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Supporting Materials

Enhanced In Vitro Cascade Catalysis of Glycerol into Pyruvate and Acetoin by Integration with Dihydroxy Acid Dehydratase from *Paralcaligenes ureilyticus*

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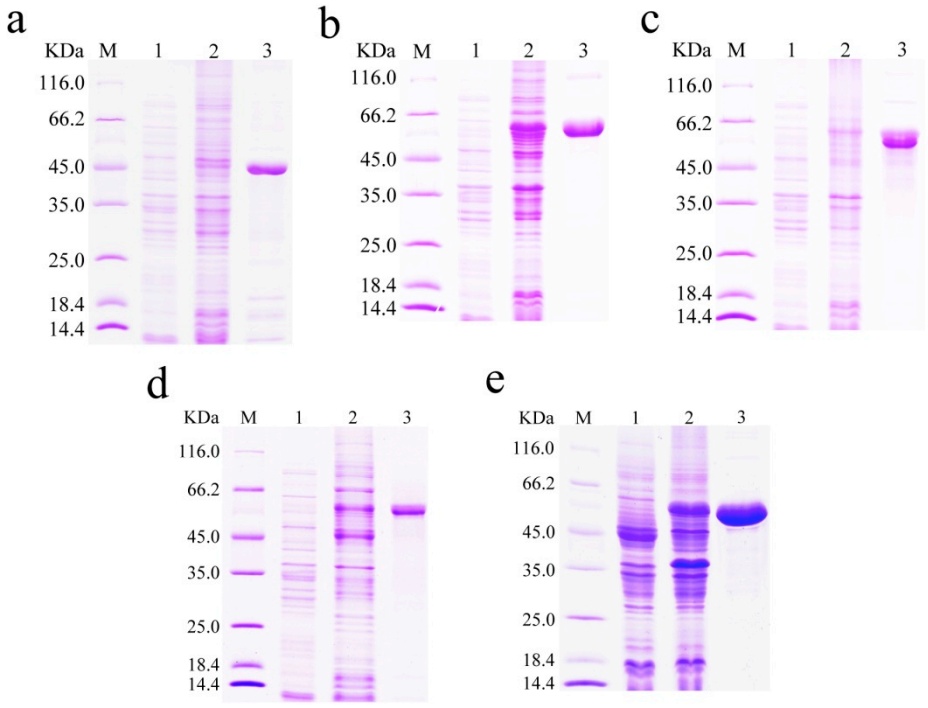

Figure S1. SDS–PAGE results of the purification of ScALDO, SsDHAD, CcXylD, PuDHT, and ZmPDC. (a) ScALDO. (b) SsDHAD. (c) CcXylD. (d) PuDHT. (e) ZmPDC. Lane M, molecular mass marker; lane 1, crude extract of *E. coli* BL21(DE3); lane 2, crude extracts of *E. coli* BL21(DE3) harboring expression vectors of different proteins; lane 3, purified target proteins.

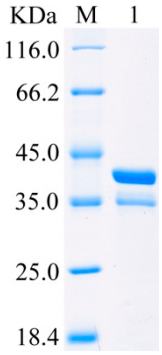

Figure S2. SDS–PAGE result of the purification of PaLdhA. Lane M, molecular mass marker; lane 1, purified PaLdhA.

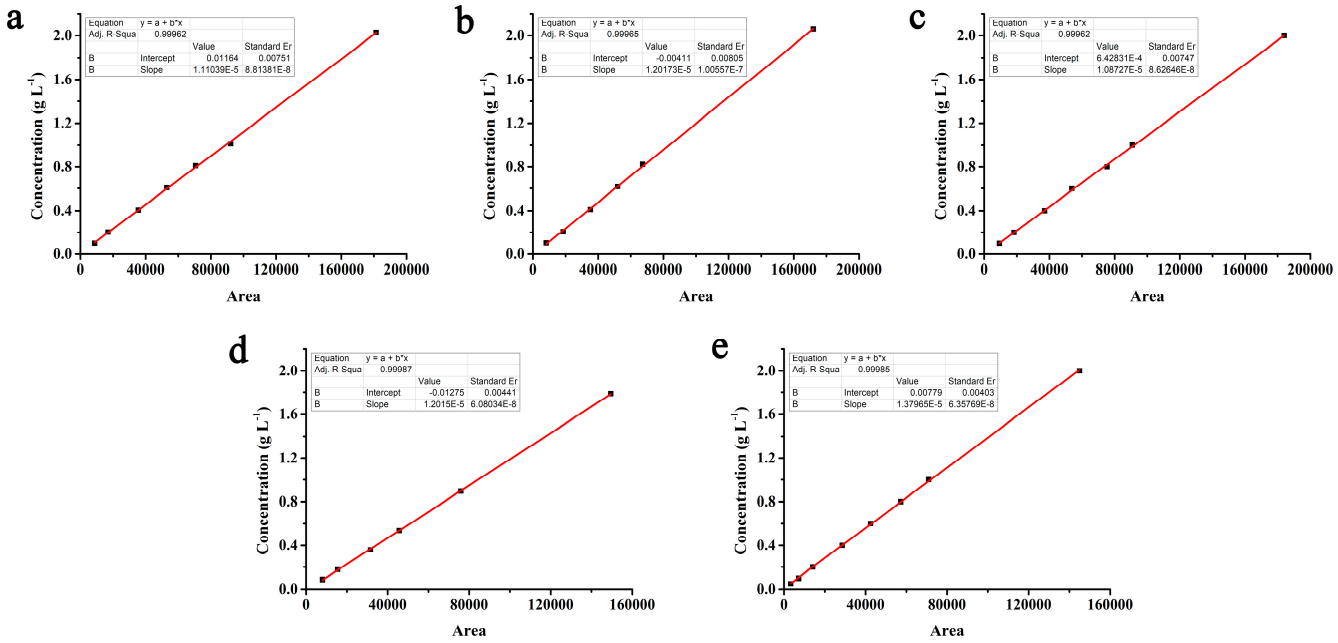

Figure S3. Calibration curves for the concentrations assayed by HPLC. (a) Glycerol. (b) Glyceraldehyde. (c) Glycerate. (d) Pyruvate. (e) Acetoin.

Table S1. Strains, plasmids, and primers used in this study.

Strain, plasmid and primer	Description	Source
Strain		
E. coli DH5α	F⁻ φ80*lacZ*ΔM15 Δ(*lacZYA-argF*)U169 *deoR recA*1 *endA*1 *hsdR*17(rκ⁻, mκ⁺) *phoA supE*44 λ⁻ *thi*-1 *gyrA*96 *relA*1	Novagen
E. coli DH5α/pETDuet–PaLdhA	*E. coli* DH5α expressing PaLdhA from *Pseudomonas aeruginosa* PAO1	This study
E. coli BL21(DE3)	F⁻ *ompT hsdSB*(*rB- mB-*) *gal*(λ *c I* 857 *ind1 Sam*7 *nin*5 *lac*UV5-T7*gene*1) *dcm* (DE3)	Novagen
E. coli BL21(DE3)/pETDuet–ScALDO	*E. coli* BL21(DE3) expressing ScALDO from *Streptomyces coelicolor* A3 with mutants (V125M/A244T)	[1]
E. coli BL21(DE3)/pETDuet–SsDHAD	*E. coli* BL21(DE3) expressing SsDHAD from *Sulfolobus solfataricus*	[1]
E. coli BL21(DE3)/pET28a–PuDHT	*E. coli* BL21(DE3) expressing PuDHT from *Paralcaligenes ureilyticus*	This study
E. coli BL21(DE3)/pET28a–CcXylD	*E. coli* BL21(DE3) expressing CcXylD from *Caulobacter crescentus*	This study
E. coli BL21(DE3)/pETDuet–ZmPDC	*E. coli* BL21(DE3) expressing ZmPDC from *Zymomonas mobile*	[2]
E. coli BL21(DE3)/pETDuet–PaLdhA	*E. coli* BL21(DE3) expressing PaLdhA from *Pseudomonas aeruginosa* PAO1	This study
Plasmid		
pETDuet-1	Vector for protein expression, Ap^r	Novagen
pET28a–PuDHT	pET28a(+) carrying *pudht* gene from *Paralcaligenes ureilyticus*	General Biology
pETDuet–PaLdhA	pETDuet carrying *ldhA* gene from *Pseudomonas aeruginosa* PAO1	This study
pET28a–CcXylD	pET28a(+) carrying *xylD* gene from *Caulobacter crescentus*	[3]
Primer	**Sequence (5′→3′)**[1]	
PaLdhA-F	CGCGGATCCGATGCGCATCCTGTTCTT (BamHI)	This study
PaLdhA-R	CCCAAGCTTTCAGGCCCGGACCCGATT (HindIII)	This study

[1] Restriction sites are underlined and the restriction enzymes are indicated in parentheses.

References

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