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Cloning and characterization of a novel NAD$^+$-dependent xylitol dehydrogenase from *Gluconobacter oxydans* CGMCC 1.637

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Abstract: [Objective] To clone the xylitol dehydrogenase gene from *Gluconobacter oxydans* CGMCC 1.637, to characterize enigmatic properties of xylitol dehydrogenase and to investigate the induction abilities of various carbon sources on the oxidative activity of xylitol dehydrogenase and the effect of various carbon sources on the bioconversion of d-xylulose to xylitol in *G. oxydans* CGMCC 1.637. **[Methods]** Touch-down polymerase chain reaction (PCR) was applied to clone the xylitol dehydrogenase gene from chromosomal DNA of *G. oxydans* CGMCC 1.637. **[Results]** The 798-bp open reading frame of xylitol dehydrogenase encoded a protein of 265 amino acids, with the molecular mass of 27.95 kDa. Sequence analysis of the putative protein revealed it to be a member of short-chain dehydrogenase/reductase family. Xylitol dehydrogenase showed oxidative activity with xylitol and sorbitol and no activity with other polyols, such as d-arabitol. K_m and V_{max} with xylitol was 78.97 mmol/L and 40.17 U/mg, respectively. The highest oxidative activity of xylitol dehydrogenase for xylitol was only 23.27 U/mg under optimum conditions (pH 10.0, 35℃). However, the activity of its reverse reaction, d-xylulose reduction, reached 255.55 U/mg under optimum conditions (pH 6.0, 30℃), 10-times higher than that of xylitol oxidation. Oxidative activity of xylitol dehydrogenase was induced when *G. oxydans* CGMCC 1.637was cultivated on d-sorbitol. D-arabitol, which supported a high cell growth, inhibited the oxidative activity of xylitol dehydrogenase and the bioconversion ability of *G. oxydans* CGMCC 1.637. **[Conclusions]** The obtained gene from *G. oxydans* CGMCC 1.637 was a novel gene encoding xylitol dehydrogenase. Oxidative activity of xylitol dehydrogenase in *G. oxydans* CGMCC 1.637 and the bioconversion ability of *G. oxydans* CGMCC 1.637 after grown on d-arabitol were inhibited, which provided a valuable clue for further study to increase xylitol yield from d-arabitol.

Keywords: *Gluconobacter oxydans*, short-chain dehydrogenase/reductase family, xylitol dehydrogenase, D-xylulose, xylitol, clone

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Xylitol is a crystalline carbohydrate with sweetening power similar to that of sucrose but lower energy. Consumption of xylitol can effectively reduce dental caries by reducing the production of lactic acid

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and enhancing ammonia formation in plaque to neutralize the lactic acid [1]. It is relatively safe to be a sweetener in diabetic foods because of its slowly absorbance and insulin-independence in initial metabolic steps. It has been reported that xylitol supplementation significantly increased the concentrations of Ca in bone and dietary xylitol protected against osteoporosis [2]. Due to its special characters, xylitol has been widely applied as a substitute of sugar in the fields of food and pharmaceuticals [3].

Xylitol is currently manufactured by chemical reduction of xylose derived from the natural polysaccharide xylan under the catalysis of Raney-nickel. In order to overcome the disadvantages such as environmental pollution and to reduce the energy consumption by chemical reduction, microbial conversion of xylitol from glucose via d-arabitol and d-xylulose was put forward [4]. *Gluconobacter oxydans*, characterized by its ability to oxidize a great variety of sugars and polyols regioselectively and incompletely, is used for many biotechnological processes. It was reported that *G. oxydans* ATCC 621 was able to converse d-arabitol to xylitol via two enzymatic oxidoreductive reactions, with membrane-bound d-arabitol dehydrogenase and xylitol dehydrogenase (XDH) [5]. The XDH responsible for the reduction of d-xylulose to xylitol has been cloned from *G. oxydans* ATCC 621 [6], some other xylose-fermenting yeast and other fungi, such as *Candida tropicalis*, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Fusarium oxysporum* [7-10]. In our previous study, *G. oxydans* CGMCC 1.637 was discovered to be able to transform d-arabitol to d-xylulose completely within 20 h, but only a small amount of xylitol produced even if the transformation time was prolonged to 48 h. So finding out the limited factors and obtained the xylitol dehydrogenase gene (*xdh*) from *G. oxydans* CGMCC 1.637 are greatly important for the metabolic engineering efforts to construct strains to increase the conversion rate of xylitol from d-xylulose in *G. oxydans* CGMCC 1.637.

In this study, we cloned *xdh* gene from *G. oxydans* CGMCC 1.637, determined its nucleotide sequence and characterized the enzyme properties of XDH. Various carbon sources were investigated for their abilities to induce the XDH activities and effect on bioconversion (d-xylulose to xylitol) in *G. oxydans* CGMCC 1.637.

1 Materials and methods

1.1 Bacterial strains, plasmids and culture conditions

G. oxydans CGMCC 1.637 was cultured on the *Acetobacter* medium-agar, containing 100 g glucose, 10 g/L yeast extract, 20 g/L $CaCO_3$ and 15 g/L agar, then inoculated into a 1000-ml flask containing 100 ml of medium consisted of 20 g/L d-sorbitol, 3 g/L yeast extract, 1 g/L $MgSO_4 \cdot 7H_2O$, 3 g/L K_2HPO_4 and cultured at 30℃, 250 r/min for 18 h [11]. *E. coli* DH5α and BL21 (DE3) were cultured in Luria-Bertani (LB) medium and used as host strain for clone and expression.

1.2 Cloning of *xdh* gene from *G. oxydans CGMCC 1.637*

The chromosomal DNA of *G. oxydans* CGMCC 1.637 was prepared using AxyPrep Bacterial Genomic DAN MiniPrep Kit (Axygen, USA). The amplification was carried out in a 50-μl reaction containing 200 μmol/L deoxynucleotide triphosphate, 50 mmol/L KCl, 5 mmol/L $MgCl_2$, 2 U *Taq* DNA polymerase (Sangon, China) and 1 μmol/L primer listed in Table 1. Ten cycles of touch-down amplification, where the annealing temperature decreased 2℃ every two cycles from 60℃ to 52℃, was followed by 20 cycles of amplification with 30 s of denaturizing at 94℃, 45 s of annealing at 50℃, and 60 s of extension at 72℃. Finally, the amplification was terminated with one extension step at 72℃ for 10 min. The PCR products were analyzed by electrophoresis using 1% agarose, and purified by using the PCR Cleanup Kit (Axygen, USA), ligated with pUC_m-T (Sangon, China), and subsequently subcloned into *E. coli* DH5α. A positive clone was selected and sequenced by Biosune (China).

Table 1 Primers for cloning *xdh* gene

Primers		Sequence (5′→3′)
Primers 1	F1	TTCATGTGGGAGCGTCAGGT
	R1	CCCGTCATGAAGCTGGAATC
Primers 2	F2	CCAGAACGGTATCGGAAATG
	R2	TTCCGGGTTGGTTGAGAAATACTG
Primers 3	F3	GCCAAGGCCAACACGCAGTAT
	R3	CGGGCTGTGGCGGAAGGAC
Primers 4	F4	CATATGGCATACGTGGTAAGTTC
	R4	GTCGACTCAGCCTCCAGAGATTTCC

1.3 Overexpression of XDH in *E. coli*

The putative *xdh* gene was amplified from genomic of *G. oxydans* CGMCC 1.637 by using primers (F4/R4) where the underlined parts in Table 1 were *Nde*I and *Xho*I sites, respectively. The target DNA was purified by using a PCR product purification kit (Axygen) and cloned into pUC_m-T, resulting in pUC_m-T-*xdh*. The resulting plasmid was then introduced into *E. coli* DH5α, prepared by AxyPrep Plasmid MiniPrep Kit (Axygen, USA). Both of pUC_m-T-*xdh* and pET-22b (+) vector were digested with *Nde*I and *Xho*I. The target gene was then ligated to the corresponding sites of the pET-22b (+) vector. The recombinant expression plasmid, named pET-22b (+) *xdh* carried a C-terminal His-tag sequence, was subsequently introduced into *E. coli* BL21 (DE3). The recombinant *E. coli* was obtained by screening on LB agar containing 50 μg ampicillin/ml and cultured in 200 ml LB medium in a 1000-ml flask at 37℃. When the optical density (OD_{600}) of the culture at 600 nm was 0.4 – 0.6, 0.2 mmol/L isopropyl-β-D-1-thiogalactopyranoside (IPTG) was added, following by cultivation at 30℃ for another 4 h.

1.4 Purification of the recombinant XDH

All the procedure of purification was performed at 4℃. The induced cells were harvested by centrifugation at 7000 r/min for 10 min and rinsed twice with 100 mmol/L Tris-HCl (pH 8.2). The cell pellets was suspended in 100 mmol/L Tris-HCl (pH 8.2) buffer and lysed by ultrasonication for 10 min. The cells debris was removed by centrifugation at 10000 r/min for 10 min. The recombinant enzyme was purified from the resulting supernatant by using His-binding-resin column (Shanghai York Biotech, China). The purified XDH was stored at – 20℃ in 100 mmol/L Tris-HCl (pH 8.2) buffer with 50% (v/v) glycerol to protect from freezing.

1.5 Protein quantification and molecular mass determination

The concentration of protein was determined as described by Bradford, using bovine serum albumin as the standard protein. The molecular mass of purified enzyme was judged by Coomassie brilliant blue staining of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The separating gel and stacking gel were 12% (w/v) and 5% (w/v) polyacrylamide, respectively.

1.6 Enzyme assay

The xylitol dehydrogenase and d-xylulose reductase activities of XDH were determined spectrophotometrically by monitoring the change in OD_{340} upon reduction of $NAD(P)^+$ and oxidation of NAD(P)H, respectively, at 30℃, unless otherwise mentioned. The reactant mixture for measuring oxidative activity of XDH consisted of 100 mmol/L CAPS-NaOH (pH 10.0), 100 mmol/L xylitol, 5 mmol/L $NAD(P)^+$ and XDH in a final volume of 500 μl, while the reactant mixture for measuring reductive activity of XDH contained 100 mmol/L sodium citrate (pH 6.0), 100 mmol/L d-xylulose, 0.125 mmol/L NAD(P)H and XDH in a final volume of 500 μl. The reactant mixture was pre-incubated for 20 min at 30℃, unless otherwise stated. The reaction was initiated by addition of XDH. One unit of enzyme was defined as the amount of enzyme catalyzing the oxidation of 1 μmol NADH or reduction of NAD^+ in 1 min. Specific activity was expressed as units of enzyme per milligram of protein (U/mg protein).

1.7 Induction of XDH in *G. oxydans CGMCC* 1.637 by various carbon sources

Glucose, d-fructose, d-mannose, xylose, glycerol, d-sorbitol, d-arabitol and xylitol were investigated for their abilities to induce the XDH activities in *G. oxydans* CGMCC 1.637. *G. oxydans* CGMCC 1.637 was grown on the medium containing 10 g/L yeast extract and various carbon sources (10 g/L)

for 18 h and the crude cell lysate obtained after sonication was used to determined the oxidative activity of XDH. The reactant mixture for measuring oxidative activity of xylitol dehydrogenase in the crude cell lysate of *G. oxydans CGMCC* 1.637 consisted of 100 mmol/L CAPS-NaOH (pH 10.0), 100 mmol/L xylitol, 5 mmol/L NAD^+ and crude cell lysate in a final volume of 500 μl. *G. oxydans* CGMCC 1.637 after grown on various carbon sources was used for bioconversion of d-xylulose to xylitol. Bioconversion was carried out in a medium (pH 6.0) containing 30 g/L d-xylulose and 10 g/L $CaCO_3$, at 30℃, 250 r/min for 24 h. *G. oxydans* CGMCC 1.637 which was grown on a medium containing only 10 g/L yeast extract was also utilized for a reference test.

1.8 Quantification of d-xylulose and xylitol

D-arabitol, d-xylulose and xylitol were quantified by using high-performance liquid chromatography (HPLC) system. The apparatus used for the HPLC analysis was Agilent 1200 series HPLC system with an evaporative light scattering detector, ELSD 2000 (Alltech, Illinois, USA). Data acquisition and analysis were performed with the ChemStation program. All determinations were performed at 80℃ using a Shodex Sugar SC1011 column (8 mm × 300 mm, 6 μm). Chromatographic separation was conducted with water as the mobile phase at 1 ml/min. The elution was monitored by an ELSD 120℃ of the drift tube temperature, 3.0 L/min of the carrier gas flow rate and 1 of the gain.

1.9 Sequence analysis and nucleotide sequence accession numbers

Nucleotide sequence similarity and identity were determined using the Blast network service (http://www.ncbi.nih.gov/BLAST/). The amino acid sequence alignment was performed by the Clustalw multiple sequence alignment program (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The molecular mass of putative XDH was predicted with the Compute pI/MW program (http://web.expasy.org/compute_pi/). The nucleotide sequence of *xdh* gene obtained from *G. oxydans* CGMCC 1.637 was submitted to the GenBank nucleotide sequence database under Accession *No. JN*676197.

2 Results and discussions

2.1 Cloning and characterization of *xdh* gene

According to the sequence of *xdh* gene (DDBJ Accession *No. AB*091690) from *G. oxydans* ATCC 621 and short-chain dehydrogenase/reductase gene from *Gluconacetobacter diazotrophicus* PAl 5 (GenBank Accession *No. ACI*52826.1), several pairs of primers (not shown), including F1/R1 were designed to amplify the partial fragment of *xdh* gene from *G. oxydans* CGMCC 1.637. A fragment about 200 bp of *xdh* gene was successfully amplified using primers (F1/R1), subcloned into vector and sequenced. In order to determine the DNA sequence of ORF, 5′ and 3′, another two pairs of primers (F2/R2, F3/R3) were designed on the sequence of the partial *xdh* gene and the chromosome sequence of *G. oxydans* ATCC 621 [12]. As a result, a 0.9-kb DNA fragment and 0.5-kb DNA fragment were obtained, subcloned, sequenced and assembled. As a result, a 798-bp open reading frame (ORF) revealed. The entire sequence was confirmed in the genomic DNA *G. oxydans* CGMCC 1.637 by PCR using primers (F4/R4), which was designed on the basis of the 5′ and 3′ ends of the revealed ORF. There was no difference between the assembled ORF and amplified product by using primers (F4, R4). The ORF of *xdh* gene encoded a putative protein of 265 amino acids. The calculated molecular mass of XDH was 27.95 kDa, in agreement with that measured by SDS-PAGE (Fig. 1).

The novel *xdh* gene sequence showed the highest identity (77%) to the sequence of *xdh* gene from *G. oxydans* ATCC 621 (GenBank Accession *No. AB06916*) and the second highest identity (72%) to dehydrogenase from *Gluconacetobacter diazotrophicus* PAl 5 (GenBank Accession *No. CP*001189.1). The amino acid sequence showed similarity to those of enzymes in the short-chain dehydrogenase/reductase (SDR) such as XDH from *G. oxydans* ATCC 621 (GenBank Accession *No. AB06916*), 3-alpha-(or 20-

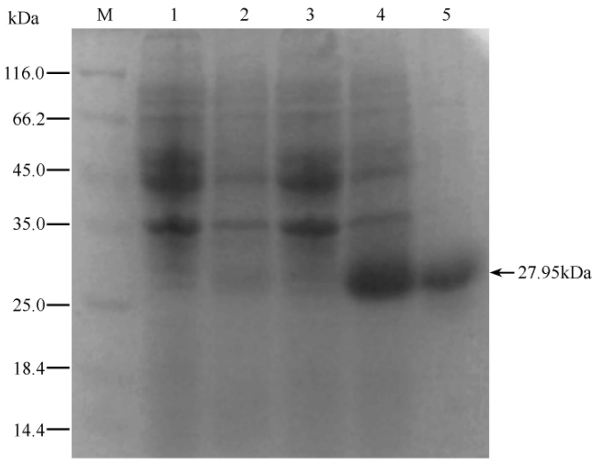

Fig. 1 SDS-PAGE analysis of XDH. Lane M stands protein marker; lane 1, crude enzyme extract of *E. coli* with pET-22 b (+); lane 2, crude enzyme extract of *E. coli* with pET-22 b (+) after 4 h induction of 0.2 mmol/L IPTG; lane 3, crude enzyme extract of *E. coli* with pET-22b (+) *xdh*; lane 4, crude enzyme extract of *E. coli* with pET-22 b (+) *xdh* after 4 h induction of 0.2 mmol/L IPTG; lane 5, purified recombinant XDH from *E. coli*.

beta)-hydroxysteroid dehydrogenase from *Streptomyces exfoliates* (*UniProtKB Accession No. P19992*), D-arabitol dehydrogenase from *Acetobacter suboxydans* (GenBank Accession *No. FJ890937*). But there was no sequence similarity between *xdh* gene from *G. oxydans* CGMCC 1.637 and those from *Candida tropicalis*, *Saccharomyces cerevisiae*, *Aspergillus niger*, *Fusarium oxysporum* [7-10].

Alignment of XDH with other SDRs was shown in Fig. 2 and revealed that there were twenty-three residues conserved among them. The glycine rich motif, which was pointed as the cofactor binding motif [13] was located in the N-terminal region (G^{17}-X-G^{19}-G^{20}-X_2-G^{23}) of XDH. The so-called catalytic tetrad [13] was fairly well conserved in XDH in the form of Asn^{144}-Ser^{147}-Tyr^{160}-Lys^{164}. Furthermore, the C-terminal motif (Pro-Gly) which was pointed to be highly conserved among SDRs [14-15], was also present in XDH.

2.2 Enzymatic properties of XDH

Fig. 3 showed the effect of pH on XDH activity and stability. The optimal pH for xylitol oxidation was around 10.0 and that for d-xylulose reduction was about 6.0. The enzyme was relatively stable for d-xylulose reductive activity from at pH 5.0 and pH 10.0 maintaining 90% and 75% residual activity, respectively and could be applied in range of pH from 5.0 to 10.0. In comparison to reductive activity, the oxidative activity of XDH was more sensitive to the change of pH. Changing of pH from 10 to 9, the oxidative activity of XDH decreased to 58%. However, after incubation of XDH at pH 11 and pH 12, the oxidative activity was enhanced by 37% and 46%, respectively, which was quite different from those of XDHs from some bacteria and fungi, such as *G. oxydans* ATCC 621, *Candida tropicalis and Aspergillus oryzae* [6-7, 16]. The precise enhancement mechanisms of oxidative activity of XDH after incubation at pH 11 and pH 12 remain unknown. But a similar phenomenon was observed by Syed and Engel in research of glutamate dehydrogenase of *Clostridium symbiosum*, which was said to undergo a slow pH-dependent transition between a high oxidative activity and a low oxidative activity [17].

The temperature profile of XDH activity was investigated by measuring the catalyzing activity of XDH at various temperatures (Fig. 4). The enzyme was most active at 30℃ for reductive activities and at 35℃ for oxidative activity. The enzyme was relatively stable for d-xylulose reduction and sensitive to temperature for xylitol oxidation. Increasing of temperature from 35℃ to 45℃, the reductive activity and the oxidative activity of XDH decreased to 89% and 52%, respectively.

Table 2 showed the effect of metal ions on XDH oxidative activity. The activity of XDH was enhanced by more than 50% by $MgCl_2$, $CaCl_2$, $FePO_4$, $CoCl_2$ and $NiCl_2$. However, $ZnCl_2$ and $BaCl_2$ inhibited XDH activity by 78%. The XDH activity was not inhibited by 1 mmol/L EDTA (Fig. 5). But the XDH activity remarkly decreased when EDTA was up to 10 mmol/L, and was completely inhibited when EDTA up to 20 mmol/L. The results were similar with that of alkaline phytase from *Bacillus sp.* MD2 [18], but were different from that of XDH from *G. oxydans* ATCC 621, which was not affected by EDTA (10 mmol/L) [6].

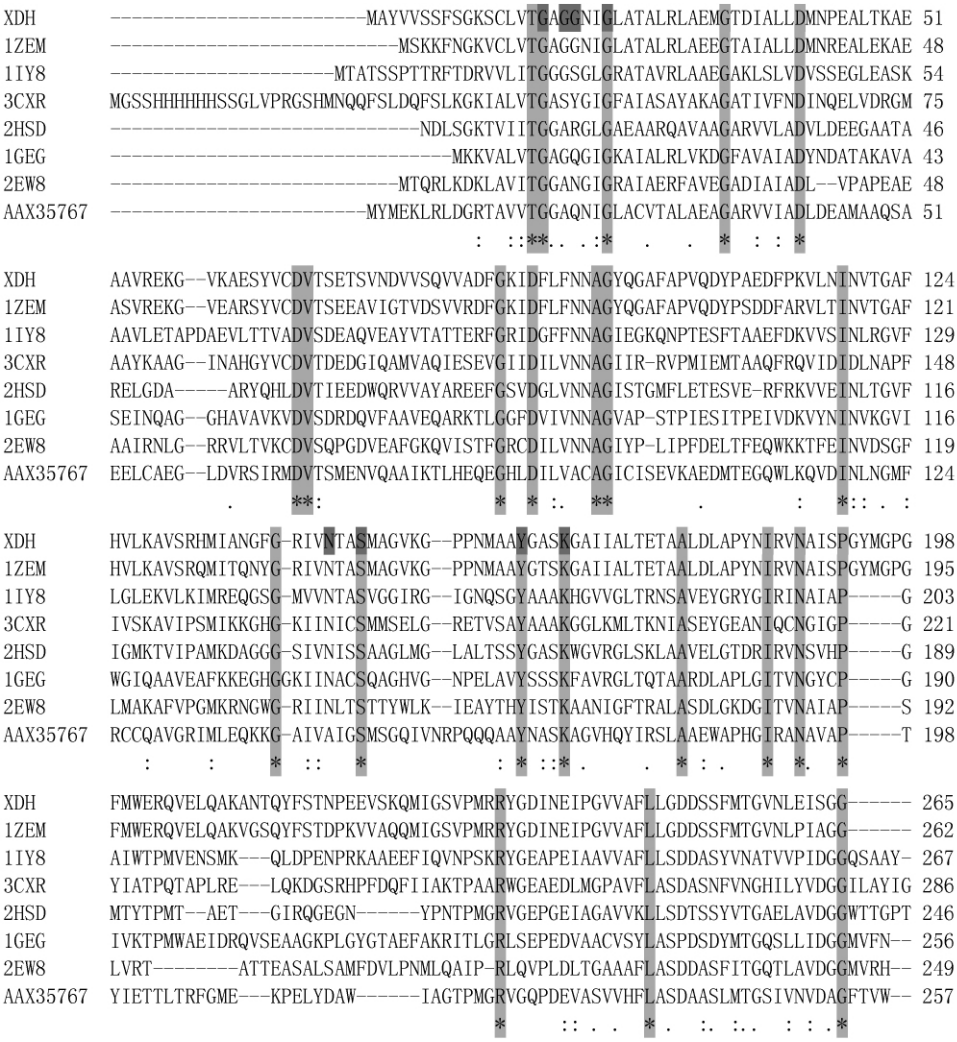

Fig. 2 Sequence alignment of GoXDH with other short-chain dehydrogenase/reductase. XDH, xylitol dehydrogenase from *G. oxydans* CGMCC 1.637; 1ZEM, xylitol dehydrogenase from *Gluconobacter oxydans* ATCC 621; 1IY8, reductase from *Leifsonia aquatica*; 3CXR, gluconate 5-dehydrogase from *Streptococcus sui*; 2HSD, 3-alpha-(or 20-beta)-hydroxysteroid dehydrogenase from *Streptomyces exfoliates*; 1GEG, diacetyl reductase from *Klebsiella pneumoniae* 342; 2EW8, (S)-specific 1-phenylethanol dehydrogenase from *Aromatoleum aromaticum* EbN1; AAX35767, d-arabitol dehydrogenase from *Gluconobacter* oxydans CGMCC 1.110. Identical amino acids were marked with "* ".

Substrate specificity of XDH for oxidation was investigated with xylitol, sorbitol, mannitol, d-arabitol and glycerol at the optimum pH of 10.0 and 35℃. No oxidative activity was detected by using d-arabitol, mannitol and glycerol as substrate. The specific oxidative activity of XDH for sorbitol was 81.92% of that for xylitol (23.27 U/mg). There was no activity observed when NADP (H) used as coenzyme. The highest oxidative activity was observed for xylitol, thus xylitol with NAD^+ as cofactor was used to determine the kinetic parameters of XDH. K_m was determined by altering xylitol concentration from 5 mmol/L to 300 mmol/L and calculated by Lineweaver-Burk plot. K_m, K_{cat} and V_{max} of XDH were 78.57 mmol/L, 18.72 s^{-1} and 40.17 U/mg, respectively.

In the reductive reaction, d-xylulose, d-fructose and d-mannose was investigated at the optimum pH of 6.0 and 30℃. Specific activity for d-fructose reducing was 125.7% of that for d-xylulose. The reductive activity of d-mannose was less than 1%. XDH was exclusively required NADH as a cofactor in the reductive reaction.

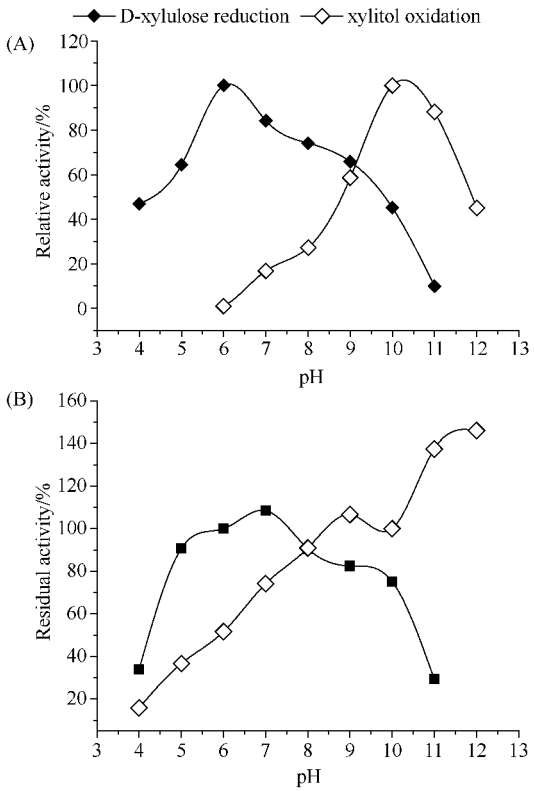

Fig. 3 Effect of pH on XDH activity (A) and stability (B). A The optimal pH was determined in 100 mmol/L sodium citrate (pH 4.0 – 6.0), 100 mmol/L potassium phosphate (pH 7.0), 100 mmol/L Tris-HCl (pH 8.0 – 9.0) or 100 mmol/L CAPS-NaOH (pH 10.0 – 12.0) buffer. The activity at the optimal pH was defined as 100%. 100% oxidative activity = 27.78 U/mg, 100% reductive activity = 253.28 U/mg. B Effect of pH on XDH stability was investigated by incubating the XDH for 20 min in various pH buffers and measuring residual activity. 100% oxidative. activity = 19.47 U/mg, 100% reductive activity = 204.53 U/mg. Each value represented the mean of triplicate measurements and varied from the mean by not more than 10%.

Table 2 Effect of metal ions and other reagent on XDH oxidative activity The effect of metal ions on XDH oxidative activity was determined in presence of various metal ions (1mmol/L) at the optimum conditions, pH 10.0, 35℃ with 100 mmol/L xylitol and 5 mmol/L NAD^+.

Reagent[a]	Specific activity (U/mg)
None	24.67 ± 0.61 (100%)
$ZnCl_2$	5.45 ± 0.36 (22%)
$BaCl_2$	18.24 ± 0.9 (74%)
$CsCl_2$	27.40 ± 2.12 (111%)
$CuSO_4$	21.04 ± 0.98 (85%)
$CaCl_2$	33.62 ± 0.05 (136%)
$FePO_4$	35.68 ± 3.38 (145%)
$MgCl_2$	33.84 ± 0.47 (137%)
$CoCl_2$	39.30 ± 1.00 (159%)
$NiCl_2$	49.71 ± 4.33 (201%)
EDTA-Na	24.88 ± 1.69 (100%)

a) All the reagents added in the assay were 1 mmol/L.

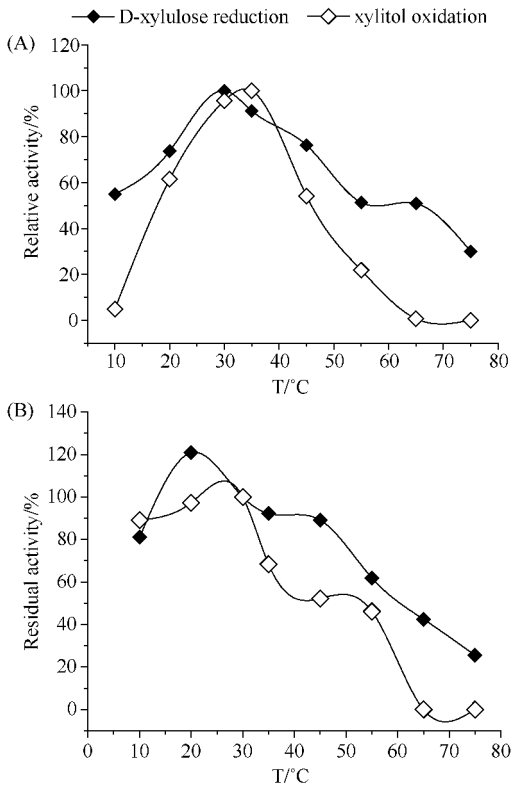

Fig. 4 Effect of temperature on XDH activity (A) and stability (B). A: The optimal temperatures were determined with reductive assay at pH 6.0 and oxidative assay at pH 10.0 from 0℃ to 85℃. The highest reductive activity of 255.55 U/mg was achieved at 30℃, while the highest oxidative activity of 23.27 U/mg was achieved at 35℃. The activity at the optimal temperature was defined as 100%. B: Thermostability of XDH was investigated by incubating purified enzyme at various temperatures for 20 min and measuring residual activity at 30℃. The reductive activity of 235.95 U/mg and oxidative activity of 24.49 U/mg at 35℃ were defined as 100%, respectively. Each value represented the mean of triplicate measurements and varied from the mean by not more than 10%.

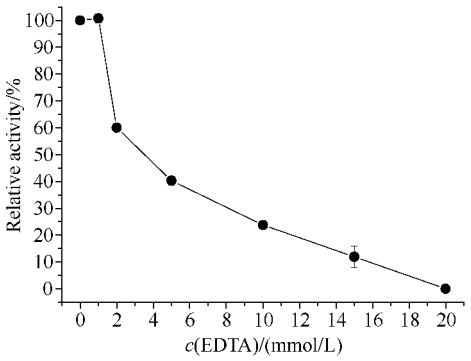

Fig. 5 Effect of EDTA concentration on the oxidative activity of XDH. The effect of EDTA concentration was investigated at 30℃, pH 10.0. 100% oxidative activity = 24.67 U/mg.

2.3 Induction of XDH by various sugars and polyols

The growth profiles of *G. oxydans* CGMCC 1.637 cultivation for 18 h on various sugars and polyols were shown in Fig. 6A. Growth of *G. oxydans* CGMCC 1.637 was significantly promoted by polyols, d-arabitol, d-sorbitol, xylitol and glycerol.

Induction of XDH oxidative activities in *G. oxydans* CGMCC 1.637 after grown on various carbon sources was shown in Fig. 6B. Relative specific activities of xylitol oxidation was significantly induced by d-sorbitol and slightly enhanced by lactose and glycerol. Glucose, d-fructose, d-mannose, xylose, d-arabitol, xylitol remarkably inhibited XDH oxidative activity, with d-arabitol inhibiting significantly. Bioconversion of d-xylulose to xylitol by *G. oxydans* CGMCC 1.637 after grown on various carbon sources was shown in Fig. 6C. It was noted that specific productivity of xylitol by *G. oxydans* CGMCC 1.637 after grown on d-mannose was remarkly higher than those on other carbon sources. D-arabitol, which supported a high cell growth, remarkly inhibited the oxidative activity of XDH and the bioconversion ability of *G. oxydans* CGMCC 1.637. From the results, it was concluded that the reduction of d-xylulose to xylitol with a pretty low yield in *G. oxydans* to some extend could be a result of the depression of d-arabitol on XDH activity in *G. oxydans*, as well as the imbalance of NADH in the *G. oxydans* [6].

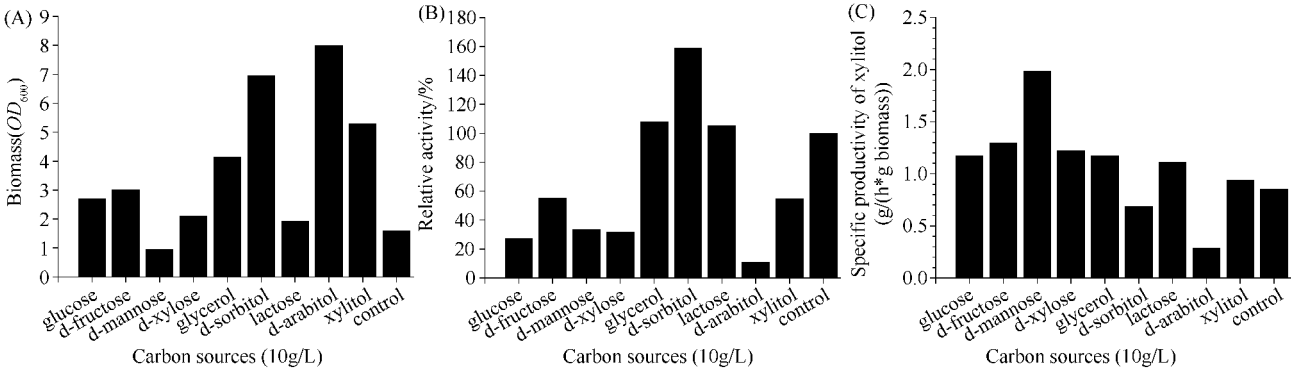

Fig. 6 Growth (A), induction (B) of XDH oxidative activities in *G. oxydans* CGMCC 1.637 and bioconversion of d-xylulose to xylitol (C) by *G. oxydans* CGMCC 1.637 after grown on various carbon sources. The 100% enzyme activity corresponding to the reference value is 8.05 U/g biomass. The specific productivity of xylitol corresponding to the reference value is 0.85 g/(h* g biomass).

In conclusion, the *xdh* gene was cloned from *G. oxydans* CGMCC 1.637 and expressed in *E. coli*. The enzyme properties of XDH, including the optimum pH and temperature and substrate specificity were investigated. The reductive activity of XDH from *G. oxydans* CGMCC 1.637 was stable in a wide range of pH. And further study will be carried out for revealing the activation mechanism of XDH under alkaline conditions. Furthermore d-arabitol was found able to remarkably inhibit the activity of XDH and the bioconversion of d-xylulose to xylitol in *G. oxydans* CGMCC 1.637, which will be an important clue for the metabolic engineering efforts for improving xylitol yield from d-arabitol.

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葡萄糖酸氧化杆菌木糖醇脱氢酶基因的克隆与表达

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摘要：【目的】获得葡萄糖酸氧化杆菌(*Gluconobacter oxydans* CGMCC 1.637)的木糖醇脱氢酶基因，研究其酶学性质及碳源特别是D-阿拉伯醇和木糖醇对该酶活性的影响。**【方法】**通过已报道序列的木糖醇脱氢酶的保守区设计引物，用聚合酶链式反应(polymerase chain reaction，PCR)扩增获得目的基因片段。根据获得的片段序列设计引物克隆目的基因的5′和3′片段，将所获得的片段拼接，获得完整的木糖醇脱氢酶基因。通过构建工程菌获得重组蛋白，并利用氧化还原反应测定重组酶的活性。用含不同碳源的培养基培养 *G. oxydans* CGMCC 1.637，并测定其破胞上清液木糖醇脱氢酶氧化木糖醇的活性；用不同碳源培养的 *G. oxydans* CGMCC 1.637 转化木酮糖，用高效液相色谱法测定木糖醇的产量。**【结果】**获得一个新的798bp的木糖醇脱氢酶基因，所编码的木糖醇脱氢酶含265个氨基酸，属于短链脱氢酶家族。酶学性质研究发现，该木糖醇脱氢酶催化木糖醇氧化的最适合条件为35℃、pH 10.0，最高活性为23.27 U/mg，催化木酮糖还原为木糖醇的最适条件为30℃、pH 6.0。最高活性为255.55 U/mg；该木糖醇脱氢酶的对木糖醇的 K_m 和 V_{max} 分别为78.97 mmol/L和40.17 U/mg。碳源诱导实验表明，d-山梨醇对 *G. oxydans* CGMCC 1.637 木糖醇脱氢酶的活性有明显的促进作用，而葡萄糖、果糖、木糖、木糖醇、D-阿拉伯醇对木糖醇脱氢酶活性有明显的抑制作用。而在转化实验中，用d-甘露糖培养的 *G. oxydans* CGMCC 1.637 的转化能力明显高于其他碳源培养的 *G. oxydans* CGMCC 1.637 的转化能力，其中，用阿拉伯醇培养的 *G. oxydans* CGMCC 1.637 的转化能力最低，仅为对照的35%。**【结论】**克隆自 *G. oxydans* CGMCC 1.637 的木糖醇脱氢酶基因是一个新的基因，用阿拉伯醇培养的 *G. oxydans* CGMCC 1.637 破胞液木糖醇脱氢酶活性低；且阿拉伯醇对 *G. oxydans* CGMCC 1.637 木酮糖的还原能力具有抑制作用。

关键词：葡萄糖酸氧化杆菌(*Gluconobacter oxydans*)，短链脱氢酶/还原酶家族，木糖醇脱氢酶，D-木酮糖，木糖醇，克隆

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