TABLE S1 Primers used in this study

Primer name	Sequence 5→3[a]	Restriction enzyme site
RxDAOP1N	GCACATATGAGGGATTGCGGGCGTGC	NdeI
RDAOP2N	AGCAAGCTTGGAGAGCGCGGCCCCGGCCAG	—
RxDAOp1-2	AGGAGGTCCAGGATCACGTA	—
RxDAONPCR1	TACAGGGCGTATCCGGAGGACC	—
RxDAOPR10	GAGCCACCCGTCGACACGAACGCTCTGATCC	—
RxDAO1R23-43p	ACTGTAAAGCGATCCGTATCG	
RDAS1B15	CGCGGATCCTTAAGGGTTCCGGTCGAGCG	BamHI

[a] Restriction enzyme site is underlined.

TABLE S2 Summary of purification of recombinant *R. xylanophilus* DAO expressed in *E. coli*

Step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Purification (fold)	Recovery (%)
Crude extract	1,540	96.9	0.0629	1	100
Heat treatment at 70°C	348	124	0.356	5.67	128
Metal-chelate affinity chromatography	0.501	10.3	21.1	336	8.31

The enzyme activity was determined by an oxygen electrode method at 60°C using 20 mM D-valine as a substrate. The starting material was 24.5 g of *E. coli* cell paste from a 3 l fermentation.

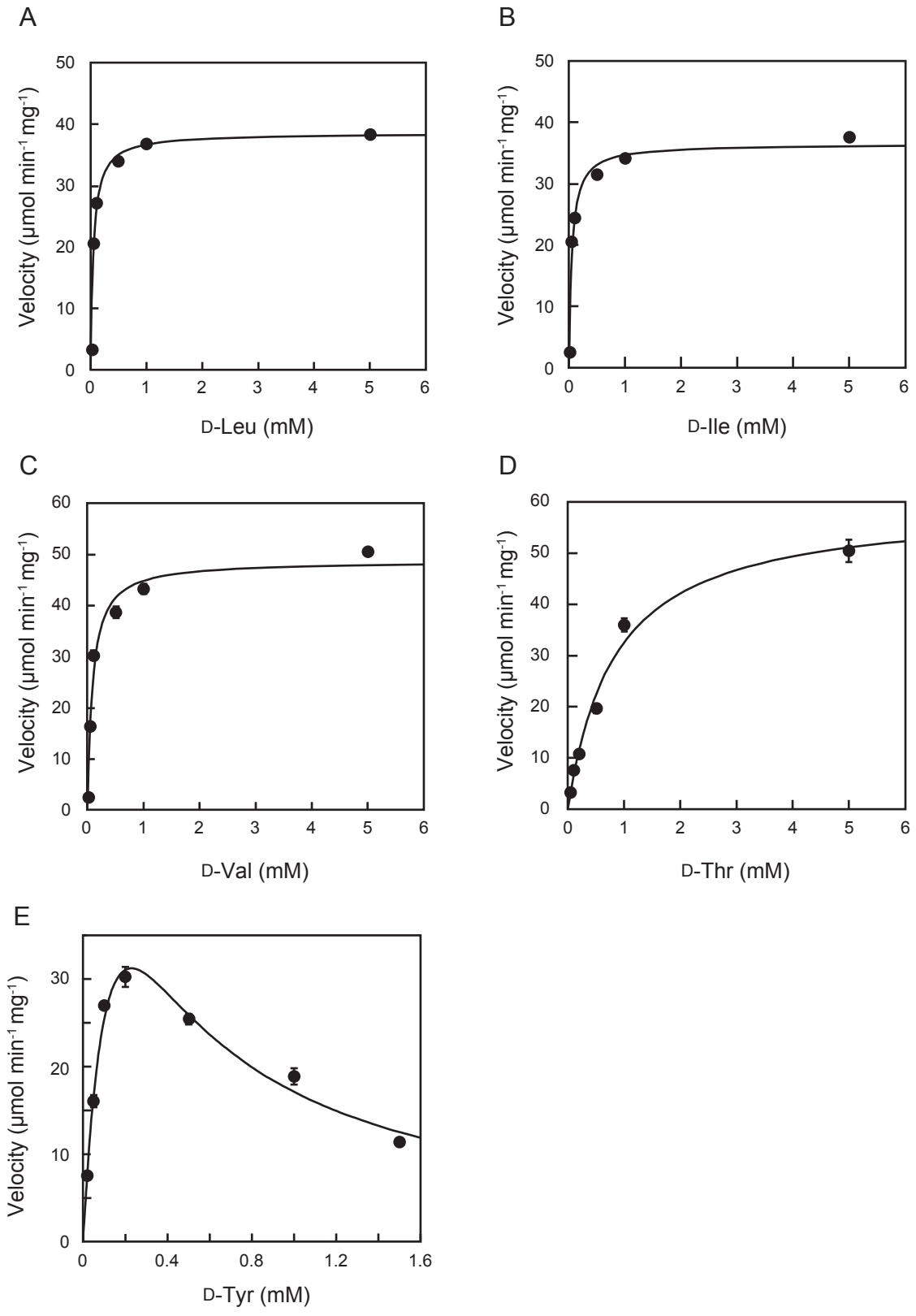

FIG. S1 Kinetic analysis of RxDAO. The kinetic analysis was performed in 50 mM potassium phosphate buffer (pH 8.0) for each D-amino acid at 60° C using a coupled *o*-dianisidine-peroxidase method. The fitting and plotting were performed using the program SigmaPlot 12.5. Each data point represents the mean ± SD of three measurements.

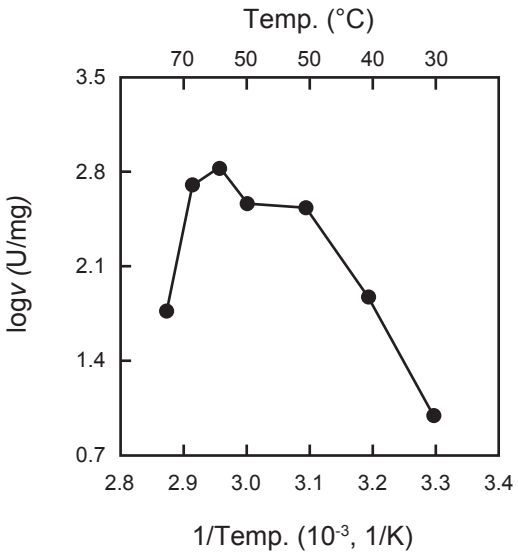

FIG. S2 Arrhenius plot of the temperature dependence of the enzyme reaction rate. This plot was made from the data of Fig. 4B. The activation energy of the reaction was calculated in the temperture range from 30°C to 50°C.