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# Cloning, expression and characterization of glycerol dehydrogenase involved in 2,3-butanediol formation in *Serratia marcescens* H30

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**Abstract** The *meso*-2,3-butanediol dehydrogenase (*meso*-BDH) from *S. marcescens* H30 is responsible for converting acetoin into 2,3-butanediol during sugar fermentation. Inactivation of the *meso*-BDH encoded by *bud*C gene does not completely abolish 2,3-butanediol production, which suggests that another similar enzyme involved in 2,3-butanediol formation exists in *S. marcescens* H30. In the present study, a glycerol dehydrogenase (GDH) encoded by *gld*A gene from *S. marcescens* H30 was expressed in *Escherichia coli* BL21(DE3), purified and characterized for its properties. In vitro conversion indicated that the purified GDH could catalyze the interconversion of (3*S*)-acetoin/*meso*-2,3-butanediol and (3*R*)-acetoin/(2*R*,3*R*)-2,3-butanediol. (2*S*,3*S*)-2,3-Butanediol was not a substrate for the GDH at all. Kinetic parameters of the GDH enzyme showed lower $K_m$ value and higher catalytic efficiency for (3*S*/3*R*)-acetoin in comparison to those for (2*R*,3*R*)-2,3-butanediol and *meso*-2,3-butanediol, implying its physiological role in favor of 2,3-butanediol formation. Maximum activity for reduction of (3*S*/3*R*)-acetoin and oxidations of *meso*-2,3-butanediol and glycerol was observed at pH 8.0, while it was pH 7.0 for diacetyl reduction. The enzyme exhibited relative high thermotolerance with optimum temperature of 60 °C in the oxidation–reduction reactions. Over 60 % of maximum activity was retained at 70 °C. Additionally, the GDH activity was significantly enhanced for *meso*-2,3-BD oxidation in the presence of $Fe^{2+}$ and for (3*S*/3*R*)-acetoin reduction in the presence of $Mn^{2+}$, while several cations inhibited its activity, particularly $Fe^{2+}$ and $Fe^{3+}$ for (3*S*/3*R*)-acetoin reduction. The properties provided potential application for single configuration production of acetoin and 2,3-butanediol.

**Keywords** *Serratia marcescens* · 2,3-Butanediol isomers · Glycerol dehydrogenase · Expression · Enzymatic properties

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## Introduction

2,3-Butanediol (2,3-BD) is one of the promising bulk chemicals due to its extensive industrial applications [3, 7]. 2,3-BD has been shown to have potential applications in the manufacture of printing inks, perfumes, fumigants, moistening and softening agents, explosives and plasticizers, and as a carrier for pharmaceuticals [8, 20, 22, 26].

2,3-BD contains two stereo centers and has three stereo isomers including (2*R*,3*R*)-, *meso*- and (2*S*,3*S*)-forms [7, 21]. All of the isomers of 2,3-BD could be produced using a variety of carbohydrates via mixed acid fermentation by many bacterial species [25, 27]. In these 2,3-BD natural producers, three key enzymes including catabolic α-acetolactate synthase (ALS), α-acetolactate decarboxylase (ALDC), and 2,3-butanediol dehydrogenase (BDH, also called acetoin/diacetyl reductase, AR) are responsible for the 2,3-BD biosynthesis [12, 23]. First, α-acetolactate is formed from pyruvate by catabolic ALS under limited oxygen supply conditions, then α-acetolactate is

decarboxylated into acetoin (AC, 3-hydroxy-2-butanone) by ALDC, which in turn is reduced in a reversible reaction into 2,3-BD by BDH. In addition, α-acetolactate also undergoes decarboxylation to form diacetyl (DA), which can be converted into 2,3-BD via AC by BDH [9, 12, 23]. Depending on various BDHs differing in their stereospecificities, the stereoisomeric composition of 2,3-BD formed by bacteria differs among strains [15]. Several BDHs with different stereospecificities have been identified and characterized in previous studies. The *meso*-BDHs from *S. marcescens* and *Klebsiella pneumoniae* belonged to the short-chain dehydrogenase/reductase (SDR) superfamily and showed the ability in the interconversion of (3*R*)-AC/*meso*-2,3-BD and (3*S*)-AC/(2*S*,3*S*)-2,3-BD [16, 25, 27]. While the (2*R*,3*R*)-BDHs from *Paenibacillus polymyxa*, *Bacillus subtilis* and *Saccharomyces cerevisiae* belonged to the medium-chain dehydrogenase/reductase (MDR) superfamily and catalyzed the interconversion of (3*R*)-AC/(2*R*,3*R*)-2,3-BD and (3*S*)-AC/*meso*-2,3-BD [5, 11, 24]. In addition, two (2*S*,3*S*)-BDHs from *Rhodococcus erythropolis* and *Brevibacterium saccharolyticum* were identified, displaying absolute stereospecificity in the reduction of DA to (2*S*,3*S*)-2,3-BD via (3*S*)-AC [14, 18]. Recent studies showed that production of 2,3-BD isomers could also be influenced by different carbon sources. Generally, a mixture of *meso*-2,3-BD and (2*S*,3*S*)-2,3-BD was produced by *K. pneumoniae* during sugar fermentation process. However, when *K. pneumoniae* utilized glycerol as a carbon source, three isomers of 2,3-BD were detected simultaneously in the fermentation supernatant [4, 19]. And two GDH enzymes were identified as having a dual role in glycerol metabolism and 2,3-BD formation in *K. pneumoniae* [4, 19]. Therefore, the existence of non-stereospecific dehydrogenases and multiple stereospecific dehydrogenases was proposed as a key factor for the mixed formation of 2,3-BD isomers.

*S. marcescens* was one of promising strains for efficient 2,3-BD production due to its high productivity and yield [13, 26]. Our previous studies showed that *S. marcescens* H30 mainly produced *meso*-2,3-BD with a small amount of (2*S*,3*S*)-2,3-BD due to the existence of a *meso*-BDH encoded by *bud*C gene [27]. Surprisingly, inactivation of *bud*C gene could not completely abolish 2,3-BD production [12]. Instead, a small amount of (2*R*,3*R*)-2,3-BD and *meso*-2,3-BD could be detected during sugar fermentation. So another similar enzyme related to (2*R*,3*R*)-2,3-BD and *meso*-2,3-BD formation exists in *S. marcescens* H30. To obtain the enzyme sequence, a search based on the genome of *S. marcescens* was carried out. Alignment results indicated that no gene exhibited high identity with any types of BDH except that *meso*-BDH reported previously. However, a GDH sequence encoded by *gld*A gene from *S. marcescens* was found to share high identities with those of *K. pneumoniae* and *Bacillus licheniformis*, which showed the abilities in the interconversion of (3*S*)-AC/*meso*-2,3-BD and (3*R*)-AC/(2*R*,3*R*)-2,3-BD [4, 8, 19]. So the *gld*A gene might play a role in *meso*-2,3-BD and (2*R*,3*R*)-2,3-BD formation in *S. marcescens*. To confirm the *gld*A gene function, we cloned and expressed the *gld*A gene from *S. marcescens* H30 in *E. coli*, and characterized substrate stereospecificities and kinetic parameters for the purified enzyme.

## Materials and methods

### Strains, plasmids, primers, and culture conditions

*S. marcescens* H30 (Deposited in the China Center for Industrial Culture Collection, accession number: CICC 20066) were grown at 30 °C. *E. coli* DH5α and *E. coli* BL21(DE3) as the cloning and expression hosts were grown at 37 °C. Plasmid pET-28a (+) (Novagen) was used for the GDH expression. All strains were cultured in Luria–Bertani (LB) medium. Antibiotics were added in the following amounts (per ml) if necessary: 50 μg kanamycin. Primers synthesis and DNA sequencing were carried out by Sangon Biotech (Shanghai, China). Enzyme and related reagents for DNA manipulation were purchased from TaKaRa Biotech (Dalian, China) and Tiangen Biotech (Shanghai, China). The plasmids and genomic DNA were prepared with Mini-prep Kit (BioTeke Corp., Beijing, China). All other chemicals, unless otherwise indicated, were obtained from Sigma-Aldrich (Shanghai, China).

### Cloning, expression and purification of GDH

The encoding sequence of *gld*A gene was amplified by PCR with the genomic DNA of *S. marcescens* H30 as template using the following primers: GDH1 (5′-TCC GAA TTC ATG TTG AGA ATC ATC CAG TC-3′) and GDH2 (5′-GAC AAG CTT TCA ATG ACG CTG CAA CCA GG-3′). The *Eco*RI and *Hin*dIII restriction sites were introduced into the primers (GDH1:*Eco*RI and GDH2:*Hin*dIII). The PCR conditions were as follows: 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 1 min at 72 °C after predenaturation for 5 min at 94 °C. The amplified product was ligated into the vector pET-28a (+) at *Eco*RI and *Hin*dIII sites, resulting in the recombinant plasmid designated as pET28a-*gld*A. The recombinant *E. coli* BL21(DE3)/pET28a-*gld*A was obtained using heat shock transformation.

The recombinant strain was cultivated at 37 °C in a 250-ml flask containing 50 ml LB medium with 50 μg $ml^{-1}$ kanamycin. The cells were induced at about 0.6 $OD_{600}$ with 0.5 mM isopropyl-beta-D-thiogalactopyranoside (IPTG) and harvested by centrifugation after 6 h. The precipitate was resuspended in binding buffer (20 mM phosphate, 500 mM NaCl, and 20 mM imidazole, pH 7.4) and

disrupted by sonication in an ice bath. The lysed cells were centrifuged at 13,000×*g* for 10 min to remove the debris. The soluble fraction was subjected to purification using a Histrap HP column according the purification protocol (GE Healthcare, USA). The eluate from the column was pooled and desalted by a Hitrap desalting column (GE Healthcare, USA). The purified enzyme was analyzed via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and stored at −80 °C for further characterization.

### Enzyme assays

The catalytic activity of GDH was measured by the substrate-dependent absorbance change of NAD(H) at 340 nm ($\varepsilon_{340}$ = 6.22 $mM^{-1}$ $cm^{-1}$). The enzyme assay was performed in duplicate at room temperature using the reaction mixture (1.0 ml) containing 50 mM potassium phosphate buffer (pH 8.0), 0.2 mM $NAD^+$ or 0.2 mM NADH, and 50 mM substrate. The addition of purified enzyme (20 μl) initiated the enzyme assay. One unit of GDH activity was defined as the amount of enzyme required to reduce 1 μmol of NAD(H) in 1 min. The protein concentrations of all samples were determined using the Bradford method, and bovine serum albumin served as the standard protein.

### Catalytic properties of GDH

The effect of temperature on GDH activity was determined at temperature from 20 °C to 70 °C. The effect of pH on GDH activity was measured in the range of pH 4–10 at room temperature using 50 mM sodium acetate (pH 4–5), potassium phosphate (pH 6–8), and glycine–NaOH buffers (pH 9–10). The substrate specificity was studied using 50 mM primary and secondary alcohols, diols and polyols or ketones and aldehydes under standard assay conditions. The effects of different cations including $NH_4^+$, $Na^+$, $Mn^{2+}$, $Mg^{2+}$, $Zn^{2+}$, $Ca^{2+}$, $Fe^{2+}$ and $Fe^{3+}$, all in the form of chloride/sulfate salts, on the GDH activity were evaluated at a final concentration of 1 mM.

To determine the kinetic constants, all parameters were kept constant, and only the substrate concentration was modified. Various concentrations of glycerol, *meso*-2,3-BD, (2*R*,3*R*)-2,3-BD, (3*S*/3*R*)-AC, and DA ranged from 10 μM to 20 mM at room temperature. The Michaelis–Menten equation was used for determination of the kinetic parameters.

### Stereospecificity of GDH

To investigate the stereospecificity of purified GDH enzyme, the interconversion of 2,3-BD, AC, and DA in the oxidation–reduction processes was studied using a mixture containing 50 mM substrate, 50 mM potassium phosphate buffer (pH 8.0), 0.2 mM $NAD^+$ or NADH, and 20 μl of purified GDH enzyme in a final volume of 1 ml. The conversion reactions were incubated at 30 °C for 2 h, and then stopped by the addition of HCl. The products in these conversion reactions were extracted by ethyl acetate and then used to check the enzyme stereospecificity using a GC system (Agilent GC9860) equipped with a chiral column (Supelco β-DEX™ 120, 30-m length, 0.25-mm inner diameter). The operation conditions were as follows: $N_2$ was used as the carrier gas at flow rate of 1.2 ml $min^{-1}$; the injector temperature and the detector temperature were 215 and 245 °C, respectively, and the column temperature was maintained at 50 °C for 1.5 min, then raised to 180 °C at a rate of 15 °C $min^{-1}$.

## Results

### Cloning and sequence analysis of the *gld*A gene

The *gld*A gene was obtained by PCR amplification using the primers (GDH1/GDH2) designed according to the GDH sequence of annotated genome from *S. marcescens* Db11 (Genbank accession number HG326223). The sequencing result showed that the ORF length of *gld*A gene was 1101 bp, which shared 95 % identity with its homolog in *S. marcescens* Db11. Its deduced amino acid sequence was compared with other reported GDHs by multiple alignment method (Table 1). The GDH showed high identities with other known functional GDHs except that from *H. polymorpha* with 17 % identity. Further, the amino acid sequence was used for searching for homologous sequences in the GenBank and PDB database with the BLAST program. The high similarities were found between *S. marcescens* H30 GDH and the enzymes of Fe-ADH (iron-dependent alcohol dehydrogenase) superfamily including *S. plymuthica* A30 GDH (93 %), *T. maritime* GDH (53 %), *B. stearothermophilus* GDH (47 %), and *S. melitoti* GDH (48 %). Alignment of *S. marcescens* H30 GDH amino acid sequence with these sequences revealed that the *S. marcescens* enzyme belonged to the type III Fe-ADH superfamily. As shown in Fig. 1, three consecutive glycine residues belonged to a 14-amino acid residue motif (GDK motif) as the coenzyme NAD(H) binding site, which was the signature of the iron-dependent ADH [6, 10]. Also, three conserved histidine residues belonged to a 16-residue segment that is homologous to the 15-residue stretch as the binding site of metal described in previous studies [1, 2].

### Expression and purification of GDH enzyme

The PCR-amplified products were ligated into the expression vector pET-28a (+) and transformed into *E. coli*

**Table 1** Alignment of the GDH sequence from *S. marcescens* H30 with other known functional GDHs from different strains

| Organism | Gene[a] | | Protein[a] | |
|---|---|---|---|---|
| | Length (bp) | Identity (%) | Length (aa) | Amino acid identity (%) |
| *S. marcescens* H30 (*gld*A) | 1,101 | 100 | 366 | 100 |
| *S. marcescens* Db11 (*gld*A) | 1,101 | 95 | 366 | 97 |
| *K. pneumoniae* (*dha*D) | 1,098 | 75 | 365 | 76 |
| *K. pneumoniae* (*gld*A) | 1,104 | 66 | 367 | 60 |
| *B. licheniformi* (*gld*A) | 1,104 | 58 | 367 | 48 |
| *H. polymorpha* (*gld*A) | 1,143 | 49 | 380 | 17 |

[a] The gene sequences used for the *gld*A gene homologous analysis are from GenBank data bank, and their deduced acids sequences were used to perform the protein identities. Accession numbers of the protein sequences are CDG14077 (*S. marcescens* Db11), WP_004206090 (*K. pneumoniae*), YP_001337887 (*K. pneumoniae*), YP_078053 (*B. licheniformi*), and BAD32688 (*H. polymorpha*)

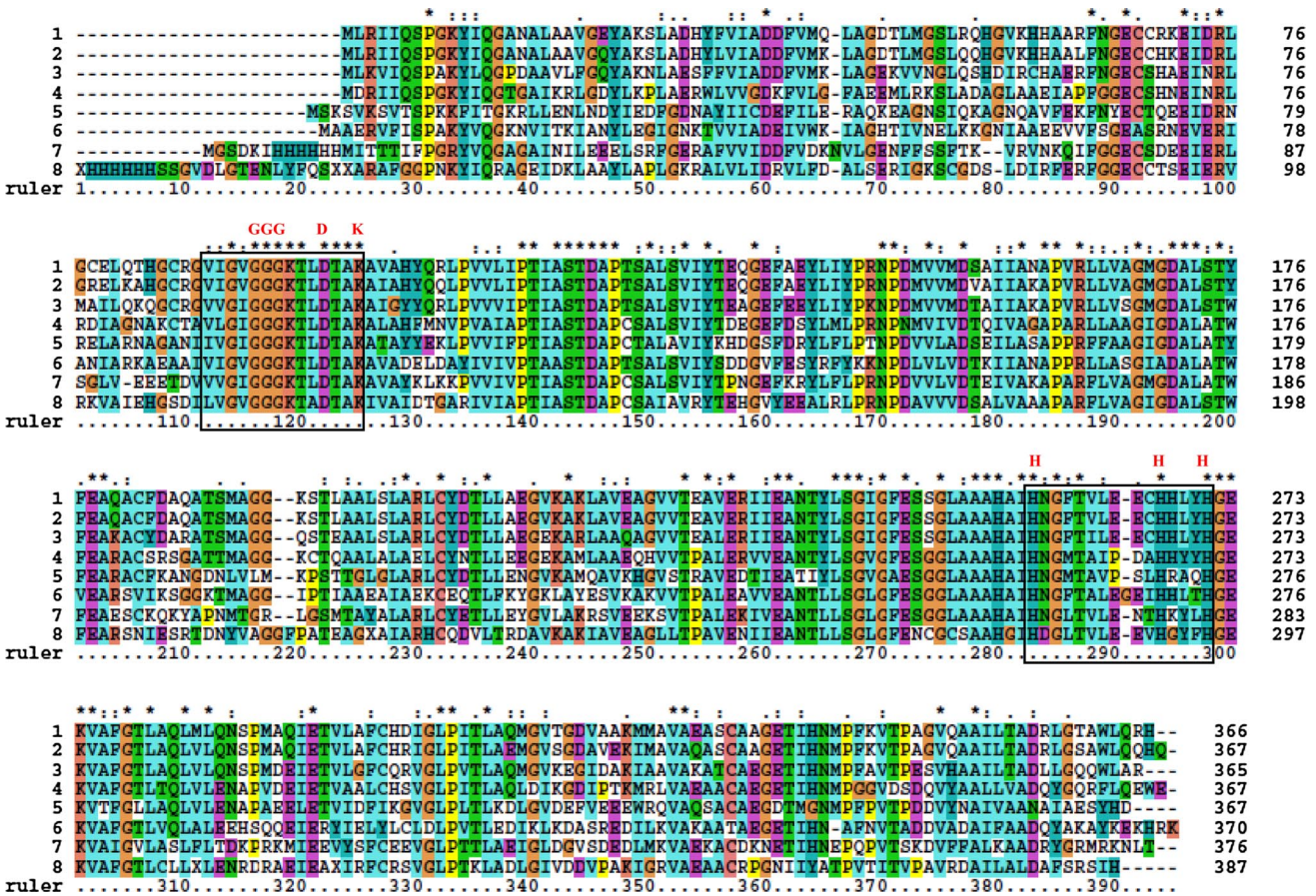

**Fig. 1** Amino acid sequence multiple alignment of GDH from *S. marcescens* H30 with other reported GDHs from different strains. *1*, *S. marcescens* H30 (GDH); *2*, *S. plymuthica* A30 (GDH); *3*, *K. pneumoniae* (DhaD); *4*, *K. pneumoniae* (GldA); *5*, *B. licheniformis* DSM 13 (GDH); *6*, *B. Stearothermophilus* (GDH); *7*, *T. Maritima* (GDH); *8*, *S. Meliloti* (GDH)

BL21(DE3) for heterologous expression. The expression and purification of GDH enzyme were carried out as described in "Materials and methods". As shown in Fig. 2, SDS-PAGE analysis on the soluble fraction from the cell lysate revealed the presence of over-expressed protein with approximate molecular weight of 39 kDa. The activity analysis of crude enzyme exhibited a maximum activity of 10.48 U $mg^{-1}$ protein with (3*S*/3*R*)-AC as a substrate and NADH as a coenzyme at room temperature (Table 2). The purified GDH was observed as a single band on SDS–polyacrylamide gel (Fig. 2), indicating that the relatively pure enzyme was obtained. The specific activity of purified GDH

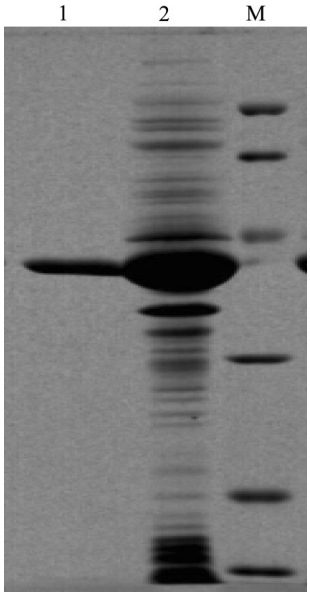


**Fig. 2** Expression and purification of the GDH protein: lane M, protein marker (97.2, 66.4, 44.3, 29, 20.01, 14.3 kDa); lane 1, purified GDH protein; lane 2, soluble protein from the cell lysate, were electrophoresed on a 12 % polyacrylamide gel under denaturing conditions

was determined to be 20.75 U $mg^{-1}$ protein for (3*S*/3*R*)-AC with NADH as a coenzyme. The purity enrichment as determined by the specific activity of GDH was 1.98-fold.

## Effects of temperature and pH on GDH activity

The effects of temperature and pH on GDH activity were studied using 50 mM DA, 50 mM (3*S*/3*R*)-AC, 50 mM *meso*-2,3-BD, and 50 mM glycerol as substrates and 0.2 mM NAD(H) as coenzymes. As shown in Fig. 3a, maximum activity for reduction of (3*S*/3*R*)-AC and oxidations of *meso*-2,3-BD and glycerol was observed at pH 8.0, while it was pH 7.0 for DA reduction. The temperature effects were determined in the range of 20–70 °C for these four substrates. The results in Fig. 3b indicated that the enzyme possessed high thermoltolerence in the oxidation–reduction reactions. The optimal temperature of the enzyme was found to be 60 °C for these substrates. More than 60 % of maximum activity was retained at 70 °C.

## Specificity of GDH for substrates

The substrate specificity of GDH enzyme in the oxidation and reduction reactions was investigated using a set of alcohols, aldehydes, and ketones (Table 3). In the oxidation reactions, the GDH could effectively catalyze the oxidization of glycerol and *meso*-2,3-BD but not (3*S*/3*R*)-AC in the presence of $NAD^+$. Although the purified enzyme exhibited low activity, the catalytic activity could still be detected in the presence of (2*R*,3*R*)-2,3-BD with GDH and $NAD^+$. While (2*S*,3*S*)-2,3-BD was not a substrate for the GDH at all. Additionally, two primary alcohols (1,4-butanediol and isopropanol) could be oxidized, though showing less activity than glycerol and *meso*-2,3-BD. In the reduction reactions, the enzyme exhibited the ability to reduce DA, (3*S*/3*R*)-AC, and formaldehyde. Maximum activity was observed for (3*S*/3*R*)-AC reduction, implying that AC was the best substrate for the GDH in the reduction reactions. These properties were similar to those of two GDHs (DhaD and GldA) from *K. pneumoniae*. The major difference was that the GDH from *S. marcescens* H30 showed its activity for DA reduction, while no activity for DA reduction was observed by the GDHs from *K. pneumoniae* [19].

The comparative data of apparent $K_m$ and $K_{cat}$ values for GDH from *S. marcescens* H30 were determined and given in Table 4. The $K_m$ and $K_{cat}$ values of GDH were 7.76 mM and 10.12 $s^{-1}$ for glycerol, 22.58 mM and 11.85 $s^{-1}$ for *meso*-2,3-BD, 154.22 mM and 2.23 $s^{-1}$ for (2*R*,3*R*)-2,3-BD, 3.14 mM and 30.21 $s^{-1}$ for (3*S*/3*R*)-AC, and 8.02 mM and 20.17 $s^{-1}$ for DA, respectively. The kinetic parameters of the enzyme showed lower $K_m$ values and higher catalytic efficiency for DA and (3*S*/3*R*)-AC in comparison to those for (2*R*,3*R*)- and *meso*-2,3-BD, suggesting its physiological role in favor of 2,3-BD formation in *S. marcescens* H30.

## Effects of cations on GDH activity

Several cations including $Mn^{2+}$, $Mg^{2+}$ and $Fe^{2+}$ have been proved to influence the activity of related enzymes involved in the interconversion between AC and 2,3-BD. So the effects of different cations on GDH activity were investigated using (3*S*/3*R*)-AC and *meso*-2,3-BD as

**Table 2** GDH activity in crude and purified enzyme extracts

| Enzyme | Total activity (U) | Total protein (mg) | Specific activity (U $mg^{-1}$) | Purification (fold) |
|---|---|---|---|---|
| Crude extract | 18.13 | 1.73 ± 0.04 | 10.48 ± 0.15 | 1.00 |
| Purified GDH | 8.30 | 0.40 ± 0.03 | 20.75 ± 0.17 | 1.98 |

Assay conditions: 50 mM potassium phosphate buffer (pH 8.0), 50 mM (3*S*/3*R*)-acetoin, 0.2 mM NADH, and appropriate amount of enzyme at room temperature

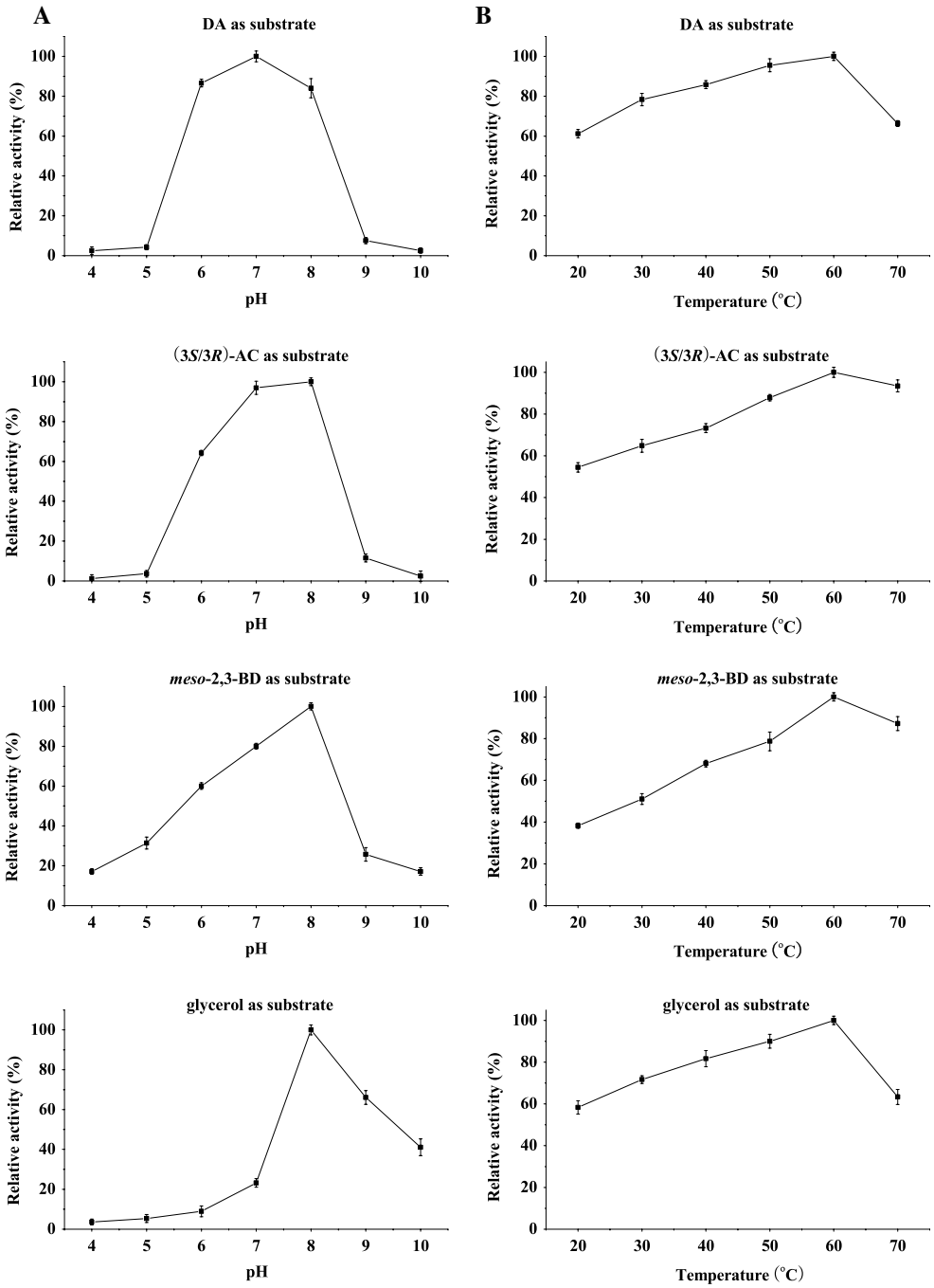


**Fig. 3** Effects of different pHs (**a**) and temperatures (**b**) on the GDH activity

substrates. As shown in Table 5, the addition of the cations such as $NH_4^+$, $Na^+$, $Mn^{2+}$, and $Mg^{2+}$ could activate and increase the GDH activity by 9–86 % in the (3*S*/3*R*)-AC reduction reaction. On the other hand, the GDH activity could be inhibited by the cations of $Zn^{2+}$, $Ca^{2+}$, $Fe^{2+}$, and $Fe^{3+}$. Especially, $Fe^{2+}$ and $Fe^{3+}$ exhibited strong inhibitions with <15 % of maximum activity for the (3*S*/3*R*)-AC reduction. In contrast, for *meso*-2,3-BD oxidation, the cation of $Fe^{2+}$ could activate and significantly increase the activity to 784 %, which suggested that the GDH enzyme was a $Fe^{2+}$-dependent dehydrogenase. Additionally, the activity of GDH also was enhanced slightly by 26 and 14 % respectively in the presence of $Zn^{2+}$ and $Fe^{3+}$. The cations of $NH_4^+$, $Na^+$, $Mn^{2+}$, $Mg^{2+}$, and $Ca^{2+}$ appeared at different inhibitions on the GDH activity in the oxidation reactions.

**Table 3** Substrate specificity of glycerol dehydrogenase from *S. marcescens* H30

| Substrates | Activity |
|---|---|
| Oxidation[a] | % |
| *meso*-2,3-Butanediol | 100 |
| (2*R*,3*R*)-2,3-Butanediol | 9 ± 2 |
| (2*S*,3*S*)-2,3-Butanediol | ND |
| Glycerol | 146 ± 3 |
| 1-Propanol | ND |
| 1,4-Butanediol | 16 ± 2 |
| Isopropanol | 5 ± 1 |
| 1-butanol | ND |
| Isopentanol | ND |
| Ethylene glycol | ND |
| Ethanol | ND |
| (3*S*/3*R*)-Acetoin | ND |
| Reduction[a] | % |
| (3*S*/3*R*)-Acetoin | 100 |
| Diacetyl | 72 ± 3 |
| Formaldehyde | 3 ± 1 |

*ND* not detected because of lower enzyme activities

[a] Assay conditions: 50 mM potassium phosphate buffer (pH 8.0), 50 mM substrate, 0.2 mM $NAD^+$ or 0.2 mM NADH for the oxidation or reduction reactions

**Table 4** Kinetic parameters of GDH from *S. marcescens* H30 for glycerol, *meso*-2,3-BD, (2*R*,3*R*)-2,3-BD, (2*S*,3*S*)-2,3-BD, (3*S*/3*R*)-AC and DA

| Substrates | $K_m$ (mM) | $K_{cat}$ ($s^{-1}$) | $K_{cat}/K_m$ ($s^{-1}$ $mM^{-1}$) |
|---|---|---|---|
| Glycerol | 7.76 ± 0.23 | 10.12 ± 0.25 | 1.30 |
| *meso*-2,3-BD | 22.58 ± 0.51 | 11.85 ± 0.33 | 0.53 |
| (2*R*,3*R*)-2,3-BD | 154.22 ± 5.84 | 2.23 ± 0.11 | 0.02 |
| (2*S*,3*S*)-2,3-BD | ND | ND | ND |
| (3*S*/3*R*)-AC | 3.14 ± 0.14 | 30.21 ± 0.25 | 9.62 |
| DA | 8.02 ± 0.54 | 20.17 ± 0.62 | 2.52 |

Assay conditions: 50 mM potassium phosphate buffer (pH 8.0), 50 mM substrate, 0.2 mM $NAD^+$ or 0.2 mM NADH for the oxidation or reduction reactions

*ND* not detected because of no activity

### Stereospecificity of the GDH

To understand the GDH stereospecificity in the oxidation–reduction processes of the 2,3-BD/AC/DA interconversion, purified GDH enzyme was used to perform the conversion described in the experimental procedures. The products of the enzymatic reactions were further analyzed using a gas chromatograph equipped with a chiral column. For 2,3-BD oxidation reactions, when *meso*-2,3-BD was used as the substrate with $NAD^+$ and GDH, (3*S*)-AC was the only product detected (Fig. 4b). Accordingly, (3*R*)-AC was, as expected, the only product obtained from (2*R*,3*R*)-2,3-BD (Fig. 4c). These results indicated that the GDH enzyme possessed the ability of selective oxidation at the *R*-carbon of *meso*-2,3-BD and (2*R*,3*R*)-2,3-BD, which was similar to the (2*R*,3*R*)-BDH characterized in previous studies [5, 11, 24]. In the reduction reactions, DA as a substrate with GDH and NADH could be converted into (3*S*)-AC and *meso*-2,3-BD (Fig. 4d). While (3*R*)-AC, (2*R*,3*R*)-2,3-BD and (2*S*,3*S*)-2,3-BD was not detected in the reaction system, implying that only (3*S*)-AC was produced from DA and further converted into *meso*-2,3-BD. When a racemic mixture of (3*S*/3*R*)-AC as substrates was incubated with GDH and NADH, a mixture of *meso*-2,3-BD and (2*R*,3*R*)-2,3-BD was detected (Fig. 4e). Considering that *meso*-2,3-BD is the only product from (3*S*)-AC, therefore, (2*R*,3*R*)-2,3-BD was produced from (3*R*)-AC as a substrate.

**Table 5** Effects of various cations on the GDH activity in the oxidation and reduction reactions

| Metals | Relative activity (%) | |
|---|---|---|
| | (3*S*/3*R*)-AC as substrate[a] | *meso*-2,3-BD as substrate[b] |
| Control | 100 | 100 |
| $NH_4Cl$ | 119 ± 4 | 66 ± 2 |
| NaCl | 122 ± 4 | 54 ± 1 |
| $MnSO_4$ | 186 ± 2 | 63 ± 4 |
| $FeSO_4$ | 11 ± 2 | 784 ± 3 |
| $FeCl_3$ | 15 ± 1 | 114 ± 3 |
| $ZnSO_4$ | 61 ± 1 | 126 ± 2 |
| $CaCl_2$ | 97 ± 3 | 40 ± 1 |
| $MgCl_2$ | 109 ± 2 | 49 ± 3 |

[a] Assay conditions: 50 mM potassium phosphate buffer (pH 8.0) with 50 mM (3*S*/3*R*)-AC as substrate and 0.2 mM NADH as coenzyme

[b] Assay conditions: 50 mM potassium phosphate buffer (pH 8.0) with 50 mM *meso*-2,3-BD as substrate and 0.2 mM $NAD^+$ as coenzyme

## Discussion

In general, GDH, which catalyzes the initial step of glycerol oxidation, is responsible for glycerol utilization in *S. marcescens*. However, our current study indicated that the GDH enzyme in *S. marcescens* also played an important role in 2,3-BD formation. Therefore, the GDH enzyme in *S. marcescens* might play a dual role in glycerol metabolism and 2,3-BD formation. Recent studies indicated that the dual role of GDH also existed in two 2,3-BD producing strains, *K. pneumoniae* and *B. licheniformis* [8, 19]. These findings suggested the GDH enzyme might have vital physiological significance to the microbes when

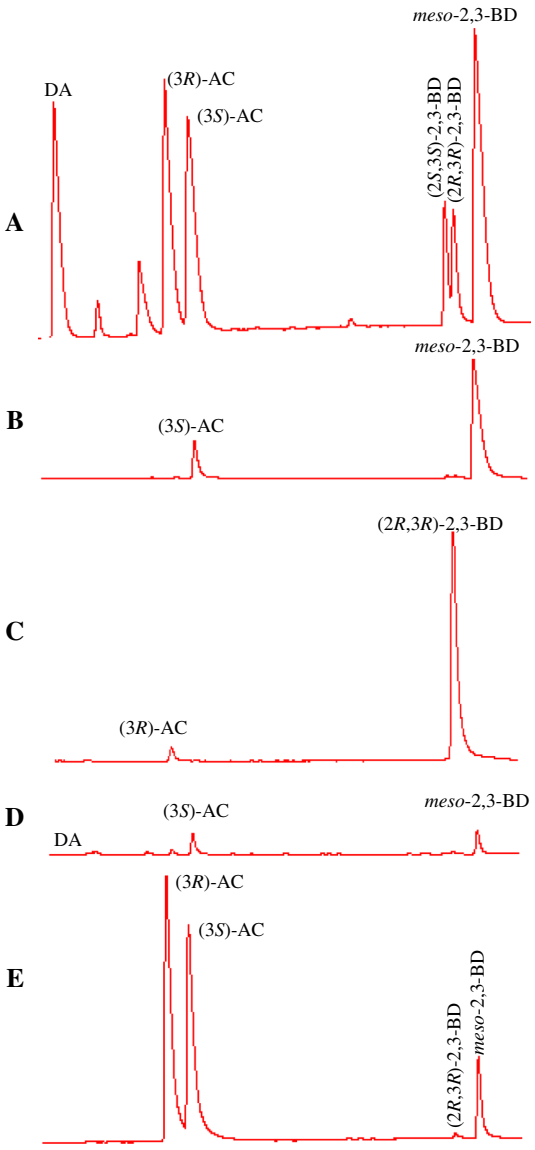


**Fig. 4** Chiral-column GC analysis of the substrates and products in the oxidation and reduction reaction catalyzed by glycerol dehydrogenase from *S. marcescens* H30. **a** Profile of mixture of standard chemicals. **b** The product from *meso*-2,3-butanediol. **c** The product from (2*R*,3*R*)-2,3-butanediol. **d** The product from diacetyl. **e** The product from (3*S*/3*R*)-acetoin (the peak of diacetyl was in front of solvent peak and not shown in the picture)

glycerol as carbon source was used. As described in previous studies, glycerol catabolism could generate excessive NADH and activate the NADH-consuming pathway (such as acid pathway and 2,3-BD pathway) when compared with glucose catabolism [17, 19]. 2,3-BD production by GDH plays a major role in oxidizing NADH, thus regulating the intracellular redox balance during glycerol metabolism. In addition, the dual role of GDH in these strains might also contribute to a suitable environmental pH and favor the survival of these strains on glycerol by shifting glycerol metabolism from acid production to the formation of neutral compounds (AC and 2,3-BD).

The enzymatic properties of GDH in this study were also determined and characterized. Its special features might lead to potential application for single configuration production of (3*S*)-AC and *meso*-2,3-BD from DA as a cheap substrate. As observed in the results section, two metal ions, $Fe^{2+}$ and $Fe^{3+}$, could strongly inhibit the reaction of (3*S*)-AC into *meso*-2,3-BD. This characteristics of the GDH from *S. marcescens* H30 are very helpful for chiral (3*S*)-AC production from DA by whole cells catalysis with over-expression of GDH in the presence of $Fe^{2+}$ and $Fe^{3+}$, which could prevent (3*S*)-AC from further forming *meso*-2,3-BD. Similarly, single configuration production of *meso*-2,3-BD could also be achieved efficiently from DA by the same catalytic system in the presence of $Mn^{2+}$, since $Mn^{2+}$ could effectively increase its activity of the reduction reaction from (3*S*)-AC to *meso*-2,3-BD. In addition, high thermotolerance of the GDH enzyme is beneficial for biocatalytic process.

In conclusion, we reported a dual role of GDH involved in glycerol metabolism and 2,3-BD formation in *S. marcescens* H30. The GDH was expressed, purified and characterized, exhibiting some special features of the interconversion between AC and 2,3-BD. These characteristics provided some potential applications for single configuration production of (3*S*)-AC and *meso*-2,3-BD.

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