

1 **Polyhydroxyalkanoate (PHA) production by thermophilic *Caldimonas thermodepolymerans***
2 **comb. nov. from xylan**

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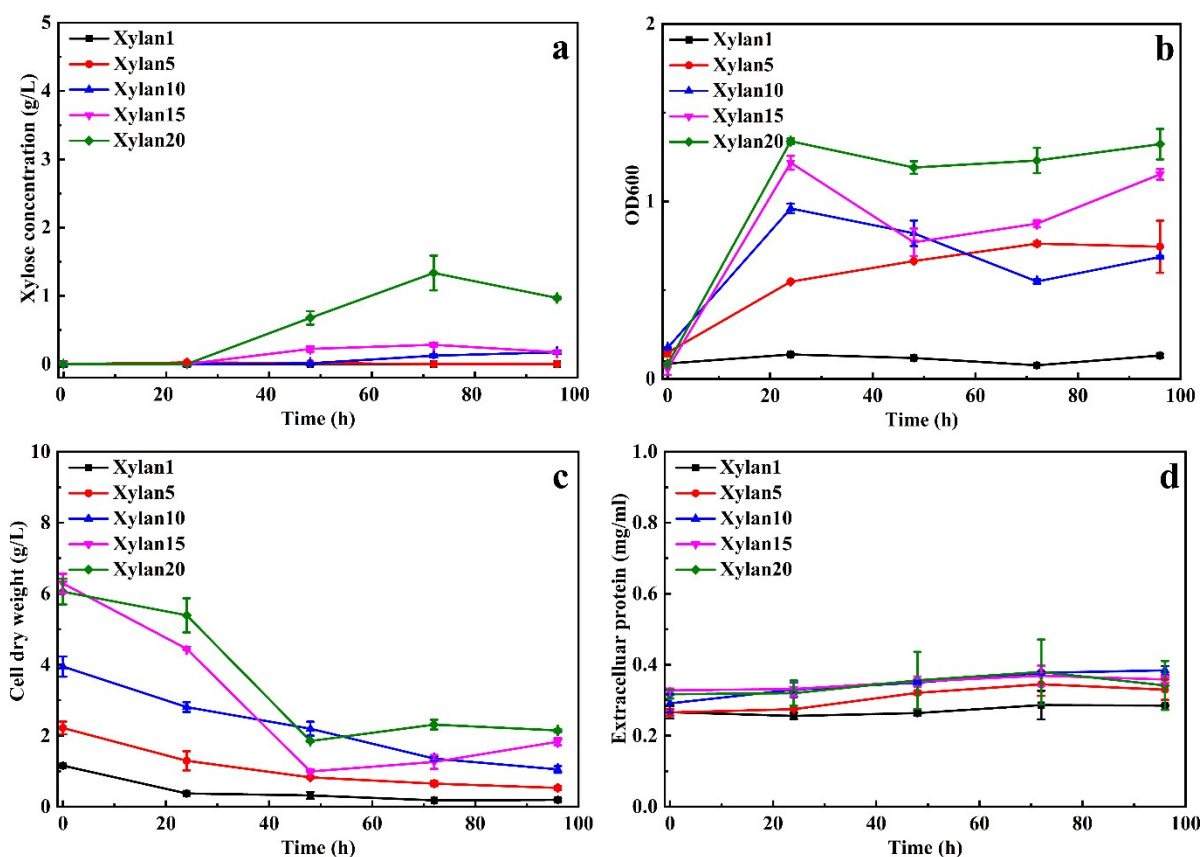
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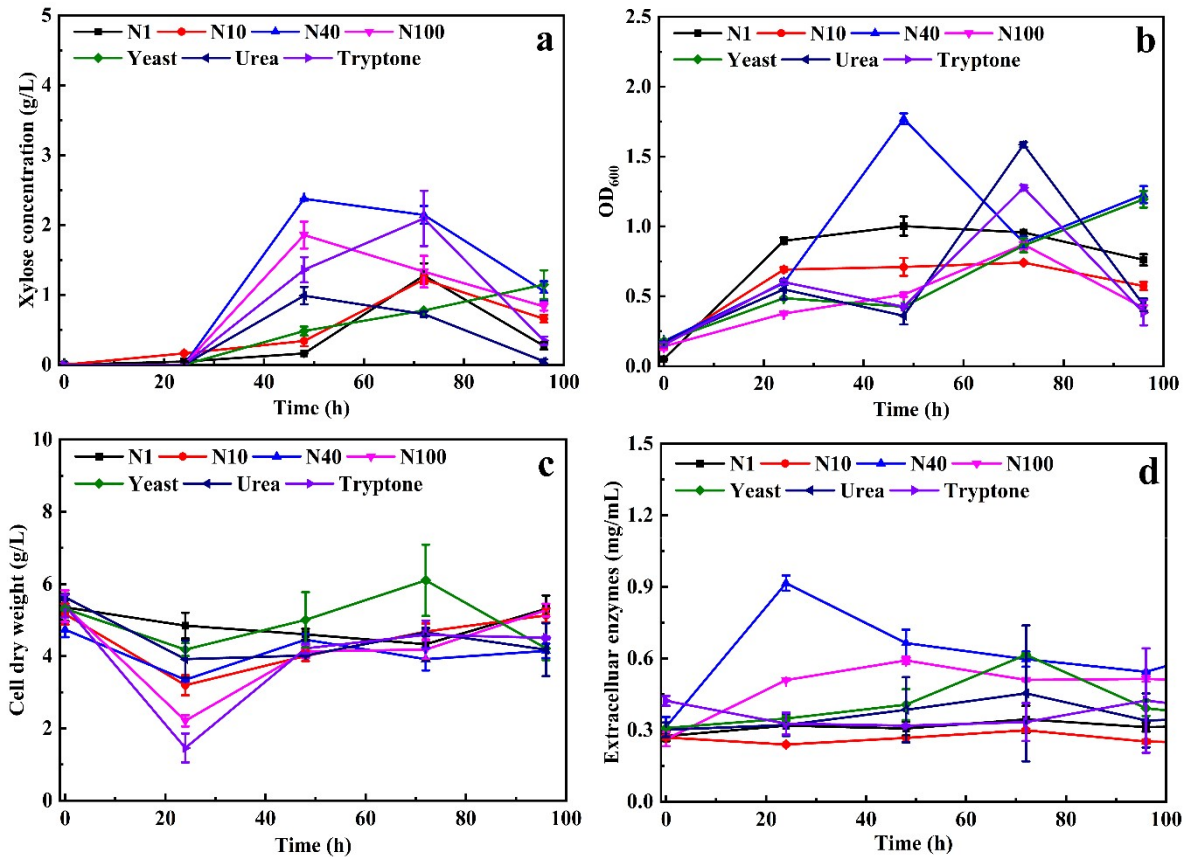
12 † These authors contributed equally to this work.

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15 **Figure S1.** The effect of xylan concentrations on xylose production and cell growth. (a) the production of xylose, (b)
16 OD₆₀₀ measurement of the cell growth, (c) the accumulation of the cell dry weight, and (d) extracellular protein
17 concentration. The basic growth conditions were 50 °C, 150 rpm, pH 7 and a C/N of 10. The errors presented in the
18 graphs represent the standard deviation (SD) of the data points, calculated from triplicate measurements of each sample.



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21 **Figure S2.** The effect of nitrogen sources and C/N ratios on xylose production and cell growth. (a) the production of
 22 xylose, (b) OD₆₀₀ measurement of the cell growth, (c) the accumulation of the cell dry weight, and (d) extracellular
 23 protein concentration. The basic growth conditions were 50 °C, 150 rpm, pH 7 and xylan concentration of 20 g/L. The
 24 errors presented in the graphs represent the standard deviation (SD) of the data points, calculated from triplicate
 25 measurements of each sample.

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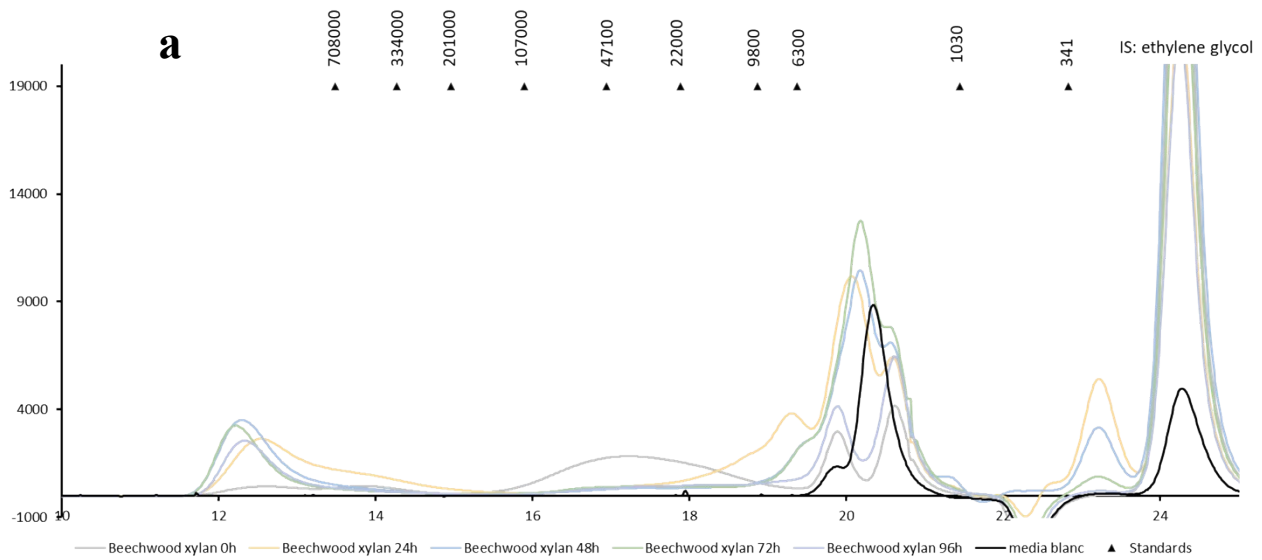
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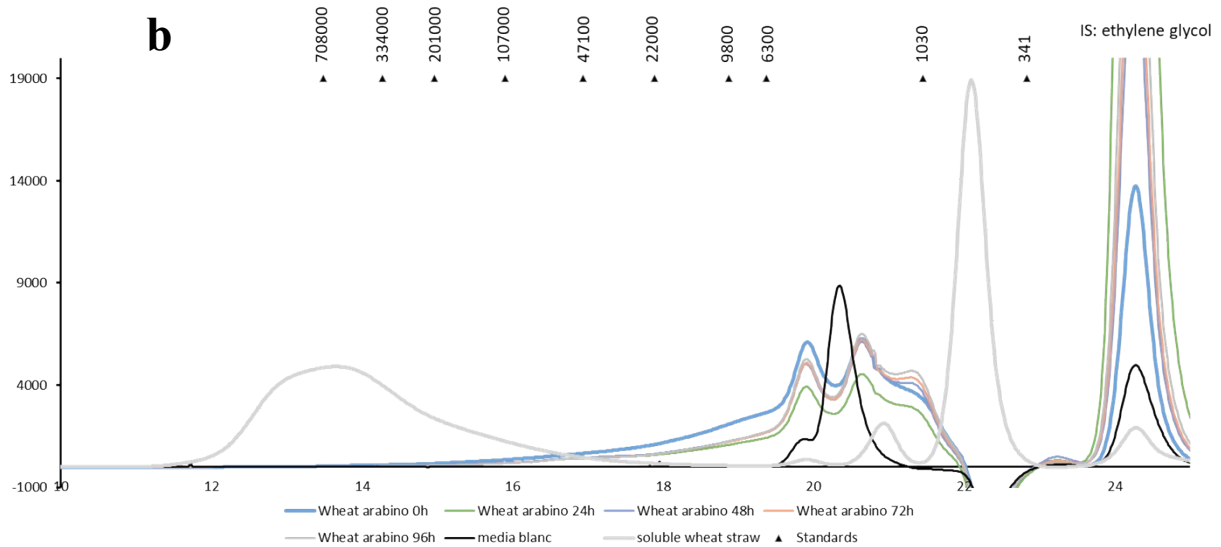
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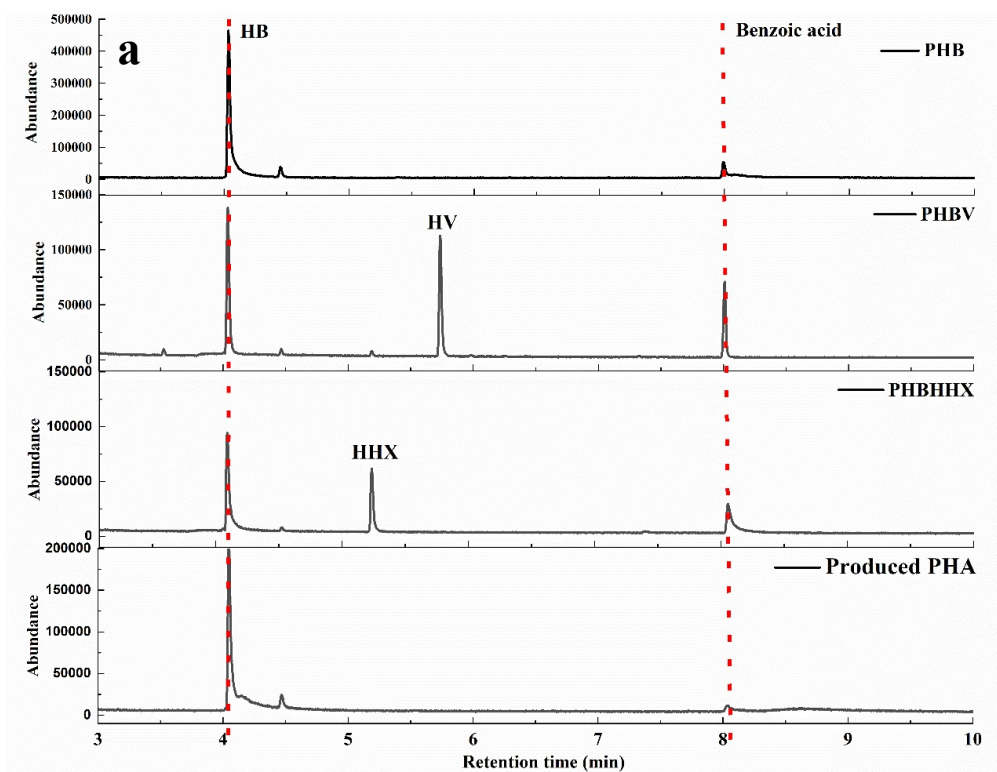
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37 **Figure S3.** The molecular weight distribution of xylans during polyhydroxyalkanoate (PHA) production process under
 38 the optimized initial conditions (20 g/L of xylan, a C/N ratio of 40, pH 7, and 50 °C). Beechwood xylan (a) and wheat
 39 arabinoxylan (b) as the sole carbon sources. The media blank is the mineral medium used for bacterial growth. The
 40 standards represent the retention time of each molecular weight of the 9 standards used. The molecular weight is
 41 expressed in Daltons.

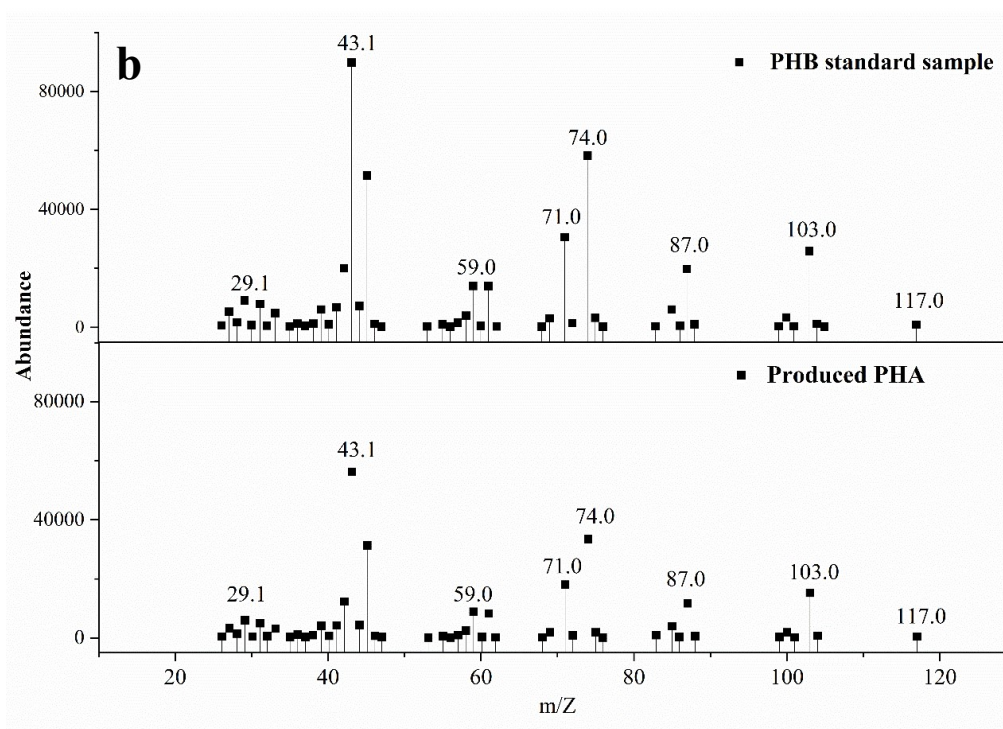
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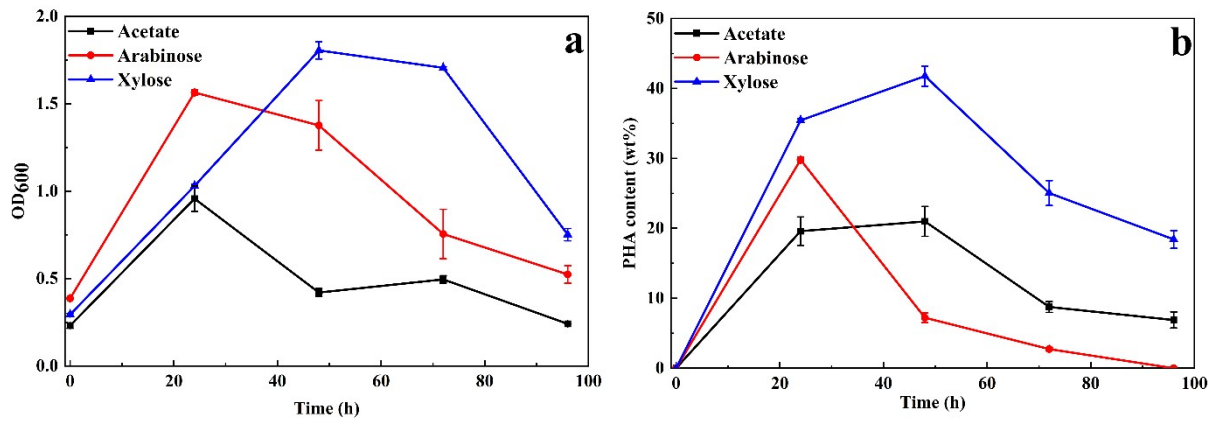
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47 **Figure S4.** Gas chromatogram (GC) of methanolized commercial poly(3-hydroxybutyrate) (P3HB), poly(3-
 48 hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), and poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) (PHBHHX), and
 49 polyhydroxyalkanoate (PHA) produced by *C. thermodepolymerans* from xylans (lower panel). (a) The GC peaks of PHB,
 50 PHBV, PHBHHX, and the produces PHA, (b). The mass pots of commercial PHB and hydroxyalkanoate (HA) produced
 51 by *C. thermodepolymerans*. Benzoic acid was added as an internal standard.

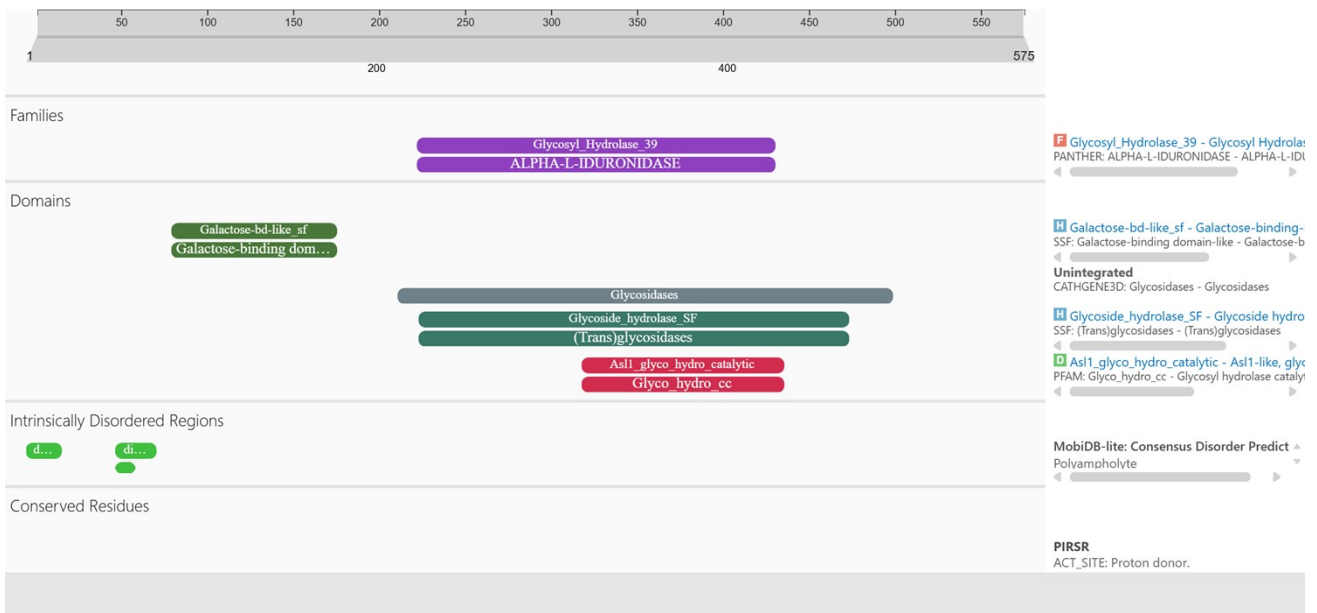
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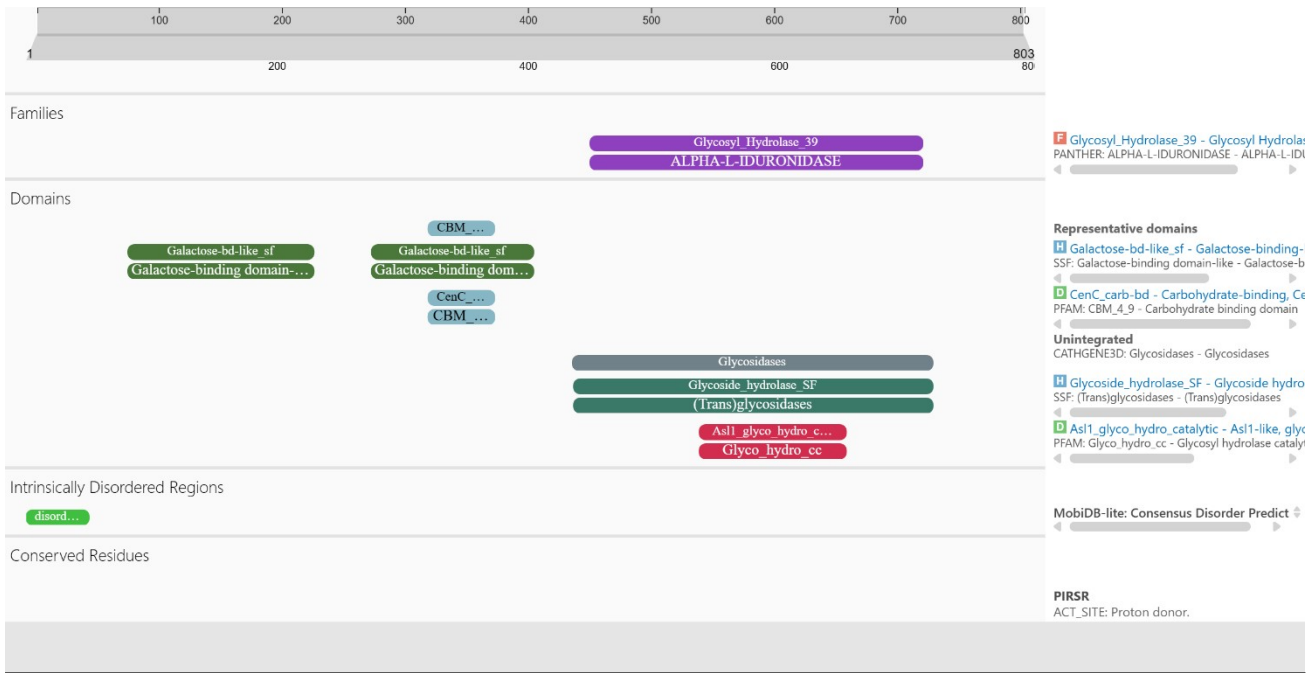
54 **Figure S5.** Cell growth (a) and PHA production (b) of *C. thermodepolymerans* using acetate, arabinose, or xylose as the
 55 sole carbon source (5 g/L). The errors presented in the graphs represent the standard deviation (SD) of the data points,
 56 calculated from triplicate measurements of each sample.

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59 **Figure S6.** InterPro search of the protein ST39S (QPC32540.1)



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Figure S7. InterPro search of the protein ST39L (QPC32539.1)

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Table S1. The predicted CAZymes that were obtained after a dbCAN with standard parameters search.

Gene ID	HMMER	dbCAN_sub	DIAMOND	SignalP
NZ_CP064338.1_1004	GT14(34-257)	GT14_e27	GT14	N
NZ_CP064338.1_1006	GT94(107-361)	GT94_e7	N	N
NZ_CP064338.1_1007	GT2(4-108)	GT2	GT2	N
NZ_CP064338.1_1009	GH39(487-674)	GH39_e38	GH39	Y(1-19)
NZ_CP064338.1_1010	GH39(274-473)	GH39_e85	GH39	Y(1-39)
NZ_CP064338.1_1011	GT4(219-354)	GT4_e1849	GT4	N
NZ_CP064338.1_1012	GT4(201-343)	GT4_e829	GT4	N
NZ_CP064338.1_1013	GT4(225-361)	GT4_e1696	GT4	N
NZ_CP064338.1_1014	GT26(51-222)	GT26_e98	GT26	N
NZ_CP064338.1_1072	GT4(180-331)	GT4_e2225	GT4	N
NZ_CP064338.1_1073	GT9(84-321)	GT9_e318	GT9	N
NZ_CP064338.1_1074	GT4(185-327)	GT4_e1924	GT4	N
NZ_CP064338.1_1075	CE4(66-204)	CE4_e274	N	N
NZ_CP064338.1_1091	GT4(214-375)	GT4_e171	GT4	N
NZ_CP064338.1_1094	GT9(86-335)	GT9_e182	GT9	N
NZ_CP064338.1_1096	GT2(63-187)	GT2	GT2	N
NZ_CP064338.1_1098	GT9(83-325)	GT9_e110	GT9	N
NZ_CP064338.1_1103	GT4(197-331)	GT4_e3402	GT4	N
NZ_CP064338.1_1106	GT4(196-334)	GT4_e1620	GT4	N
NZ_CP064338.1_1440	CE4(60-185)	CE4_e300	N	N
NZ_CP064338.1_1441	GT2(46-204)	GT2	GT2	N
NZ_CP064338.1_1442	GT4(218-369)	GT4_e3700	GT4	N

NZ_CP064338.1_1443	GT4(204-351)	GT4_e2314	GT4	N
NZ_CP064338.1_1445	GT4(216-350)	GT4_e1064	GT4	N
NZ_CP064338.1_1447	CE4(93-304)	CE4_e298	N	N
NZ_CP064338.1_1500	GT104(3-357)	GT104_e0	GT104	N
NZ_CP064338.1_1728	GT2(155-337)+GT2(250-463)	GT2	GT2	N
NZ_CP064338.1_1764	GT51(81-255)	GT51_e153	GT51	N
NZ_CP064338.1_1779	CBM48(21-111)+GH13_11(191-540)	CBM48_e3+GH13_e48	CBM48+GH13_11	N
NZ_CP064338.1_1781	GH13_16(49-402)	GH13_e0	GH13_16	N
NZ_CP064338.1_1782	GH13_3(239-460)	GH13_e41	GH13_3	N
NZ_CP064338.1_1783	CBM48(33-118)+GH13_9(182-482)	CBM48_e2+GH13_e200	CBM48+GH13_9	N
NZ_CP064338.1_1792	CBM48(14-105)+GH13_11(184-533)	CBM48_e3+GH13_e48	CBM48+GH13_11	N
NZ_CP064338.1_1793	GH13_10(121-435)	CBM48_e0+GH13_e120	CBM48+GH13_10	N
NZ_CP064338.1_1794	GH77(164-706)+GH13_26(750-1121)	GH77_e27+GH13_e105	GH13_26+GH77	N
NZ_CP064338.1_186	GH1(24-455)	GH1_e107	GH1	N
NZ_CP064338.1_1942	GT19(8-363)	GT19_e37	GT19	N
NZ_CP064338.1_2005	GH23(150-287)	GH23_e326	CBM50+GH23	Y(1-39)
NZ_CP064338.1_2010	GT51(56-223)	GT51_e71	GT51	N
NZ_CP064338.1_2263	GH103(52-348)	GH103_e12	GH103	Y(1-34)
NZ_CP064338.1_2306	GT30(41-216)	GT30_e26	GT30	N
NZ_CP064338.1_2484	AA6(4-202)	AA6_e3	N	N
NZ_CP064338.1_2517	GH23(147-248)	GH23_e807	GH23	N
NZ_CP064338.1_2622	GH23(174-282)	GH23_e526	GH23	Y(1-26)
NZ_CP064338.1_264	GT107(1-254)+GT107(239-591)	GT107_e0+GT107_e2	GT107	N
NZ_CP064338.1_265	GT2(14-288)	GT2	GT2	N
NZ_CP064338.1_2665	GH73(145-280)	GH73_e233	GH73	N
NZ_CP064338.1_2694	GT41(29-638)	GT41_e27	GT41	N
NZ_CP064338.1_2786	CE11(4-283)	CE11_e22	CE11	N
NZ_CP064338.1_2792	GT28(186-349)	GT28_e46	GT28	N
NZ_CP064338.1_2869	GT51(62-237)	GT51_e98	GT51	Y(1-47)
NZ_CP064338.1_3186	GH102(93-233)	GH102_e7	GH102	N
NZ_CP064338.1_3223	GT51(243-376)	GT51_e135	GT51	Y(1-29)
NZ_CP064338.1_3536	AA3_2(11-556)	AA3_e57	AA3	N
NZ_CP064338.1_3558	GH15(228-590)	GH15_e57	GH15	N
NZ_CP064338.1_3559	GT20(3-455)	GT20_e1	GT20	N
NZ_CP064338.1_3575	AA4(57-252)	AA4_e1	N	N
NZ_CP064338.1_436	GH23(507-633)	GH23_e952	GH23	Y(1-33)
NZ_CP064338.1_697	GT51(81-253)	GT51_e153	GT51	N
NZ_CP064338.1_770	GH23(97-194)	GH23_e653	GH23	N
NZ_CP064338.1_809	GH24(59-182)	GH24_e280	GH24	Y(1-21)

NZ_CP064338.1_857	GH78(370-598)	N	GH0	N
NZ_CP064338.1_858	GT4(168-302)	GT4_e907	GT4	N
NZ_CP064338.1_902	GT4(179-321)	GT4_e2553	GT4	N
NZ_CP064338.1_903	GT2(6-140)	GT2	GT2	N
NZ_CP064338.1_932	GH3(55-286)	GH3_e78	GH3	N
NZ_CP064338.1_989	GT41(26-564)	GT41_e36	GT41	N
NZ_CP064338.1_1099	N	GT4_e1925	GT4	N
NZ_CP064338.1_3213	N	AA2_e1	AA0	N
NZ_CP064338.1_427	N	CBM50_e780	CBM50	Y(1-38)
NZ_CP064338.1_969	N	CE4_e177	CE4	N
NZ_CP064338.1_1560	N	CBM50_e330	CBM50	Y(1-25)

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Table S2. Results of the blast against the Swissprot database of the protein QPC32539.1

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. ident
RecName: Full=Endo-1,4-beta-xylanase C; AltName: Full=Xylanase X34; Flags: Precursor	<i>Aspergillus nidulans</i> FGSC A4	48.1	48.1	16%	0.0002	32.37
RecName: Full=Endo-1,4-beta-xylanase; Full=FIA-xylanase;	<i>Aspergillus aculeatus</i>	45.1	45.1	16%	0.002	27.01
RecName: Full=Endo-1,4-beta-xylanase Full=Endo-1,4-beta-xylanase AltName: Full=TAXI	<i>Claviceps purpurea</i>	44.3	44.3	15%	0.003	31.54
RecName: Full=Endo-1,4-beta-xylanase Full=1,4-beta-D-xylan xylanohydrolase F3	<i>Thermoascus aurantiacus</i>	43.1	43.1	16%	0.006	28.57
RecName: Full=Endo-1,4-beta-xylanase Full=1,4-beta-D-xylan xylanohydrolase F3	<i>Aspergillus oryzae</i> RIB40	43.1	43.1	16%	0.006	26.62
RecName: Full=Alpha-L-iduronidase; Flags: Precursor	<i>Homo sapiens</i>	42.4	42.4	16%	0.017	27.27

RecName: Full=Alpha-L-
iduronidase; Flags: *Mus musculus* 41.6 41.6 16% 0.029 28.57
Precursor

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73 **Table S3.** Results of the blast against the PDB database of the protein QPC32539.1

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per.ident
Crystal Structure of xylanase (GH10) in complex with inhibitor (XIP)	<i>Aspergillus nidulans</i>	47.8	47.8	16%	0.00004	32.37
Ultra high resolution structure of a Thermostable Xylanase from <i>Thermoascus aurantiacus</i>	<i>Thermoascus aurantiacus</i>	43.5	43.5	16%	1	28.57
The xylanase TA from <i>Thermoascus aurantiacus</i> utilizes arabinose decorations of xylan as significant substrate specificity determinants	<i>Thermoascus aurantiacus</i>	43.5	43.5	16%	1	28.57
Structural studies on the mobility in the active site of the <i>Thermoascus aurantiacus</i> xylanase I	<i>Thermoascus aurantiacus</i>	43.5	43.5	16%	1	28.57
Thermostable xylanase I from <i>Thermoascus aurantiacus</i> - Crystal form II	<i>Thermoascus aurantiacus</i>	43.5	43.5	16%	1	28.57
Crystal structure of apo-iduronidase in the R3 form <i>Homo sapiens</i>	<i>Homo sapiens</i>	42.4	42.4	16%	3	27.27
New Irreversible α -L-Iduronidase Inhibitors and Activity-Based Probes	<i>Homo sapiens</i>	42.4	42.4	16%	4	27.27
Human α -L-iduronidase	<i>Homo sapiens</i>	42.4	42.4	16%	4	27.27
Crystal structure of human α -L-iduronidase complex with 5-fluoro- α -L-idopyranosyluronic acid fluoride	<i>Homo sapiens</i>	42.0	42.0	16%	4	27.27
High Resolution Crystal Structure Of A Thermostable Xylanase From <i>Thermoascus Aurantiacus</i>	<i>Thermoascus aurantiacus</i>	41.6	41.6	16%	4	28.68
Crystal Structure of an Inactive Kemp Elimination Design HG-1	<i>Thermoascus aurantiacus</i>	41.2	41.2	16%	6	25.53
Xylanase from <i>Penicillium simplicissimum</i> , complex with <i>simplicissimum</i> , complex with 1,2-(4-deoxy- β -L-thero-hex-4-enopyranosyluronic-acid)- β -1,4-xylotriose	<i>Penicillium simplicissimum</i>	39.3	39.3	18%	22	27.06

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75 **Table S4.** Proteomic analysis of total protein from the extracellular culture liquid at t=0 and after growth for 48 hours on
76 beechwood xylan or wheat arabinoxylan

Enzymes		0 h	Beechwood xylan-48 h	Wheat arabinoxylan- 48 h
Transporter	Transporter	0	17309.17	72317.352
	ABC transporter substrate-binding protein	139705.7	1125489	225303237
	ABC transporter ATP-binding protein	15830.6	222673	2258912.1
	ABC transporter permease	133517.8	482578.9	79575975
	ABC transporter	0	1208.621	1312726.8
	BMP family ABC transporter substrate-binding protein	554914.3	88489.13	5849114.3
	C4-dicarboxylate ABC transporter substrate-binding protein	0	255137.2	50154997
	C4-dicarboxylate ABC transporter	55756.83	286737.4	33042947
	C4-dicarboxylate ABC transporter permease	0	26155.35	0
	Amino acid/amide ABC transporter substrate-binding protein	1331315	538700.9	122887903
	Carbohydrate ABC transporter substrate-binding protein	30018.55	441776.9	14227445
	Branched-chain amino acid ABC transporter permease	0	12370.23	92559.859
	Ammonium transporter	0	21286.03	34918.34
	Efflux transporter periplasmic adaptor subunit	227388.3	1691.521	150210.5
	sugar transporter subunit IIA	0	21844.94	4161114.3
	LPS export ABC transporter permease	0	9241.949	0
	Lysophospholipid transporter	0	17614.61	0
	Sodium-independent anion transporter	0	20137.29	121645.81
	Fe (3+) ABC transporter substrate-binding protein	0	44726.75	528021.75
	Sulfate ABC transporter substrate-binding protein	0	38142.63	9300577
	Cobalamin ABC transporter ATP-binding protein	0	24699.37	144565.44
	Tripartite-type tricarboxylate transporter receptor subunit	37122.06	238572	84035509
	Tripartite tricarboxylate transporter substrate binding protein	0	8665.682	320510.13
	MFS transporter	0	3331.7	186677.02
	TRAP transporter permease	0	26275.34	53178.809
	Drug/metabolite transporter (DMT)-like permease	0	11521.64	0
	EmrB/QacA subfamily drug resistance transporter	0	2277.696	0
	AI-2E family transporter	0	29949.5	73228.438
	Efflux RND transporter periplasmic adaptor subunit	0	9253.369	118919.13
	Dipeptide ABC transporter ATP-binding protein	55375.15	0	0
	Membrane protein	Outer membrane protein	1281631	4347670
Putative membrane protein		235801.3	221017.9	1251239.4
Amino acid ABC transporter membrane protein		38465.6	188113.4	1698956.3
Putative tricarboxylic transport membrane protein		0	30200.94	19376066
Putative salt-induced outer membrane protein		0	5918.958	77274.719
Carbohydrate ABC transporter membrane	0	32688.42	0	

protein				
	Membrane protein	6725.177	10172.73	0
	Protein-export membrane protein SecE	0	11953.55	0
	Membrane protein insertase	0	4054.811	839012.19
	PEP-CTERM system-associated outer membrane protein	156642	89958.48	626162.06
	O-antigen/teichoic acid export membrane protein	0	5163.34	0
	Type VI secretion system membrane subunit	0	29144.91	209504.38
	Succinate dehydrogenase hydrophobic membrane anchor subunit	0	0	131542.56
	Protein-export membrane protein	0	14610.46	53715.914
	Putative RDD family membrane protein	0	4935.892	0
	NodT family efflux transporter outer membrane factor (OMF) lipoprotein	0	7608.985	83046.758
	Efflux pump membrane transporter	13771.63	33397.38	119916.85
	Uncharacterized membrane protein	0	7955.448	0
	Glucose-6-phosphate isomerase	0	11096.74	563280.25
	6-phosphofructokinase	0	16988.08	397465.69
	ADP-dependent glucokinase/phosphofructokinase	0	16988.08	397465.69
	Fructose-bisphosphate aldolase class Ia, DhnA family	10494.68	50971.35	8028198
	Triosephosphate isomerase	0	16549.23	2035152.8
Glycolysis	Glyceraldehyde-3-phosphate dehydrogenase/erythrose-4-phosphate dehydrogenase	617521.1	40543.21	16066581
	3-phosphoglycerate kinase	0	75889.34	35991748
	Phosphoglycerate mutase (BPG-dependent)	0	68539.97	5923951.5
	2,3-bisphosphoglycerate-independent phosphoglycerate mutase, archaeal type	0	68539.97	5923951.5
	Enolase	113322.1	32661.18	2205944.8
	Pyruvate kinase	0	4895.187	1751279.9
Entner-Doudoroff pathway	2-dehydro-3-deoxy-phosphogluconate aldolase	0	17298.79	1903680.1
	Glucose-6-phosphate 1-dehydrogenase	0	18456.01	739141.06
Pentose phosphate pathway	Ribose 5-phosphate isomerase	0	73970.46	4452923
	Transketolase	0	22956.66	693371.19
	Transaldolase/fructose-6-phosphate aldolase	8107.817	47216.61	4322302.5
	Acetyl-CoA carboxylase alpha subunit	0	15661.53	108470.2
	Biotin carboxyl carrier protein	0	0	379910.31
	Biotin carboxylase	0	26050.21	197681.47
	Acetyl-CoA carboxylase beta subunit	0	15063.8	266006.03
Fatty acid synthesis	Malonyl CoA-acyl carrier protein transacylase	0	229619.4	24986800
	3-oxoacyl-[acyl-carrier-protein] synthase III	0	26893.13	1412349.4
	NAD(P)-dependent dehydrogenase, short-chain alcohol dehydrogenase family	0	68868.23	16740414
	3-hydroxymyristoyl/3-hydroxydecanoyl-(acyl carrier protein) dehydratase	0	13599.37	5313491

	Enoyl-[acyl-carrier-protein] reductase FabI	0	33608.79	1070500
	3-oxoacyl-(acyl-carrier-protein) synthase	0	26893.13	1412349.4
	Acetyl-CoA acetyltransferase	0	9995.566	4880144.5
	3-hydroxyacyl-CoA dehydrogenase	0	90561.81	9627799.8
	Enoyl-CoA hydratase/carnithine racemase	0	93012.52	5296138.1
	Acyl-CoA dehydrogenase related to the alkylation response protein AidB	131242.5	1004792	32107102
	Acyl carrier protein	0	13599.37	5313491
	3-Ketoacyl CoA thiolase	0	0	654933.19
	D-xylose ABC transporter	0	643372	360684928
Xylose utilization	Xylose isomerase	0	29866.35	4176567
	Xylulose kinase	0	0	93138.625
	Glucose/arabinose dehydrogenase	0	0	244401.05
	Arabinose-5-phosphate isomerase	0	0	1233705
Arabinose utilization	Aldose 1-epimerase	0	35474.29	2920257.3
	Ribulose-phosphate 3-epimerase	138586.1	43139.48	1805026.1
	4-amino-4-deoxy-L-arabinose transferase-like glycosyltransferase	0	9186.413	17453.438
Xylan degradation	Carbohydrate binding protein (QPC32539)	0	0	9441.758
	Uncharacterized protein (QPC32540)	0	0	23396.098

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