

Supplementary material

# Comparative analysis and biochemical characterization of two endo- $\beta$ -1,3-glucanases from the thermophilic bacterium *Fervidobacterium* sp.

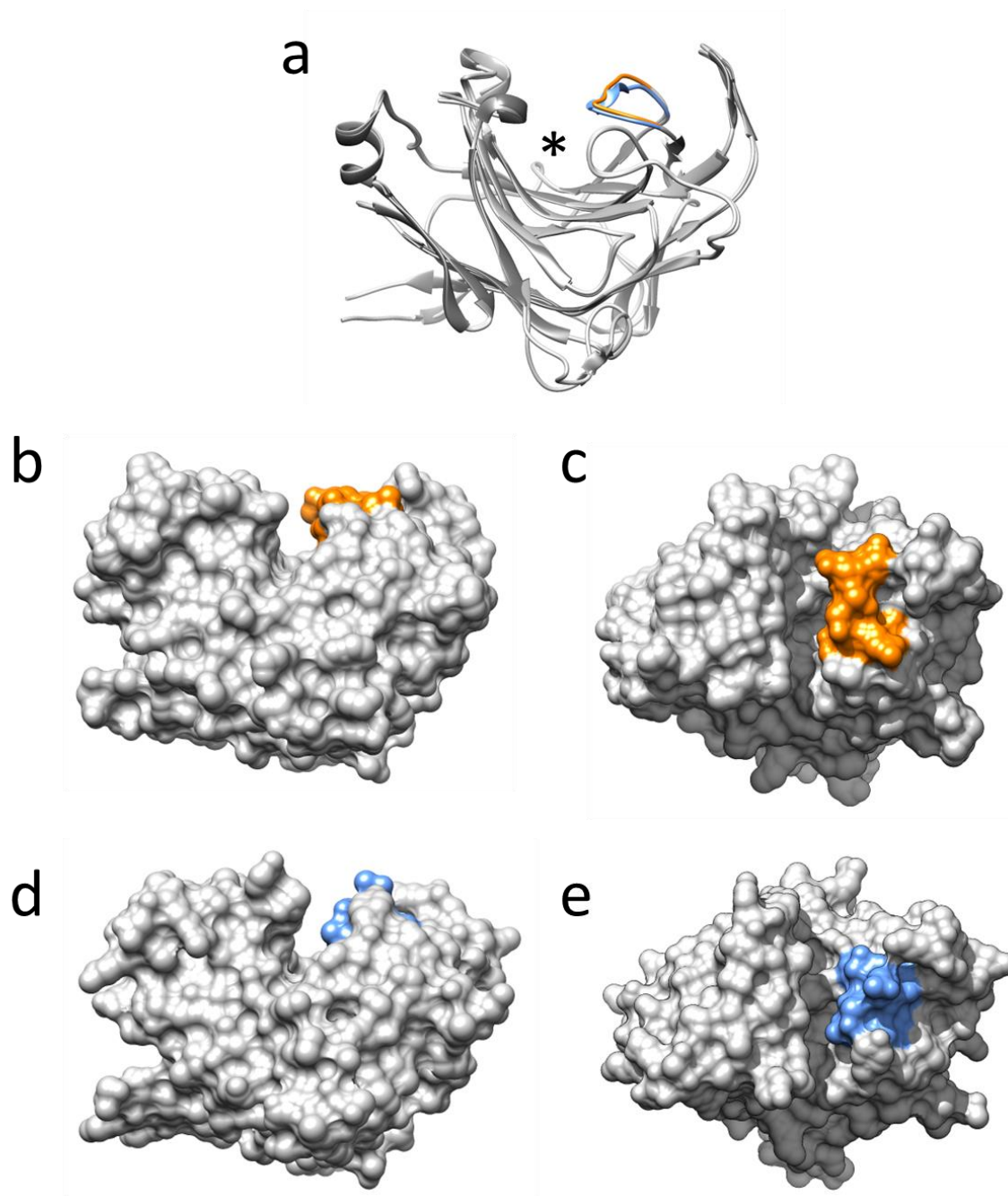
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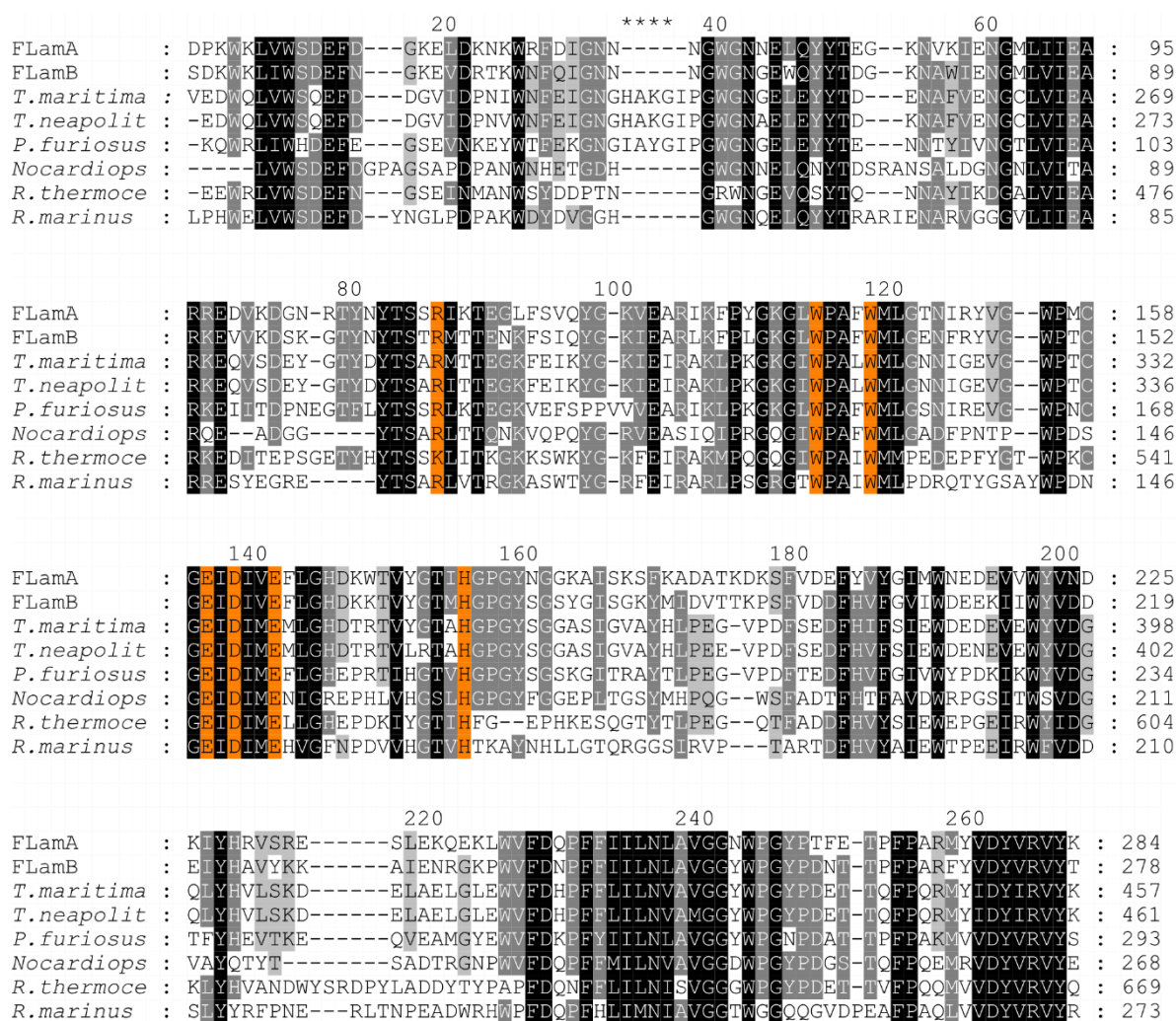
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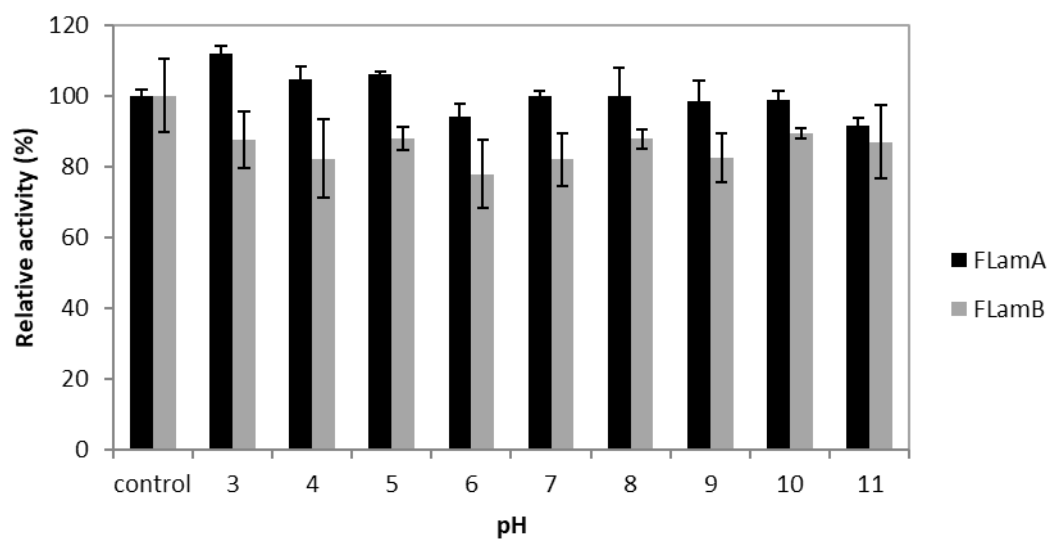
**Figure S1.** Structural models of FLamA and FLamB. Models based on the catalytic residue of *T. maritima* (PDB ID: 3azx). (a) Overlay of fold predictions of both proteins. The loops, possibly cause structural differences next to the catalytic cleft (\*), are indicated in orange (FLamA) and blue (FLamB). (b,c) Side and top view of the surface of FLamA with the catalytic cleft. (d,e) Side and top view of the surface of FLamB with the catalytic cleft which seems to be upward more open.

**Table S1.** Purification of the recombinant FLamA and FLamB from *E. coli*.

Protein	Purification step	Total protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification (fold)
<b>FLamA</b>	Crude extract	672.67	2083	3	100	1
	Ni-NTA	3.30	651	197	42	71
	Superose 12	1.12	617	552	30	178
<b>FLamB</b>	Crude extract	466.99	676	1	100	1
	Ni-NTA	3.59	464	130	69	90
	Superose 12	0.14	119	858	18	593



**Figure S2.** Amino acid alignment of the catalytic domains of FLamA and FLamB with other members of GH 16. Conserved residues are highlighted in black (100 %) dark grey (80 %) and light grey (60 %). Active sites are indicated in orange. The asterisk region corresponds to amino acid residues in the kink which possibly generate higher preference for mixed-linked glucans. The sequences used are listed below: *Thermotoga maritima* MSB8 laminarinase (AAD35118.1), *Thermotoga neapolitana* laminarinase LamA (CAA88008.1), *Pyrococcus furiosus* DSM3638 endo-beta-1,3-glucanase (AAC25554.2), *Nocardioopsis* sp. F96 beta-1,3-glucanase BglF (BAE54302.1), *Ruminiclostridium thermocellum* endo-1,3(4)-beta-glucanase Lic16a (CAC27412.2), *Rhodothermus marinus* laminarinase LamR (AAC69707.1).



**Figure S3.** Laminarinase activity of FLamA and FLamB after incubation at pH 3-11 and 4 °C for 24 h. Activity before incubation was defined as 100 %.