

3-Hydroxypropionaldehyde-specific aldehyde dehydrogenase from *Bacillus subtilis* catalyzes 3-hydroxypropionic acid production in *Klebsiella pneumoniae*

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Abstract In *Klebsiella pneumoniae*, aldehyde dehydrogenases (ALDH) convert 3-hydroxypropionaldehyde (3-HPA) into 3-hydroxypropionic acid (3-HP). Although ALDHs can increase the production of 3-HP in *K. pneumoniae*, the substrate specificity of ALDH homologues from other microorganisms toward 3-HPA is less documented. Here we report that DhaS, a putative ALDH from *Bacillus subtilis*, shows high specificity toward 3-HPA when heterologously expressed in *K. pneumoniae*. Using NAD^+ as a cofactor, DhaS exhibited higher catalytic activity (2.3 U mg^{-1}) and lower K_m value (0.4 mmol l^{-1}) toward 3-HPA than that toward other aldehydes. Under shake-flask conditions, the recombinant strain produced $2.1 \text{ g 3-HP l}^{-1}$ in 24 h, which is 3.9-fold of that in a control harboring a blank vector. Under non-optimized bioreactor conditions, the recombinant strain produced 18 g 3-HP l^{-1} and 1,3-propanediol (1,3-PDO) at 27 g l^{-1} in 24 h. The overall conversion rate from glycerol to 3-HP and 1,3-PDO reached

$59.4 \text{ mol mol}^{-1}$. Homology modeling of DhaS illustrates substrate specificity and NAD^+ -binding site. DhaS is thus a 3-HPA-specific enzyme useful for production of 3-HP.

Keywords Aldehyde dehydrogenase · Glycerol · 3-Hydroxypropionaldehyde · 3-Hydroxypropionic acid · *Klebsiella pneumoniae* · Substrate specificity

Introduction

3-Hydroxypropionic acid (3-HP) has attracted significant attention because it can be converted into a variety of commercially-valuable compounds, including acrylic acid, 3-hydroxypropionaldehyde (3-HPA), 1,3-propanediol (1,3-PDO) and malonic acid (Werpy et al. Werpy and Petersen 2004; Valdehuesa et al. 2013; Kumar et al. 2013). *Klebsiella pneumoniae* is a promising 3-HP-synthesizing strain due to active cell proliferation and high capacity to metabolize glycerol (Huang et al. 2013). Under anaerobic conditions, glycerol dissimilation in *K. pneumoniae* is mediated by the *dha* regulon which involves parallel oxidation and reduction pathways (Forge and Lin Forge and Lin 1982). In the glycerol oxidation pathway, glycerol is converted into dihydroxyacetone phosphate, which subsequently enters glycolic pathway. In the glycerol reduction pathway, glycerol is converted into 3-HPA by glycerol dehydratase (*dhaB*) (Forge and Foster 1982). Next, 3-HPA is catalyzed into 1,3-PDO by 1,3-

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propanediol oxidoreductase (*dhaT*) (Forage and Lin 1982) and 3-HP by aldehyde dehydrogenases (ALDH) (Ashok et al. 2011) respectively.

3-HP production has been achieved by overexpression of ALDHs, including ALD4 from *Saccharomyces cerevisiae* (Li et al. 2013), KGSADH from *Azospirillum brasilense* (Rathnasingh et al. 2009), and isozymes PucC, YdcW and YneI from *K. pneumoniae* (Raj et al. 2010; Luo et al. 2013). However, the yield of 3-HP is too low for commercialization. The reasons behind include but are not limited to the following: (i) massive consumption of NAD⁺, which slows cell growth and thereby hinders 3-HP production; (ii) low specificity of ALDHs toward 3-HPA, that is, other aldehydes beyond 3-HPA in cytoplasm were also catalyzed into corresponding acids by ALDHs, leading to metabolic perturbation and the decrease of 3-HP biosynthesis. In fact, in wild-type strains, ALDHs usually demonstrate low activity to maintain cofactor balance (Ko et al. 2012). Based on these information, we speculate that in addition to catalytic activity, the ALDH specificity toward 3-HPA might be crucial for overproduction of 3-HP.

In this study, DhaS, a putative ALDH from *B. subtilis*, was heterologously expressed in *K. pneumoniae* to produce 3-HP. Both the in vitro activities toward different aldehydes and the specificity toward 3-HPA were examined. Homology modeling and sequence analysis of DhaS were carried out to predict its spatial structure. Shake-flask and bioreactor cultivation in micro-anaerobic conditions were performed to comprehensively evaluate the efficiency of DhaS for the production of 3-HP.

Materials and methods

Strains, plasmids and reagents

Klebsiella pneumoniae DSM 2026 and *Bacillus subtilis* subsp. 168 were from DSMZ GmbH, Germany. The vector pET-28a (Novagen) was used in this study with minor modification. The original T7 promoter in vector pET-28a was replaced by *pk*, the native promoter of *dhaB1* gene (GenBank: YP_001337151.1) in *K. pneumoniae* DSM 2026. The resulting vector was named as pET-pk (Li et al. 2013). Restriction enzymes and Taq DNA polymerase were purchased from TaKaRa (Dalian, China). Other

chemicals for enzymatic activity assay, gel electrophoresis and HPLC analysis were products of Sigma. DNA synthesis and sequencing were performed by Biomed Co. Ltd., China.

Cultivation conditions

B. subtilis subsp. 168 was grown in LB medium. The medium for producing 3-HP by *K. pneumoniae* contained the following components (per liter): K₂HPO₄·3H₂O, 3.4 g; KH₂PO₄, 1.3 g; (NH₄)₂SO₄, 4 g; MgSO₄·7H₂O, 0.5 g; CaCO₃, 0.1 g; yeast extract, 3 g; glycerol, 40 g; and 1.25 ml trace element solution. The trace element solution contained (per liter): FeSO₄, 32 g; ZnCl₂·6H₂O, 2.72 g; MnCl₂·4H₂O, 0.68 g; CoCl₂·6H₂O, 1.88 g; H₃BO₃, 0.24 g; Na₂MoO₄, 0.02 g; CuCl₂·2H₂O, 1.88 g; and 40 ml conc. HCl. The recombinant strain was grown microaerobically in 50 ml Erlenmeyer flasks containing 20 ml medium and 50 µg kanamycin ml⁻¹, and shaken at 150 rpm for 24 h.

Construction of the recombinants

The native *pk* promoter of *dhaB1* gene was used to drive gene expression. The gene *dhaS* (GenBank: NC_000964.3) was amplified from the genomic DNA of *B. subtilis* by using the following primers. Forward: 5'-CGCGGA TCCATGAGTTCTTTAACGAT-3', Reverse: 5'-TCC GAGCTCTTAGTCTTCAAGGTTTACC-3', the underlined nucleotides indicate *Bam*HI and *Sac*I restriction sites, respectively. The PCR procedures were: initial denaturation at 94 °C for 4 min; followed by 30 cycles of 94 °C for 1 min, 55 °C for 45 s, 72 °C for 90 s, and 72 °C for 10 min. All other molecular manipulations followed the standard protocol. The PCR product of *dhaS* was cloned into vector pET-pk, leading to recombinant vector pET-pk-*dhaS*. It was then transformed into *K. pneumoniae* DSM 2026, the positive recombinants were screened by LB kanamycin plate and further confirmed by sequencing.

Determination of enzyme activities

The recombinant strains were grown in fermentation medium containing kanamycin of 50 µg ml⁻¹. After 9 h cultivation, cells were centrifuged at 10,000×g for 10 min and washed by 5 ml phosphate buffer solution (PBS, pH 7.0). 50 µl PMSF (10 mg ml⁻¹) was used to inhibit the protease activity. The cells were sonicated

and the resulting solution was centrifuged at 17,000g for 15 min. Protein concentration was determined using the Bradford reagent (BioRad). 100 µl cell-free solutions were added into 2 ml centrifugation tube containing 0.2 mmol NADH (or NADPH) and 5 mmol aldehyde, and incubated at 37 °C for 5 min. The amount of consumed NADH (or NADPH) was determined by measuring the decrease of absorbance at 340 nm. The experiment was performed in triplicate. One unit activity of ALDH was defined as the amount of enzyme used in consuming 1 µmol NADH per min.

Homology modeling of DhaS

The online Basic Local Alignment Search Tool (BLAST) was used to search suitable template structure from Protein Data Bank (PDB). Based on the crystal structure of human mitochondrial ALDH (PDB: 3inj_H) (Steinmetz et al. 1997), the DhaS model was generated using Swiss-Model software at the ExPASy website (<http://swissmodel.expasy.org/>). The predicted structures were evaluated by VERIFY-3D (a structure evaluation server), a program developed to verify the average distance between the backbones of superimposed proteins, thus enabling a quantitative comparison between the protein structures (Luthy et al. 1992). These models were used to identify the active site and cofactor-binding site.

3-HP production by recombinant *K. pneumoniae*

In shake-flask cultivations, the recombinants were grown in LB medium containing: yeast extract 5 g, NaCl 10 g, peptone 10 g, and kanamycin 50 mg. 1 % overnight culture was inoculated to the medium containing the same concentration of antibiotics. To maintain microaerobic conditions, the flasks were plugged with an O₂-permeable cotton stopper and incubated at 37 °C with shaking at 150 rpm.

Fed-batch cultivation of the recombinant *K. pneumoniae* strain Kp(pET-pk-*dhaS*) was carried out at 37 °C in a 5 l bioreactor (Baoding, China) containing 3 l fermentation medium. The fermentation conditions were the same as those previously reported (Huang et al. 2013). The strain was pre-cultivated in a 100 ml fermentation medium overnight at 37 °C and then added into bioreactor. The agitation speed was 400 rpm and the air was supplied at 1.5 vvm. pH

was maintained at 7 by automatic addition of 5 M NaOH. Residual glycerol was maintained at 25 g l⁻¹. Dissolved oxygen (DO) was monitored automatically. Samples were taken every 3 h, and metabolites, cell concentration and residual glycerol were examined.

Analytical methods

Cell concentrations were measured by using microplate reader at 600 nm. 3-HP, lactic acid and acetic acid were determined by HPLC equipped with a C18 column with the eluate being monitored at 210 nm. The mobile phase was 0.05 % phosphoric acid 0.8 ml min⁻¹. Residual glycerol concentration was measured every 3 h by a titration method with NaIO₄ (for control of glycerol). 1,3-PDO was quantitatively analyzed by HPLC equipped with a column of Aminex HPX-87H Ion Exclusion particles (300 × 7.8 mm, Bio-Rad, Hercules, CA, USA) using a differential refractive index detector. The column was maintained at 65 °C and the mobile phase was 5 mM H₂SO₄ (in Milli-Q water) at 0.6 ml min⁻¹. All samples were filtered through 0.22 µm membrane filter.

Results and discussion

Characterization of the recombinants

The recombinant vectors were electro-transformed into *K. pneumoniae* DSM 2026. All positive recombinants were screened by a LB kanamycin plate and further confirmed by sequencing. The *dhaS* gene in recombinant vector pET-pk-*dhaS* showed 100 % sequence identity with the reported sequence in the GenBank, suggesting that it was correctly acquired. The recombinant strain Kp(pET-pk-*dhaS*) was used for subsequent activity assay and production of 3-HP. By using constitutive promoter *pk*, the enzyme DhaS was constitutively induced by dihydroxyacetone (DHA), the metabolite of glycerol oxidization.

DhaS activity and specificity

The in vitro DhaS catalytic activities toward different aldehydes were investigated. When NADP⁺ was the cofactor, DhaS did not show any activity toward propionaldehyde, benzaldehyde, valeraldehyde, butyraldehyde and acetaldehyde, with exception of only 0.1 U mg⁻¹ activity toward propionaldehyde. In

Table 1 Catalytic activities of DhaS toward different aldehydes using NAD⁺ or NADP⁺ as the cofactor

Substrates	Cofactors	Catalytic activity (U mg ⁻¹)
3-HPA	NAD ⁺	2.3
	NADP ⁺	0
PA	NAD ⁺	0.3
	NADP ⁺	0.2
BA	NAD ⁺	0.2
	NADP ⁺	0
VA	NAD ⁺	0
	NADP ⁺	0
BVA	NAD ⁺	0.1
	NADP ⁺	0
AA	NAD ⁺	0.1
	NADP ⁺	0

3-HPA 3-hydroxypropionaldehyde, PA propionaldehyde, BA benzaldehyde, VA valeraldehyde, BVA butyraldehyde, AA acetaldehyde

contrast, when NAD⁺ was the cofactor, the activity of DhaS toward 3-HPA reached up to 2.3 U mg⁻¹, and the recombinant *K. pneumoniae* harboring vector pET-pk (as the control) showed activity of 1.3 U mg⁻¹ which was contributed by the inherent ALDHs in *K. pneumoniae*. Interestingly, except 3-HPA, the activities of *K. pneumoniae* (pET-pk-*dhaS*) toward other five aldehydes were nearly equal to that of *K. pneumoniae* (pET-pk), implying that other aldehydes are not the preferred substrates of DhaS. Indeed, DhaS activities toward other aldehydes are much lower than that toward 3-HPA when NAD⁺ was the cofactor. The relative activities of DhaS toward distinct aldehydes were shown in Table 1. The K_m value of DhaS for 3-HPA was 0.4 mM, which is much lower than that toward other aldehydes. Collectively, 3-HPA is an appropriate substrate of DhaS, and DhaS is an ideal enzyme for catalyzing 3-HPA into 3-HP.

Homology modeling and sequence analysis of DhaS

To better understand the spatial structure of DhaS, homology modeling and sequence analysis were performed. Homology modeling of DhaS was accomplished based on the crystal structure of human mitochondrial ALDH (3inj_H). The protein structure checking tool, Verify 3D, showed that the model of

DhaS is reliable, because the statistics of non-bonded interactions analysis displayed that the overall quality factor is 87.9, which is higher than the standard (>85). Amino acid sequence alignment showed 42 % identity between the *dhaS* from *B. subtilis* and the *aldH* from *E. coli*. Despite low sequence similarity between two ALDHs, there exist conserved domains, such as Glu267 and Cys302 residues, related to the catalytic activities toward aldehydes and a glycine-rich motif G-x-G-x-x-x-G (GFGEDAG in DhaS) involved in binding NAD(P)⁺ (Fig. 1). Interestingly, most of NAD⁺-dependent ALDHs contain lysine and glutamate residues adjacent to adenosine ribose (Perozich et al. 2000). A lysine residue, also conserved in NADP⁺-dependent ALDHs, interacts with the adenosine ribose in NAD⁺, or the 2'-phosphate in NADP⁺. Unlike the lysine residue, the glutamate residue likely participates in the exclusion of NADP⁺. In view of putative structure of DhaS, Lys191 and Glu194 residues adjacent to glycine motif are postulated to bind NAD⁺. Overall, 3D-structure model of DhaS revealed that NAD⁺ is the appropriate cofactor.

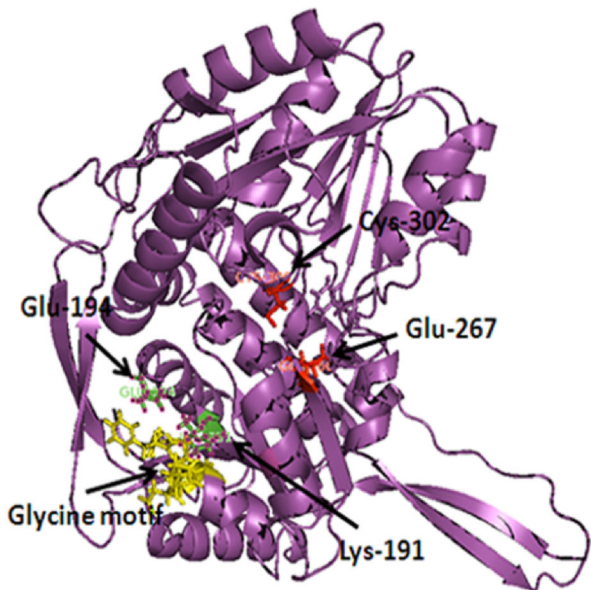
Flask cultivation of the recombinant *K. pneumoniae* expressing *dhaS*

Since DhaS showed catalytic activity for in vitro conversion of 3-HPA into 3-HP, its in vivo performance was investigated. For shake-flask cultivation, 40 g glycerol was initially added to the medium. After 24 h cultivation, more than 20 g glycerol l⁻¹ was left in the broth of the strain *K. pneumoniae* (pET-pk-*dhaS*), while less than 10 g glycerol l⁻¹ was left in that of wild-type *K. pneumoniae* (Kp-WT) (Fig. 2a). In accordance with glycerol consumption, the recombinant strain Kp(pET-pk-*dhaS*) grew more slowly than wild-type *K. pneumoniae* (Fig. 2b).

The decreased cell growth and glycerol consumption may be largely ascribed to plasmid replication which overburdened the cells. To determine the influences of DhaS overexpression on glycerol allocation, the two major byproducts, lactic acid and acetic acid, were measured. In the strain Kp(pET-pk-*dhaS*), the production of lactic acid was slightly affected compared to that in wild-type, however, acetic acid reached up to 1.5 g l⁻¹ at 24 h, which was 2.5-fold of that in wild-type strain (Fig. 2c, d). Strain Kp(pET-pk-*dhaS*) yielded 2.1 g 3-HP l⁻¹ at 24 h, which was 3.9-fold of that in control harboring a blank

Fig. 1 Sequence analysis and homology modeling of DhaS. **a** Amino acid sequence alignment between DhaS from *B. subtilis* and AldH from *E. coli* K12 (GenBank AAA23428.1). Arrows indicate the putative active site catalyzing aldehydes into acids. The glycine motif, G-X-G-X-X-G, is the NAD(P)⁺-binding site. **b** The predicted 3D structure of DhaS. Cys 302 and Glu267 residues highlight the active site. Lys191 and Glu194 residues in glycine motif indicate cofactor-binding site

dhas	MSSLTMQVTKRLETFLQGTKKLYIDGKFVPSASGATFDTNPATGETLMTLYEAQAADV	60
aldh	MNFHHLAYWQDKALSLAIENRLFINGEYTAAEENETFTVDVPTQAPLAKIARGKSVDID	60
	*. : : * :*:*:*:*:*:*. **:* :*.* .* : : : : : * : *	
dhas	KAVKAARKAFDQGEWRTMSPASRSRLMYKLADLMEEHKTLELAQLETLDNKPINETTINGD	120
aldh	RAMSAARGVFERGDWLSLSPAKRKAVLNKLADLMEAHAEELALLETLDTGKPIRHSRDD	120
	:*:**.* :*:*: * **.* : : ***** * ** * ** ** * ** * : : . *	
dhas	IPLAIEHMRYAGWCTKKTIGQTI PVSGAYFNYTRHEPVGVVQIIPWNFPLLMAMWKMG	180
aldh	IPGAARAIRWYAEADIKVYGEVATTSSHELAMIVREPVGVIAAIVPWNFPLLLTCWKLG	180
	** * . :*:** * : : : : ***** : * : ** * : : : *	
	GXGXXXG	
dhas	ALATGCTIVLKPAEQTPLSALYLAELIDQAGFPAGVINIIPGFGEADAGEALTNHEAVDKI	240
aldh	ALAAGNSVILKPKSEKSPLSAIRLAGLAKEAGLPDGVNVTGFGHEAGQALSRHNDIDAI	240
	***:* : :*:*:*:*:*: * * : : **.* ** :*: : : : : : : : : * : *	
	↓	
dhas	AFTGSTEIGKKIMSTAAKS- IKRVTTLELGGKSPNILLPDA-NLKKAI PGALNGVMFNQGG	298
aldh	AFTGSTRTGKQLLDAGDSNMKRVWLEAGGKSANIVFADCPDLQQAASATAAGIFYNQGG	300
	*****. ** : : . . . * : ** * * ** * . * : : . : : * : : : : *	
	↓	
dhas	VCCAGSRVFIHKDQYDEVDEMASYAESLRQGAGLHKDTQIGPLVSKEQHERVLSYIQKG	358
aldh	VCIAGTRLLLEESIADEFIALLKQQAQNWQPGHPLDPATMGTLLIDCAHADSVHSFIREG	360
	** ** : * : : : . . . ** : : . . . * * . * : * : . . : : * * : * : *	
dhas	KDEGAKAVTGGSCPFEAGYFVAPTVFANVEDEMTIAKEEIFGPVLTAIPYETVDEVIERA	418
aldh	ESKGQLLDGRNAGLAA--AIGPTIFVDVDPNASLSREEIFGPVLVVTRFTSEEQALQLA	418
	: : * : * . . : * : : * : * : : : : * : : : : : * : : : : : *	
dhas	NHSEYGLAAGLWTEENVKQAHYIADRLQAGTVVWVNCYNVFDAA SPFGGKQSGLGREMGSY	478
aldh	NDSQYGLGAAVWTRDLSRAHRMSRRLKAGSVFVNNYNDGDMTVPFGGYKQSGNGRDKSLH	478
	*. : * : * : * : * : : : * : : * : * : * : * * * : * : * : * : * : * : . :	
dhas	ALDNYTEVKSVMVNL	495
aldh	ALEKFTELKTIWISLEA	495
	** : : * : * : * : * : *	



vector. In addition, Kp(pET-pk-*dhaS*) yielded 7 g 1,3-PDO l⁻¹ at 24 h, while wild-type strain produced 14 g 1,3-PDO l⁻¹. The overall conversion rate from

glycerol to 3-HP and 1,3-PDO of strain Kp(pET-pk-*dhaS*) was 59.4 mol mol⁻¹, indicating that glycerol was efficiently utilized (Table 2).

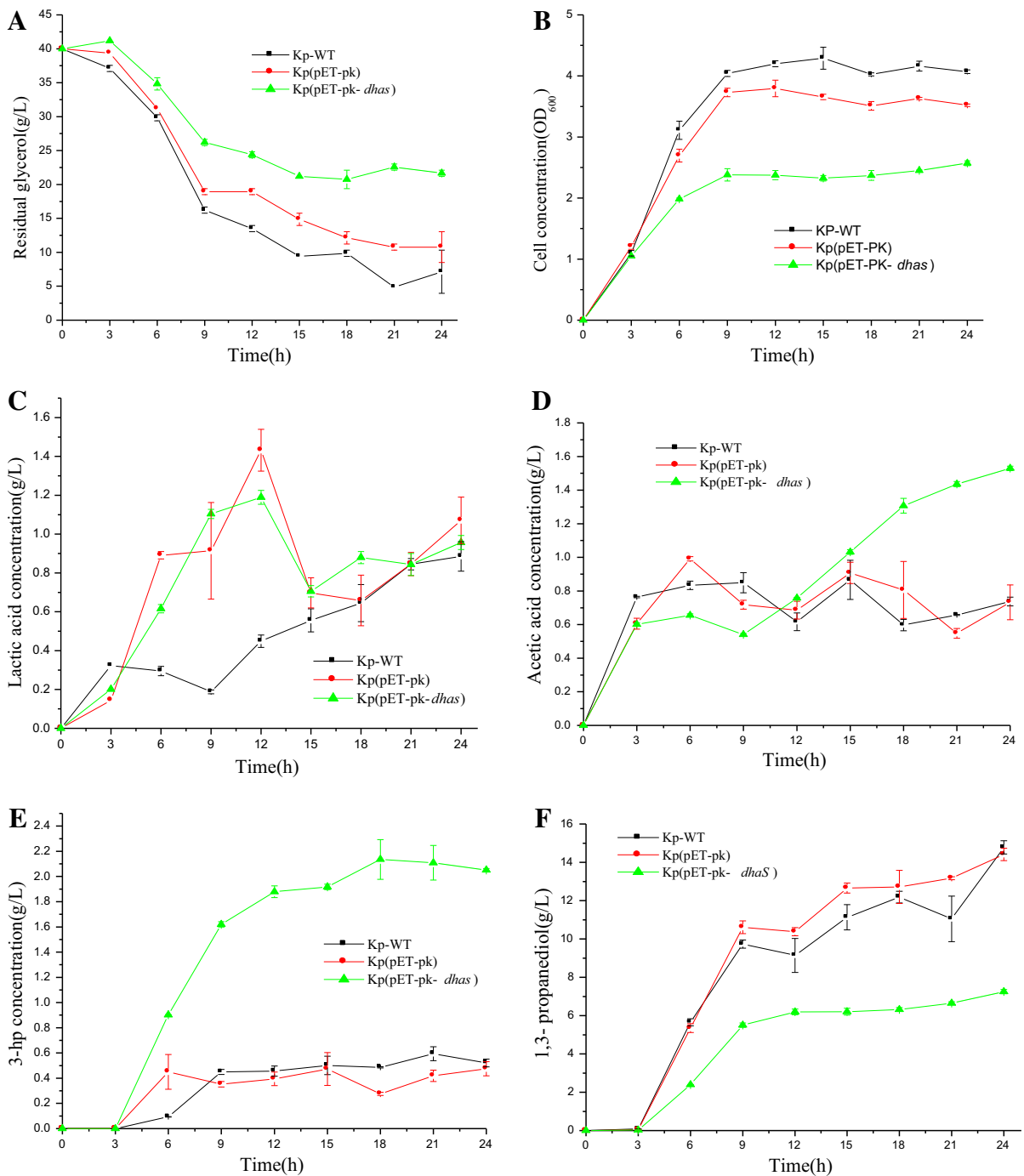


Fig. 2 Shake-flask cultivation of the strains. **a** Residual glycerol, **b** cell concentration, **c** lactic acid, **d** acetic acid, **e** 3-hydroxypropionic acid, **f** 1,3-propanediol. Kp-WT: Wild-type *K. pneumoniae*; Kp(pET-pk): Recombinant *K. pneumoniae*

harboring blank vector pET-pk; Kp(pET-pk-dhaS): Recombinant *K. pneumoniae* harboring vector pET-pk-dhaS. Error bars represent standard deviation from three independent experiments

Table 2 Carbon distribution for glycerol metabolism in recombinant strain Kp(pET-pk-dhaS)^a cultivated in shake-flask

Substrate and metabolites	Kp(pET-pk-dhaS)		
	g l ⁻¹	mM	C (%)
Substrate			
Glycerol	18.3	199	100
Metabolites			
3-HP	2.1	23	11.6
1,3-PDO	7.3	95.3	47.9
Lactate	1	10.6	5.3
Acetate	1.5	25.5	12.8
Carbon recovery (%)			77.5

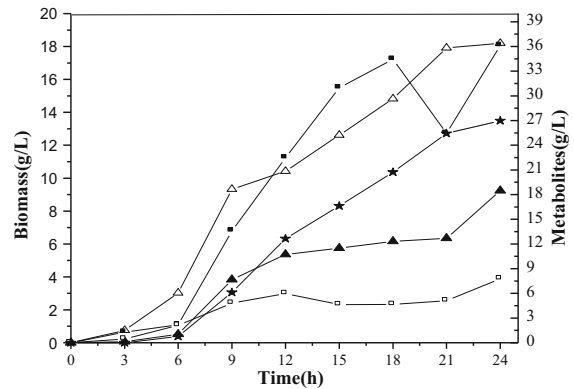
The standard deviation of the measurements was less than 5 %
Kp(pET-pk-dhaS) recombinant *K. pneumoniae* expressing DhaS

^a The calculation did not consider CO₂ evolution

Bioreactor cultivation of the recombinant *K. pneumoniae* expressing *dhaS*

To further investigate the performance of Kp(pET-pk-dhaS), fed-batch fermentation was carried out in a 5 l bioreactor containing 3 l medium. Under O₂-sufficient conditions, the recombinant strain Kp(pET-pk-dhaS) produced 18.5 g 3-HP l⁻¹ and 27 g 1,3-PDO l⁻¹ in 24 h (Fig. 3). Although air supply was efficient for aerobic fermentation, the dissolved O₂ (DO) dropped dramatically to zero at the initial 1 h and remained unchanged during the entire fermentation process. Along with the low level of DO, lactic acid reached 36.1 g l⁻¹. The overall conversion rate from glycerol to 3-HP and 1,3-PDO of the strain Kp(pET-pk-dhaS) was 37.1 mol mol⁻¹ (Table 3), which is lower than that in the shake-flask condition. The decreased glycerol conversion rate may be ascribed to the environmental differences between shake-flask and bioreactor. Nevertheless, DhaS is shown to be a 3-HPA-specific enzyme and promising for conversion of 3-HPA into 3-HP.

Although DhaS shows strict specificity toward 3-HPA and NAD⁺, the current 3-HP yield is far from commercialization. The reasons behind may be multifaceted. One reason is cofactor imbalance. 3-HP biosynthesis is positively correlated with cell growth, and there exists a competition between 3-HP production and cell growth for NAD⁺. Even though *K. pneumoniae* can regenerate NAD⁺ by the formation of

**Fig. 3** Fed-batch fermentation of the recombinant strain Kp(pET-pk-dhaS) in bioreactor. The left hand Y axis only indicates biomass (dry cell weight). Biomass *open triangles*, lactic acid *closed squares*, acetic acid *open squares*, 3-hydroxypropionic acid *closed triangles*, 1,3-propanediol *five-pointed star***Table 3** Carbon distribution for glycerol metabolism in recombinant strain Kp(pET-pk-dhaS) cultivated in bioreactor

Substrate and metabolites	Kp(pET-pk-dhaS)		
	g l ⁻¹	mmol l ⁻¹	C (%)
Substrate			
Glycerol	139	1509.4	100
Metabolites			
3-HP	18.5	205.2	13.6
1,3-PDO	27.0	354.6	23.5
Lactate	36.1	401.6	26.6
Acetate	7.8	129.7	8.6
Carbon recovery (%)			72.3

Kp(pET-pk-dhaS) recombinant *K. pneumoniae* expressing DhaS

lactic acid, whereby lactate dehydrogenase converts NADH into NAD⁺ (Laval et al. 1984), the amount of NAD⁺ may not be sufficient for overproduction of 3-HP. In addition to cofactor imbalance, 3-HP toxicity to the cell may be the second reason for hindering 3-HP accumulation. In fact, excessive 3-HP impeded cell growth which in turn retarded the formation of 3-HP. The third reason hindering 3-HP accumulation is the low activity of ALDH toward 3-HPA. Therefore, we suggest the enhancement of catalytic activity via directed evolution but without compromising substrate specificity. In summary, high 3-HP yield depends on cofactor availability, cell tolerance, enzymatic properties, or beyond.

Conclusion

A variety of aldehydes are catalyzed into corresponding acids by ALDHs and 3-HP is one of the enzymatic products of ALDHs. To preferentially produce 3-HP, a 3-HPA-specific enzyme is required. In this study, DhaS, an ALDH from *B. subtilis*, was shown to be a 3-HPA-specific enzyme. In a recombinant *K. pneumoniae* expressing *dhaS*, DhaS exhibited higher activity (2.3 U mg^{-1}) toward 3-HPA than toward other aldehydes, indicating that 3-HPA is the preferred substrate of DhaS (Table 1). Furthermore, a predicted model of DhaS demonstrated the spatial structure for binding 3-HPA and NAD^+ (Fig. 1). In shake-flasks and in a 5 l bioreactor, the recombinant strain *Kp*(pET-pk-dhaS) produced 2.1 and 18 g l^{-1} of 3-HP, respectively (Figs. 2, 3). Overall these results indicate that DhaS is a promising enzyme for production of 3-HP.

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