Supporting information for

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

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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.

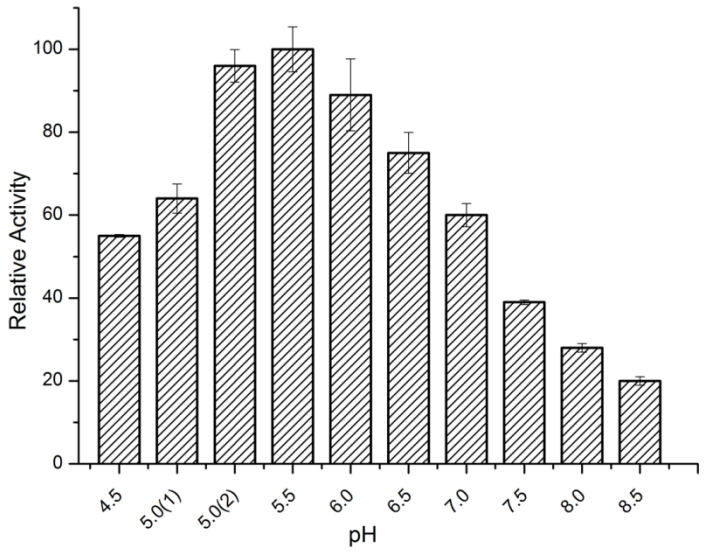
Table S1 Diffraction data statistics.

Values in parentheses are for the highest resolution shell.

Data Collection	
Cell parameters (Å)	a = 61.26, b = 109.51 c = 113.00 $\alpha = \beta = \gamma = 90^o$
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/σI	5.1 (1.6)
R_{merge} [†] (%)	13.6 (34.5)
Refinement	
Total No. of reflections	13121
No. of reflections used	11818
R_{work} [§] / R_{free} [¶](%)	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

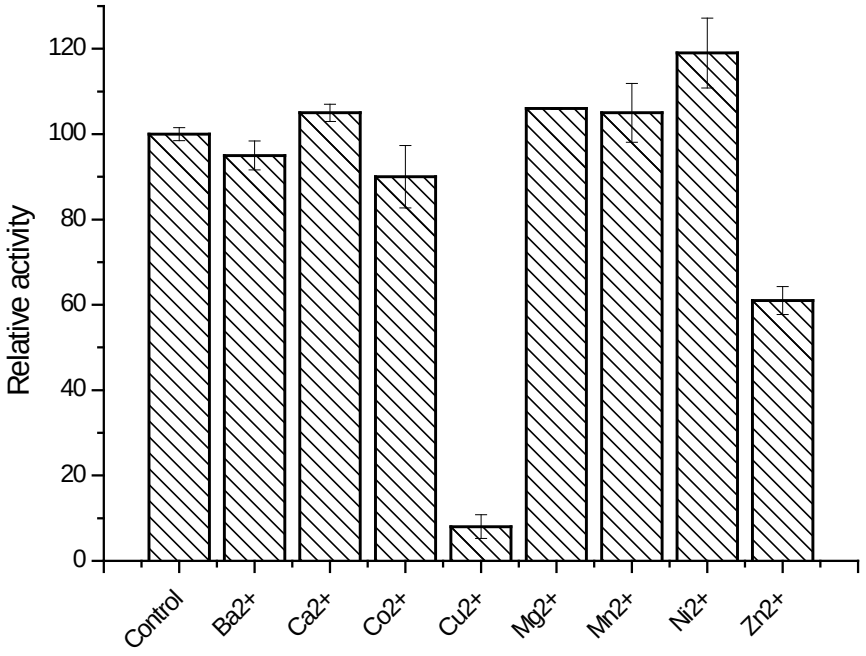
[†] $R_{merge}=\sum_{hkl}\sum_i\left|I_i(hkl)-\langle I(hkl)\rangle\right|/\sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of the $i$thobservation and $\langle I(hkl)\rangle$ is the mean intensity of reflections. [§] $R_{work}=\sum\left||F_o|-|F_c|\right|/\sum|F_o|$, where F_o and F_c are observed and calculated structure factors, respectively. [¶] R_{free} was calculated using 5% of data excluded from refinement.

Figure S1



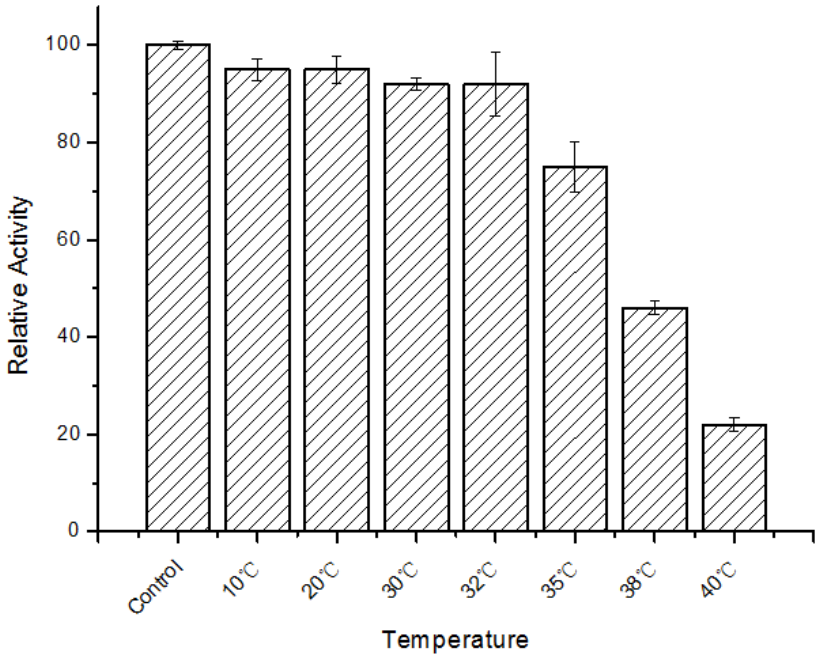
Effect of pH on D-lactate dehydrogenase activity of DLDH744

Fig. S2



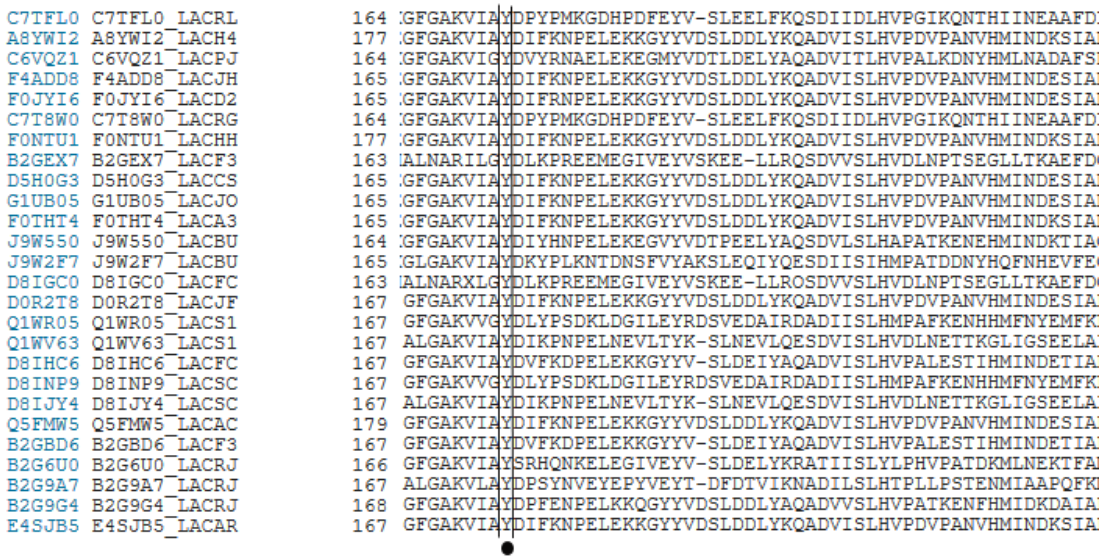
Effect of metal ions on D-lactate dehydrogenase activity of DLDH744

Fig. S3



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

Fig. S4



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.