Title: Engineering the epoxide hydrolase from *Agromyces mediolanus* for enhanced enantioselectivity and activity in the kinetic resolution of racemic epichlorohydrin

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1. Materials and methods

1.1 Isolation and identification of (S)-ECH

The synthesized (*S*)-ECH was extracted twice with the equal volume of diethylether, dried with anhydrous sodium sulfate. A reduced pressure distillation was used to recover the diethylether at 40 °C. Then a pure (*S*)-ECH (>99%) was gained after the reduced pressure distillation at 80 °C.

Identification of (*S*)-ECH was performed by ^{1}HNMR and ^{13}C NMR spectrometry (Bruker 500-MHz NMR spectrometer, Switzerland, 1% (*S*)-ECH in CD_3OD) and gas chromatography mass spectra (Agilent 7890A/5970N GC-MS system).

Table S1 Oligonucleotide sequences used in this study

Primer	Oligonucleotide sequences (5'-3')
EN240NF	AGGGTGCGTATNNNAGTCTGCAGTCGACGAAG
EN240NR	ACTGCAGACTNNNATACGCACCCTCGCGC
ER338NF	CCGCCGAAATTNNNCGACCGCCGGCCGAG
ER338NR	CCGGCGGTCGNNNAATTTCGGCGGGGAATCG
ER313NF	CCGAACGAGCGNNNGAAGGGTGGACTTTTCCTC
ER313NR	AGTCCACCCTTCNNNCGCTCGTTCGGCGTAC
ES233NF	AACCAGGAATGGNNNGCGCGCGAGGGTGCG
ES233NR	CCCTCGCGCGCNNNCCATTCCTGGTTCGCAC
ES207NF	TTAACTACAGCNNNGTACAGCCCAGCATGATC
ES207NR	TGGGCTGTACNNNGCTGTAGTTAAGGTGCAG
EF318NF	AAGGGTGGACTNNNCCTCCGGGCGAGATC
EF318NR	GCCCGGAGGNNNAGTCCACCCTTCCCGC
EW182NF	AGGGCGGCGACNNNGGTTCTACCGTCCTCACC
EW182NR	ACGGTAGAACCNNNGTCGCCGCCCTGCCC
EW182FF	AGGGCGGCGACTTTGGTTCTACCGTCCTCACC
EW182FR	ACGGTAGAACCAAAGTCGCCGCCCTGCCC
EN240Df	AGGGTGCGTATGACAGTCTGCAGTCGACG
EN240Dr	ACTGCAGACTGTCATACGCACCCTCGCG

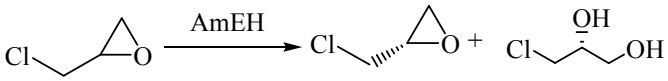
N stands for A, T, G or C

Table S2 Results of docking experiments

mutant	$d_{R\text{-}ECH}$ [a]	$d_{s\text{-}ECH}$	α_{1R} [deg][b]	α_{2R} [deg][b]	α_{1S} [deg][b]	α_{2S} [deg][b]	Reference
WT	3.5	3.8	125.0	118.8	94.3	88.3	1
N240D	3.2	3.9	121.0	118.3	69.9	63.9	This study
VD	3.2	4.1	137.9	112.7	82.9	62.8	This study
VDF	3.3	4.4	142.0	136.8	55.2	64.7	This study

[a] *d*, the distance between the Asp181 oxygen and the attacked epoxide carbon;

[b] α_1, the angle from Asp181 oxygen *via* the attacked epoxide carbon to the epoxide oxygen; α_2, the angle Asp181 oxygen *via* the attacked epoxide carbon to the other epoxide carbon.



(*R*,*S*)-epichlorohydrin (*S*)-epichlorohydrin (*R*)-3-chloro-1,2-propanediol

Fig S1 Synthesis of (*S*)-epichlorohydrin by epoxide hydrolase from *Agromyces mediolanus* ZJB120203 (AmEH).

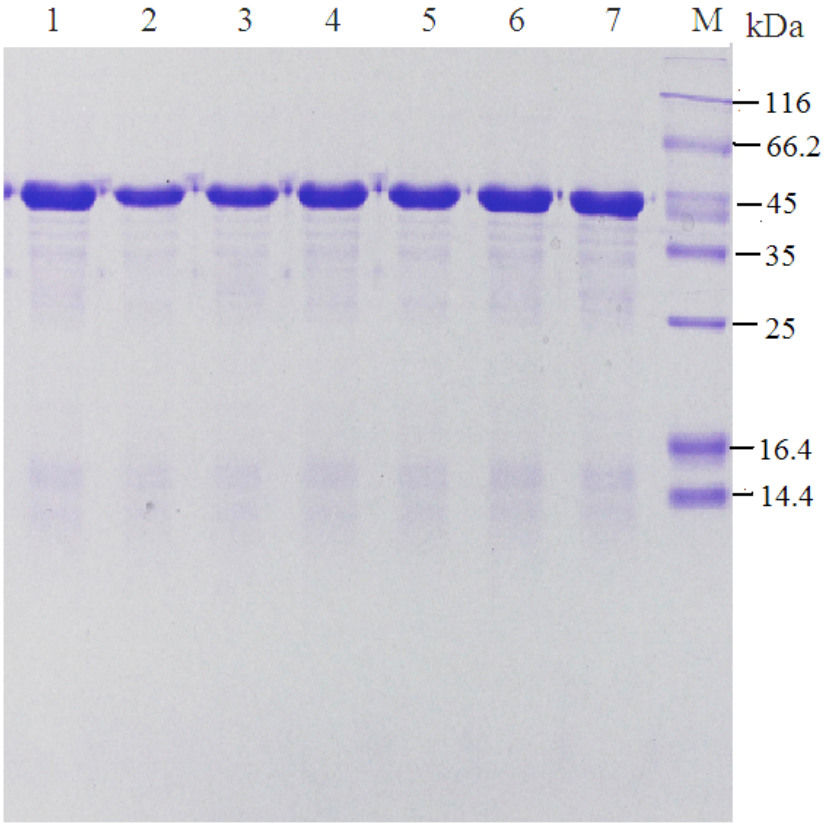


Fig. S2 SDS-PAGE analysis of the purified wild-type AmEH and its variants.Lane 1: the purified wild-type AmEH; Lane 2: the purified variant W182F; Lane 3: the purified variant S207V; Lane 4: the purified variant N240D; Lane 5: the purified variant W182F/S207V; Lane 6: the purified variant VD; Lane 7: the purified variant VDF; Lane M: protein marker.

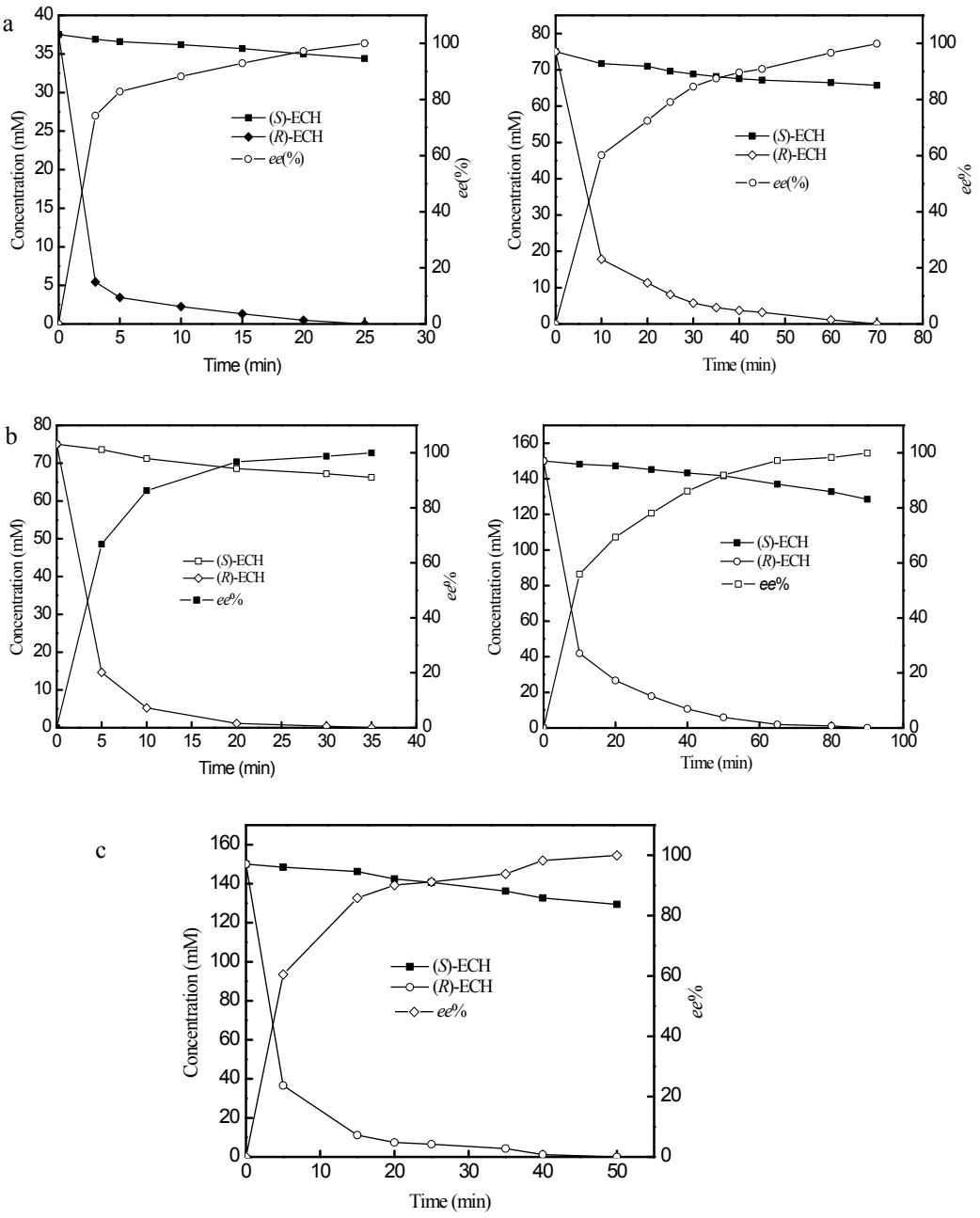


Fig. S3 Enantioselective hydrolysis of racemic ECH by the recombinant *E. coli* variant VDF. The reaction was performed at 30 °C in 200 mM sodium phosphate buffer (pH 8.0), 75-300 mM ECH and 4.5-13.5 g dcw/L recombinant *E. coli*. Samples were removed at time intervals, the ECH concentration and the optical purity of the (*S*)-ECH were determined by chiral GC.

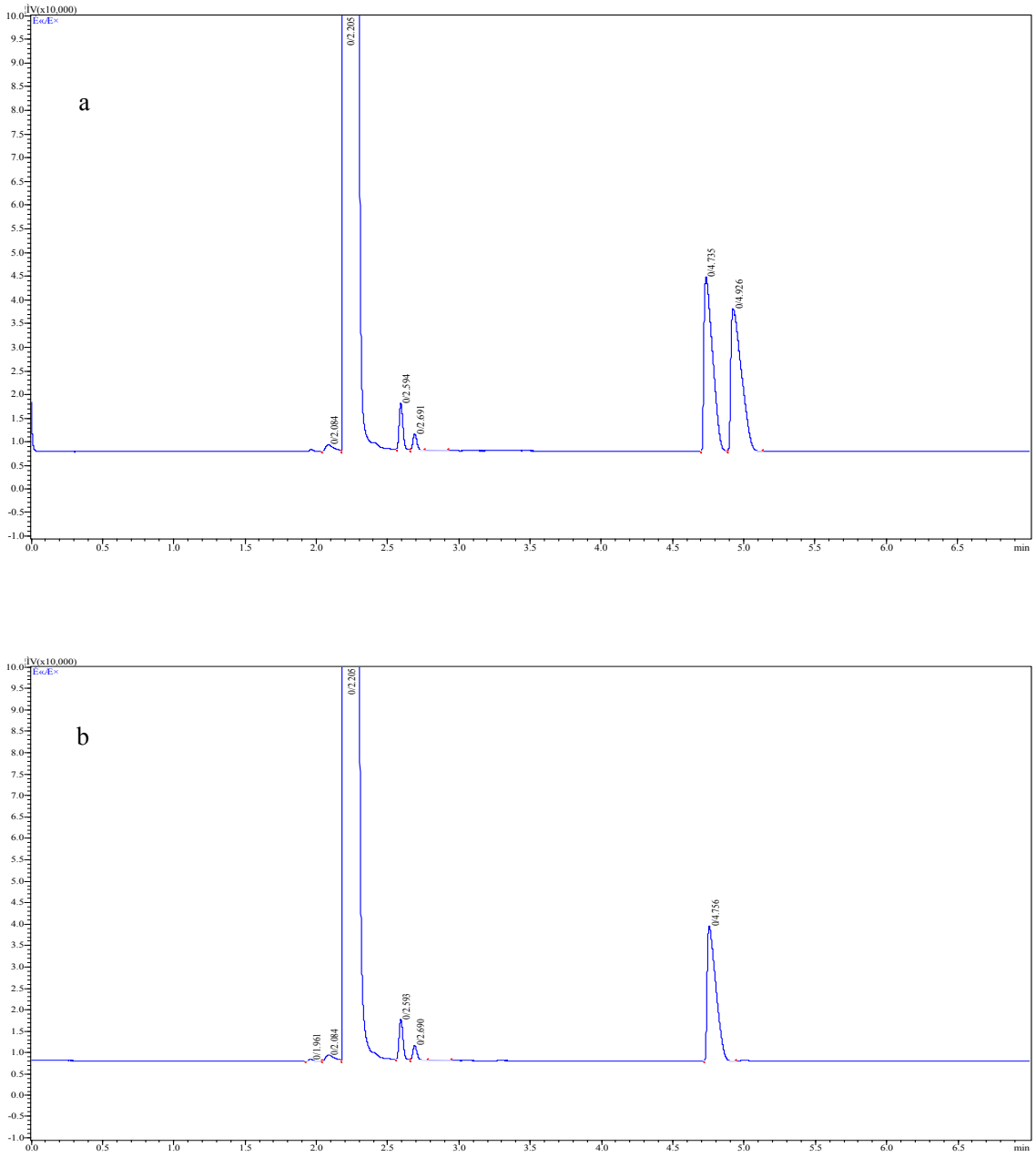


Fig. S4. Estimation of optical purity of (*S*)-ECH by gas chromatography. Samples: a (*R*,*S*)-ECH, b (*S*)-ECH.

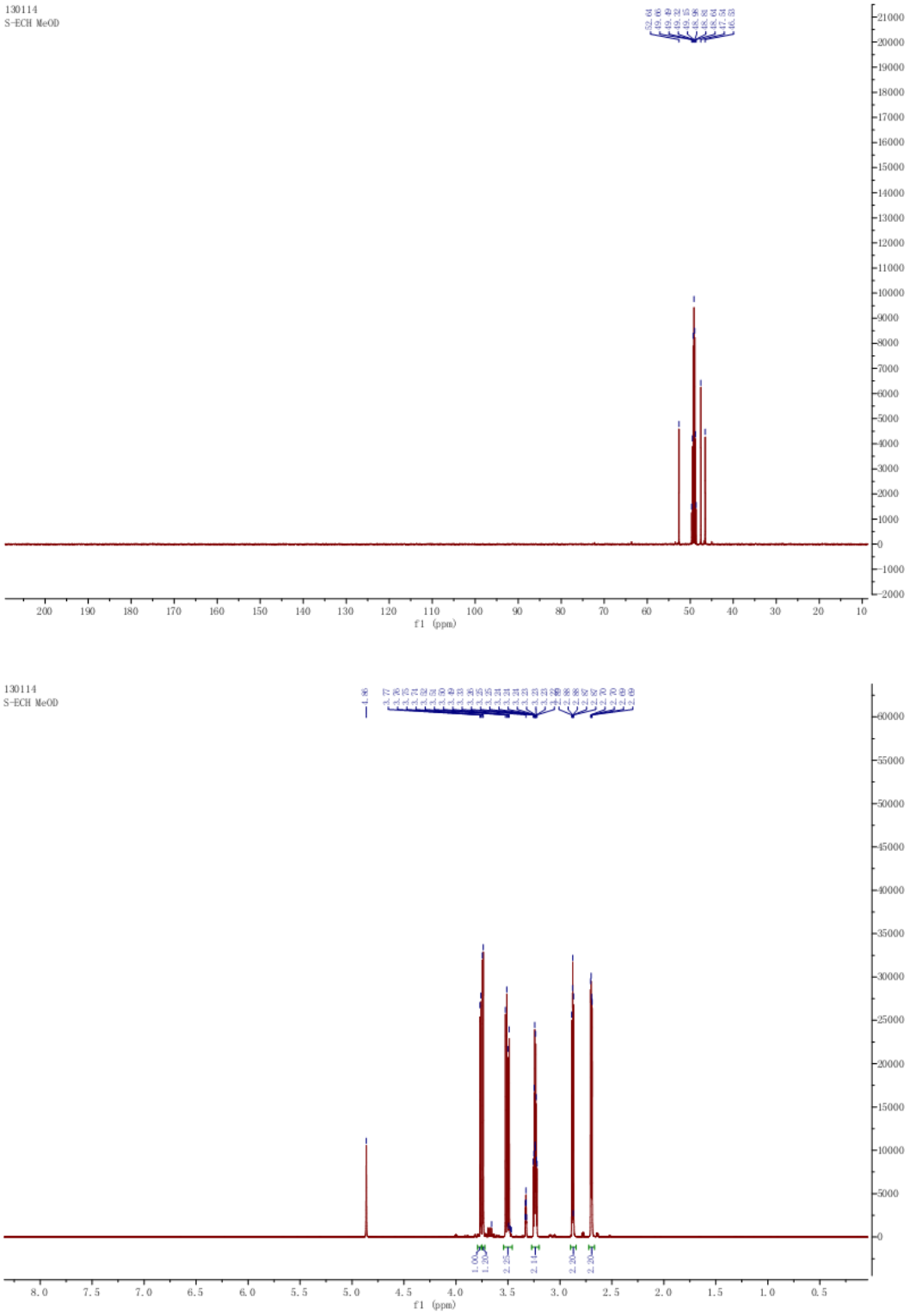


Fig. S5. ^{13}C-NMR(a) and ^{1}H-NMR(b) spectra of (*S*)-ECH.

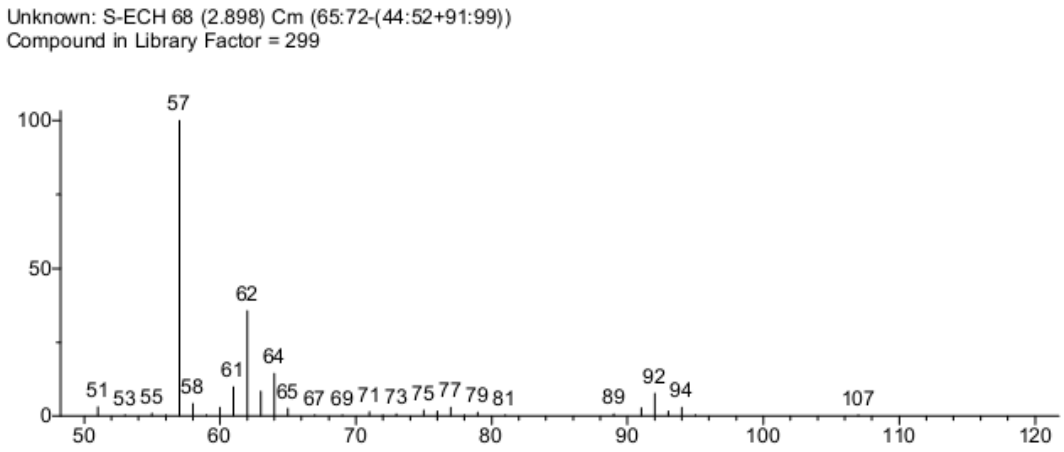


Fig. S6. Mass spectroscopy chromatogram of of (*S*)-ECH.

References

1. F. Xue, Z. Q. Liu, S. P. Zou, N. W. Wan, W. Y. Zhu, Q. Zhu and Y. G. Zheng, *Process Biochemistry*, 2014, **49**, 409-417.