Supplemental Material

Applied Microbiology and Biotechnology

Clustered surface amino acid residues modulate the acid stability of GH10 xylanase in fungi

Yanwei Xia[1,#], Wei Wang[1,#], Yaning Wei[1], Chuanxu Guo[1], Sisi Song[1], Siqi Cai[1] and Youzhi Miao[1,*]

[1]Jiangsu Provincial Key Lab for Organic Solid Waste Utilization, National Engineering Research Center for Organic-based Fertilizers, Jiangsu Collaborative Innovation Center for Solid Organic Waste Resource Utilization, Nanjing Agricultural University, Nanjing, 210095, China.

[#]These authors contribute equally to this article.

[*]Address correspondence to: Youzhi Miao, yzmiao@njau.edu.cn

Mailing address: College of Resources & Environmental Science, Nanjing Agricultural University, 210095, Nanjing, China, Tel: 86-25-84396477; Fax: 86-25-84396260;

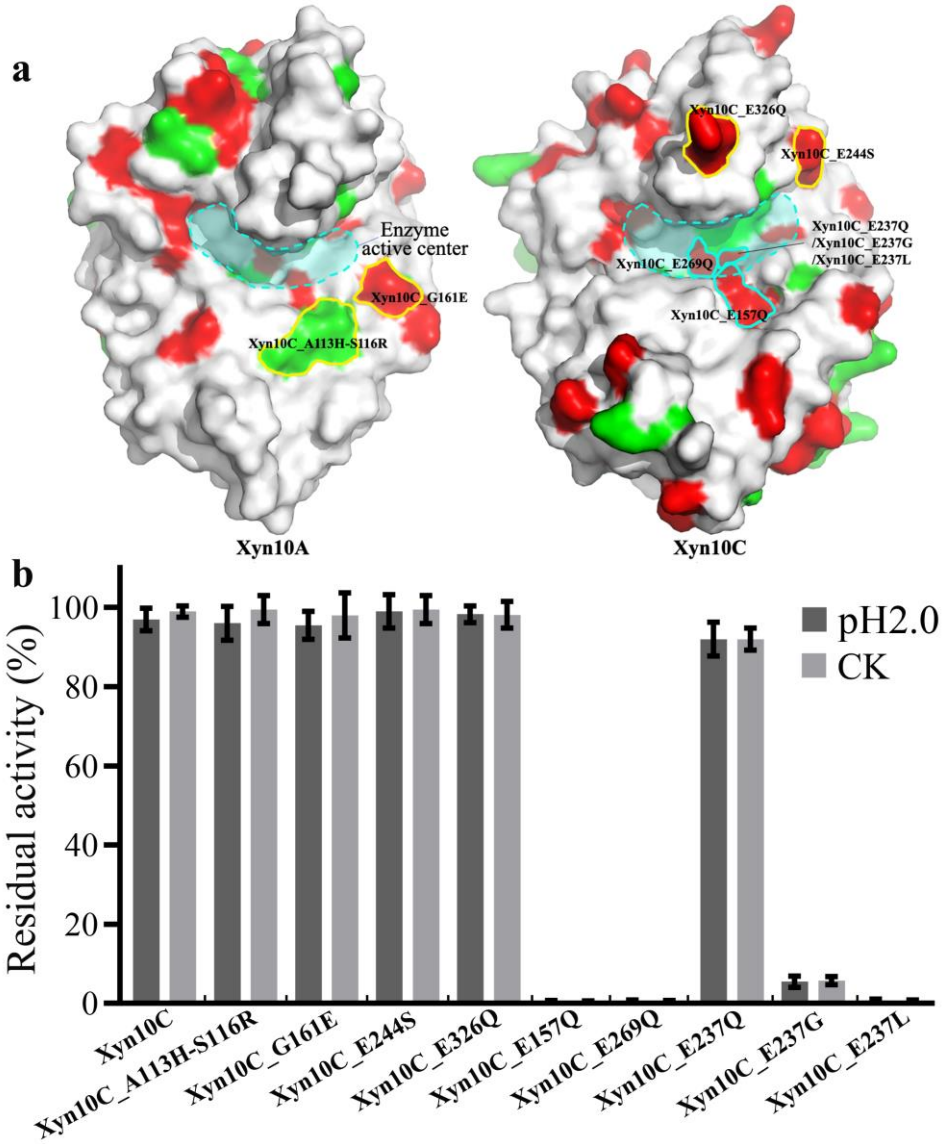

Fig. S1 Detecting the acid stability of Xyn10C and its different mutants at pH 2.0. The color of red and green, respectively, on the protein structures of Xyn10A and Xyn10C, corresponds to the acidic residues (D and E) and the basic residues (R, K and H) (**a**). By comparison, a total of 7 different residues located in or around the enzyme active center were used to construct 9 Xyn10C mutants (Xyn10C_A113H-S116R, Xyn10C_G161E, Xyn10C_E244S and Xyn10C_E326Q, Xyn10C_E237Q, Xyn10C_E237G, Xyn10C_E237L, Xyn10C_E157Q and Xyn10C_E269Q). The residual xylanase activity of Xyn10C and these mutants was detected at the optimal conditions after incubation at pH 2.0 for 1 h (**b**). CK means a direct detection without acid treatment.

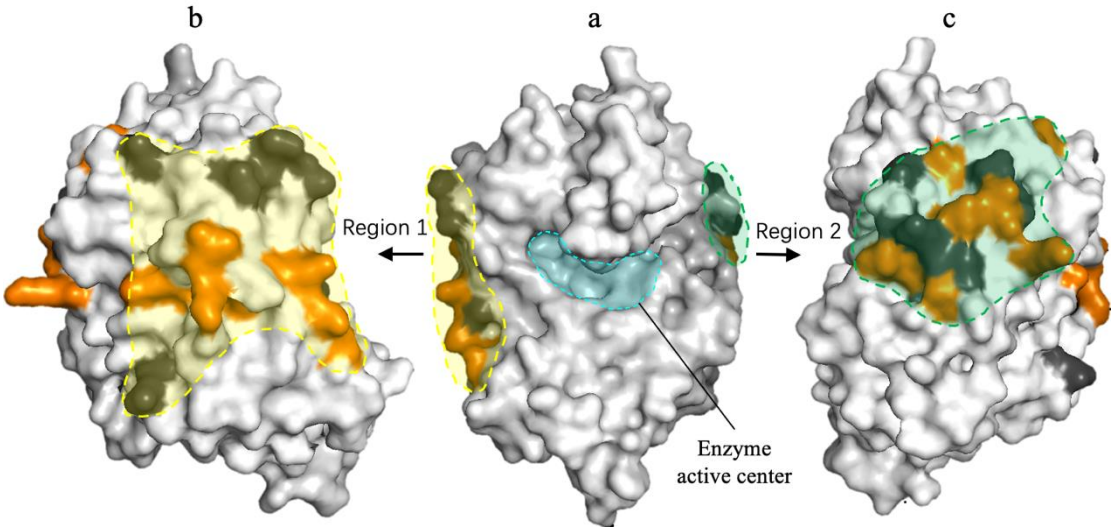

Fig. S2 Key amino acid residues influencing acid stability of Xyn10RE. A total of 34 amino acids, including all substitutions in the Xyn10RE_9, Xyn10RE_11, and Xyn10RE_12 mutants (refer to **Supplemental Table S3**), are predominantly located on the backside of the Xyn10RE's 3D structure, opposite the enzyme active center (**a**). These residues form two distinct areas: region 1 (**b**) and region 2 (**c**). The brown and grey colors in the figure represent the amino acid residues of Group A and Group B, respectively.

Table S1 The enzymatic properties of different GH10 xylanases.

		Xyn10A	Xyn10AR (EYE97139.1)	Xyn10AO (AFP43760.1)	Xyn10PR (CDM37719.1)	Xyn10PP (AVA16958.1)	Xyn10TC (BAN82655.1)	Xyn10RE (CAD34597.1)	Xyn10BS (ACS96449.1)	Xyn10PC (ACP27611.1)
Clusters		I	II	II	III	nc	IV	IV	IV	IV
Optimal Temperatures		90℃	80℃	80℃	80℃	75℃	70℃	80℃	85℃	70℃
Optimal pH		6.0	5.0	6.0	4.0	4.0	4.0	4.0	6.0	5.0
Specific activity ($U \cdot mg^{-1}$)		553.6±36.4	168.5±16.5	236.1±17.8	676.5±14.8	407.9±21.3	425.9±12.7	386.5±12.9	938.0±5.9	245.9±17.5
K_m value ($mg \cdot mL^{-1}$)		11.3±1.8	13.7±2.9	24.3±2.3	14.7±1.6	12.1±1.8	23.4±2.6	56.9±6.3	8.38±0.6	15.1±3.4
Residual activities after incubation at 70℃	10 min	87.8±3.6	73.8±1.5	83.2±3.2	71.9±0.9	79.1±3.2	36.1±7.8	92.2±1.4	90.9±2.5	11.2±0.9
	30 min	69.2±1.1	48.6±2.6	62.1±1.4	33.9±1.6	39.0±3.4	24.9±1.1	67.3±2.0	75.1±0.4	2.3±0.4
	1 h	37.1±2.8	21.3±0.7	30.9±0.3	10.3±0.6	11.2±0.6	10.4±5.7	44.6±0.8	67.7±0.7	0.0±0.0
	3 h	11.4±1.3	1.5±0.4	4.8±0.7	0.0±0.0	0.4±0.7	4.8±1.3	5.2±0.8	63.5±0.7	0.0±0.0
	6 h	1.3±0.7	0.0±0.0	0.7±0.4	0.0±0.0	0.0±0.0	1.0±0.5	1.2±0.2	52.1±0.4	0.0±0.0

For these enzymes, both optimal temperature and thermostability are assessed at each optimal pH, while optimal pH is determined at each optimal temperature. nc, no cluster.

Table S2 The distribution of π-π stacking types between GH10 xylanases in different clusters.

Clusters	Names	π-π stacking types								
		His-Phe	His-Trp	His-Tyr	Phe-Phe	Phe-Trp	Phe-Tyr	Trp-Trp	Trp-Tyr	Tyr-Tyr
I	Y699_04481 (**Xyn10A**)	2	4	0	0	4	3	3	3	3
	KEY79926.1	2	4	0	0	4	3	3	3	3
	XP_001258500.1	2	4	0	0	4	3	3	3	3
	GAQ02815.1	2	4	0	0	4	3	3	3	3
	XP_024683552.1	2	4	0	0	4	3	3	3	3
	GAO88483.1	2	4	0	0	4	3	3	3	3
	RLL99221.1	2	4	0	0	4	3	3	3	3
	RHZ56388.1	2	4	0	0	4	3	3	3	3
	XP_026615005.1	2	4	0	0	4	3	3	3	3
II	EYE97139.1 (**Xyn10AR**)	2	4	0	0	4	2	3	3	4
	ODM22525.1	2	4	0	0	4	2	3	3	4
	AFP43760.1 (**Xyn10AO**)	2	4	0	0	4	2	4	3	3
	XP_022484985.1	2	4	0	0	3	3	2	2	4
III	XP_022399347.1	2	4	0	0	4	3	3	3	4
	OQD79351.1	2	4	0	1	3	2	2	2	4
	OQE83864.1	1	4	1	0	3	2	2	2	5
	OQE17690.1	1	4	1	0	3	2	2	2	5
	OQE01923.1	1	4	1	0	3	2	2	2	5
	CRL21214.1	1	4	1	0	3	2	2	2	5
	XP_016600950.1	1	4	1	0	3	2	2	2	5
	CDM37719.1 (**Xyn10PR**)	1	4	1	0	3	2	2	2	5
	OQE05466.1	1	4	1	0	3	2	2	2	5
	OQE18216.1	1	4	1	0	4	1	3	3	5
IV	ACP27611.1 (**Xyn10PC**)	3	3	1	7	1	6	0	1	2

	Y699_06333 (**Xyn10C**)	3	3	0	6	2	6	0	1	2
	ACS96449.1 (**Xyn10BS**)	3	3	1	4	3	4	1	2	2
	BAN82655.1 (**Xyn10TC**)	1	4	1	1	3	3	1	2	7
	CAG25554.1	1	5	1	1	3	4	1	2	7
	CAD34597.1 (**Xyn10RE**)	2	4	0	0	4	2	3	3	4

Table S3 The substitution detail and enzymatic properties of all Xyn10RE mutants

Names	Substitution details	Specific activity ($U \cdot mg^{-1}$)	K_m value ($mg \cdot mL^{-1}$)	Optimal pH
Xyn10RE_1	I32K,T38S,S47T,T49S,E52V,T53A,N56S,Q59D,L64I,A67G,E78S,V81S,T83S,S85A,A86N,Q89A,I90V,A91V,K95N,A96K,M100L,L101M,N105T,Y109H,N110S,S114N,T117S,E124A,Q144K,D157E,Y161F,S163N,N164S,Y169I,E172P,N188D,A189V,P201S,L211I,L214M,V215I,Q216K,S217A,R221K,S229A,E235S,T239Q,S241D,Q242L,Q243T,Q244T,N245V,M246L,A247K,A248G,F249Y,I258Y,E269S,E271A,L273K,T275A,A278S,Y281F,S283G,T284V,V285A,Q286A,A289V,N290S,K292T,I297V,V299I,T312V,S314Q,D318A,A319P,C320L,A324E,Q327V,E333D,I335L,L336M,T337A	266.2±11.4	4.6±0.3	5.0
Xyn10RE_2	I32K,T38S,T83S,S85A,Q89A,A91V,K95N,A96K,M100L,L101M,N105T,T117S,Q144K,Y169I,P201S,L214M,S229A,Q242L,Q243T,N245V,M246L,A248G,I258Y,L273K,Y281F,S283G,T284V,V285A,N290S,K292T,T312V,S314Q,A319P,C320L,I335L,L336M	256.3±12.0	7.9±1.5	5.0
Xyn10RE_3	32K,T38S,S47T,T49S,E52V,T53A,N56S,Q59D,L64I,A67G,E78S,V81S,T83S,S85A,A86N,Q89A,I90V,A91V,K95N,A96K,M100L,L101M,N105T,Y109H,N110S,S114N,T117S,E124A,Q144K,D157E,Y161F,S163N,N164S,Y169I,E172P,N188D,A189V	226.4±5.4	2.7±0.1	5.0
Xyn10RE_4	P201S,L211I,L214M,V215I,Q216K,S217A,R221K,S229A,E235S,T239Q,S241D,Q242L,Q243T,Q244T,N245V,M246L,A247K,A248G,F249Y,I258Y,E269S,E271A,L273K,T275A,A278S,Y281F,S283G,T284V,V285A,Q286A,A289V,N290S,K292T,I297V,V299I,T312V,S314Q,D318A,A319P,C320L,A324E,Q327V,E333D,I335L,L336M,T337A	429.8±16.2	5.1±0.5	5.0
Xyn10RE_5	S47T,T49S,E52V,T53A,N56S,Q59D,L64I,A67G,E78S,V81S,A86N,I90V,Y109H,N110S,S114N,E124A,D157E,Y161F,S163N,N164S,E172P,N188D,A189V,L211I,V215I,Q216K,S217A,R221K,E235S,T239Q,S241	478.7±17.8	33.8±5.8	6.0

	D,Q244T,A247K,F249Y,E269S,E271A,T275A,A278S,Q286A,A289V,I297V,V299I,D318A,A324E,Q327V,E333D,T337A			
Xyn10RE_6	I32K,T38S,T83S,S85A,Q89A,A91V,K95N,A96K,M100L,L101M,N105T,T117S,Q144K,Y169I	290.1±11.2	29.6±5.4	4.0
Xyn10RE_7	P201S,L214M,S229A,Q242L,Q243T,N245V,M246L,A248G,I258Y,L273K,Y281F,S283G,T284V,V285A,N290S,K292T,T312V,S314Q,A319P,C320L,I335L,L336M	180.9±7.3	25.1±3.2	4.0
Xyn10RE_8	T83S,S85A,Q89A,A91V,K95N,A96K,M100L,L101M	421.8±10.5	18.7±4.1	4.0
Xyn10RE_9	P201S,Q243T,N245V,M246L,A248G,S283G,N290S,K292T,L336M	335.0±6.8	8.6±1.8	5.0
Xyn10RE_10	T83S,S85A,Q89A,A91V,K95N,A96K,M100L,L101M,P201S,Q243T,N245V,M246L,A248G,S283G,N290S,K292T,L336M	342.2±11.9	26.9±1.9	4.0
Xyn10RE_11	S47T,T49S,E52V,T53A,N56S,Q59D,T83S,S85A,A86N,Q89A,I90V,A91V,K95N,A96K,M100L,L101M,N188D,A189V	260.5±6.6	21.7±5.2	4.0
Xyn10RE_12	T239Q,S241D,Q244T,A247K,F249Y,Q286A,A289V	277.3±3.5	28.1±4.3	4.0
Xyn10RE_13	A67G,E78S,V81S,N105T,Y109H,N110S,S114N,T117S,E124A,D157E,Y161F,S163N,N164S,Y169I,E172P	223.8±3.6	47.2±6.5	4.0
Xyn10RE_14	L214M,V215I,Q216K,S217A,R221K,S229A,I258Y,E269S,E271A,L273K,T275A,A278S,T312V,S314Q,D318A,A319P,C320L,A324E,Q327V,E333D,I335L	924.9±37.8	7.1±0.2	6.0

Table S4 The changes of non-covalent interactions in Xyn10RE and its mutants.

	Hydrogen bonds	π-π Stack	π-Cation	Ionic bonds	Disulphide bonds	Van der Waals forces
Xyn10RE	295	22	3	5	2	330
Xyn10RE_1	292	22	3	6	2	327
Xyn10RE_2	289	22	3	6	2	328
Xyn10RE_3	293	22	3	6	2	313
Xyn10RE_4	293	22	3	5	2	332
Xyn10RE_5	293	22	3	6	2	330
Xyn10RE_6	294	22	3	5	2	317
Xyn10RE_7	292	22	3	5	2	337
Xyn10RE_8	294	22	3	5	2	331
Xyn10RE_9	294	22	3	5	2	327
Xyn10RE_10	293	22	3	5	2	328
Xyn10RE_11	294	22	3	5	2	330
Xyn10RE_12	294	22	3	6	2	323
Xyn10RE_13	294	22	3	5	2	334

Xyn10RE_14	294	22	3	5	2	327