One-Pot Biocatalytic Preparation of Enantiopure Unusual α-Amino Acids from α-Hydroxy Acids via a Hydrogen-Borrowing Dual-Enzyme Cascade

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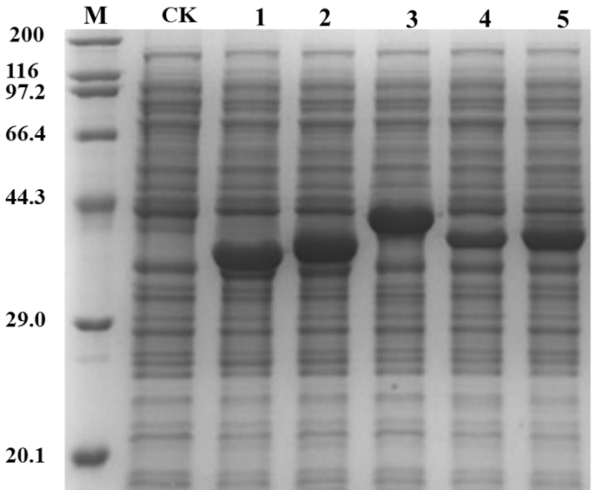

Figure S1: SDS-PAGE analysis of the expression of D-hydroxy acid dehydrogenases from different sources. Lane M is the standard marker protein. CK *E. coli*-pET-28a, Line 1 *E. coli*-pET-28a-*Lb*MDH, Line 2 *E. coli*-pET-28a-*Ef*MDH, Line 3 *E. coli*-pET-28a-*Pa*DLacDH, Line 4 *E. coli*-pET-28a-*Pa*2D2R, Line 5 *E. coli*-pET-28a-*Sa*DLacDH.

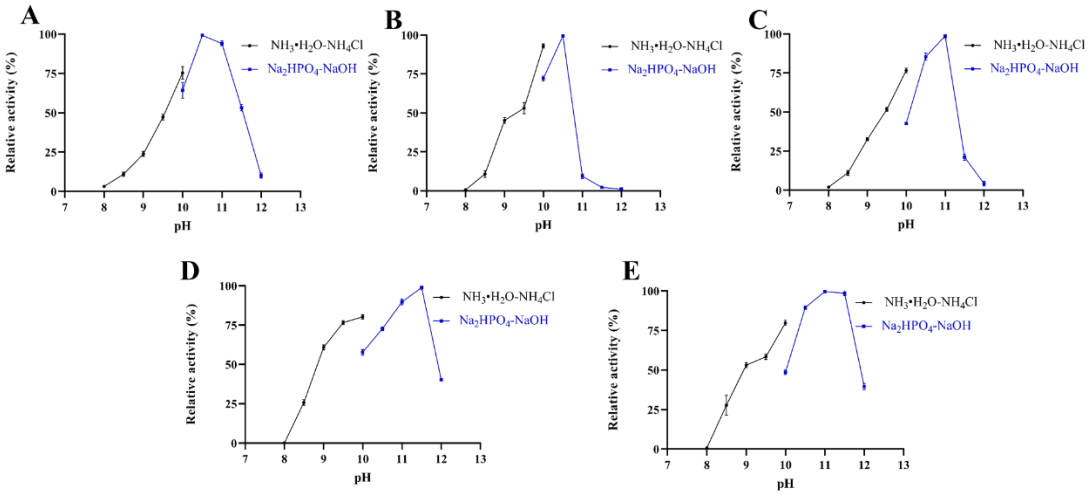

Figure S2: Optimum pH of D-hydroxy acid dehydrogenase from different sources. (**A**) *Lb*MDH from *Lactobacillus brevis*; (**B**) *Ef*2D2R from *Enterococcus faecalis;* (**C**) *Sa*DlacDH from *Staphylococcus aureus;* (**D**) *Pa*DlacDH and (**E**) *Pa*2D2R from *Pseudomonas aeruginosa*.

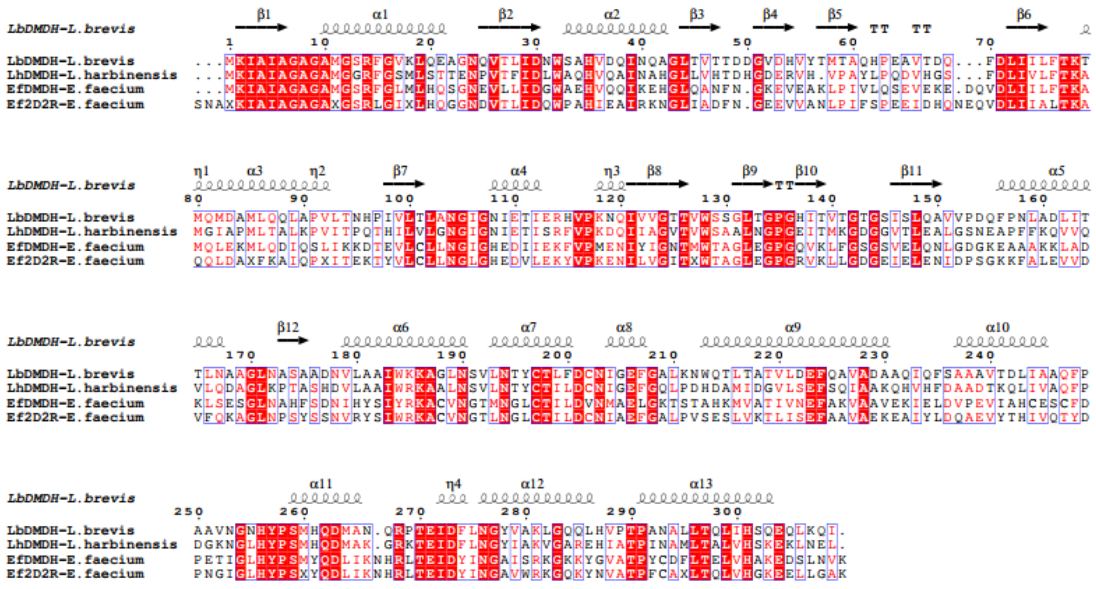

Figure S3: Multiple-sequence alignment of D-mandelate dehydrogenase with other D-hydroxy acid dehydrogenase from *Lactobacillus harbinensi* and *E. faecium* by the help of software Clustal X and Espript 3. Conserved residues were indicated by intense red color.

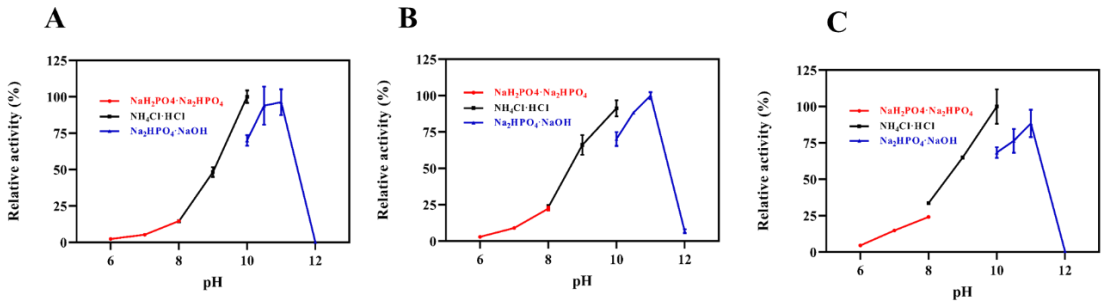

Figure S4: Optimum pH of variants of *Lb*MDH. (**A**) Mutation L243M; (**B**) L243I; (**C**) L243W

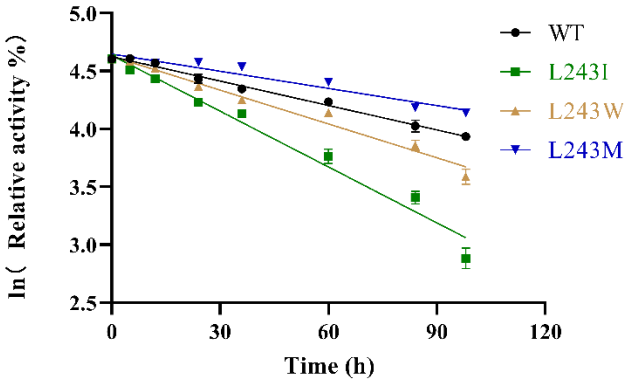

Figure S5: The exponential fitting curves of the data points of thermostability analysis. Thermal stability analysis of wild-type *Lb*MDH and variants were performed at 40 °C. Error bars showed the standard deviation and data were collected from three independent experiments.

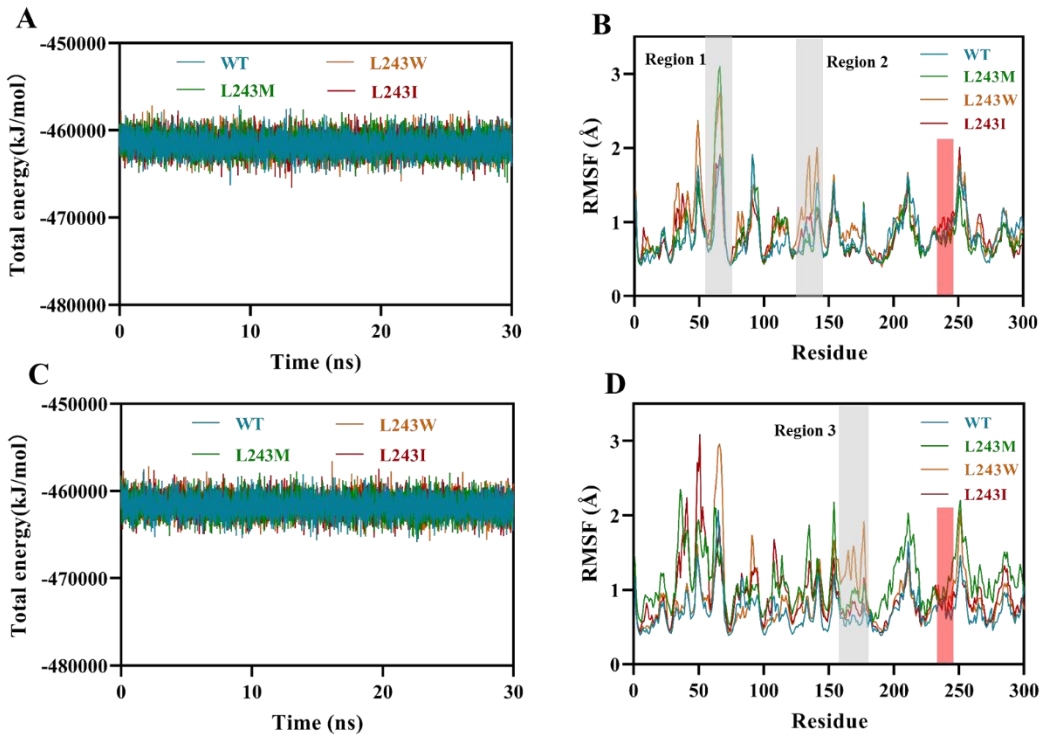

Figure S6: Structure analysis of *Lb*MDH and the variants by MD simulation. (A) Total energy of *Lb*MDH and the variants with NAD and D-mandelic acid; (B) RMSF of *Lb*MDH and the variants with NAD and D-mandelic acid; (C) Total energy of *Lb*MDH and the variants with NAD and D-α-hydroxybutyric acid; (D) RMSF of *Lb*MDH and the variants with NAD and D-α-hydroxybutyric acid. The grey shadowed regions 1, 2, 3 represent the loops. The red shadowed region was where the 243 residue is.

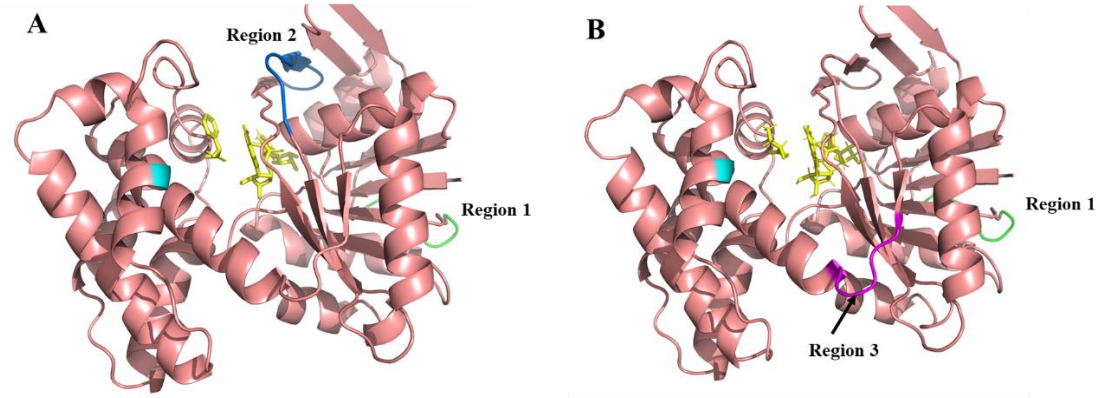

Figure S7: Structure analysis of *Lb*MDH and the variants. (A) The structure of *Lb*MDH compound with NAD and D-mandelic acid; (B) the structure of *Lb*MDH compound with NAD and D-α-hydroxybutyric acid. The structure was displayed with different colors: the carbon backbone marked in salmon, the 243 residue marked in cyan, region 1 (residues from 62-68) marked in green, region 2 (residues from 135-144) marked in marine, region 3 (residues from 175-179) marked in magenta.

Table S1: Primers used for cloning of D-hydroxy acid dehydrogenase

Primers	Primer sequences 5′-3′
*Lb*MDH-F	GGGTCGCGGATCC**GAATTC**ATGAAAATCGCCATTGCGGG
*Lb*MDH-R	CTCGAGTGCGGCCGC**AAGCTT**TCAAATCTGCTTCAGCTG
*Ef*2D2R-F	TGGGTCGCGGATCC**GAATTC**ATGAAAATAGCAATTGCAGG
*Ef*2D2R-R	CGAGTGCGGCCGC**AAGCTT**TTATTTTACGTTCAAACTA
*Pa*DlacDH-F	TGGGTCGCGGATCC**GAATTC**ATGCGCATCCTGTTCTTCAG
*Pa*DlacDH-R	CGAGTGCGGCCGC**AAGCTT**TTAGGCCCGGACCCGATTGCG
*Pa*2D2R-F	TGGGTCGCGGATCC**GAATTC**ATGACCTGGCATATCCTCGG
*Pa*2D2R-R	CGAGTGCGGCCGC**AAGCTT**TTAGCGGTCGGGCAAACCGCG
*Sa*DlacDH-F	GGGTCGCGGATCC**GAATTC**ATGACAAAAATTATGTTCTTTG
*Sa*DlacDH-R	CTCGAGTGCGGCCGC**AAGCTT**TTAATTTAAACGTGTTTC

Note: The bold type shows the site of restriction enzymes *EcoR* I and *Hind* III.

Table S2: Primers used for site-directed and saturated mutagenesis

Primers	Primer sequences 5′-3′
V127A-F	CGGCACGACG***GCC***TGGTCCTCCG
L189A-F	GAAGGCGGGC***GCC***AACAGCGTGC
L189W-F	GAAGGCGGGC***TGG***AACAGCGTGC
L193A-F	CAACAGCGTG***GCG***AATACGTACTG
L193W-F	CAACAGCGTG***TGG***AATACGTACTG
I204A-F	CGACTGCAAT***GCC***GGCGAGTTCGG
L243A-F	CGTCACCGAT***GCC***ATTGCCGCGC
L243W-F	CGTCACCGAT***TGG***ATTGCCGCGC
Q247A-F	CTGATTGCCGCG***GCC***TTTCCGGCCG
Q247W-F	CTGATTGCCGCG***TGG***TTTCCGGCCG
L243X-F	CGTCACCGAT***NNN***ATTGCCGCGC
28a-R	GCCTTACTGGTTAGCAGAATG

Note: Mutation sites are shown in bold and italic. Saturated mutation sites were substituted NNN (N=A/G/C/T).