Supplementary Information

Table.S1 all of the PCR primers used for construction of mutants

Round	Primer name	Nucleotide sequence
First-round	L127-F	GTGTTACCNNNTGGACCTCTTCTCTGG
	L127-R	GAGGTCCANNNGGTAACACCAGCAAC
	T129-F	CCCTGTGGNNNTCTTCTCTGGTTA
	T129-R	CAGAGAAGANNNCCACAGGGTAAC
	L244-F	CTGTCTCGTNNNCTGAAACCGG
	L244-R	GTTTCAGNNNACGAGACAGGTCAG
	A250-F	GAAAACNNNGGTAACCACTACCCG
	A250-R	GTTACCNNNGTTTTCCGGTTTCAG
	L245-F	CGTGGTNNNAAACCGGAAAAC
	L245-R	CGGTTTNNNACCACGAGACAG
Second-round	L127I-F	GGTGTTACCATTTGGACCTCTTCTCTGG
	L127I-R	GAGGTCCAAATGGTAACACCAGCAAC
	L244G-F	GTCTCGTGGTCTGAAACCGG
	L244G-R	GTTTCAGACCACGAGACAGGTC
	A250G-F	GGAAAACGGTGGTAACCACTAC
	A250G-R	GTTACCACCGTTTTCCGGTTTC
Third-round	L245-F	CGTGGTNNNAAACCGGAAAAC
	L245-R	CGGTTTNNNACCACGAGACAG
	L245R-F	CGTGGTCCGAAACCGGAAAAC
	L245R-R	CGGTTTCGGACCACGAGACAG

Table S2 Comparison of Asymmetric reduction of OPBA to (R)-HPBA through bio-reduction with the reported works

Enzyme	Product (mM)	Productivity ($mM \cdot h^{-1}$)	ee_p (%)	references
D-LDH from S*taphylococcus epiderimidis* and FDH	182	38.2	>99	[3]
D-LDH (EC 1.1.1.28) and FDH	56.7	49.9	No report	[26]
YiaE from *E. Coli* K12 and GDH	100	4.2	98	[27]
LDH from *Lactobacillus bulgaricus* and FDH	71.8	47.9	>99	[28]
D-MDH from *Enterococcus faecali* and GDH	224	85.7	>99	This study

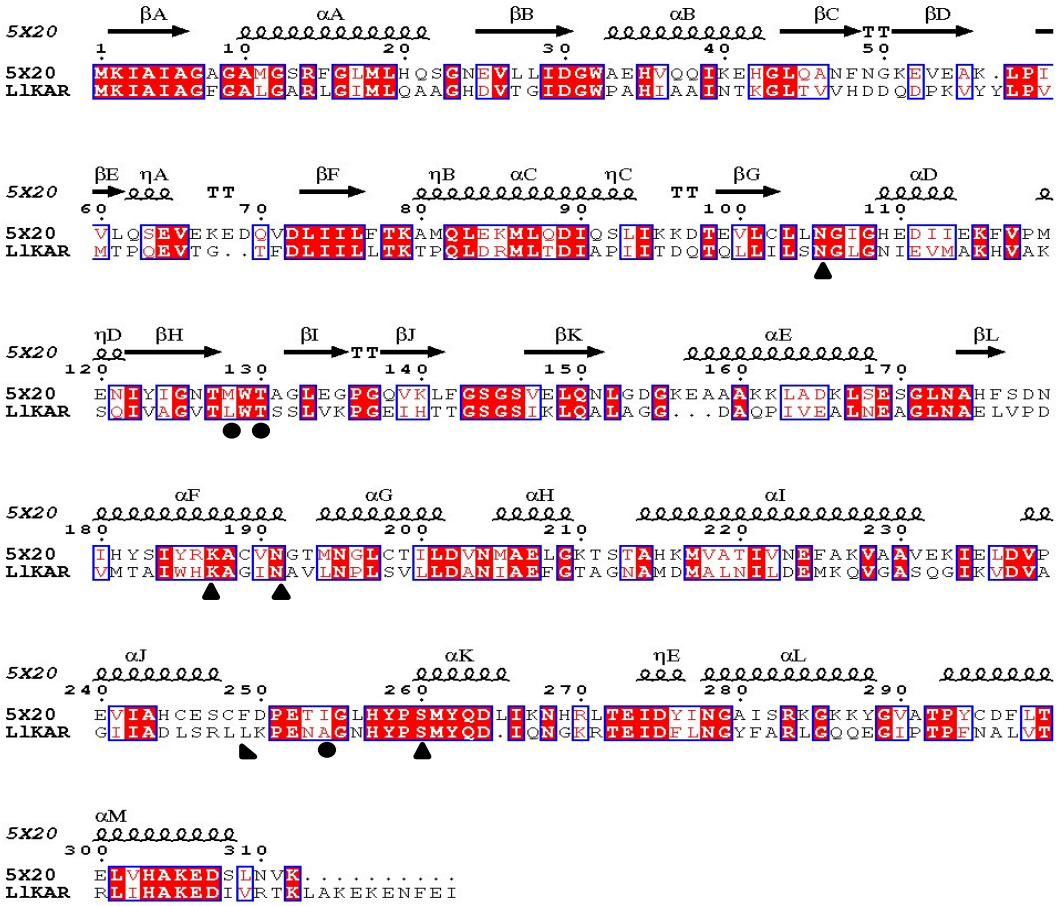

Figure S1. Structure-related sequence alignment between *Ll*KAR and its close homologue: D-mandelate dehydrogenases (D-MDH) from *Leuconostoc lactis*. The homologue of LlKAR, D-mandelate dehydrogenases (D-MDH) with a PDB code of 5X20, 3WFI and 3WFJ, was identified by performing a BLASTP search, and the alignment was carried out using the program ClustalW. The sequence alignment was subsequently visualized using ESPript 3.2. Above the alignments are elements of the secondary structure of 5X20. The numbering shown is from 5X20. Triangles, critical coenzyme binding residues; stars, putative catalytic residues located at the corresponding positions of the conserved catalytic residues Asn104, Asn183, and Glu263. Strictly conserved residues are highlighted with red boxes.

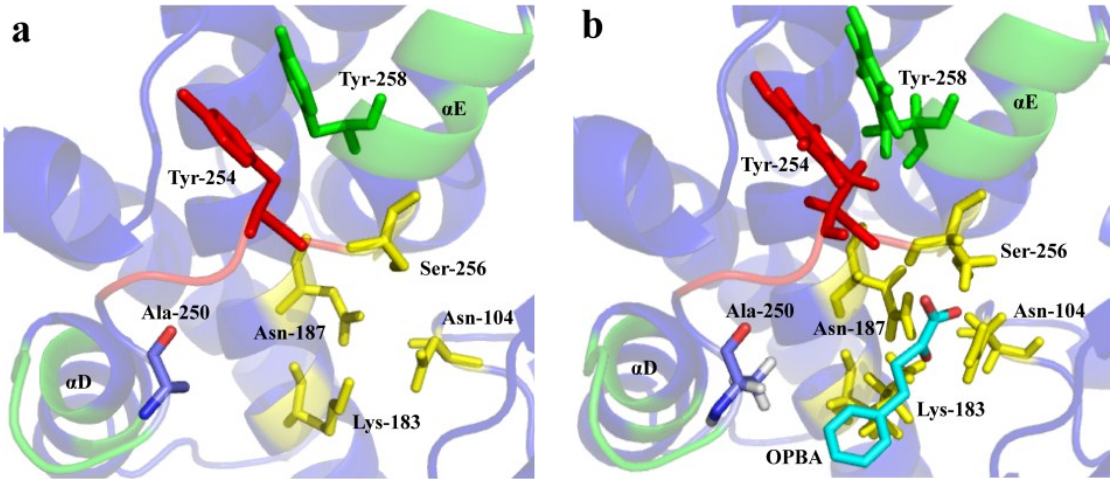

Figure S2. The clear spatial structure of the *Ll*KAR catalytic and binding pocket. (a) The catalytic tetrad, Asn187, Lys183, and Ser256 are shown in yellow sticks. And the hydrophilic domains are shown in green and red cartoon; (b) the clear Spatial structure-activity relationship between α-helices and loop αD-αE. OPBA are shown as cyans sticks. All of the α-helices, β-sheets and loops are shown in blue cartoon.

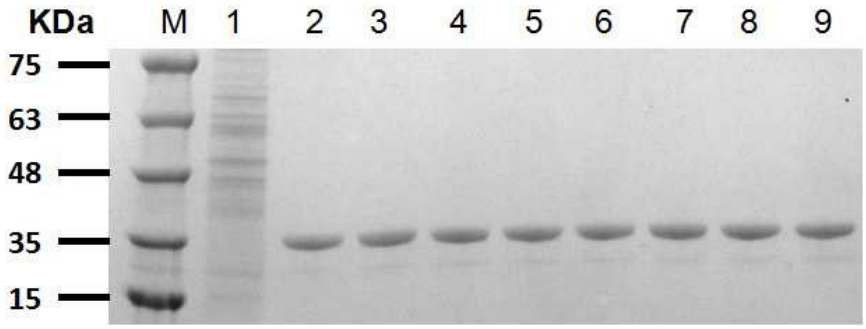

Figure S3 SDS-PAGE analysis of the wild-type *Ll*KAR and its mutants. 10 µL sample which contained approximately 40 µg protein was loaded for each lane. Lane M: protein marker; Lane 1: the supernatant of the recombination *E.coli* BL21 (harboring pET-28b); Lane 2: the purified wild-type *Ll*KAR; Lane 3: the purified mut-L127I; Lane 4: the purified mut-L244G; Lane 5: the purified mut-A250G; Lane 6: the purified mut-L127I/L244G; Lane 7: the purified mut-L127I/A250G; Lane 8: the purified mut-L244G/A250G; Lane 9: the purified mut-L244G/A250G/L245R.

References

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