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Supporting information for article:

Crystal structure of a novel homodimeric D-allulose 3-epimerase from a Clostridia bacterium

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Table S1 Biochemical properties of KEases (Adapted from Zhang et al. (2021)).

KEases	Organism	Genbank accession	T_{opt}[a]	Half-life (min)	Metal ion[b]	k_{cat}/K_m ($mM^{-1}\cdot min^{-1}$)[c]
Agtu-DAE	*Agrobacterium tumefaciens*	AAK88700.1	50	8.9 (55°C)	Mn^{2+}	85
Clce-DAE	*Clostridium cellulolyticum* H10	ACL75304.1	55	24 (55°C)	Co^{2+}	62.7
Agsp-DAE	*Agrobacterium* sp. ATCC 31749	EGL65884.1	55	0.75 (55°C)	Co^{2+}	58.6
Clsi-DAE	*Clostridium scindens* ATCC 35704	EDS06411.1	60	50 (60°C)	Mn^{2+}	8.72
Dosp-DAE	*Dorea* sp. CAG317	WP_022318236.1	70	30 (60°C)	Co^{2+}	199
Clsp-DAE	*Clostridium* sp. BNL1100	WP_014314767.1	65	15 (60°C)	Co^{2+}	58.7
Rusp-DAE	*Ruminococcus* sp. 5_1_39BFAA	ZP_04858451	60	96 (60°C)	Mn^{2+}	16
Clbo-DAE	*Clostridium bolteae* BAA-613	EDP19602.1	55	156 (55°C)	Co^{2+}	59.4
Trpr-DAE	*Treponema primitia* ZAS-1	ZP_09717154.1	70	30 (50°C)	Co^{2+}	63
Thca-DAE	*Thermoclostridium caenicola*	SHI77623.1	65	816 (55°C)	Co^{2+}	132
Pase-DAE	*Paenibacillus senegalensis*	WP_010270828.1	55	140 (55°C)	Mn^{2+}	6.7
Basp-DAE	*Bacillus* sp. KCTC 13219	KYG89858.1	55	1320 (55°C)	Mn^{2+}	168.6
Desp-DAE	*Desmospora* sp. 8347	WP_009711885.1	60	120 (50°C)	Co^{2+}	116
Noth-DAE	*Novibacillus thermophilus*	WP_077721022.1	70	47.8 (60°C)	Co^{2+}	312.3
DeaM	-	QHD25651.1	80	3240 (70°C)	Co^{2+}	17.4
Haco-DAE	*Halanaerobium congolense*	WP_110301365.1	70	66 (70°C)	Mg^{2+}	NRc
Flpl-DAE	*Flavonifractor plautii* ATCC 29863	EHM40452.1	65	40 (65°C)	Co^{2+}	64
Pisp-DAE	*Pirellula* sp. SH-Sr6A	WP_146677337.1	60	360 (60°C)	Co^{2+}	37.8
Sifr-DAE	*Sinorhizobium fredii* CCBAU 83666	ASY72161.1	70	600 (60°C)	Mg^{2+}	55.8
Rhba-DAE	*Rhodopirellula baltica*	WP_007330622.1	60	52.3 (60°C)	Mn^{2+}	11.45
Chmi-DTE	*Christensenella minuta*	WP_066519968.1	50	40 (50°C)	Ni^{2+}	45
Psci-DTE	*Pseudomonas cichorii*	BAA24429.1	60	NR	None	NR
Cafo-DTE	*Caballeronia fortuita*	WP_061137998.1	65	63 (60°C)	Co^{2+}	78.5
Cesp-DTE	*Cereibacter sphaeroides*	ACO59490.1	40	30 (60°C)	Mn^{2+}	NR
Sisp-DTE	*Sinorhizobium* sp. RAC02	WP_069063284.1	50	NR	Mn^{2+}	54.6

Melo-LRE	*Mesorhizobium loti*	BAB50456.1	60	138 (70°C)	Mn^{2+}	NR
Mesp-LRE	*Methylomonas* sp. DH-1	WP_064020855.1	70	208 (60°C)	NR	NR
Argl-LRE	*Arthrobacter globiformis*	BAW27657.1	70	NR	Mg^{2+}	66.9
Laen-LRE	*Labedella endophytica*	WP_127049469.1	80	276 (70°C)	Ni^{2+}	82.3
Thma-LRE	*Thermotoga maritima* MSB8	AAD35501.1	80	NR	Mn^{2+}	NR

[a] The optimum temperature for KEases.

[b] The optimum metal ion for KEases.

[c] The k_{cat}/K_m values of KEases towards D-fructose.

Table S2 Comparison of the active sites in KEases bound with D-fructose

KEases		**Metal-binding**	**Substrate-binding (hydrogen bonds)**		**Hydrophobic pocket**
			O-1, O-2, O-3	**O-4, O-5, O-6**	
DAEase	Agtu-DAE	Glu150, Asp183 His209, Glu244	Glu150, Glu244, Glu156, His186, Arg215	Ile66, Ala107	Trp14, Ile66, Ala107, Trp112, Phe246
	Clce-DAE			Tyr6	Trp14, Ala107, Trp112, Phe246
	CDAE			--	Trp14, Gly107, Trp112, Phe246
DTEase	Psci-DTE	Glu152, Asp185 His211, Glu246	Glu152, Glu246, Glu158, His188, Arg217	Cys66	Phe7, Trp15, Ile67, Leu108, Trp113, Phe248
LREase	Argl-LRE	Glu146, Asp180 His206, Glu241	Glu146, Glu241, Glu152, His182, Arg211	--	Met39, Leu64, Val105, Met110, Phe242, Phe251
	Mesp-LRE	Glu152, Asp185 His211, Glu246	Glu152, Glu246, Glu158, His188, Arg217	His12, Ser69	Leu45, Leu70, Val111, Leu116, Phe248, Leu257
	Melo-RLE	Glu147, Asp180 His206, Glu241	Glu147, Glu241, Glu153, His183, Arg212	--	Ile65, Ile106, Phe243, Leu252

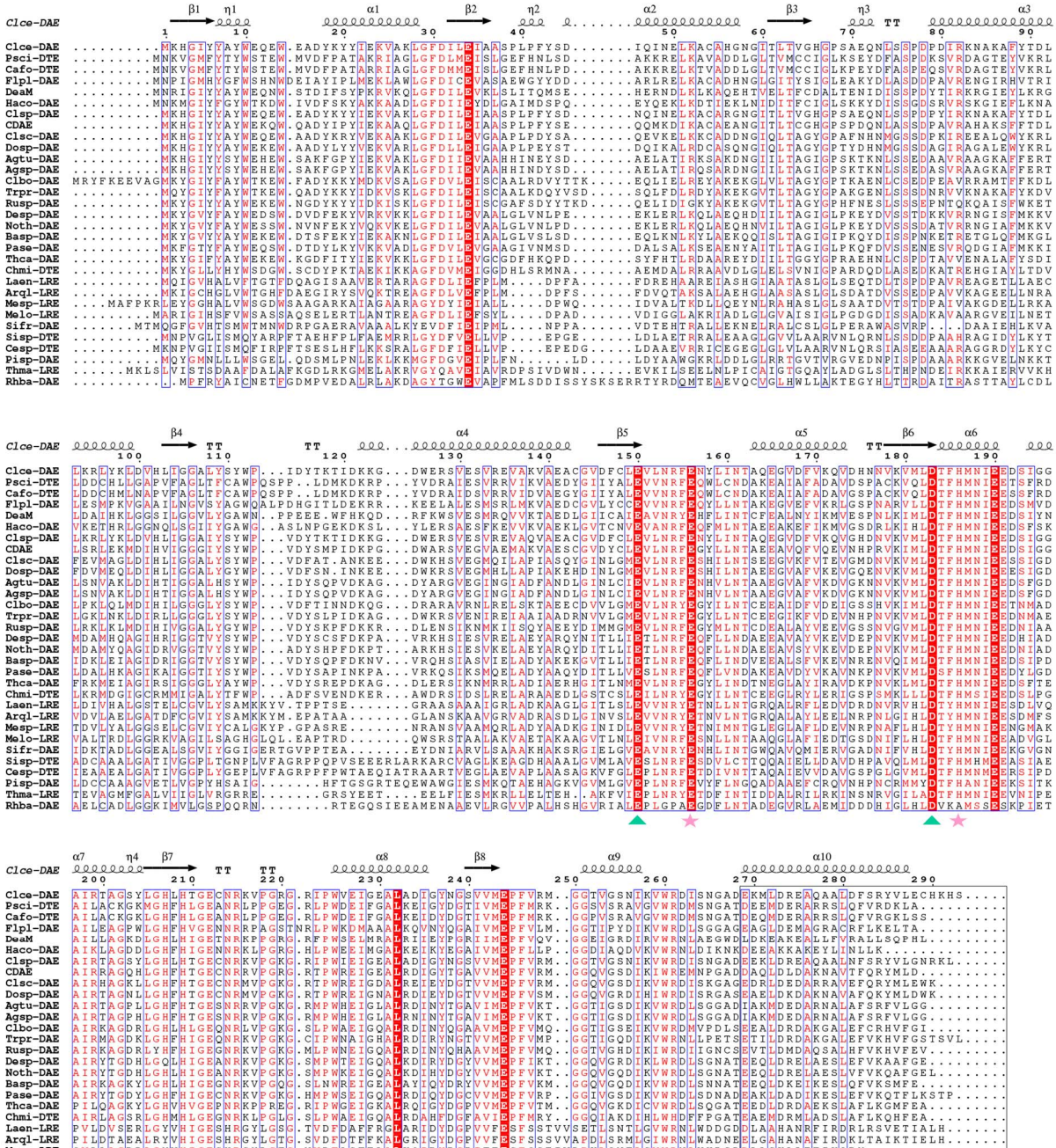

Figure S1 Multiple sequence alignment of amino acids from KEase family. Conserved residues are designated with red squares and white letters, and similar residues are designated with red letters. The residues involved in metal-binding and substrate-binding are marked with green triangles and pink pentagram, respectively. Secondary structure assignments for DAEase from *Clostridium cellulolyticum* H10 (PDB ID: 3VNK) are presented above the alignment. The black frame highlights the C-terminal α-helix of KEases. Sequence alignments were performed using ClustalW and ESPript v3.0.

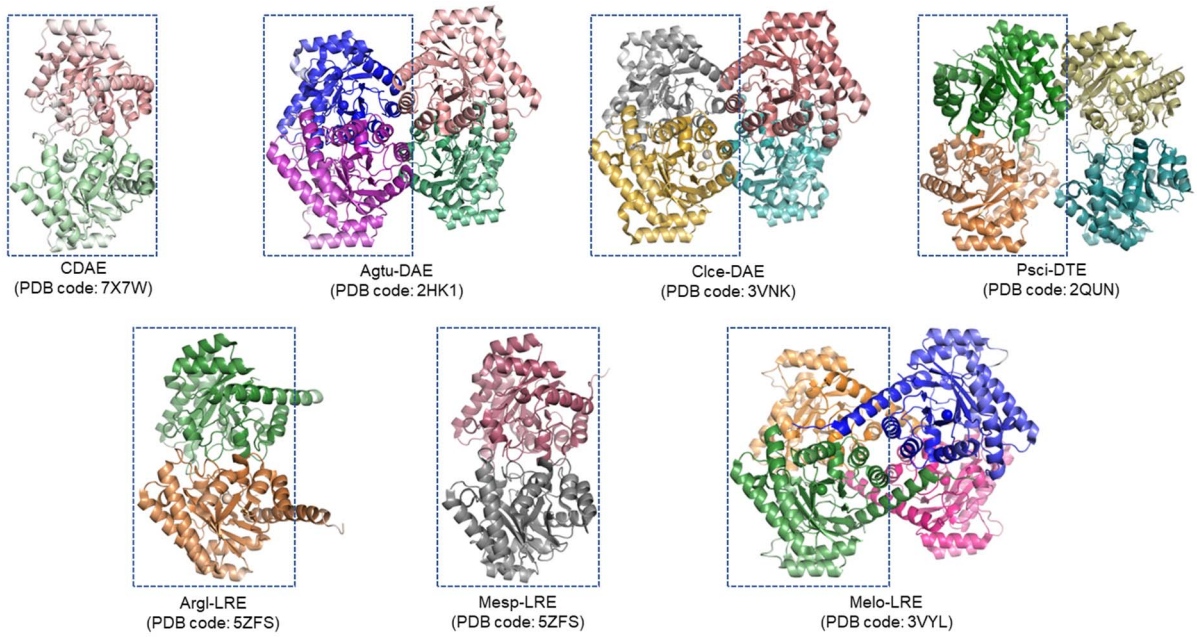

Figure S2 Overall structure of KEases in cartoon representations. Dimeric formation is designated with blue dotted square.

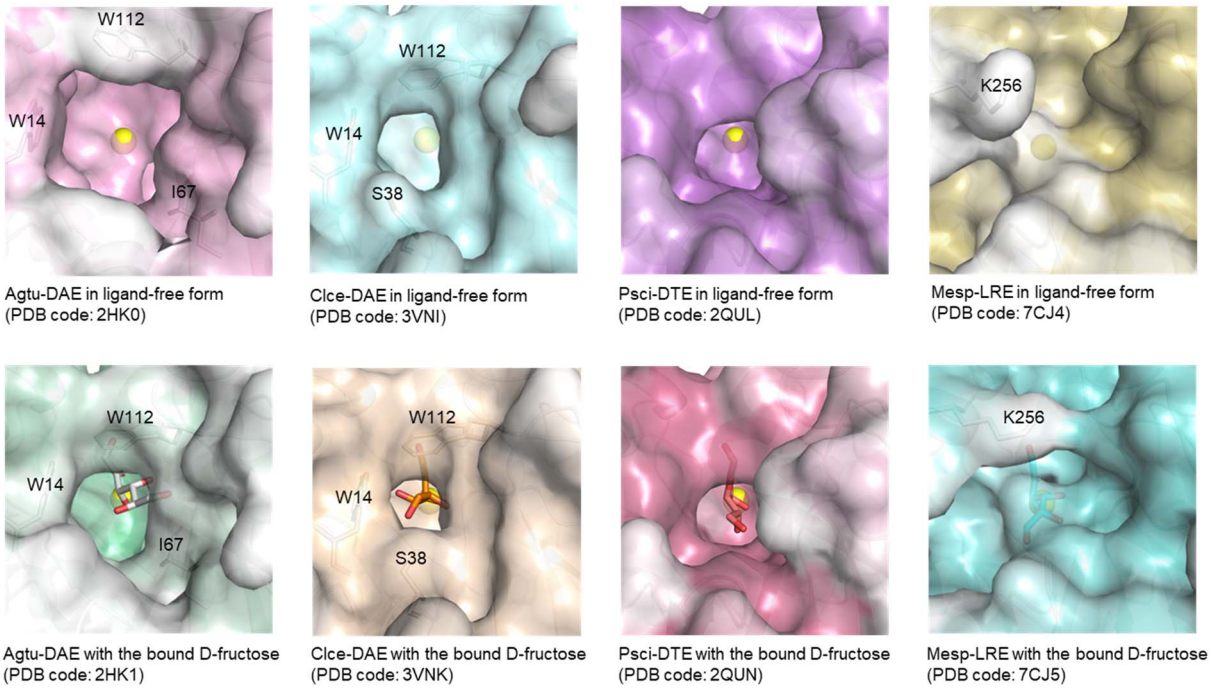

Figure S3 The change of channel to the active site upon substrate binding. Comparison is conducted between KEase in apo-form and in complex with D-fructose.