

Supporting information for

NADP⁺-Preferring D-Lactate Dehydrogenase from *Sporolactobacillus inulinus*

Lingfeng Zhu^{1,5}, Xiaoling Xu³, Limin Wang¹, Hui Dong^{2,*}, Bo Yu^{1,*}, Yanhe Ma⁴

¹ *CAS Key Laboratory of Microbial Physiological and Metabolic Engineering, Institute of Microbiology, Chinese Academy of Sciences, Beijing 100101, PR China*

² *Key Laboratory of Tianjin Radiation and Molecular Nuclear Medicine, Institute of Radiation Medicine, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300192, PR China*

³ *Institute of Ageing Research, School of Medicine, Hangzhou Normal University, Hangzhou, 311121, China*

⁴ *Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, PR China*

⁵ *University of Chinese Academy of Sciences, Beijing 100049, PR China*

* Corresponding authors.

E-mails: yub@im.ac.cn (B. Yu) and donghui@irm-cams.ac.cn (H. Dong)

Phone/Fax: +86-10-64806132

METHODS

Analysis of enzyme characteristic of DLDH744

The enzyme characteristic of DLDH744 was determined using 10 μg purified enzyme in 400 μL of the following mixture: 20 mM sodium pyruvate and 0.2 mM NADH. The effect of pH on the activity of DLDH744 was tested under standard conditions except that the reacting pH was changed between 4.5 and 8.5. Reactions were performed in sodium citrate and sodium phosphate buffers for pH below and above 5.0 respectively, and the activities of DLDH744 at pH 5.0 in these two different buffers were compared. To investigate the enzymatic stability under different temperature, the enzyme was pre-incubated at different temperatures for 10 minutes and then the activity was tested at pH 5.5 and 30°C. The activity of enzyme pre-incubated at 0°C was defined as 100% and the activities of DLDH744 pre-incubated at other temperatures were compared. Different metal ions (2 mM) were added in the enzyme reaction system to test the effect of metal ions on D-lactate dehydrogenase activity. The reaction was performed under the optimum assay condition (pH 5.5, 30°C). The enzyme activity in the absence of metals was the control, with activity defined as 100%. The error bars in the Figure S1, S2 and S3 indicated the standard deviations of three parallel replicates.

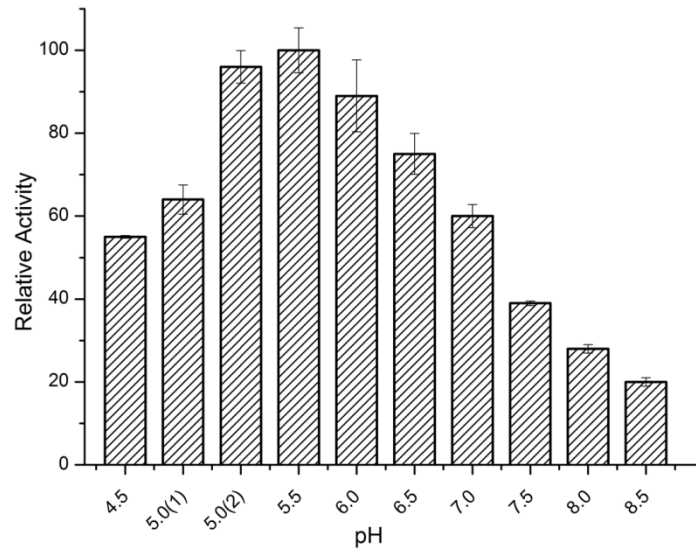
Table S1 Diffraction data statistics.

Values in parentheses are for the highest resolution shell.

Data Collection	
	$a = 61.26, b = 109.51$
Cell parameters (Å)	$c = 113.00$
	$\alpha = \beta = \gamma = 90^\circ$
Space group	$P2_12_12_1$
Resolution (Å)	50 (3.20) – 3.15
No. of all reflections	80807
No. of unique reflections	13247
Completeness (%)	96.4 (65.4)
Redundancy	6.1 (2.7)
$I/\sigma I$	5.1 (1.6)
R_{merge}^\dagger (%)	13.6 (34.5)
Refinement	
Total No. of reflections	13121
No. of reflections used	11818
$R_{\text{work}}^\S / R_{\text{free}}^\P$ (%)	20.4 / 29.2
No. of atoms	5208
Protein	5114
Water	0
NAD ⁺	2
R.m.s. deviations	
Bond lengths (Å)	0.009
Bond angle (°)	1.439
Average B-factors (Å ²)	57.3

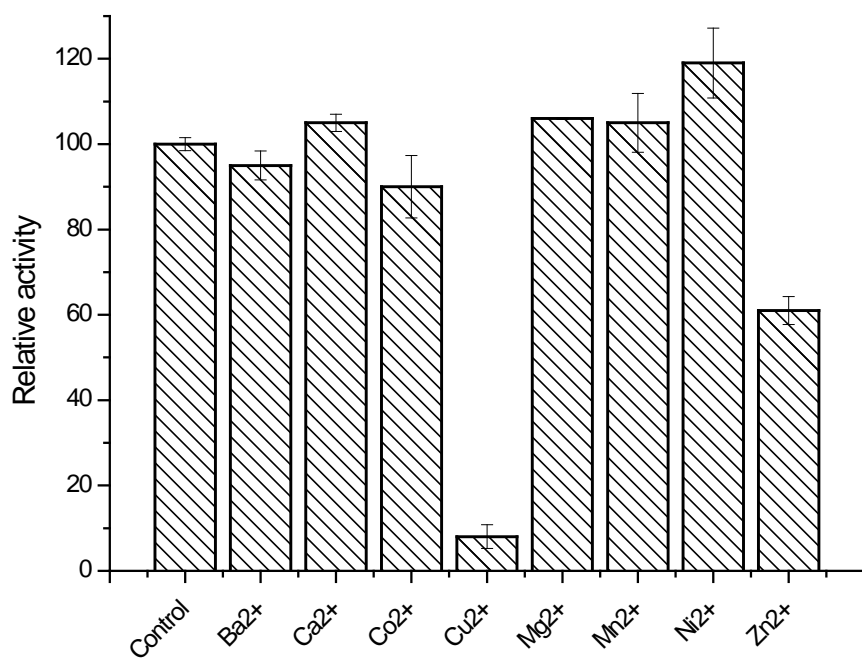
$^\dagger R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$, where $I_i(hkl)$ is the intensity of the i th observation and $\langle I(hkl) \rangle$ is the mean intensity of reflections. $^\S R_{\text{work}} = \frac{\sum |F_o| - |F_c|}{\sum |F_o|}$, where F_o and F_c are observed and calculated structure factors, respectively. $^\P R_{\text{free}}$ was calculated using 5% of data excluded from refinement.

Figure S1



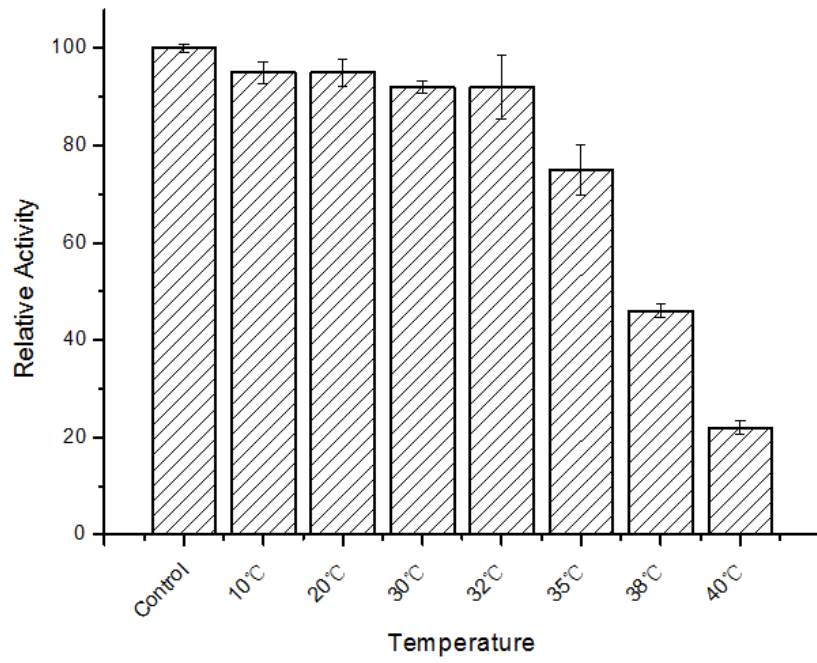
Effect of pH on D-lactate dehydrogenase activity of DLDH744

Fig. S2



Effect of metal ions on D-lactate dehydrogenase activity of DLDH744

Fig. S3



Effect of temperature on the D-lactate dehydrogenase activity of DLDH744

Fig. S4

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C7TFLO C7TFLO_LACRL 164 :GFGAKVIAIDPPYPMKGDHPDFEYV-SLEELFKQSDIIDLHVPGIKQNTHIINEAAFD:
A8YW12 A8YW12_LACH4 177 :GFGAKVIAIDIFKNPELEKKGYYVDSLDDLYKQADVISLHVPDVPANVHMINDKSI:
C6VQ21 C6VQ21_LACPJ 164 :GFGAKVIGDQVYRNAELEKEGMYVDTLDELYAQADVITLHVPALKDNYHMLNADAFS:
F4ADD8 F4ADD8_LACJH 165 :GFGAKVIAIDIFKNPELEKKGYYVDSLDDLYKQADVISLHVPDVPANVHMINDESI:
FOJY16 FOJY16_LACD2 165 :GFGAKVIAIDIFRNPELEKKGYYVDSLDDLYKQADVISLHVPDVPANVHMINDESI:
C7T8W0 C7T8W0_LACRG 164 :GFGAKVIAIDPPYPMKGDHPDFEYV-SLEELFKQSDIIDLHVPGIKQNTHIINEAAFD:
FONTU1 FONTU1_LACHH 177 :GFGAKVIAIDIFKNPELEKKGYYVDSLDDLYKQADVISLHVPDVPANVHMINDKSI:
B2GEX7 B2GEX7_LACF3 163 :ALNARILGVDLKPREEEMEGIVEYVSKEE-LLRQSDVVS LHVDLNPTSEGLLTKAEFD:
D5H0G3 D5H0G3_LACCS 165 :GFGAKVIAIDIFKNPELEKKGYYVDSLDDLYKQADVISLHVPDVPANVHMINDESI:
G1UB05 G1UB05_LACJO 165 :GFGAKVIAIDIFKNPELEKKGYYVDSLDDLYKQADVISLHVPDVPANVHMINDESI:
F0THT4 F0THT4_LACA3 165 :GFGAKVIAIDIFKNPELEKKGYYVDSLDDLYKQADVISLHVPDVPANVHMINDKSI:
J9W550 J9W550_LACBU 164 :GFGAKVIAIDIIYHNPELEKEGVYVDTPEELYAQSDVLSLHAPATKENEHMINDKTI:
J9W2F7 J9W2F7_LACBU 165 :GLGAKVIAIDKYPLKNTDNSFVYAKSLEQIYQESDIISIHMPATDDNYHQFNHEVFE:
D8IGC0 D8IGC0_LACFC 163 :ALNARXLGVDLKPREEEMEGIVEYVSKEE-LLROSDVVS LHVDLNPTSEGLLTKAEFD:
D0R2T8 D0R2T8_LACJF 167 :GFGAKVIAIDIFKNPELEKKGYYVDSLDDLYKQADVISLHVPDVPANVHMINDESI:
Q1WR05 Q1WR05_LACS1 167 :GFGAKVVGVDLYPSDKLDGILEYRDSVEDAIRDADIISLHMPAFKENHMFNYEMFK:
Q1WV63 Q1WV63_LACS1 167 :ALGAKVIAIDIKPNPELNEVLTYK-SLNEVLQESDVISLHVDLNETT KGLIGSEELA:
D8IHC6 D8IHC6_LACFC 167 :GFGAKVIAIDVFKDPELEKKGYYV-SLDEIYAQADVISLHVPALRESTIHMINDETIA:
D8INP9 D8INP9_LACSC 167 :GFGAKVVGVDLYPSDKLDGILEYRDSVEDAIRDADIISLHMPAFKENHMFNYEMFK:
D8IJY4 D8IJY4_LACSC 167 :ALGAKVIAIDIKPNPELNEVLTYK-SLNEVLQESDVISLHVDLNETT KGLIGSEELA:
Q5FMW5 Q5FMW5_LACAC 179 :GFGAKVIAIDIFKNPELEKKGYYVDSLDDLYKQADVISLHVPDVPANVHMINDESI:
B2GBD6 B2GBD6_LACF3 167 :GFGAKVIAIDVFKDPELEKKGYYV-SLDEIYAQADVISLHVPALRESTIHMINDETIA:
B2G6U0 B2G6U0_LACRJ 166 :GFGAKVIAYSRHQNKLEGEIYVEYV-SLDELYKRATIIISLYLPHVPATDKMLNEKTF:
B2G9A7 B2G9A7_LACRJ 167 :ALGAKVLAIDPSYNVEYEPYVEYT-DFDTVIKNADILSLHTPLLPSTENMIAAPQFK:
B2G9G4 B2G9G4_LACRJ 168 :GFGAKVIAIDPFENPELKKQGYVDSLDDLYAQADVVS LHVPATKENFHMIDKDAIA:
E4SJB5 E4SJB5_LACAR 167 :GFGAKVIAIDIFKNPELEKKGYYVDSLDDLYKQADVISLHVPDVPANVHMINDKSI:
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Sequence alignment of DLDH744 with uncharacterized lactate dehydrogenases from *Lactobacillus* genome sequences

These amino acid sequences were chosen from the UniProt database

(<http://www.uniprot.org/>), the accession number is in front of each sequence. The

corresponding amino acid residue in 174 is identified by a black circle.