAGAAAGGAGG-3’ | Sac I |
| Down-F      | 5’-ACGCGTGCAAGCAGACTGTATTACTGCGAGGCTGGTCTCCTGTTGTA | Sal I |
| Down-R      | 5’-ATAGTCTAGCCCGGCTTACCGACAGGGCTATGAGCTCGCGTGGCTAGT | Not I |

| *poxB*       |                     |                   |
| Up-F         | 5’-GGAAGATCTCGGCGGCGCAGCAGCTG-3’ | Bgl II |
| Up-R         | 5’-TCCGAGCTCTGCCTTTTTTTTTTTTGAATTACGCCCTTC-3’ | Sac I |
| Down-F       | 5’-ACGCGTGCAAGAAGCAGATACGTTGACCAGG-3’ | Sal I |
| Down-F       | 5’-TAAAGCGGCCGCGCAGCTCGTCTAGGTTTTG-3’ | Not I |

(Continues)
TABLE 1 (Continued)

| Primer name | Sequence (5’ to 3’) | Restriction enzyme |
|-------------|---------------------|--------------------|
| *frdB*      |                     |                    |
| Up-F        | 5’-GGAGATCTCCCGAGCGCATGGATGAGGATG-3’ | Bgl II |
| Up-R        | 5’-TCCGAGCTCGACATCGAGGAGGCGCGCGCGTT-3’ | Sac I |
| Down-F      | 5’-ACGGCTGACGAGTTCATTAATGAGCAGCG-3’ | Sal I |
| Down-R      | 5’-TAAAGCGGCGCGCTTAAGCTCAGAAGGACG-3’ | Not I |
| *fumC*      |                     |                    |
| Up-F        | 5’-GGAGATCTCCCGAGCGCATGGATGAGGATG-3’ | Bgl II |
| Up-R        | 5’-TCCGAGCTCGACATCGAGGAGGCGCGCGCGTT-3’ | Sac I |
| Down-F      | 5’-CCCAAGCTTATAAGCAATTCAGCAGCTGCG-3’ | Hind III |
| Down-R      | 5’-ATAGTTTAAAGCAGCGCATGCGAGCCAGGCGA-3’ | Not I |
| *dhaT*      |                     |                    |
| Up-F        | 5’-GGAGATCTTGGTCCACTTGTACTGAAGGACG-3’ | Bgl II |
| Up-R        | 5’-TCCGAGCTCAATACGAGGCGCCGTCATGCTCCTTCACC | Sac I |
| Down-F      | 5’-ACGGCTGAGGAGAATCAGGCG-3’ | Sal I |
| Down-R      | 5’-TAAAGCGGCGCGCTTAAGCTCAGAAGGACG-3’ | Not I |
| *ilvH*      |                     |                    |
| Up-F        | 5’-GGAGATCTCAGCTGCGCCTTCCCAGG-3’ | Bgl II |
| Up-R        | 5’-TCCGAGCTCAATACGAGGCGCCGTCATGCTCCTTCACC | Sac I |
| Down-F      | 5’-ACGGCTGACGAGTTCATTAATGAGCAGCG-3’ | Sal I |
| Down-R      | 5’-TAAAGCGGCGCGCTTAAGCTCAGAAGGACG-3’ | Not I |
| *adhP*      |                     |                    |
| Up-F        | 5’-CGCGGATCCCGCGCGCTTAAAGCCG-3’ | Bam HI |
| Up-R        | 5’-TCCGAGCTCGACATCGAGGCGCCGTCATGCTCCTTCACC | Sac I |
| Down-F      | 5’-CCCAAGCTTATAAGCAATTCAGCAGCTGCG-3’ | Hind III |
| Down-R      | 5’-TAAAGCGGCGCGCTTAAGCTCAGAAGGACG-3’ | Not I |
| *pflB*      |                     |                    |
| Up-F        | 5’-GGAGATCTTGGTCCACTTGTACTGAAGGATG-3’ | Bgl II |
| Up-R        | 5’-TCCGAGCTCAATACGAGGCGCCGTCATGCTCCTTCACC | Sac I |
| Down-F      | 5’-ACGGCTGACGAGTTCATTAATGAGCAGCG-3’ | Sal I |
| Down-R      | 5’-ATAGTTTAAAGCAGCGCATGCGAGCCAGGCGA-3’ | Not I |

Note: The underlined sequence indicates the restriction enzyme site.
Abbreviations: F, forward; R, reverse; Down, downstream homologous arm; Up, upstream homologous arm.

2.2 Construction of the recombinants

To investigate the impacts of byproducts on 3-HP production, a total of eight genes including *pmd, poxB, frdB, fumC, dhaT, ilvH, adhP*, and *pflB* were individually disrupted from the *K. pneumoniae* genome. To achieve this, their upstream and downstream homologous arms of 1,000 bp each were cloned by polymerase chain reaction (PCR) from the genomic DNA of *K. pneumoniae*. The PCR protocol used was: 94°C for 3 min, followed by 30 cycles of 94°C for 1 min, 55°C for 45 s, 72°C for X min, where X depends on the length of the amplified gene. The upstream and downstream homologous arms as well as chloramphenicol resistance gene were ligated and cloned into the vector pTAC, resulting in the vectors pET-UD, pET-poxBUD, pET-frdBUD, pET-fumCUD, pET-dhaTUD, pET-ilvHUD, pET-adhPUD, and pET-pflBUD, respectively (“UD” indicates upstream and downstream homologous arms). These vectors were separately transformed into *K. pneumoniae* and cultivated in an LB plate containing 170 μg/ml chloramphenicol for screening mutants. The mutants were further confirmed by colony PCR and sequencing. To dispel the vectors out of cells, the *K. pneumoniae* mutants were grown in LB medium containing 50 mM CaCl2 for 3 days. The strains unable
to survive in LB chloramphenicol plates were the strains devoid of vectors and named \( K.\ pneumoniae\Delta rpmd, K.\ pneumoniae\Delta poxB, K.\ pneumoniae\Delta frdB, K.\ pneumoniae\Delta fumC, K.\ pneumoniae\Delta dhaT, K.\ pneumoniae\Delta ilvH, K.\ pneumoniae\Delta adhP, \) and \( K.\ pneumoniae\Delta pfIB, \) respectively. These strains, devoid of vectors, were further confirmed by plasmid extraction and agarose gel electrophoresis. Double deletion of genes followed the protocol of RecA homologous recombination. The recombinants were confirmed by colony PCR and sequencing.

### 2.3 Shake-flask and bioreactor cultivation

Before shake-flask cultivation, the strains were precultivated in LB medium containing the following components per liter: 10 g peptone, 10 g NaCl, 5 g yeast extract, and 170 µg/ml chloramphenicol. One percent of overnight broth with \( OD_{600} \) of 4.0 was inoculated to a 250 ml Erlenmeyer flask containing 100 ml medium for producing 3-HP (see aforementioned) and antibiotics as appropriate at 37°C and 150-rpm shaking. To maintain microaerobic conditions, the flasks were plugged with an O2-permeable cotton stopper and incubated in an orbital shaker at 180 rpm and 37°C. Samples were collected every 3 h to measure residual glycerol, biomass, and metabolites.

Fed-batch cultivation of the strains was carried out in a 5 L bioreactor (Baoxing, China) containing 3-L fermentation medium and 0.5 mM IPTG (isopropyl-\( \beta \)-d-thiogalactoside). The fermentation conditions were followed according to the previously reported method [10]. The strain was precultivated in 100 ml fermentation medium overnight at 37°C and then transferred to a bioreactor. The agitation speed was 400 rpm and air was supplied at 1.5 vvm. The temperature was 37°C and pH value was maintained at 7.0 by adding 5 M NaOH. Dissolved oxygen was monitored automatically. Samples were taken out every 3 h to examine cell concentration, residual glycerol, and metabolites.

### 2.4 Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis

\( K.\ pneumoniae \) strain was precultured in a test tube containing 4 ml LB medium at 37°C and rotated at 180 rpm for 12 h. One percent of overnight broth with \( OD_{600} \) of 4.0 was inoculated in a 250-ml shake-flask containing 100 ml medium (for production of 3-HP), 170 mg/L chloramphenicol and 0.5 mM IPTG. After 16-h cultivation, the cell samples were harvested by centrifugation at 10,000 rpm for 5 min, and the supernatant was discarded, as the target protein was intracellularly expressed. The cells were mixed with loading buffer (Takara, China) and boiled for 5 min. Protein expression was analyzed by 12% (v/w) SDS-PAGE with cell-free extract under denaturing conditions. The pH values of stacking gel and resolving gel were 6.8 and 8.8, respectively. A Mini-Protein III Electrophoresis System (Bio-Rad) was employed to perform the operation. Coomassie Brilliant Blue R-250 (0.2%, w/v) was utilized to stain proteins on the gel. Upon decolorization, the protein concentration was determined by the Bradford method with bovine serum albumin as standard protein.

### 2.5 Analytical methods

Cell density was measured by using a microplate reader at 600 nm with 200 µl fermentation broth added in a cuvette. Residual glycerol was measured by the titration method with NaIO4 (for control of glycerol). Metabolites 3-HP, lactic acid and acetic acid were measured by using a HPLC system (Shimazu, Kyoto, Japan) equipped with a C18 column and a SPD-20A UV detector at 210 nm. The initial GC oven temperature was maintained at 60°C for 3 min, and increased to 180°C at a rate of 10°C/min. Column temperature was 25°C. 1,3-PD was determined by gas chromatography (GC; PerSee, China) equipped with a capillary column of TR-WAX (30 m × 0.25 mm ID, 0.25 µm). Nitrogen was used as carrier gas, and the column flow rate was maintained at 0.5 ml/min. Injector and detector temperatures were maintained at 230°C. The initial GC oven temperature was maintained at 60°C for 3 min, and increased to 180°C at a rate of 10°C/min and maintained for 5 min. All samples were filtered through a 0.22 µm membrane filter.

### 3 RESULTS

#### 3.1 Characterization of recombinants

To dissect the influences of byproducts on 3-HP production, a total of eight genes \( pmd, poxB, frdB, fumC, dhaT, ilvH, adhP, \) and \( pfIB \) were independently deleted through the RecA homologous recombination technique. Colony PCR analysis showed that the above eight genes were independently but not simultaneously deleted from the \( K.\ pneumoniae \) genome, and the eight mutants were designated as \( K.\ pneumoniae\Delta rpmd, K.\ pneumoniae\Delta poxB, K.\ pneumoniae\Delta frdB, K.\ pneumoniae\Delta fumC, K.\ pneumoniae\Delta dhaT, K.\ pneumoniae\Delta ilvH, K.\ pneumoniae\Delta adhP, \) and \( K. pneumoniae\Delta pfIB. \) In addition, the two genes \( adhP \) and \( pfIB \) were shown to be double-disrupted from the \( K. pneumoniae \) genome, and the recombinant strain was named as \( K. pneumoniae\Delta adhP\Delta pfIB. \) Subsequently, this strain was
transformed with vector pTAC-puuC to overproduce 3-HP, and the resulting strain was designated as K. pneumoniaeΔadhPΔpflB(pTAC-puuC). Plasmid extraction, restriction digestion and DNA sequencing showed that the vector pTAC-puuC was correctly constructed and transformed into K. pneumoniaeΔadhPΔpflB.

3.2 SDS-PAGE analysis

To investigate the influences of gene deletion on protein expression, K. pneumoniaeΔadhPΔpflB(pTAC-puuC) was analyzed by SDS-PAGE, and wild-type K. pneumonia, K. pneumoniaeΔadhPΔpflB(pET-28a), and K. pneumoniaeΔadhPΔpflB(pTAC-puuC) were used as reference strains. As shown in Figure 2, lane 1–4 indicate wild-type K. pneumoniae (lane 1) and K. pneumoniaeΔadhPΔpflB (lane 2), respectively. Due to overexpression of PuuC, a 53 kDa band was observed in K. pneumoniae(pTAC-puuC) and K. pneumoniaeΔadhPΔpflB(pTAC-puuC) strains, which was much stronger than those in wild-type and K. pneumoniae(pET-28a) strains. Moreover, the 38 kDa protein in K. pneumoniaeΔadhPΔpflB(pTAC-puuC) was significantly weaker than those in wild-type K. pneumoniae (lane 1) and K. pneumoniaeΔadhPΔpflB (lane 2), indicating that the adhP gene was successfully disrupted. Interestingly, no difference was observed for the 85 kDa band (pflB) in all four strains. This may be explained by the innate low expression of pflB and probable existence of proteins having a molecular weight similar to PflB. Colony PCR validated the fact that the pflB gene was deleted from K. pneumoniae genome. Overall, the above results suggested that the recombinant strain K. pneumoniaeΔadhPΔpflB(pTAC-puuC) was successfully engineered and the two genes adhP and pflB were double-deleted from the K. pneumoniae genome.

3.3 Shake-flask cultivation of single gene deletion mutants

To clarify the influences of byproduct-producing enzymes on 3-HP production, a total of eight genes were individually deleted, resulting in eight mutants K. pneumoniaeΔpmd, K. pneumoniaeΔpoxB, K. pneumoniaeΔfdrB, K. pneumoniaeΔfumC, K. pneumoniaeΔdhaT, K. pneumoniaeΔsilH, K. pneumoniaeΔadhP, and K. pneumoniaeΔpflB (Figure 3). Considering K. pneumoniae cells had vectors dispelled from them, the wild-type strain instead of the recombinant strain-harboring the empty vector was used as the control. Compared with the wild-type strain, all mutants showed increased growth in the first 12 h, however, this situation changed in the next 12 h (Figure 3). The mutants K. pneumoniaeΔpmd, K. pneumoniaeΔpoxB, K. pneumoniaeΔfdrB, and K. pneumoniaeΔfumC displayed expedited growth, while the mutants K. pneumoniaeΔdhaT, K. pneumoniaeΔsilH, K. pneumoniaeΔadhP, and K. pneumoniaeΔpflB demonstrated retarded growth. Consistent with cell growth, all strains consumed glycerol and produced 3-HP in the first 12-h fermentation. Interestingly, compared with the wild-type strain, all eight mutants produced less 1,3-PD and 2,3-BD in the first 12 h, except the strain K. pneumoniaeΔpmd which produced more 2,3-BD during the entire 24-h fermentation. More importantly, the mutants K. pneumoniaeΔadhP and K. pneumoniaeΔpflB produced higher levels of 3-HP (0.40 and 0.44 g/L, respectively) than other mutants in the first 12 h. During the entire fermentation process, K. pneumoniaeΔadhP and K. pneumoniaeΔpflB were the best-acting strains for production of 3-HP. Hence, the genes adhP and ΔpflB were double-deleted, and the resulting strain
K. pneumoniaeΔadhPΔpflB was used as a chassis strain to express PuuC.

3.4 Shake-flask cultivation of double deletion gene mutants

Now that PCR and SDS-PAGE analysis had experimentally validated the double deletion of adhP and pflB genes from the K. pneumoniae genome, the mutant K. pneumoniaeΔadhPΔpflB(pTAC-puuC) was grown in shake-flasks to investigate 3-HP production and cell growth. To do so, wild-type K. pneumoniae, K. pneumoniae(pET-28a), and K. pneumoniae(pTAC-puuC) were used as control strains. As shown in Figure 4a, the strain K. pneumoniaeΔadhPΔpflB presented similar levels of biomass and glycerol consumption to the wild-type strain, indicating that double deletion of adhP and pflB had no significant impacts on cell growth. In contrast, the three strains K. pneumoniae(pET-28a), K. pneumoniae(pTAC-puuC), and K. pneumoniaeΔadhPΔpflB(pTAC-puuC) showed retarded growth. This may be explained by the metabolic burden imposed by the vector pET-28a or pTAC-puuC. For 3-HP production, no significant difference was observed between K. pneumoniae and K. pneumoniaeΔadhPΔpflB. However, K. pneumoniaeΔadhPΔpflB(pTAC-puuC) produced 2.09 g/L 3-HP in 24 h, which was 1.2-fold of that produced by K. pneumoniae(pTAC-puuC). It should be noted that the lactic acid level showed irregular fluctuation, and no difference was observed for the strains K. pneumoniaeΔadhPΔpflB and K. pneumoniaeΔadhPΔpflB (pTAC-puuC). Notably, although PflB catalyzes the formation of formic acid, the pflB-deletion mutant produced a similar level of formic acid to the wild-type strain. This may be due to the existence of multiple formic acid pathways. In view of 24-h shake-flask fermentation, we found that double deletion of adhP and pflB imposed no significant influences on cell growth.

3.5 Bioreactor cultivation

To further elucidate the influences of double deletion of genes adhP and pflB on 3-HP production, the strain K. pneumoniaeΔadhPΔpflB(pTAC-puuC) was cultivated in a 5-L bioreactor, where the pH value was maintained at 7.0 by adding NaOH. The wild-type K. pneumoniae, K. pneumoniaeΔadhPΔpflB and K. pneumoniae(pTAC-puuC) were referenced as control. The strain K. pneumoniae(pTAC-puuC) presented a cumulative 3-HP titer in 54 h with a titer of 67.47 g/L and 65.66% yield on glycerol (Figure 5a; Table 2), while the strain K. pneumoniaeΔadhPΔpflB(pTAC-puuC) presented a 3-HP peak in 60 h with a titer of 66.91 g/L and 70.84% yield on glycerol (Figure 5b; Table 2). Importantly, the strain K. pneumoniaeΔadhPΔpflB(pTAC-puuC) produced much less lactic acid, formic acid, 1,3-PD and 2,3-BD.
FIGURE 4  Continued.
compared with *K. pneumoniae*(pTAC-puuC). These results indicated that although double deletion of *adhP* and *pflB* has little influences on 3-HP production, most byproducts were largely attenuated.

### 4 | DISCUSSION

Biosynthesis of 3-HP, 1,3-PD, and 2,3-BD in *K. pneumoniae* encounters buildup of byproducts, which not only take up the carbon source but also entangle downstream separation [10,16]. To decipher the influences of byproducts on 3-HP production, here, a total of eight byproduct-synthesizing genes including *pmd, poxB, frdB, fumC, dhaT, ilvH, adhP*, and *pflB* were individually deleted from *K. pneumoniae* genome, and the resultant eight mutants were cultivated in shake-flasks for metabolic analysis. Results showed that the eight mutants demonstrated different levels of cell growth, glycerol consumption, and the formation of 3-HP, 1,3-PD, and 2,3-BD. Although all mutants produced more 3-HP than wild-type *K. pneumoniae* in the first 12 h, this situation changed in the next 12 h. As shown in Figure 3, compared to wild-type *K. pneumoniae*, the mutants *K. pneumoniaeΔfrdB, K. pneumoniaeΔilvH, K. pneumoniaeΔadhP*, and *K. pneumoniaeΔpflB* produced more 3-HP, while the other mutants produced less 3-HP (Figure 3). Notably, the mutants *K. pneumoniaeΔadhP* and *K. pneumoniaeΔpflB* generated 0.42 and 0.45 g/L 3-HP, respectively, which were higher than that of other mutants. To further enhance 3-HP production, the two genes *adhP* and *pflB* were double-deleted. The resulting mutant *K. pneumoniaeΔadhPΔpflB* generated 1.58 g/L 3-HP in 24 h shake-flask cultivation. When this strain was transformed with vector pTAC-puuC that catalyzes the formation of 3-HP [10], the recombinant strain *K. pneumoniaeΔadhPΔpflB*(pTAC-puuC) was engineered. In a 5-L bioreactor, this strain produced 66.91 g/L 3-HP in 60 h with 70.84% yield on glycerol (Figure 5b and Table 2). Although this 3-HP titer (66.91 g/L) was lower than that produced by *K. pneumoniae*(pTAC-puuC) (67.47 g/L; Figure 5a and Table 2), the byproducts acetic acid, 1,3-PD, and 2,3-BD in *K. pneumoniaeΔadhPΔpflB*(pTAC-puuC) were significantly attenuated, indicating that double disruption of *adhP* and *pflB* remarkably altered metabolic flux. Overall, these results indicated that byproduct-producing genes exhibited differential influences on 3-HP production. For glycerol pathways (Figure 1), the enzyme genes far away from 3-HP imposed minimal impacts on glycerol consumption and 3-HP formation, while the enzyme genes in the vicinity of 3-HP such as *dhaT* and *puuC* substantially affected 3-HP production. To the best of our knowledge, this is the first systematic exploration on how byproducts influence glycerol metabolism in *K. pneumoniae*.

Deletion of competing pathways is a common strategy to minimize byproducts [17]. However, this strategy in most cases compromises cell viability and thereby hinders biosynthesis of desired metabolites [26]. To date, 3-HP production remains to be improved. This is mainly due to incomplete understanding of the *dha* regulon that governs 3-HP biosynthesis. In view of our study and the work of other groups, we realize that the *dha* regulon in *K. pneumoniae* manifests both structural “plasticity” and “rigidity”. The “plasticity” indicates that disruption of partial pathways has no significant impacts on cell growth. This viewpoint is evidenced by the *pflB*- and *pmd*-deletion mutants which showed similar levels of biomass to wild-type strain in 12-h cultivation (Figure 3). This phenomenon may be attributed to tailored metabolic compensation. For instance, although DhaT catalyzes the formation of 1,3-PD, the *dhaT*-deletion mutant still produced 1,3-PD because of the expression of NAD(P)H-dependent hypothetical oxidoreductase, an isoenzyme of DhaT [17]. In contrast to “plasticity”, the structural “rigidity” of the *dha* regulon indicates that some pathways are essential for cell viability, and deleting them may substantially halt cell growth or even trigger cell death. For instance, compared with wild-type strain, the *dhaT*-deletion mutant presented slower growth and produced less 1,3-PD and 3-HP in 24-h fermentation (Figure 3). The reason behind this is that DhaT catalyzes 3-HPA into 1,3-PD, and this reaction is a central metabolism in *K. pneumoniae* when glycerol is the sole carbon source (Figure 1). In addition, this reaction converts NADH to NAD+, and NAD+ is the cofactor of ALDH that catalyzes 3-HPA into 3-HP. Presumably, 1,3-PD biosynthesis is coupled with 3-HP production via cofactor recycling [27], implying the feasible coproduction of 1,3-PD and 3-HP [20,21]. In addition to supplying cofactor, byproduct pathways

---

**FIGURE 4** Shake-flask cultivation of Klebsiella pneumoniae strains for production of 3-hydroxypropionic acid. *K. pneumoniae* WT, wild-type *K. pneumoniae; K. pneumoniae*(pET-28a), recombinant *K. pneumoniae* harboring empty vector pET-28a; *K. pneumoniae*(pTAC-puuC), recombinant *K. pneumoniae* harboring PuuC expression vector pTAC-puuC; *K. pneumoniaeΔadhPΔpflB*(pTAC-puuC), the mutant *K. pneumoniaeΔadhPΔpflB* harboring PuuC expression vector pTAC-puuC. PuuC, an aldehyde dehydrogenase native to *K. pneumoniae*. 3-HP, 3-hydroxypropionic acid; 1,3-PD, 1,3-propanediol; 2,3-BD, 2,3-butanediol
provide ATP for the cells. For example, the enzyme genes pmddd, poxB, adhP, fumC, and frdB participate in glycolysis and tricarboxylic acid cycle and thus supply energy for the cells (Figure 1). In summary, glycerol metabolism relies on both core and subsidiary pathways. Although all pathways affect 3-HP production, only a small part of them are pivotal.

From the viewpoint of genetics, the fermentation titer is a quantitative trait that depends on multiple factors. Of the myriad factors affecting 3-HP production, ALDH is most influential because it directly catalyzes 3-HPA to 3-HP. Previously, PuuC as a native ALDH was overexpressed in K. pneumoniae, and 83.8 g/L 3-HP was produced in a 5-L bioreactor [10]. This study suggests that optimizing key enzymes instead of multiple enzymes also enables overproduction of desired metabolites. This “pathway-focused approach” is popular in most labs, even though rational design-dependent systems metabolic engineering may be more powerful in strain engineering. In fact, systems metabolic engineering is time-consuming due to its reliance on global reprogramming of cell metabolism and subsequent high throughput screening. Hence, the “pathway-focused strategy” is more applicable in most cases. Following this thinking, three strategies might be feasible for boosting 3-HP production in K. pneumoniae. The first is directed evolution of ALDH [15], aiming to improve its specificity toward 3-HPA which is toxic to cells. A research team in Korea altered the substrate specificity of α-ketoglutaric semialdehyde dehydrogenase (KGSADH), an enzyme from Azospirillum brasilense catalyzing 3-HPA to 3-HP [28]. The improved KGSADHs exhibited lower $K_m$ values for both 3-HPA and NAD+. The enzymes also displayed higher substrate specificities for aldehyde and NAD+ and weaker inhibition by NADH. Furthermore, the recombinant Pseudomonas denitrificans strain carrying one of KGSADH variants exhibited less 3-HPA and higher cell growth compared with the wild-type KGSADH [29]. The second approach for improving 3-HP production may be promoter engineering, by which sufficient RNA polymerases (RNAPs) can be recruited and thus facilitate PuuC expression and boost 3-HP.

### TABLE 2 Carbon distribution of Klebsiella pneumoniae(pTAC-puuC) and K. pneumoniaeΔadhPΔpfIB(pTAC-puuC) in bioreactor cultivation (54 and 60 h, respectively)

| Strains                                      | 3-HP (g/L) | AA (%) | 1,3-PD (g/L) | GCR (%) | 2,3-BD (g/L) | GCR (%) |
|----------------------------------------------|------------|--------|--------------|---------|--------------|---------|
| K. pneumoniae(pTAC-puuC)                     | 67.47      | 65.66  | 9.25         | 9.80    | 15.21        | 14.80   |
| K. pneumoniaeΔadhPΔpfIB(pTAC-puuC)           | 66.91      | 70.84  | 7.23         | 7.40    | 4.61         | 4.88    |

Abbreviations: 1,3-PD, 1,3-propanediol; 2,3-BD, 2,3-butanediol; 3-HP, 3-hydroxypropionic acid; AA, acetic acid; GCR, glycerol conversion rate. K. pneumoniae (pTAC-puuC), recombinant K. pneumoniae strain-harboring vector pTAC-puuC, where puuC is controlled by the tac promoter; K. pneumoniaeΔadhPΔpfIB (pTAC-puuC), mutant K. pneumoniaeΔadhPΔpfIB-harboring PuuC expression vector pTAC-puuC.
production [30]. It has been shown that RNAP as an intracellular "resource allocator" significantly affects metabolic flux [31,32]. The third strategy is mitigation of metabolite inhibition on the host cell. It has been shown that adjusting the pH value with NaOH benefits cell growth and thus facilitates 3-HP production [10]. This may be ascribed to the close coupling between 3-HP production and cell growth especially in the exponential phase [27]. Judging from this, measures facilitating cell growth might benefit 3-HP production. In addition to cell growth, many other factors also contribute to 3-HP production, such as substrate provision, cofactor availability, redox balance, and cell tolerance to substrate and metabolites. In this study, we systematically investigated the influences of byproduct pathways on the production of 3-HP, 1,3-PD, and 2,3-BD. We believe that this study offers valuable insights for basic research and metabolic engineering of K. pneumoniae.

ACKNOWLEDGMENTS

This study was funded by grants from the National Natural Science Foundation of China (No. 21476011), the National Key Research and Development Program of China (No. 2018YFA0901800), the National High Technology Research and Development Program (863 Program; No. 2015AA021003), the National Basic Research Program of China (973 Program; No. 2012CB725200), and the Fundamental Research Funds for the Central Universities (YS1407). We appreciate Geran Tian for polishing this manuscript.

CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

ORCID

Pingfang Tian ▼ http://orcid.org/0000-0001-5261-9516

REFERENCES

[1] Werpy T, Petersen G. Top value added chemicals from biomass. Washington, DC: U.S.DOE; 2004.
[2] Karp EM, Eaton TR, Sánchez I Nogué V, Vorotnikov V, Biddy MJ, Tan ECD, et al. Renewable acrylonitrile production. Science. 2017;358:1307-10.
[3] Kumar V, Ashok S, Park S. Recent advances in biological production of 3-hydroxypropionic acid. Biotech Adv. 2013;31:945-61.
[4] Andreessen B, Lange AB, Robenek H, Steinbuchel A. Conversion of glycerol to poly(3-hydroxypropionate) in recombinant Escherichia coli. Appl Environ Microbiol. 2010;76:622-6.
[5] Chu HS, Kim YS, Lee CM, Lee JH, Jung WS, Ahn JH, et al. Metabolic engineering of 3-hydroxypropionic acid biosynthesis in Escherichia coli. Biotechnol Bioeng. 2015;112:356-64.
[6] Cheng Z, Jiang J, Wu H, Li Z, Ye Q. Enhanced production of 3-hydroxypropionic acid from glucose via malonyl-CoA pathway by engineered Escherichia coli. Bioreour Technol. 2016;200:897-904.
[7] Chen Z, Huang J, Wu Y, Wu W, Zhang Y, Liu D. Metabolic engineering of Corynebacterium glutamicum for the production of 3-hydroxypropionic acid from glucose and xylose. Metab Eng. 2017;39:151-8.
[8] Kildegaard KR, Jensen NB, Schneider K, Czarnotta E, Özdemir E, Klein T, et al. Engineering and systems-level analysis of Saccharomyces cerevisiae for production of 3-hydroxypropionic acid via malonyl-CoA reductase-dependent pathway. Microb Cell Fact. 2016;15:53.
[9] Yang YM, Chen WJ, Yang J, Zhou YM, Hu B, Zhang M, et al. Production of 3-hydroxypropionic acid in engineered Methylobacterium extorquens AM1 and its reassimilation through a reductive route. Microb Cell Fact. 2017;16:179.
[10] Li Y, Wang X, Ge X, Tian P. High production of 3-hydroxypropionic acid in Klebsiella pneumoniae by systematic optimization of glycerol metabolism. Sci Rep. 2016;6:26932.
[11] Forage RG, Lin EC. DHA system mediating aerobic and anaerobic dissimilation of glycerol in Klebsiella pneumoniae NCIB 418. J Bacteriol. 1982;151:591-9.
[12] Kumar V, Park S. Potential and limitations of Klebsiella pneumoniae as a microbial cell factory utilizing glycerol as the carbon source. Biotech Adv. 2018;36:150-67.
[13] Johnson EA, Lin EC. Klebsiella pneumoniae 1,3-propanediol: NAD+ oxidoreductase. J Bacteriol. 1987;169:2050-4.
[14] Wang Y, Tao F, Xu P. Glycerol dehydrogenase plays a dual role in glycerol metabolism and 2,3-butanediol formation in Klebsiella pneumoniae. J Biol Chem. 2014;289:6080-90.
[15] Seok JY, Yang J, Choi SJ, Lim HG, Choi UJ, Kim KJ, et al. Directed evolution of the 3-hydroxypropionic acid production pathway by engineering aldehyde dehydrogenase using a synthetic selection device. Metab Eng. 2018;47:113-20.
[16] Wang J, Zhao P, Li Y, Xu L, Tian P. Engineering CRISPR interference system in Klebsiella pneumoniae for attenuating lactic acid synthesis. Microb Cell Fact. 2018;17:56-67.
[17] Ashok S, Raj SM, Rathnasingh C, Park S. Development of recombinant Klebsiella pneumoniae ΔdhaT strain for the co-production of 3-hydroxypropionic acid and 1,3-propanediol from glycerol. Appl Microbiol Biotechnol. 2011;90:1253-65.
[18] Ashok S, Mohanraj S, Ko Y, Sankaranarayanan M, Zhou S, Kumar V. Effect of puuC overexpression and nitrate addition on glycerol metabolism and anaerobic 3-hydroxypropionic acid production in recombinant Klebsiella pneumoniaeΔgkpΔdhaT. Metab Eng. 2013;15:10-24.
[19] Maris AJA, Konings WN, Dijken JP, Pronk JT. Microbial export of lactic and 3-hydroxypropanoic acid: implications for industrial fermentation processes. Metab Eng. 2004;6:245-55.
[20] Kumar V, Sankaranarayanan M, Jae K, Durgapal M, Ashok S, Ko Y, et al. Co-production of 3-hydroxypropionic acid and 1,3-propanediol from glycerol using resting cells of recombinant
Klebsiella pneumoniae J2B strain overexpressing aldehyde dehydrogenase. Appl Microbiol Biotechnol. 2012;96:373-83.

[21] Huang Y, Li Z, Shimizu K, Ye Q. Co-production of 3-hydroxypropionic acid and 1, 3-propanediol by Klebsiella pneumoniae expressing aldH under microaerobic conditions. Bioresour Technol. 2013;128:505-12.

[22] Rahman MS, Xu C, Ma K, Guo H, Qin W. Utilization of by-product glycerol from bio-diesel plants as feedstock for 2,3-butanediol accumulation and biosynthesis genes response of Klebsiella variicola SW3. Renew Energ. 2017;114:1272-80.

[23] Yang Z, Zhang Z. Recent advances on production of 2,3-butanediol using engineered microbes. Biotech Adv. 2019;37:569-78.

[24] Li Y, Li S, Ge X, Tian P. Development of a Red recombinase system and antisense RNA technology in Klebsiella pneumoniae for the production of chemicals. RSC Adv. 2016;6:79920-27.

[25] Raj SM, Rathnasingh C, Jung WC, Selvakumar E, Park S. A novel NAD+-dependent aldehyde dehydrogenase encoded by the puuC gene of Klebsiella pneumoniae DSM 2026 that utilizes 3-hydroxyxpropanaldehyde as a substrate. Biotechnol Bioprocess Eng. 2010;15:131-8.

[26] Leonard E, Ajikumar PK, Thayer K, Xiao WH, Mo JD, Tidor B, et al. Combining metabolic and protein engineering of a terpenoid biosynthetic pathway for overproduction and selectivity control. Proc Natl Acad Sci USA. 2010;107:13654-9.

[27] Wang X, Sa N, Wang F, Tian P. Engineered constitutive pathway in Klebsiella pneumoniae for 3-hydroxypropionic acid production and implications for decoupling glycerol dissimilation pathways. Curr Microbiol. 2013;66:293-9.

[28] Son HF, Park S, Yoo TH, Jung GY, Kim KJ. Structural insights into the production of 3-hydroxypropionic acid by aldehyde dehydrogenase from Azospirillum brasilense. Sci Rep. 2017;7:46005.

[29] Park YS, Choi UJ, Nam NH, Choi SJ, Nasir A, Lee SG, et al. Engineering an aldehyde dehydrogenase toward its substrates, 3-hydroxypropanal and NAD+, for enhancing the production of 3-hydroxypropionic acid. Sci Rep. 2017;7:17155.

[30] Zhao P, Wang W, Tian P. Development of cyclic AMP receptor protein-based artificial transcription factor for intensifying gene expression. Appl Microbiol Biotechnol. 2018;102:1673-85.

[31] Klumpp S, Hwa T. Growth-rate-dependent partitioning of RNA polymerases in bacteria. Proc Natl Acad Sci USA. 2008;105:20245-50.

[32] Segall-Shapiro TH, Meyer AJ, Ellington AD, Sontag ED. A ‘resource allocator’ for transcription based on a highly fragmented T7RNA polymerase. Mol Syst Biol. 2014;10:742.

How to cite this article: Li X, Chen L, Wang X, Tian P. Physiological investigations of the influences of byproduct pathways on 3-hydroxypropionic acid production in Klebsiella pneumoniae. J Basic Microbiol. 2019;59:1195–1207. https://doi.org/10.1002/jobm.201800640