Supplementary information

Enhanced production of optical (*S*)-acetoin by recombinant *Escherichia coli* whole-cell biocatalyst with NADH regeneration

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Table S1. Sequences of codon-optimized genes

> *fdh*

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> *gdh*

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> Kp*dar*

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> *ardII*

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> *adh*

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> *adr*

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Table S2. Substrate specificities of KpDAR

Reaction	Substrate	Relative activity (%)
Reductive reaction [a]	Diacetyl	100 ± 9.07
	(*R*)/(*S*)-Acetoin	33.3 ± 4.02
	Ethyl pyruvate	14.9 ± 1.30
	Acetophenone	0.4 ± 0.02
Oxidative reaction [b]	*meso*-2,3-Butanediol	100 ± 5.6
	(2*S*,3*S*)-2,3-Butanediol	0.6 ± 0.06
	(2*R*,3*R*)-2,3-Butanediol	0.3 ± 0.01
	(*R*)/(*S*)-Acetoin	0.7 ± 0.07
	Phenethyl alcohol	0.7 ± 0.02
	Ethyl acetate	0.7± 0.03

[a] Enzyme activities in ketone reduction were measured with 5 mM substrate and 0.2 mM NADH in 100 mM phosphate buffer, and the relative activity of 100% represents 2887.6 U/mg for diacetyl reduction. [b] Alcohol oxidation reactions were measured with 5 mM substrate and 0.2 mM NAD^+ in 100 mM phosphate buffer, and the relative activity of 100% represents 108.8 U/mg for *meso*-2,3-butanediol oxidation. All reactions were carried out at 45°C and pH 6.0. Data are the averages standard deviations (n=3).

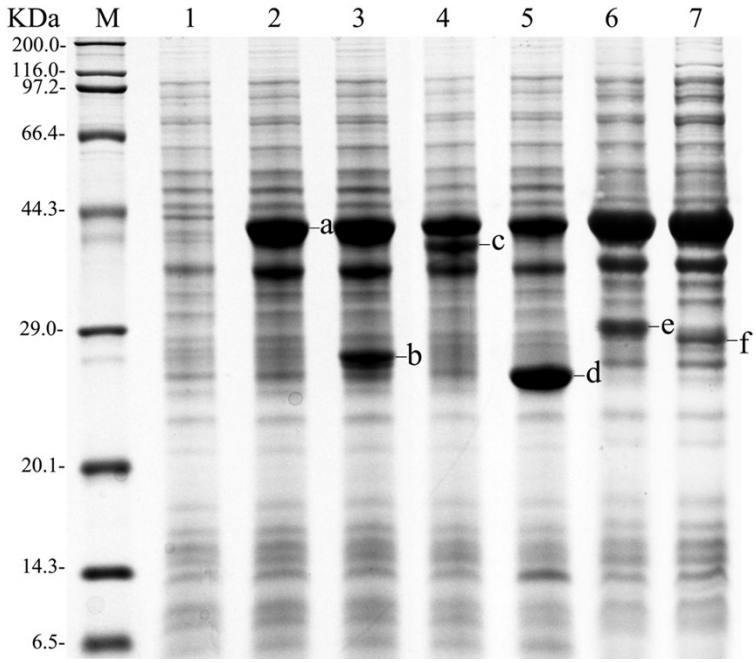

Fig. S1 SDS-PAGE analyses the expression of various DARs. Lane M, marker proteins; lane 1, *E. coli* BL21 (DE3) /pETDuet-1; lane 2, *E. coli* BL21/ pETDuet-*fdh*; lane 3, *E. coli* BL21/ pETDuet-*adr-fdh*; lane 4, *E. coli* BL21/ pETDuet-*adh-fdh*; lane 5, *E. coli* BL21/ pETDuet-Pp*dar-fdh*; lane 6, *E. coli* BL21/ pETDuet-*ard*II-*fdh*; lane 7, *E. coli* BL21/ pETDuet-Kp*dar-fdh*. a: FDH; b-f: DAR encoed by *adr*, *adh*, Pp*dar*, *ard*II and Kp*dar*, respectively.

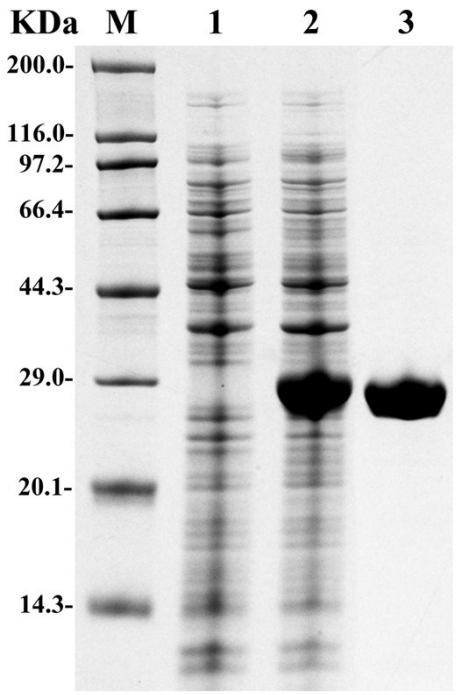

Fig. S2 SDS-PAGE analysis of purified KpDAR. Lane M, maker; lane 1, crude extract of *E. coli* BL21 (DE3)/pET-28a(+); lane 2, crude extract of E. coli BL21/pET 28a(+)-Kp*dar*; lane 3, purified KpDAR.

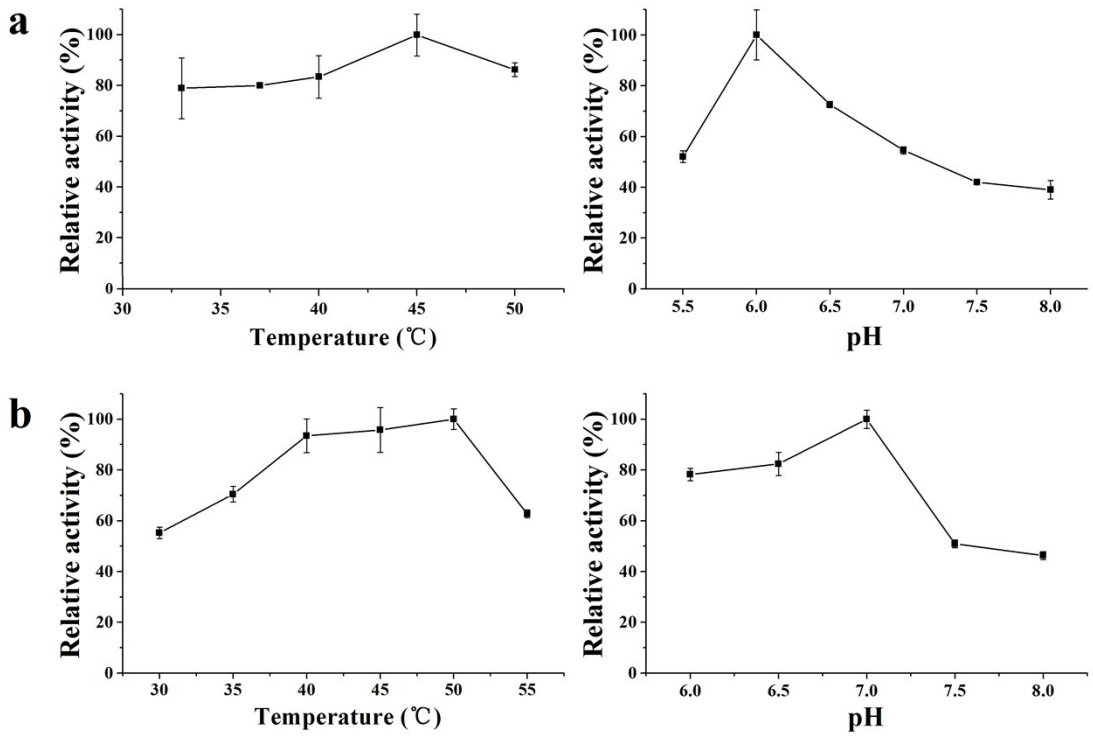

Fig. S3 Effect temperature and pH on the activities of KpDAR. **(a)** Reductive reaction towards diacetyl; **(b)** Reductive reaction towards (*R*)/(*S*)-acetoin. The optimal temperature for diacetyl and (*R*)/(*S*)-acetoin were 45°C and 50°C, respectively. Maximum activities were observed at pH 6.0 and pH 7.0 respectively.