Supporting information

An in vitro cascade with four enzymes for the production of D-3,4-dihydroxybutyric acid from D-xylose

Yipeng Zhang,[a,b,1] Liting Ma,[a,1] Weikang Sun,[a] Dan Xiao,[a] Chuanjuan Lü,[a] Qian Wang,[a] Ping Xu,[c] Cuiqing Ma,[a] and Chao Gao[a,*]

[a] *State Key Laboratory of Microbial Technology, Shandong University, Qingdao 266237, China*

[b] *State Key Laboratory of Military Stomatology and National Clinical Research Center for Oral Diseases and Shaanxi Key Laboratory of Stomatology, School of Stomatology, The Fourth Military Medical University, Xi'an, Shaanxi 710032, China*

[c] *State Key Laboratory of Microbial Metabolism, Shanghai Jiao Tong University, Shanghai 200240, China*

*Corresponding Author: Chao Gao, E-mail: jieerbu@sdu.edu.cn, Address: NO.72 Binhai Road, 266237, Qingdao, P. R. China, Tel.: +86-532-58631561

[1] These authors contributed equally: Yipeng Zhang, Liting Ma.

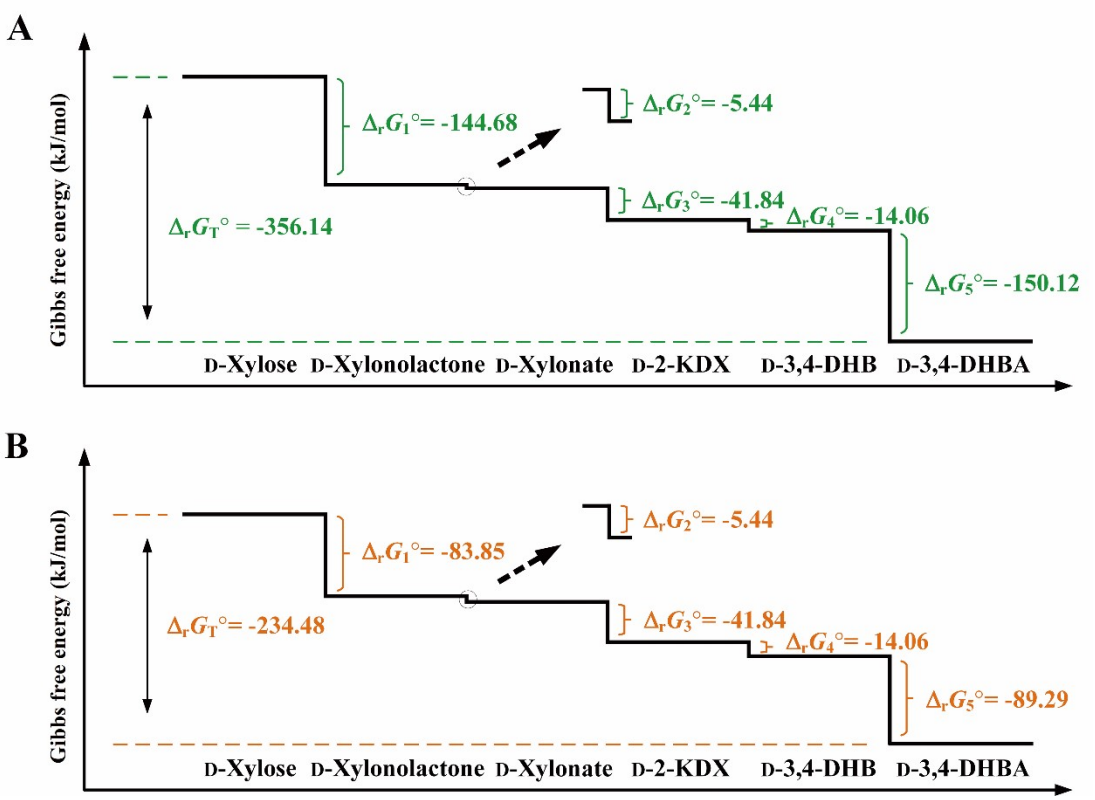

Fig. S1 Changes of Gibbs free energy during the production of D-3,4-DHBA from xylose. (A) D-3,4-DHBA production from D-xylose using the designed *in vitro* enzymatic cascade. (B) D-3,4-DHBA production from D-xylose through the natural nonphosphorylative D-xylose metabolic pathway involves two dehydrogenation reactions.

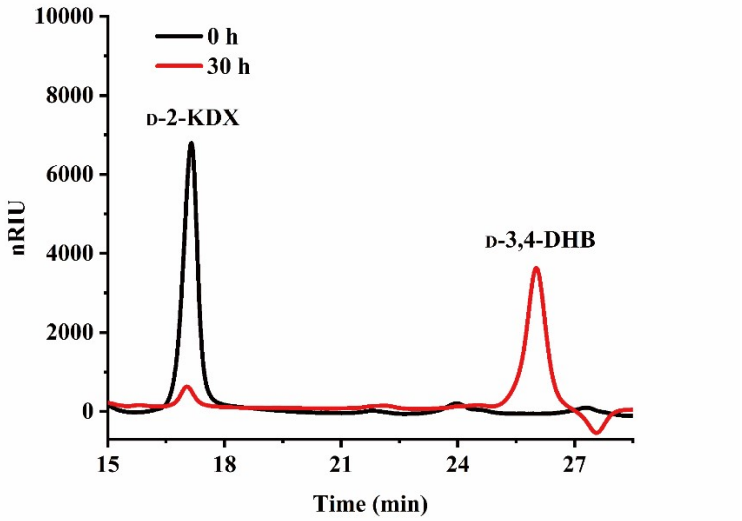

Fig. S2 Chromatograms illustrating D-3,4-DHB production by LlKdcA from D-2-KDX. The reaction mixture containing 100 mM HEPES-KOH (pH 7.0), 40 mM D-2-KDX, 25 μM LlKdcA, 1mM TPP, and 1mM $MgCl_2$ was incubated at 30 °C and 50 rpm for 30 h. D-2-KDX, 2-keto-3-deoxy-D-xylonate; D-3,4-DHB, D-3,4-dihydroxybutanal.

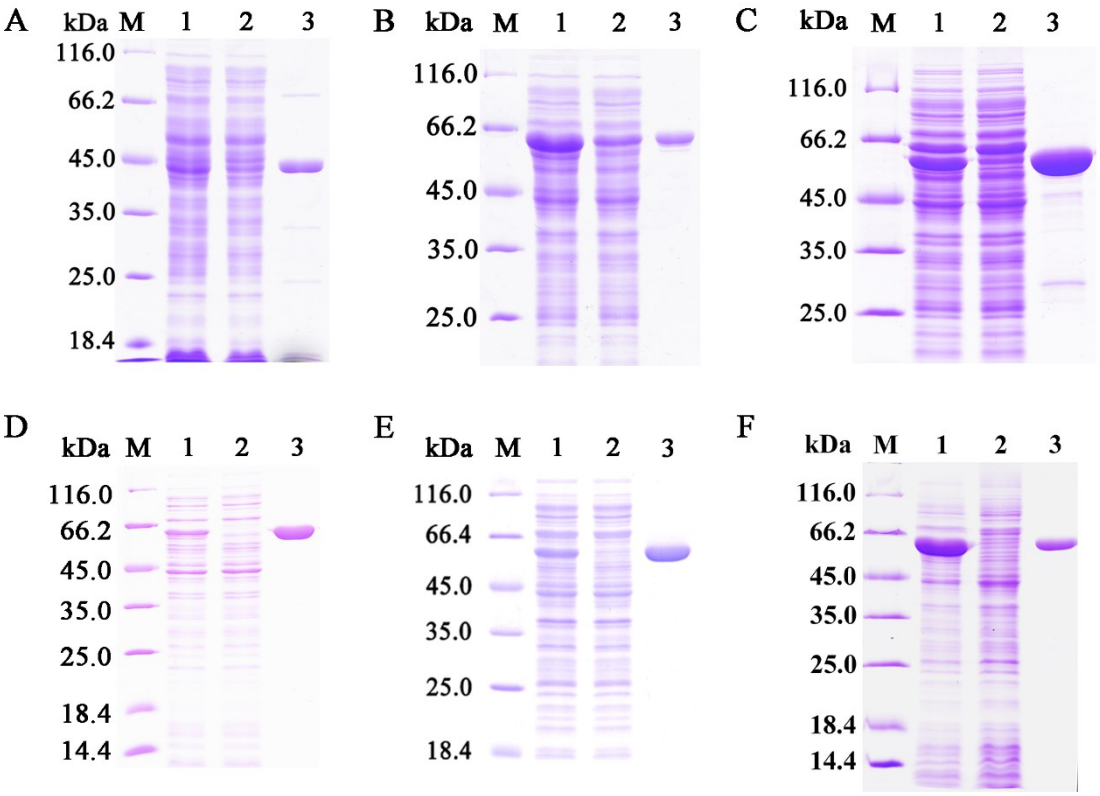

Fig. S3 SDS-PAGE results of purified enzymes. (A) ScAldO; (B) CcXylD; (C) LlKivD; (D) SsDHAD; (E) PuDHT; (F) LlKdcA. Lane M, molecular weight markers; lane 1, crude extract of recombinant expression strain; lane 2, the unbound protein of the HisTrap HP column; lane 3, the purified target protein.

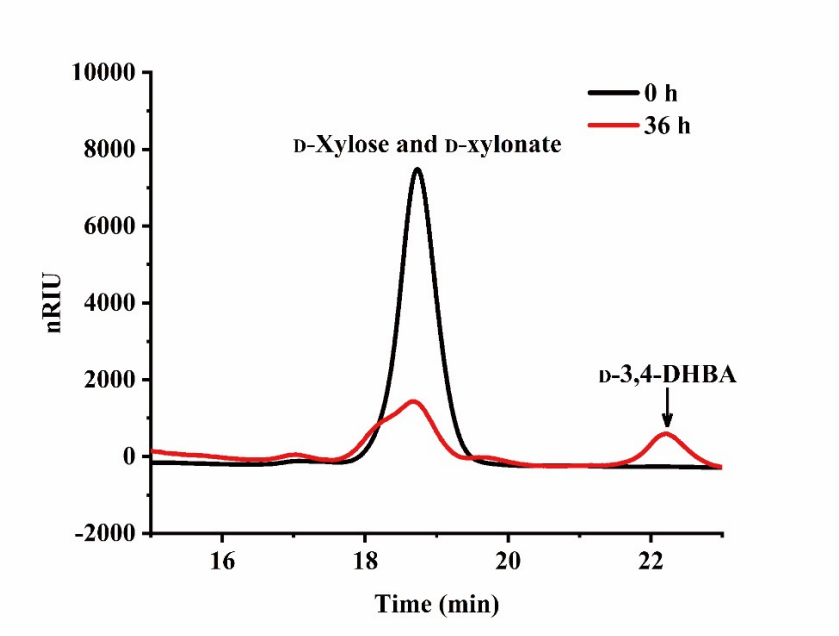

Fig. S4 HPLC analysis of D-3,4-DHBA production from D-xylose by *in vitro* enzymatic cascade comprised by ScAldO, CcXylD, LlKivD and AnCAT. Reaction was carried out in a mixture containing 6 g/L D-xylose, 50 μM ScAldO, 25 μM CcXylD, 25 μM LlKivD, and 500 U/mL AnCAT at 30 °C and 50 rpm for 36 h.

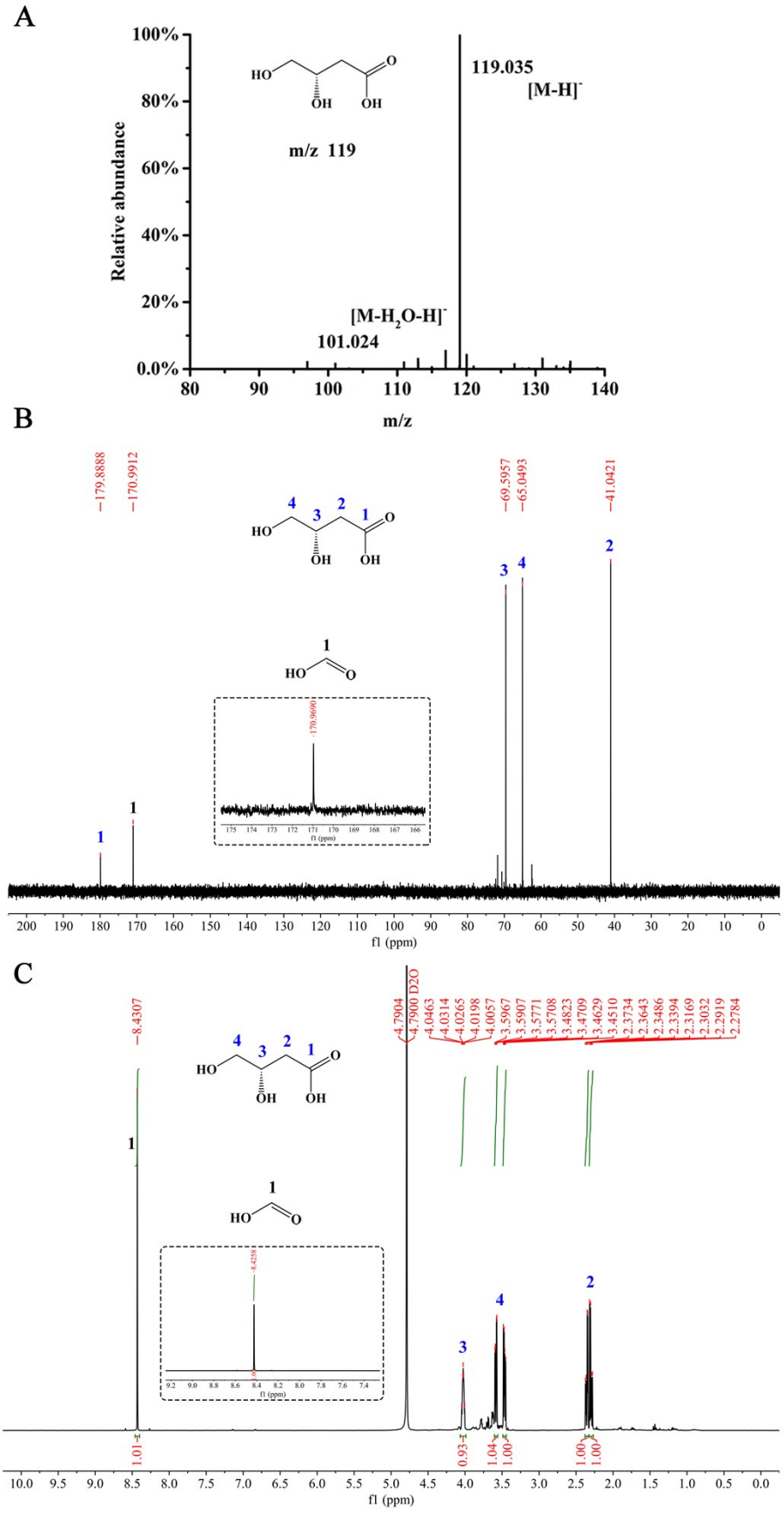

Fig. S5 Identification of D-3,4-DHBA produced by enzymatic cascade via LC-MS and NMR. (A) LC-MS analysis of D-3,4-DHBA produced through enzymatic cascade composed with ScAldO,

CcXylD, LlKivD, and AnCAT. (B, C) ^{13}C NMR (B) and ^{1}H NMR (C) spectra of D-3,4-DHBA (blue) isolated through preparative liquid chromatography with formic acid (black) as the mobile phase. The ^{13}C NMR and ^{1}H NMR spectra of formic acid standard are indicated in dotted boxes. Chemical shifts of D-3,4-DHBA: ^{13}C NMR (150 MHz, D_2O) δ 41.0 (C-2), 65.0 (C-4), 69.6 (C-3), 179.9 (C-1). ^{1}H NMR (600 MHz, D_2O) δ 2.30 (dd, J = 8.22, 15.00 Hz, 1H), 2.36 (dd, J = 5.46, 14.88 Hz, 1H), 3.47 (dd, J = 6.84, 11.64 Hz, 1H), 3.58 (dd, J = 3.6, 11.76 Hz, 1H), 4.01~4.05 (m, 1H). Chemical shifts of formic acid: ^{13}C NMR (150 MHz, D_2O) δ 170.97 (C-1). ^{1}H NMR (600 MHz, D_2O) δ 8.43 (s, 1H).

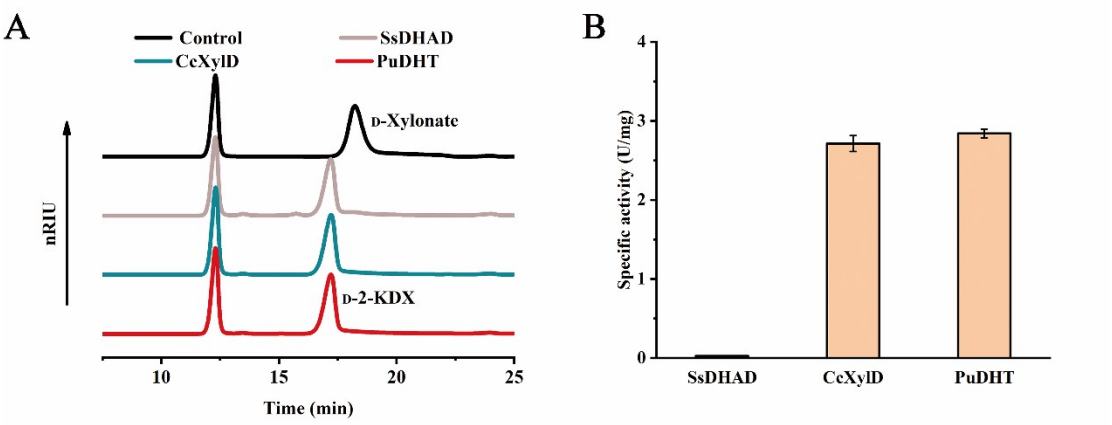

Fig. S6 Selection of optimal dehydratase for D-xylonate dehydration. (A) HPLC analysis of the D-xylonate dehydration catalyzed by CcXylD, SsDHAD and PuDHT. The reaction system containing 100 mM HEPES-KOH, 25 μM dehydratase (SsDHAD, CcXylD, or PuDHT), 40 mM D-xylonate, and 1 mM $MgCl_2$ was performed at 30 °C and 50 rpm for 30 h. (B) Specific activity of SsDHAD, CcXylD and PuDHT toward D-xylonate.

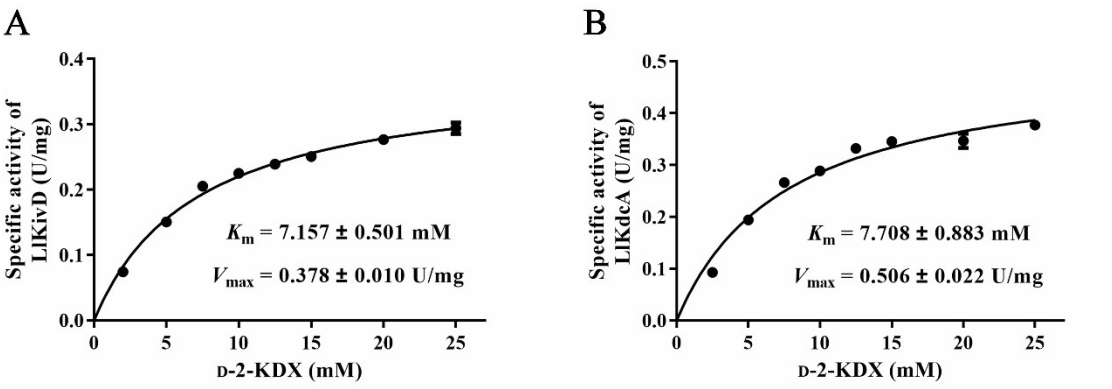

Fig. S7 Steady-state kinetic parameters of purified LlKivD (A) and LlKdcA (B) toward D-2-KDX. For the calculation of K_m and V_{max} values, the data were analyzed with GraphPad Prism 5.0 by using a Michaelis-Menten equation. D-2-KDX, 2-keto-3-deoxy-D-xylonate.