Supplementary material to the manuscript

Non-specific GH30_7 endo-β-1,4-xylanase from *Talaromyces leycettanus*

authored by

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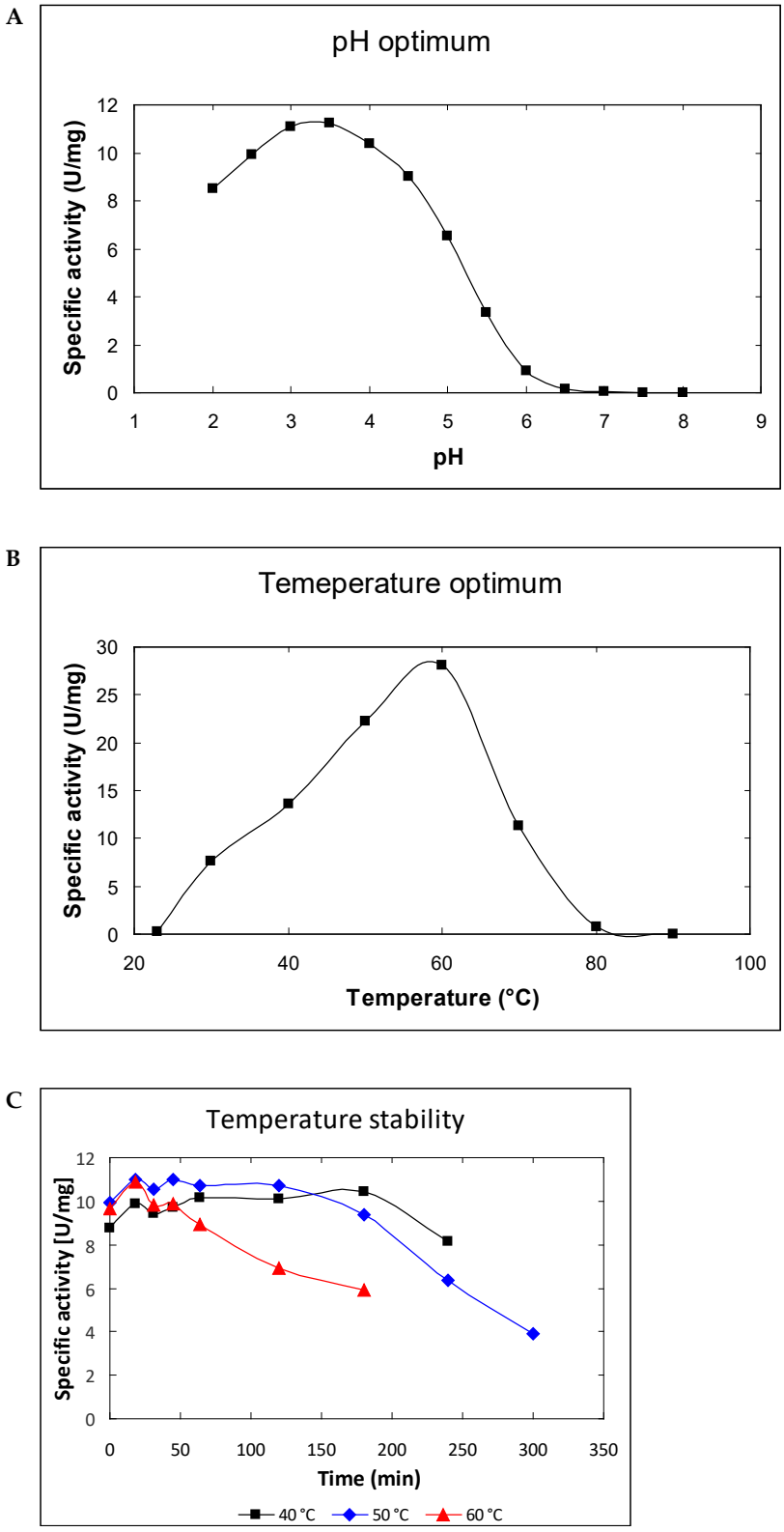
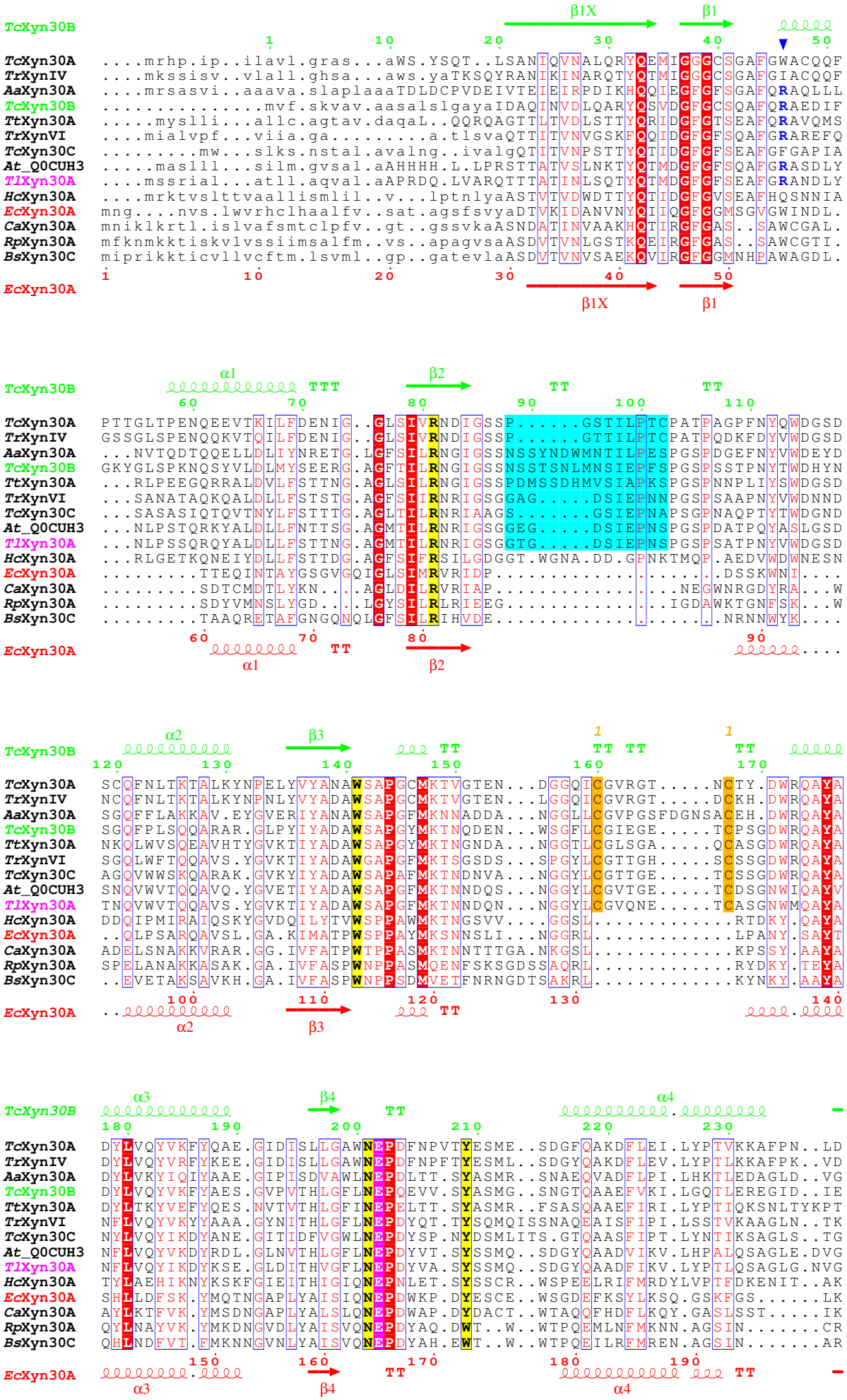


Figure S1. pH and temperature optimum and temperature stability of *Tl*Xyn30A. **A**) pH optimum was determined at 40 °C using a 15 min incubation of the enzyme with 1% GX as a substrate in either 40 mM Britton-Robinson (pH 3.5-5.5) or 50 mM sodium phosphate buffers (pH 6.0-8.0). **B**) Temperature optimum was determined under the same conditions using 40 mM Britton-Robinson buffer, pH 3.5, and temperatures ranging from 23 °C to 90 °C. **C**) Temperature stability was tested as follows: the enzyme was heated at 40, 50 or 60 °C for up to 5 hours in 40 mM Britton-Robinson buffer, pH 3.5 (in the absence of the substrate), and then assayed at 40 °C.


TcXyn30B
β1X
β1
TcXyn30A
TrXynIV
AaXyn30A
TcXyn30B
TtXyn30A
TrXynVI
TcXyn30C
At_Q0CUH3
TlXyn30A
HcXyn30A
EcXyn30A
CaXyn30A
RpXyn30A
BsXyn30C
α1
β2
α2
β3
α3
β4
α4

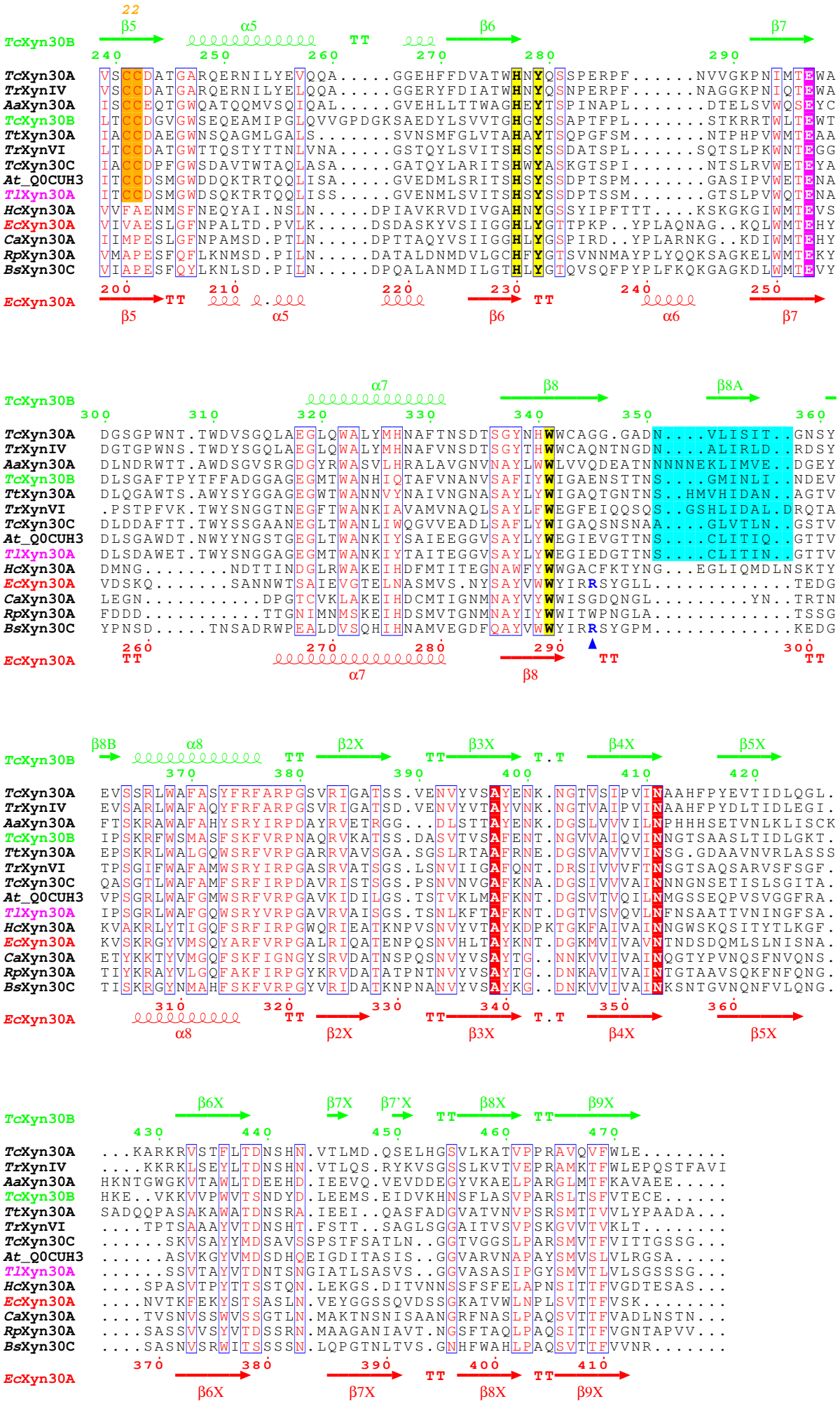

TcXyn30B
TcXyn30A
TrXynIV
AaXyn30A
TcXyn30B
TtXyn30A
TrXynVI
TcXyn30C
At_Q0CUH3
TlXyn30A
HcXyn30A
EcXyn30A
CaXyn30A
RpXyn30A
BsXyn30C
EcXyn30A

Figure S2. Multiple sequence alignment of GH30 catalytic domain of *Tl*Xyn30A and selected GH30 xylanases. The first two sequences, *Tc*Xyn30A (Uniprot ID: A0A0B8MZ29) and *Tr*XynIV (A0A024RWW9), represent reducing-end xylose releasing enzymes. For next three sequences, *Aa*Xyn30A [Ref. 5], *Tc*Xyn30B (A0A4V8H018) and *Tt*Xyn30A (G2Q1N4), the non-reducing end xylobiohydrolase activity has been demonstrated. The latter two enzymes are specific glucuronoxylanases, similarly to *Tr*XynVI (G0RV92). In contrast, *Tc*Xyn30C (A0A6N4SL16) is a non-specific endoxylanase. The next sequence, *At*_QUCUH3, corresponding to an uncharacterized *Aspergillus terreus* protein, shows the highest similarity to *Tl*Xyn30A. The last 5 sequences are of bacterial origin and belong to GH30_8 subfamily. *Hc*Xyn30A (G8LU16) is a unique prokaryotic non-reducing end xylobiohydrolase. *Ec*Xyn30A (Q46961) and *Bs*XynC (Q45070) are canonical GH30_8 glucuronoxylanases. *Ca*Xyn30A (Q97TI2) and *Rp*Xyn30A (F1TBY8) are exceptional GH30_8 non-specific endoxylanases. Secondary structure elements identified in the structures of *Tc*Xyn30B (green) and *Ec*Xyn30A (red) are shown on top and bottom. Catalytic acid and catalytic base are typed on violet background. Another amino acids that are invariant in GH30 xylanases are typed on yellow background. Cyan-highlighted are β2-α2 loop and β8-α8 region (both are shown in detail in **Figure 6**) where there are the greatest differences between GH30_8 and GH30_7 members. Prokaryotic arginine typed in blue and marked at the bottom by an up triangle is responsible for glucuronoxylanases specificity of the bacterial glucuronoxylanases. Eukaryotic arginine, which is believed to contribute to the MeGlcA moiety recognition by the eukaryotic xylanases and is rather well conserved in the GH30_7 enzymes, is also found in *Tl*Xyn30A and is marked on the top by a down triangle Four cysteín residues conserved in the eukaryotic enzymes form a pair of disulphide bonds.