

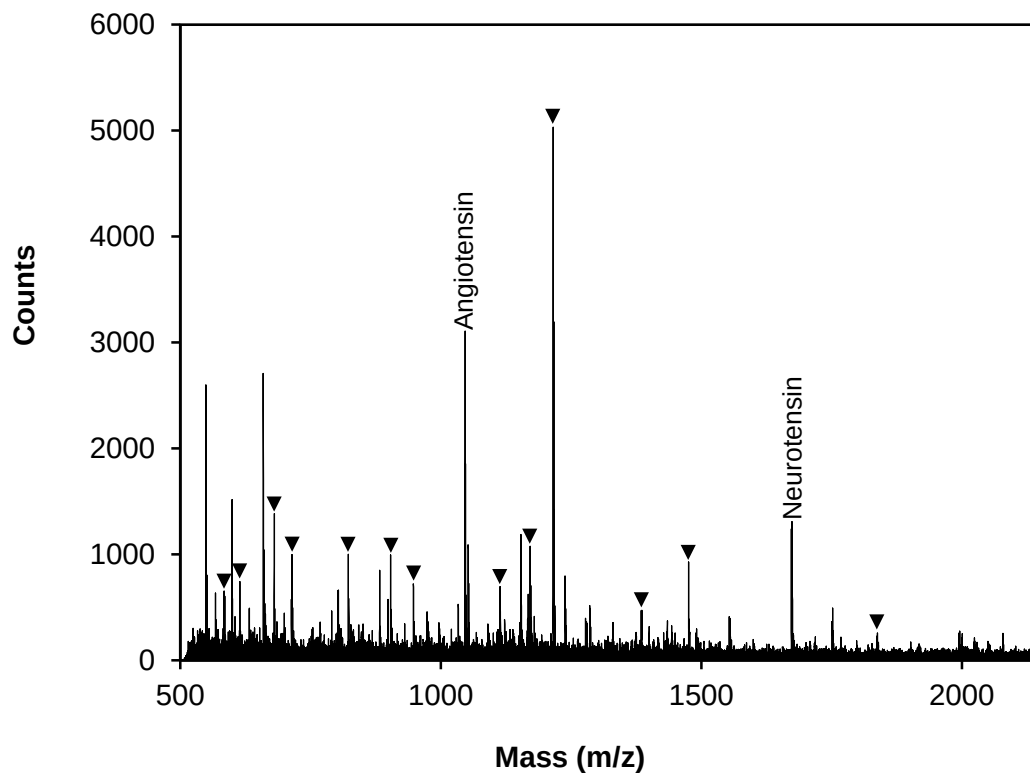


Title	Biochemical Characterization of a Thermophilic Cellobiose 2-Epimerase from a Thermohalophilic Bacterium, <i>Rhodothermus marinus</i> JCM9785
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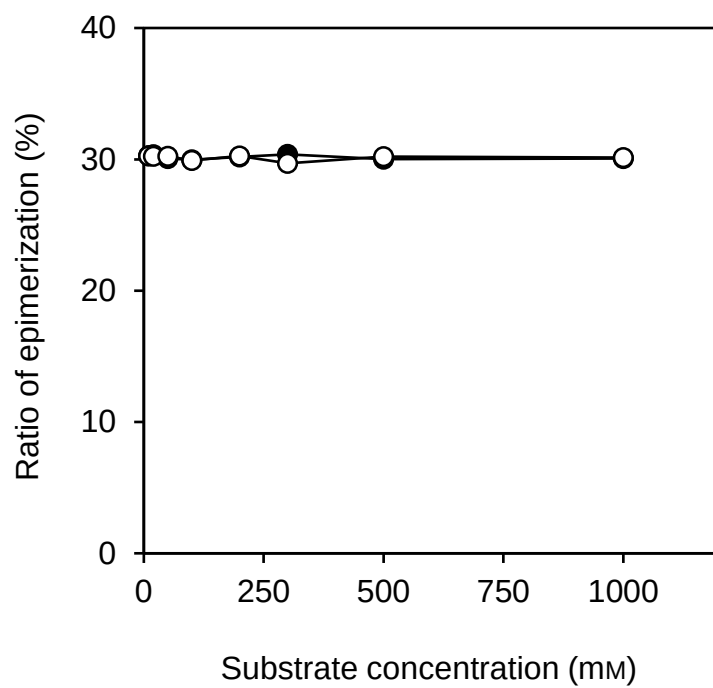
Supplemental Figures and Tables

Supplemental Fig. 1, MALDI-TOF MS of tryptic peptide mixture of RmCE. Supplemental Fig. 2, Conversion ratios of RmCE reaction to various substrate concentrations. Supplemental Fig. 3, Comparison of model structures of RmCE and RaCE. Supplemental Table 1, MALDI-TOF MS of tryptic peptide mixture of RmCE. Supplemental Table 2, Numbers of acidic and basic amino acids in RmCE and RaCE.



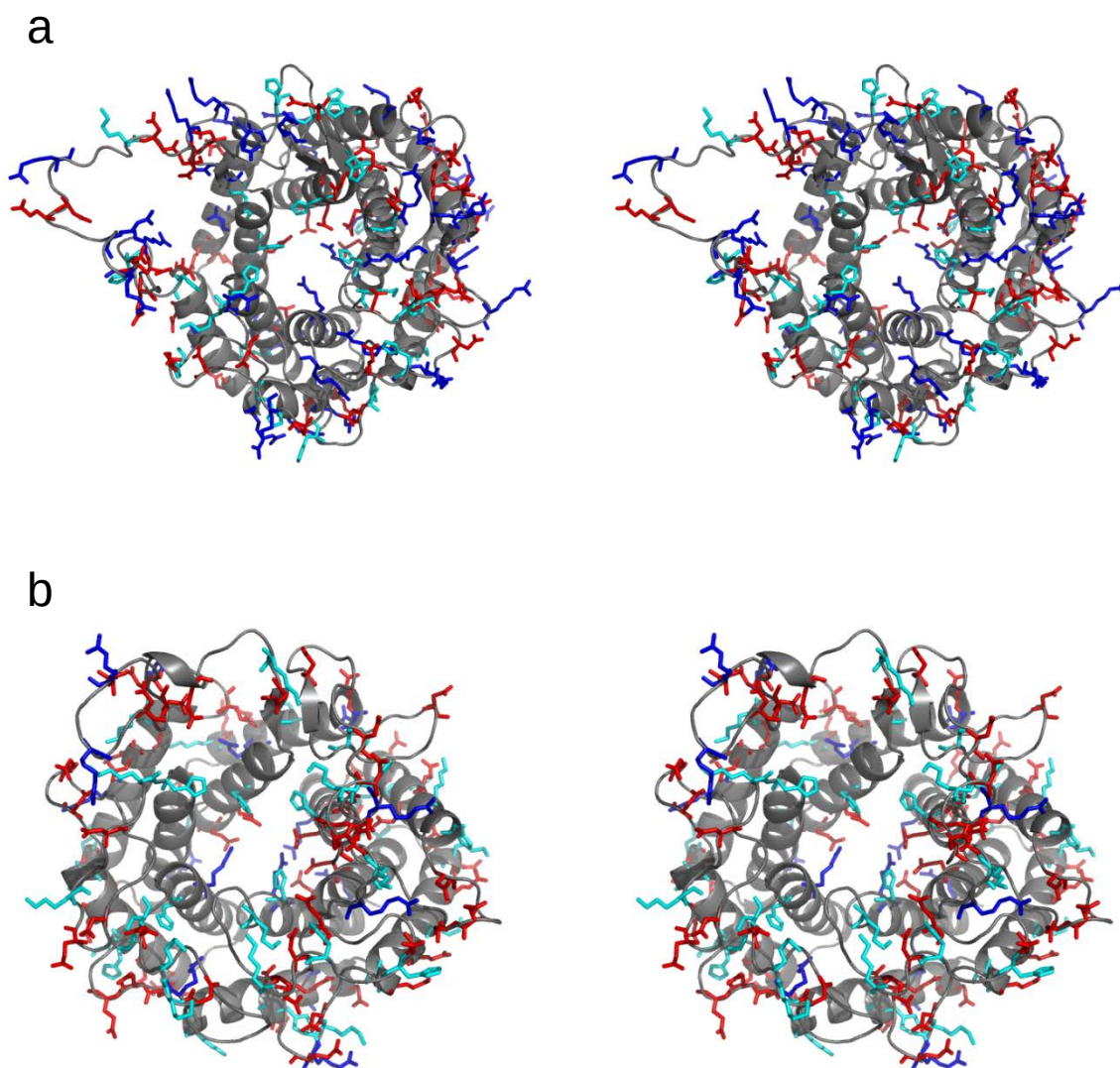
Supplemental Fig. 1. MALDI-TOF MS of Tryptic Peptide Mixture of RmCE.

Purified RmCE of 1 μ g was subjected to SDS-PAGE and protein band was excised from the gel. In-gel-digestion of separated RmCE with trypsin and MALDI-TOF MS analysis of the resultant peptides were performed. Angiotensin (molecular mass, 1046.54 Da) and neurotensin (molecular mass, 1672.92 Da) were used as the internal standard. Marked signals match the theoretical values calculated from the deduced amino acid sequence of RmCE.



Supplemental Fig. 2. Conversion Ratios of RmCE Reaction at Various Substrate Concentrations.

The epimerization ratios at equilibrium in the reactions to varying concentrations of substrate were assessed. The reaction mixtures consisting of 10-1000 mM lactose or cellobiose, 5 mM potassium phosphate buffer (pH 6.5), and RmCE (30 U/g-substrate solid) were incubated at 60°C for 24 h. The amount of products was measured by HPLC after the reactions had fully reached equilibrium. Close and open circles indicate lactose and cellobiose, respectively.



Supplemental Fig. 3. Comparison of Model Structures of RmCE and RaCE.

Model structures of RmCE and RaCE were constructed with CPHmodels-3.0 Server.²⁴⁾ Three dimensional structure images were created with PyMOL (www.pymol.org). Arg, Lys and His, and Glu and Asp residues are shown in blue, cyan, and red, respectively. a, RmCE. b, RaCE.

Supplemental Table 1. MALDI-TOF MS of Tryptic Peptide Mixture of RmCE

Mass measured	Mass calculated	Start residue	End residue	Sequence
584.34	584.32	93	96	YFVR
614.36	614.35	248	251	WRPR
680.36	680.35	30	34	FWATR
714.43	714.43	60	66	GAILNAR
822.40	822.39	363	369	GGEWFAR
903.51	903.52	404	410	HLLEHVR
947.53	947.53	77	84	QLGTPLYR
1113.56	1113.58	175	183	AWRPLEDAR
1171.64	1171.61	211	220	VWPETELAAR
1215.75	1215.75	221	230	LQALIELFLR
1384.67	1384.71	47	59	VGPDGRPHPEAPR
1475.74	1475.72	382	393	VDFWKGPYHNGR
1836.94	1836.97	14	29	ALQAEVHEELTENILK

Peptides are marked as in Supplemental Fig. 1. Measured and calculated molecular masses are given as protonated mono-isotopic masses.

Supplemental Table 2. Numbers of Acidic and Basic Amino Acids in RmCE and RaCE

Amino acid	RmCE	RaCE
Asp	24 (5.8)	36 (9.3)
Glu	32 (8.3)	33 (8.5)
Lys	15 (1.2)	27 (6.9)
Arg	46 (11.2)	16 (4.1)
His	21 (5.1)	12 (3.1)
Total	412	389

Amino acid content (%) is provided in parentheses.