

Supplementary materials

Xylan Deconstruction by Thermophilic *Thermoanaerobacterium bryantii* Hemicellulases is Stimulated by Two Oxidoreductases

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Supplemental Table S1. Characteristics that differentiate strain mel9^T from other species of *Thermoanaerobacterium*.

Characteristics	Strain mel9 ^T	<i>T.</i> <i>thermosaccharolyticum</i> [#]	<i>T.</i> <i>aotearoense</i> [#]	<i>T.</i> <i>saccharolyticum</i> [#]	<i>T.</i> <i>thermosulfurigenes</i> [#]	<i>T.</i> <i>xylanolyticum</i> [#]
G + C content (mol%)	37.7	29–32	34.5–35	36	32.6	36.1
pH range	5.0–8.0	6.5–8.5	3.8–6.8	5.0–7.5	4.0–7.6	5.0–7.5
Optimum pH	6.8–7.0	7.8	5.2	6.0	5.5–6.5	6.0
Optimum temp (°C)	62–65	55–60	60.1–62.6	60	60	60
Maximum temp (°C)	69	62	65.7	68–70	75	70
Spore formation	–	+	+	+	+	+
Fermentation						
End Products*						
Lactate	–	+	+	+	+	–
Acetate	+	+	+	+	+	+
Butyrate	+	+	–	–	–	–
Ethanol	+	+	+	+	+	+
Butanol	+	–	–	–	–	–
CO ₂	+	+	+	+	+	+
H ₂	+	+	+	+	+	+

[#]From liu et al. [63], ^{*}From Lee et al. [44], except data on *T. thermosaccharolyticum* from Jones and Woods [64].

Supplemental Table S2. Amino acid sequence identities among the enzymes in the hemi-cellulase gene clusters from *T. bryantii* (*T.b*), *T. xylanolyticum* (*T.x*) and *T. thermosaccharolyticum* (*T.t*).

Sequence Identity	Agu67	HEOA	HEOB	Xyl52	Xyl39	Axe1
<i>T.b–T.x</i>	86.5%	91.5%	95.8%	89.3%	86.6%	92.5%
<i>T.b–T.t</i>	91.3%	95.1%	91.9%	92.0%	95.0%	99.4%
<i>T.b–T.s</i>	84.8%	90.2%	91.6%	86.0%	89.0%	92.5%

Supplemental Table S3. End products of synergistic hydrolysis of different xylan-containing plant biomass by the seven enzymes encoded by the *T. bryantii* hemicellulase gene cluster^{a,b,c}

Enzymes Combination	Substrates	End-Products (mM)			Substrates	End-Products (mM)		
		X1	X2	X3		X1	X2	X3
1	BeeWX	0.1 ± 0.0	3.5 ± 0.0	2.0 ± 0.2	BWV	ND	0.5 ± 0.2	0.4 ± 0.1
2		0.8 ± 0.0	3.9 ± 0.1	1.6 ± 0.1		1.7 ± 0.2	1.2 ± 0.2	0.5 ± 0.1
3		10.6 ± 0.4	0.8 ± 0.0	<0.1		2.1 ± 0.6	0.7 ± 0.5	0.3 ± 0.2
4		11.5 ± 0.6	0.6 ± 0.1	<0.1		4.3 ± 0.2	0.5 ± 0.0	0.2 ± 0.0
5		12.9 ± 1.5	0.4 ± 0.0	ND		7.6 ± 0.6	1.1 ± 0.2	0.3 ± 0.2
6		12.5 ± 1.6	0.3 ± 0.0	ND		9.7 ± 0.1	1.3 ± 0.0	0.3 ± 0.0
7		13.8 ± 0.9	0.3 ± 0.0	ND		12.5 ± 0.5	1.3 ± 0.1	0.2 ± 0.0
8		18.8 ± 1.1	0.4 ± 0.1	ND		16.1 ± 0.3	1.0 ± 0.2	0.2 ± 0.1
9		14.4 ± 0.8	0.2 ± 0.1	ND		15.2 ± 0.7	0.6 ± 0.0	<0.1
10		17.8 ± 1.2	0.7 ± 0.1	ND		17.0 ± 0.4	1.1 ± 0.2	0.2 ± 0.0
1	OSX	ND	0.8 ± 0.1	0.5 ± 0.0	LAX	<0.1	1.7 ± 0.2	1.0 ± 0.1
2		1.0 ± 0.1	0.7 ± 0.3	0.2 ± 0.0		0.9 ± 0.1	1.7 ± 0.2	0.7 ± 0.0
3		2.3 ± 0.3	0.3 ± 0.0	<0.1		2.9 ± 0.1	0.7 ± 0.0	0.3 ± 0.0
4		3.0 ± 0.5	0.3 ± 0.0	<0.1		3.3 ± 0.0	0.7 ± 0.0	0.2 ± 0.0
5		5.7 ± 0.1	0.3 ± 0.0	ND		5.5 ± 0.2	0.7 ± 0.1	0.2 ± 0.0
6		5.6 ± 0.1	0.3 ± 0.0	ND		5.7 ± 0.6	0.9 ± 0.1	0.2 ± 0.0
7		6.4 ± 0.8	0.2 ± 0.0	ND		5.9 ± 0.2	0.7 ± 0.0	0.1 ± 0.0
8		6.6 ± 0.3	0.2 ± 0.0	ND		7.6 ± 0.7	0.5 ± 0.1	0.1 ± 0.1
9		8.3 ± 0.0	0.2 ± 0.0	ND		7.3 ± 0.1	0.6 ± 0.0	0.2 ± 0.0
10		7.4 ± 1.1	0.2 ± 0.0	ND		6.3 ± 0.2	0.6 ± 0.1	0.1 ± 0.0
1	WAX	0.1 ± 0.1	1.1 ± 0.1	0.5 ± 0.0	Miscanthus	0.2 ± 0.0	0.7 ± 0.0	ND
2		0.4 ± 0.2	1.1 ± 0.3	0.3 ± 0.0		1.2 ± 0.2	<0.1	ND
3		1.2 ± 0.2	1.0 ± 0.2	0.2 ± 0.1		1.4 ± 0.2	ND	ND
4		1.3 ± 0.0	0.8 ± 0.0	0.2 ± 0.0		2.0 ± 0.0	ND	ND
5		1.3 ± 0.3	0.9 ± 0.1	0.2 ± 0.0		2.2 ± 0.2	ND	ND
6		1.6 ± 0.1	1.0 ± 0.0	0.2 ± 0.0		2.0 ± 0.2	ND	ND
7		1.9 ± 0.2	1.1 ± 0.1	0.3 ± 0.0		2.1 ± 0.1	ND	ND
8		1.9 ± 0.1	0.9 ± 0.1	0.1 ± 0.0		3.0 ± 0.3	ND	ND
9		1.8 ± 0.1	1.0 ± 0.0	0.1 ± 0.0		2.5 ± 0.2	ND	ND
10		2.0 ± 0.3	1.1 ± 0.0	0.2 ± 0.0		2.9 ± 0.1	ND	ND

^a Enzymes combination 1: TbXyn10A; 2: TbXyn10A and TbXyl39A; 3: TbXyn10A and TbXyl52A; 4: TbXyn10A, TbXyl39A and TbXyl52A; 5: TbXyn10A, TbXyl39A, TbXyl52A and TbHEOA; 6: TbXyn10A, TbXyl39A, TbXyl52A and TbHEOB; 7: TbXyn10A, TbXyl39A, TbXyl52A, TbHEOA and TbHEOB; 8: TbXyn10A, TbXyl39A, TbXyl52A, TbHEOA, TbHEOB and TbAgu67A; 9: TbXyn10A, TbXyl39A, TbXyl52A, TbHEOA, TbHEOB and TbAxe1A; 10: TbXyn10A, TbXyl39A, TbXyl52A, TbHEOA, TbHEOB, TbAgu67A and TbAxe1A; ^b ND: not detected. ^c Abbreviations were X1: xylose; X2: xylobiose; X3: xylotriose.

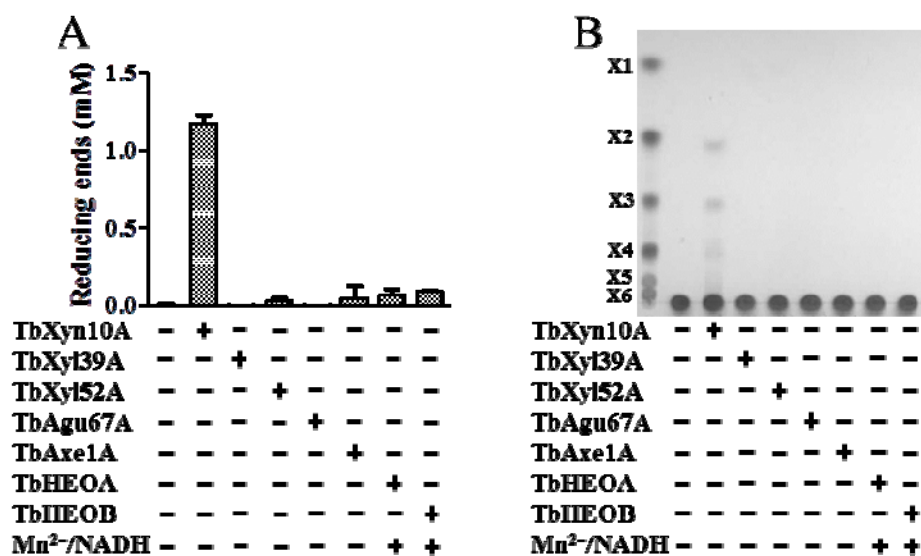
Supplemental Table S4. Conservations of two adjacent oxidoreductases (TbHEOA and TbHEOB) from *T. bryantii* in other bacteria.

Source		Predicted Function	Amino Acid Identities	
Strains	GenBank no.		TbHEOA	TbHEOB
<i>Thermoanaerobacterium thermosaccharolyticum</i> DSM 571	ADL68510.1	oxidoreductase domain protein	95%	/
	ADL68511.1		/	96%
<i>Thermoanaerobacterium xylanolyticum</i> LX-11	AEF17767.1	oxidoreductase domain protein	91%	/
	AEF17766.1		/	92%
<i>Thermoanaerobacterium saccharolyticum</i> JW/SL-YS485	AFK86456.1	oxidoreductase domain protein	90%	/
	AFK86457.1		/	92%
<i>Thermoanaerobacter italicus</i> Ab9	ADD01506.1	oxidoreductase domain protein	75%	/
	ADD01507.1		/	81%
<i>Thermoanaerobacter mathranii</i> subsp.	ADH60019.1	oxidoreductase domain protein	74%	/
	ADH60020.1		/	81%
<i>Paenibacillus</i> sp. oral taxon 786 str. D14	EES74063.1	oxidoreductase	63%	/
	EES74062.1		/	64%
<i>Paenibacillus</i> sp. JDR-2	ACT03815.1	oxidoreductase domain protein	63%	/
	ACT03814.1		/	63%
<i>Rahnella</i> sp. Y9602	ADW72798.1	oxidoreductase domain protein	60%	/
	ADW72799.1		/	64%
<i>Hafnia alvei</i> ATCC 51873	EHM43730.1	putative dehydrogenase	60%	/
	EHM43731.1		/	62%
<i>Enterobacteriaceae</i> bacterium 9_2_54FAA	EFV40373.1	oxidoreductase	60%	/
	EFV40372.1		/	62%
<i>Enterobacter cancerogenus</i> ATCC 35316	EFC53861.1	oxidoreductase	60%	/
	EFC53860.1		/	64%
<i>Enterobacter mori</i> LMG 25706	ZP_09036120.1	hypothetical protein	59%	/
	ZP_09036121.1	EmorL2_03392	/	65%
<i>Enterobacter hormaechei</i> ATCC 49162	EGK61318.1	oxidoreductase	58%	/
	EGK61319.1		/	65%
<i>Enterobacter cloacae</i> subsp. <i>cloacae</i> ATCC 13047	ADF60186.1	hypothetical protein ECL_00620	58%	/
	ADF60185.1		/	65%
<i>Enterobacter cloacae</i> EcWSU1	AEW71857.1	glucose--fructose oxidoreductase	58%	/
	AEW71856.1		/	64%
<i>Pantoea</i> sp. Sc1	EIB96373.1	oxidoreductase	57%	/
	EIB96374.1		/	63%
<i>Pantoea stewartii</i> subsp. <i>stewartii</i> DC283	EHU00915.1	oxidoreductase domain protein	56%	/
	EHU00916.1		/	62%
<i>Lachnospiraceae</i> bacterium 3_1_57FAA_CT1	EGN35098.1	hypothetical protein	53%	/
	EGN35097.1	HMPREF0994_04650	/	63%
<i>Clostridiales</i> bacterium 1_7_47FAA	EEQ58458.1	oxidoreductase domain-containing protein	48%	/
	EEQ58457.1		/	54%
<i>Clostridium citroniae</i> WAL-17108	EHE96106.1	hypothetical protein	48%	/
	EHE96107.1	HMPREF9469_04981	/	56%
<i>Kineococcus radiotolerans</i> SRS30216	ABS02075.1	Oxidoreductase domain	49%	/
	ABS02074.1		/	57%
<i>Xylanimonas cellulosilytica</i> DSM 15894	ACZ30672.1	oxidoreductase domain protein	49%	/
	ACZ30671.1		/	60%
<i>Clostridium phytofermentans</i> ISDg	ABX40986.1	oxidoreductase domain protein	46%	/
	ABX40987.1		/	57%
<i>Sphaerochaeta globus</i> str. Buddy	ADY11936.1	oxidoreductase domain protein	45%	/
	ADY11935.1		/	52%

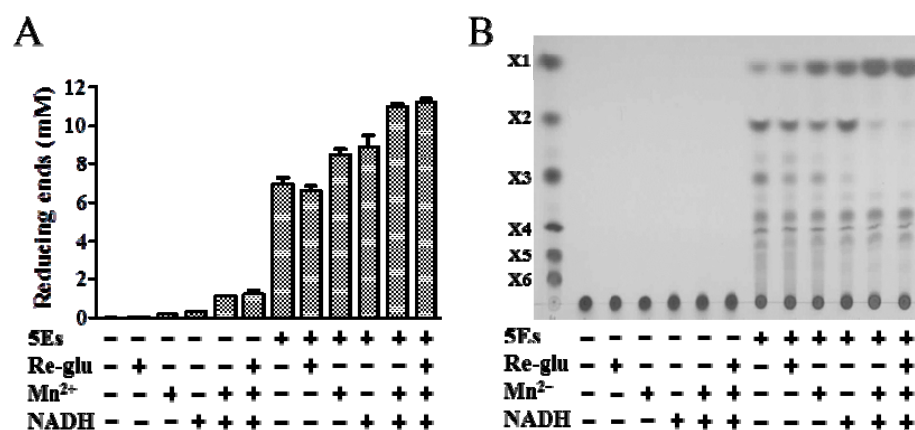
Supplemental Table S5. Nucleotide sequences of primers used for cloning of the genes in the hemicellulase gene cluster.

Gene	Primer direction	Primer sequences ^a
TbXyn10A	Forward	5'- <u>GACGACGACAAGAT</u> GCCTTCAAAGGGAAAAATG-3'
	Reverse	5'-GAGGAGAAGCCCGGTAAAAATCAACTATGCTGTAAAAG-3'
TbAgu67A	Forward	5'- <u>GACGACGACAAGAT</u> GTACGACTGCTGGCTTAGGTATAAG-3'
	Reverse	5'-GAGGAGAAGCCCGGTATTCATAAATTTTCCTCCATG-3'
TbHEOA	Forward	5'- <u>GACGACGACAAGAT</u> GATTAATATAGCCATTATTGGCGC-3'
	Reverse	5'-GAGGAGAAGCCCGGTACTTAATATCTCTTCCAAATG-3'
TbHEOB	Forward	5'- <u>GACGACGACAAGAT</u> GAGCAAAGAAAACGGCATGTAC-3'
	Reverse	5'-GAGGAGAAGCCCGGTCATTCTACCTTTATTGCATTTG-3'
TbXyl52A	Forward	5'- <u>GACGACGACAAGAT</u> GATAAGTAAATCTTTTATGCGC-3'
	Reverse	5'-GAGGAGAAGCCCGGTATTTTCATCCACAGTATGCTGG-3'
TbXyl39A	Forward	5'- <u>GACGACGACAAGAT</u> GATAAAAAATAAAGATACCAAAAAATTC-3'
	Reverse	5'-GAGGAGAAGCCCGGTCAATATCCGTTTATCTTGCTATC-3'
TbAxe1A	Forward	5'- <u>GACGACGACAAGAT</u> TGGGACTTTTTGATATGCCATTAC-3'
	Reverse	5'-GAGGAGAAGCCCGGTAAAGCTCCAGTAAAAATTGC-3'

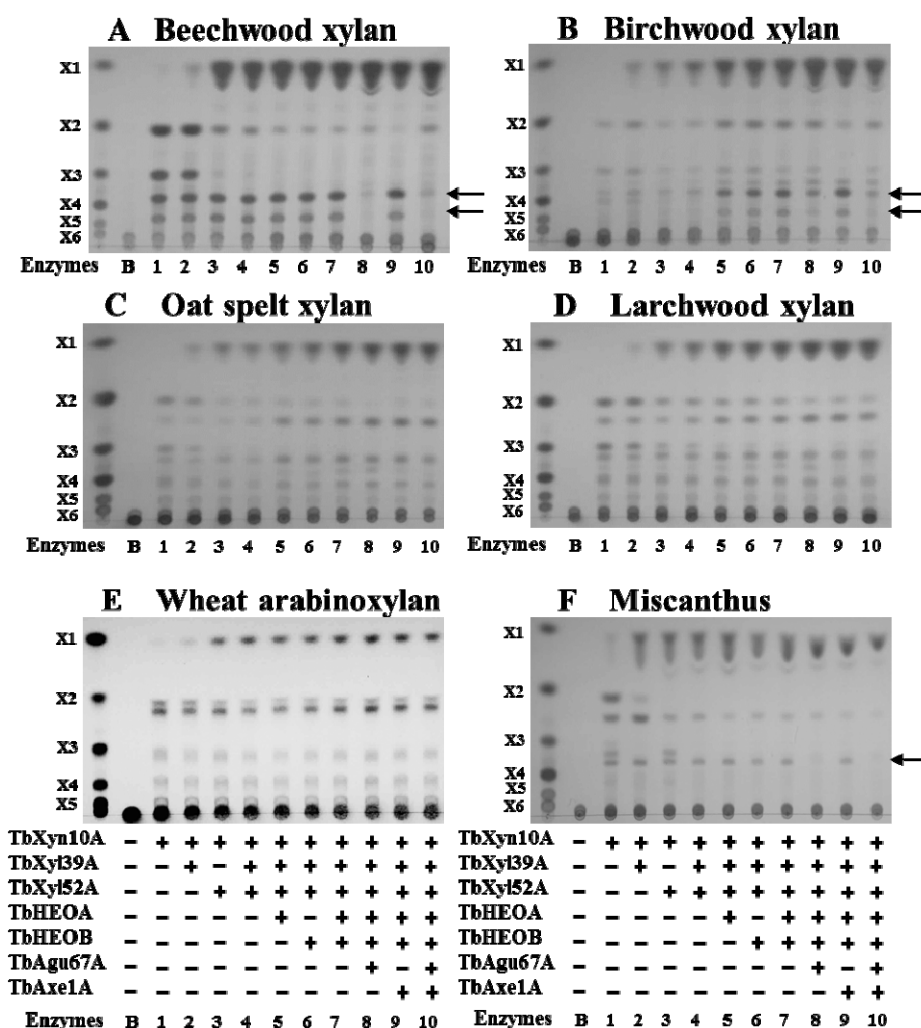
^a: Underlined nucleotides are added to facilitate ligation-independent cloning into a pET46-Ek/LIC vector.



Supplemental Figure S1. Hydrolysis of birchwood xylan (BWV) by a single enzyme (TbXyn10A, TbXyl39A, TbXyl52A, TbHEOA, TbHEOB, TbAgu67A or TbAxe1A) in the gene cluster as analyzed by reducing sugar assay (A) and TLC (B). Five mg/mL of BWV was incubated with each enzyme (10 nM TbXyn10A, 10 nM TbXyl39A, 5 nM TbXyl52A, 100 nM TbAgu67A, 100 nM TbAxe1A, 100 nM TbHEOA or 100 nM TbHEOB) at 60 °C in a citrate buffer (pH 5.5) for 16 h, in the presence or absence of cofactors (1 mM NADH and 1 mM MnSO₄).



Supplemental Figure S2. Effects of redox-active cofactors and Mn²⁺ ion on the capacity of two new oxidoreductase-like proteins (TbHEOA and TbHEOB) to enhance hydrolysis of BWX by three enzymes mixture composed of TbXyn10A, TbXyl39A, and TbXyl52A. The end products were analyzed by reducing sugar assay (A) and TLC (B). Five mg/mL BWX was incubated with and without five enzymes mixture (10 nM TbXyn10A, 10 nM TbXyl39A, 5 nM TbXyl52A, 50 nM TbHEOA, and 50 nM TbHEOB) at 60 °C in a citrate buffer (pH 5.5) in the presence or absence of cofactors (1 mM NADH, 1 mM Mn²⁺ or 0.5 mM Re-glu) for 20 h. 5Es: 5 enzymes mixtures (TbXyn10A, TbXyl39A, TbXyl52A, TbHEOA, and TbHEOB). Other abbreviations were as follows: X1: xylose; X2: xylobiose; X3: xylotriose; X4: xylotetraose; X5: xlopentaose; X6: xylohexaose.



Supplemental Figure S3. The synergistic effect of the seven enzymes encoded in the *T. bryantii* hemicellulase gene cluster during hydrolysis of heterogeneous xylans (A–E) and Miscanthus (F). The enzymatic reactions were carried out at 60 °C in a pH 5.5 citrate buffer supplemented with 1.0 mM NADH and 1.0 mM Mn²⁺. The substrates (BeeWX, BWX, LWX, and OSX) at 5 mg/mL were incubated with different combinations of the seven enzymes for 16 h. In the case of WAX, a lower concentration of substrate (2.5 mg/mL) was incubated with a lower concentration of enzymes (5 nM TbXyn10A, 5 nM TbXyl39A, 2.5 nM TbXyl52A, 50 nM TbAgu67A, 50 nM TbAxe1A, 25 nM TbHEOA, and 25 nM TbHEOB) for 16 h. Miscanthus was incubated at a higher concentration of substrate loading (10 mg/mL) with higher concentrations of enzymes (1 μM TbXyn10A, 0.5 μM TbXyl39A, 0.5 μM TbXyl52A, 2 μM TbHEOA, 2 μM TbHEOB, 2 μM TbAgu67A, and 2 μM TbAxe1A) for a longer period of 21 h. The end products were analyzed by TLC. (B): is a control of substrate without enzyme.

TbHEOA	MIN.....IAIIGAGNISSAHLQGFLEFKDRCKIVISDIYKDKAEKKRRFGLNDATVY	55
TbHEOB	MSKENGMYMPESRAKKVCS.....EGDFFFAAIGLDHGHYGMTKGLIEAGAEVKWVYDHDPEKVERFIKAFPIAK	72
GFO	ATLPAGASQVFTTFAGREMPFYAIREMPEDRRFGYAIVGLGKYALNQILPGFAGCQHSRIEALVSGNAEKAKIVAAEYGVDPKRIY	85
IDH	MSLR.....IGVIGTGAIGKEHINRITNKLSGAEIVVTDVNQEAAQKVVEQYQLN.ATVY	55
MocA	MTFRFR.....LGLVGAGRMGQVFEVR.....AAESSLVEIAAVADPIAASRLNLAGNGIK	50
Consensus		
TbHEOA	..SDYREILKREDVINDICTEPYTHADIAVESLNAGKNVIVKPMASLEECDRMIEASRKN.KKLLSVIAQNRFRTQFMKIKK	137
TbHEOB	KARDEDEILMNSVKLVASAAIPSECAIGLKAMDAGKDYFAKPEPMTTREQLEQAKDKVKKTKRKYAVVYGERLHNEASVYAGQ	157
GFO	DYSNFDKIAKDPKIDAYIILFNSLHAEFAIRAFKAGKHVMCKPMATSVADCCQRMIDAAKAA.NKKLMIGYRCHYDPMNRAAVK	169
IDH	..PNDDSLLADENVDALVTSWGPAAHESSVLKAIKCKYVFCBKPLATTAE.GCMRIVEEEIKVGKRLVQVGFMRRYDSGYVQLKE	138
MocA	TYETAGDMIEAGEVGLIATFSNTHVDTVADIAARGLPILOKPCGVTAFEARKAAVAERY.KVHLQIGYWRRFVPELKQLRD	134
Consensus v kp		
TbHEOA	IVESGLAGDIVHAQVDSFWWRGHSYDYLWWRGTWEKEGGCTLNHAIHHIDMLIWLIG.MFEEVQAVMNNVAHDN..AEVEDISI	219
TbHEOB	LVEKGATGRVIQVIGMGPHREKGRPDWFYE...KDKFGGILCIGSHQIEQFLYYTGAKDARVQSAKVANYNHKQYPTFEDFGD	239
GFO	LIRENQIKGLGMVITDNSDVMQNDPAQQWRLRRELAGGSLMDIGIYGLNGTRYLLGEEPIEVRAYTYS.DNDERFVEVEDRII	254
IDH	ALDNHVNGEPLMIHCAHRNPTVGDN.....YTTIMAVVDTLVHEIDVLHVLVN.DDYESVQVIYPKKSKNALPHLKDPQI	212
MocA	DIRAGLLGNLYLVSCFQWDEAPPAN.....SFRATGGGAFIDMGVHEFLQMRWLITQEETNFRVATSKTTFAGAVKGDPLAVQ	212
Consensus g d		
TbHEOA	AILKYKSGALTQITSSV.VHHGEEQQIILQGGKARISVFWKVVASTASSNGFFPSGRDEELEKKIQDYYDSLEETKYSGHTPCIDD	303
TbHEOB	VTLIGDNGATGYFRLDWFTPDGLG...TWGDGRFLILGADGYIELR.....KYIDVARENTTDHVYLANKDG	303
GFO	WQMRFRSGALSHGASSY.STTTTSRFSVQGGKAVLLMDPATGYYNLISVQTPGHAN.....QSMMPQFIMPANNQFSAQLDH	331
IDH	VVIETKGGIVINAEIYVNCKYGYDIQCEIVGEDGIKLEPSSISLRKEGRFS.....TDILMDWQRRFVAAYDVEIQD	286
MocA	LLCDLSGSSGLVSLGRRFPFGDACWTQVFGTSGFAEARFFWP.....PDGEAVFLNALQAQLED	272
Consensus g		
TbHEOA	VLKALESEHEIL.VKGS.DGRNALELITAIYKAATTREVVKLPINKDDPFYTVDGIMSSVPHFYERKTFVENFNDERITFGRDI	385
TbHEOB	EYHMDVKRGVGFPPFGELILDSINRTENAMTQEHIFKAIELALEAQTNAIKVE.....	356
GFO	LAEAVINNKPV.RFSGEEGMQDVRLIQIYEART...GRPVNTD.WGYVRQGGY.....	381
IDH	FIDSIQKKGEVSGPTAWDGYIAAVITDA CVKQESGQKEKVELKEKEPEFYQSFTTVQN.....	344
MocA	FVQAARGGAPRG.ATASDAVAALTIATATALLSR.....DINKAEGNNVR.....	317
Consensus		

Supplemental Figure S4. Multiple sequence alignment of TbHEOA and TbHEOB with three characterized Oxidoreductases. GFO (PDB accession number: 1OFG): a glucose-fructose oxidoreductase from *Zymomonas mobilis*; IDH: an inositol dehydrogenase from *Bacillus subtilis*; MocA: a rhizopine catabolism protein from *Rhizobium meliloti* L5-30. The conserved motif EKP is highlighted by rectangle.

AD76185.1/1-355	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
AEW71856.1/1-355	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EFC53660.1/1-355	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EGK61319.1/1-355	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
TP_09036120.1/1-355	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ETB96374.1/1-354	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EHU00916.1/1-354	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EHM43731.1/1-357	ACHTBASRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
AEF70372.1/1-357	ACHTBASRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ADW27299.1/1-355	ACHTBASRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ABS02074.1/1-362	AATDAGRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
AC230671.1/1-360	AATVTSRAALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EGN35097.1/1-359	ACVTSRAALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ACT03814.1/1-357	AATVTSRAALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
THE080/1-356	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ADL68511.1/1-356	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
AEF17766.1/1-356	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
AFK86455.1/1-356	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
AD015007.1/1-356	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ADH60020.1/1-356	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EES74602.1/1-360	ACVTSRAALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EH696107.1/1-359	ACVTSRAALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EES8457.1/1-363	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ABX40987.1/1-362	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
AEU11935.1/1-361	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
THE080/1-386	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ADL68510.1/1-386	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
AEF17767.1/1-386	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
AFK86456.1/1-386	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
AD015006.1/1-385	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ADH60019.1/1-385	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EES74603.1/1-383	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ACT03815.1/1-385	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EHM43730.1/1-397	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EFY40373.1/1-397	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ADW27298.1/1-391	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
TP_09036120.1/1-389	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ZF_006186.1/1-394	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
AEW71857.1/1-405	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EFC53661.1/1-404	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EHU66373.1/1-389	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EHU00915.1/1-389	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
EGN35098.1/1-387	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
ABS02075.1/1-391	AATPSSRCALCIKVMAGKQVDFDKAPITITLEOLADKAMAEKKCKKAYVYSERLIVSSAVAGLQVCAIGVYVITCTGPHRE-GTGRDNFYD	1889
AC230672.		

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ADF60185.1/1-355	-----	355
AEW71856.1/1-355	-----	355
EFC53860.1/1-355	-----	355
EGK61319.1/1-355	-----	355
ZP_09036121.1/1-355	-----	355
EIB96374.1/1-354	-----	354
EHU00916.1/1-354	-----	354
EHM43731.1/1-357	-----	357
EFV40372.1/1-357	-----	357
ADW72799.1/1-355	-----	355
ABS02074.1/1-362	-----	362
ACZ30671.1/1-360	-----	360
EGN35097.1/1-359	-----	359
ACT03814.1/1-357	-----	357
TbHEOB/1-356	-----	356
ADL68511.1/1-356	-----	356
AEF17766.1/1-356	-----	356
AFK86457.1/1-356	-----	356
ADD01507.1/1-356	-----	356
ADH60020.1/1-356	-----	356
EES74062.1/1-360	-----	360
EHE96107.1/1-359	-----	359
EEQ58457.1/1-363	-----	363
ABX40987.1/1-362	-----	362
ADY11935.1/1-361	-----	361
TbHEOA/1-386	HFYEKKTFVENFNDER-ITFGRDIK-----	386
ADL68510.1/1-386	HFYEKKTFVENFNDER-ITFGREIK-----	386
AEF17767.1/1-386	HFYEKKTFVENFNDER-ITFGRDIK-----	386
AFK86456.1/1-386	HFYEKKTFVENFNDER-ITFGREIK-----	386
ADD01506.1/1-385	YFHKKTFIENFDEN-ITFGRII-----	385
ADH60019.1/1-385	YFYKKTFIENFDEN-ITFGRII-----	385
EES74063.1/1-383	YFYKKTSIENFSDNT-ITSGGNS-----	383
ACT03815.1/1-385	HFYEKASVENFSDRA-ITGSGNLRG-----	385
EHM43730.1/1-397	HFYEKASVENFSDGEIPLGKMDKMSSEVSA	397
EFV40373.1/1-397	HFHEKASVENFSDGEIPLGKMDKMSSEVSA	397
ADW72798.1/1-391	HFYEKASVENFANEDAIPLGKNMDKGA-----	391
ZP_09036120.1/1-389	RFYEKASVANFSEVGAIPLGKDL-----	389
ADF60186.1/1-394	RFYEKASVANFSEVGAIPLGKDLDEGVTP-----	394
AEW71857.1/1-405	RFHEKASVANFSEVGAIPLGKDLDRGI-----	405
EFC53861.1/1-404	RFYEKSTSVANFSEVGAIPLGKDLDRGV-----	404
EIB96373.1/1-389	HFYEKQCSVENFADVDDIPLGKNFA-----	389
EHU00915.1/1-389	HFYEKQASVENFADEDAIPLGKNFA-----	389
EGN35098.1/1-387	HFYEKASVENFTSGE-ITVGNV-----	387
ABS02075.1/1-391	RFPAKSASVTDLAGSISVPGSGAGIGAGR-----	391
ACZ30672.1/1-388	RYFTKTGSVDE-----PGEPTYTYGGSPAPTF	388
EEQ58458.1/1-383	RFNEKKKSVENYADTG-IKVGCTL-----	383
ABX40986.1/1-383	KPNKTKSVENFQDIG-ISVGGTL-----	383
EHE96106.1/1-385	HFPEKTKSVENFEKVE-ITLGRDVCK-----	385
ADY11936.1/1-384	RFHTKGKSVDNFSTSK-ITLGRDFNA-----	384

Supplemental Figure S5. Multiple sequence alignment using Clustal Omega of the two HEOs from *T. bryantii* with putative functional homologs occurring as adjacent genes in other bacteria. GenBank accession numbers of the oxidoreductase-like proteins are the same as listed in Supplemental Table 5. The amino acids are shaded based on conservation. Residues with 50% or more identity in the aligned sequences are shaded in black. Amino acids with similar properties (grouped as GAVLI, FYW, CM, ST, KRH, DENQ, P) in 50% or more of the aligned sequences are shaded in grey.