Identification and characterization of novel cellulolytic and hemicellulolytic genes and enzymes derived from German grassland soil metagenomes

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Supplementary materials (Tables and Figures)

Contents

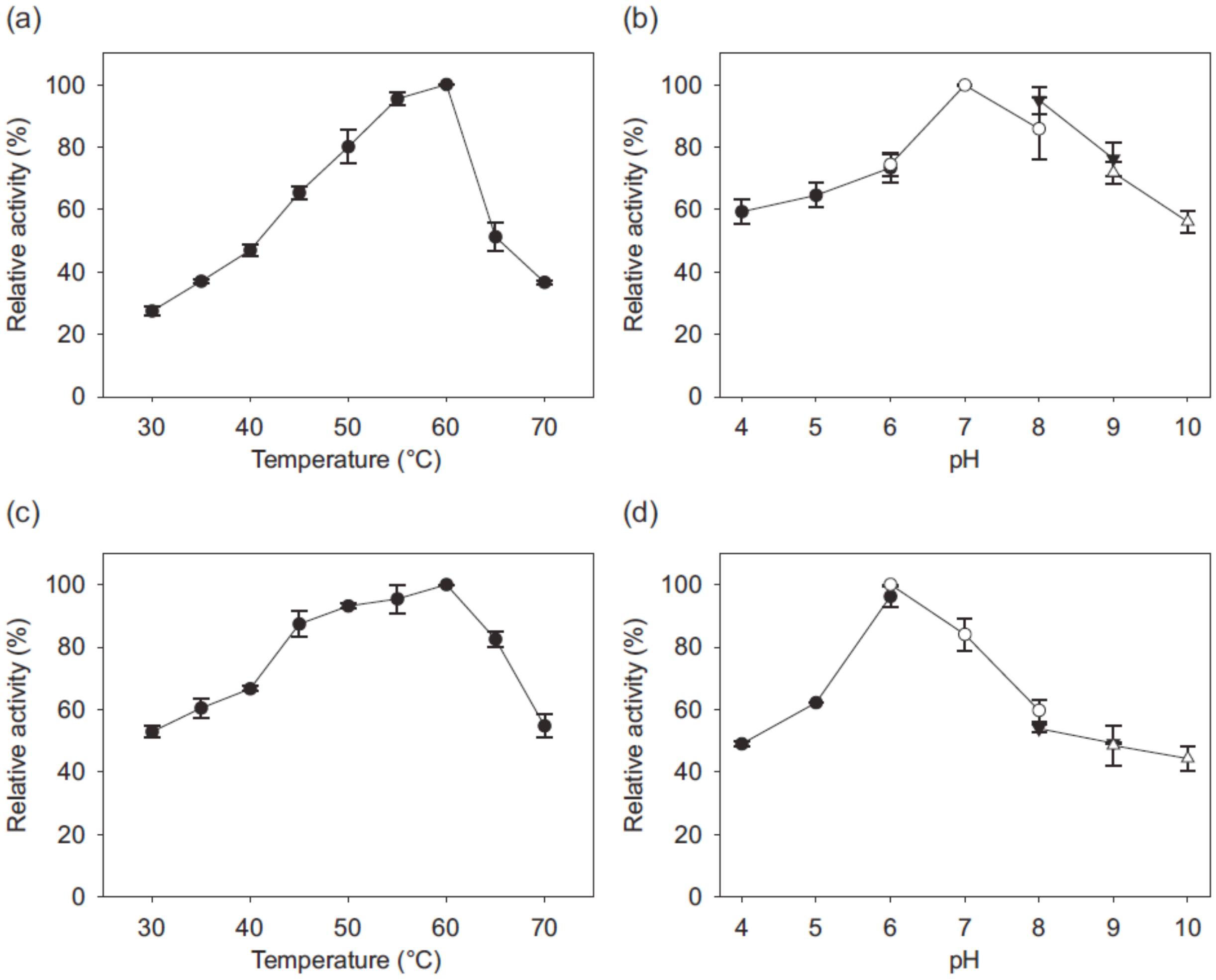

Fig. S1. Effects of temperature and pH on activity of Xyn01 and Xyn02. The effects were measured by using crude cell-free extracts prepared from *E. coli* TOP10 carrying pLX01 (Xyn01) or pLX02 (Xyn02). Activity was determined using the standard assay with xylan from birch wood as a substrate at temperatures between 30 and 70°C. The dependence of activity on pH was measured at pH values between 4 and 10 using sodium acetate buffer (black circles), sodium phosphate buffer (white circles), Tris-HCl buffer (black triangles), and glycine-NaOH buffer (white triangles). (a) Effect of temperature on activity of Xyn01, (b) effect of pH on the activity of Xyn01, (c) effect of temperature on activity of Xyn02, and (d) effect of pH on the activity of Xyn02. The average of triplicate experiments is presented.

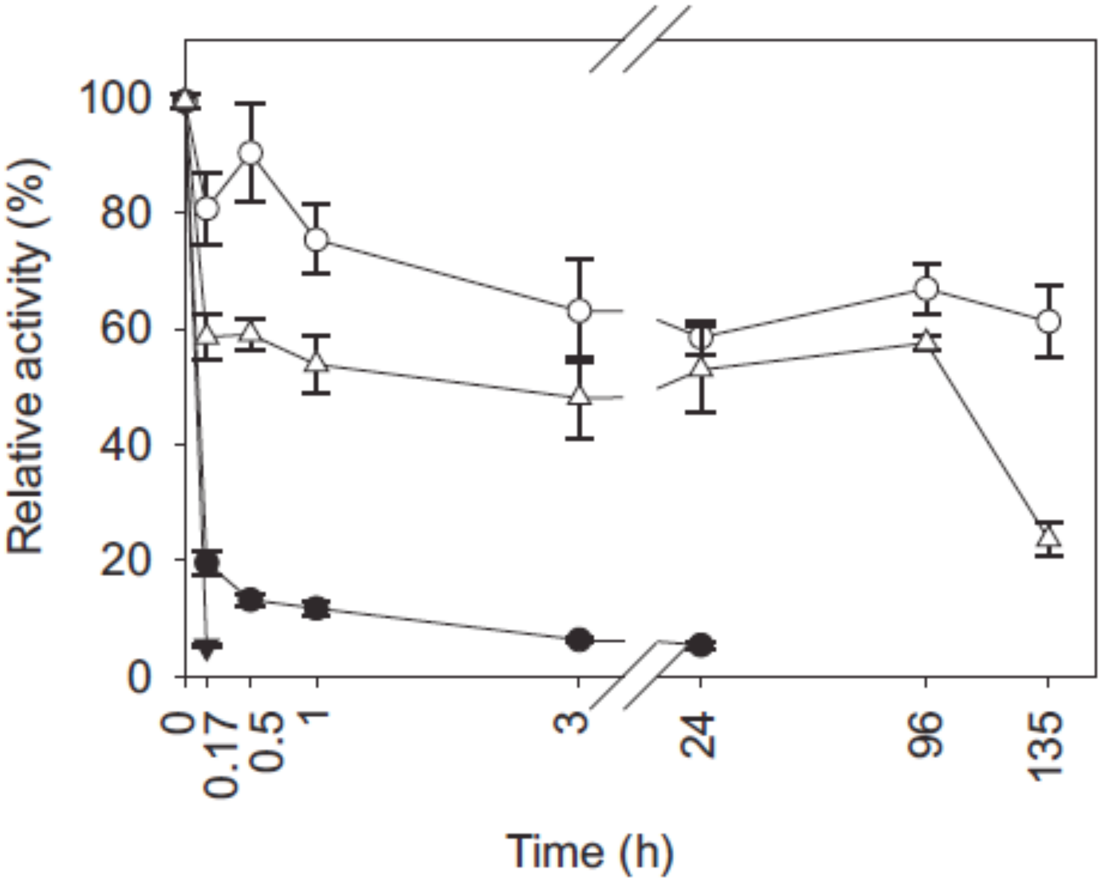

Fig. S2. Thermal stability of Cel01. The thermal stability of Cel01 was investigated after incubation of the enzyme at different temperatures (30°C, white circles; 40°C, white triangles; 50°C, black circles; 60°C, black triangles), and then measuring the residual activity with the standard assay. The average of triplicate experiments is presented.

Table S1. DNA yields and characteristics of the analyzed grassland soil samples.

Sample	DNA yield (µg/g soil)	Soil group	pH	OC[a] (g kg^{-1})	Total N (g kg^{-1})	C:N ratio	Gravimetric water content (%)
SEG9	53.6	Histosol	6.2	229.7	18.7	12.3	114.8
AEG3	25.3	Leptosol	6.3	53.7	5.2	10.4	57.2
AEG6	56.7	Leptosol	6.1	68.2	6.7	10.2	64.0

[a], Organic carbon content

Table S2. Open reading frames identified on pLC01, pLX01, and pLX02 and description of corresponding gene products and their observed sequence similarities.

Plasmid (accession no.)	ORF (Position)	No. of encoded amino acids	Closest similar protein, accession no. (no. of encoded amino acids), organism	E value	Amino acid homology to closest similar protein (% identity)
pLC01 (JF799947)	*cel01* (3138-643)	831	Cellulase, YP_001612873 (772), *Sorangium cellulosum* 'So ce 56'	7e-150	288/581 (50)
pLX01 (JF799945)	*xyn01* (2839-2327)	170	Xylanase, ABL11222 (226), uncultured bacterium	2e-71	132/171 (78)
	orf01 (3226-4524)	432	Transcriptional regulator of DeoR family, YP_004119403 (322), *Pantoea* sp. At-9b	0.001	62/249 (25)
pLX02 (JF799946)	*xyn02* (757-1524)	255	Xylanase, ABL11222 (226), uncultured bacterium	1e-69	140/203 (69)
	orf01 (1762-2961)	398	Ser/Thr protein phosphatase family protein, ZP_05031548 (321), *Brevundimonas* sp. BAL3	2e-29	88/277 (32)

Table S3. Properties of Cel01 and other soil metagenome-derived cellulases.

Cellulase	Source	No. of amino acids	GH family	CBM	Substrate profile	Temperature optimum (°C)	pH optimum	pH range[a]	Reference
Cel01	German grassland soil	831	9	+	β-1,4-linked glucans	50	7.0	5.0-9.0	This study
Umcel9B	Aerated compost soil	579	9	-	β-linked glucose	25	7.0	6.5-8.5	Pang et al. 2009
CelM2	Korean soil	662	44	-	β-linked glucose and xylose	45	4.0	3.5-5.5	Kim et al. 2008
Cel5G	South Chinese red soil	443	5	-	β-linked glucose and xylose	50	4.8	4.0-6.6	Liu et al. 2011
Cel5A	Enrichment culture of soil	363	5	-	β-1,4-linked glucans	45	6.5	5.5-9.0	Voget et al. 2006

[a], relative activity ≥40%; +, detected; -, not detected;; ND, not determined; GH, glycoside hydrolase; CBM, carbohydrate-binding module.

References

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