

SUPPORTING INFORMATION

Glycosynthase-based Synthesis of Xylo-oligosaccharides using an Engineered Retaining Xylanase from *Cellulomonas fimi*

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Construction strategy for CFXcd and its nucleophile mutants

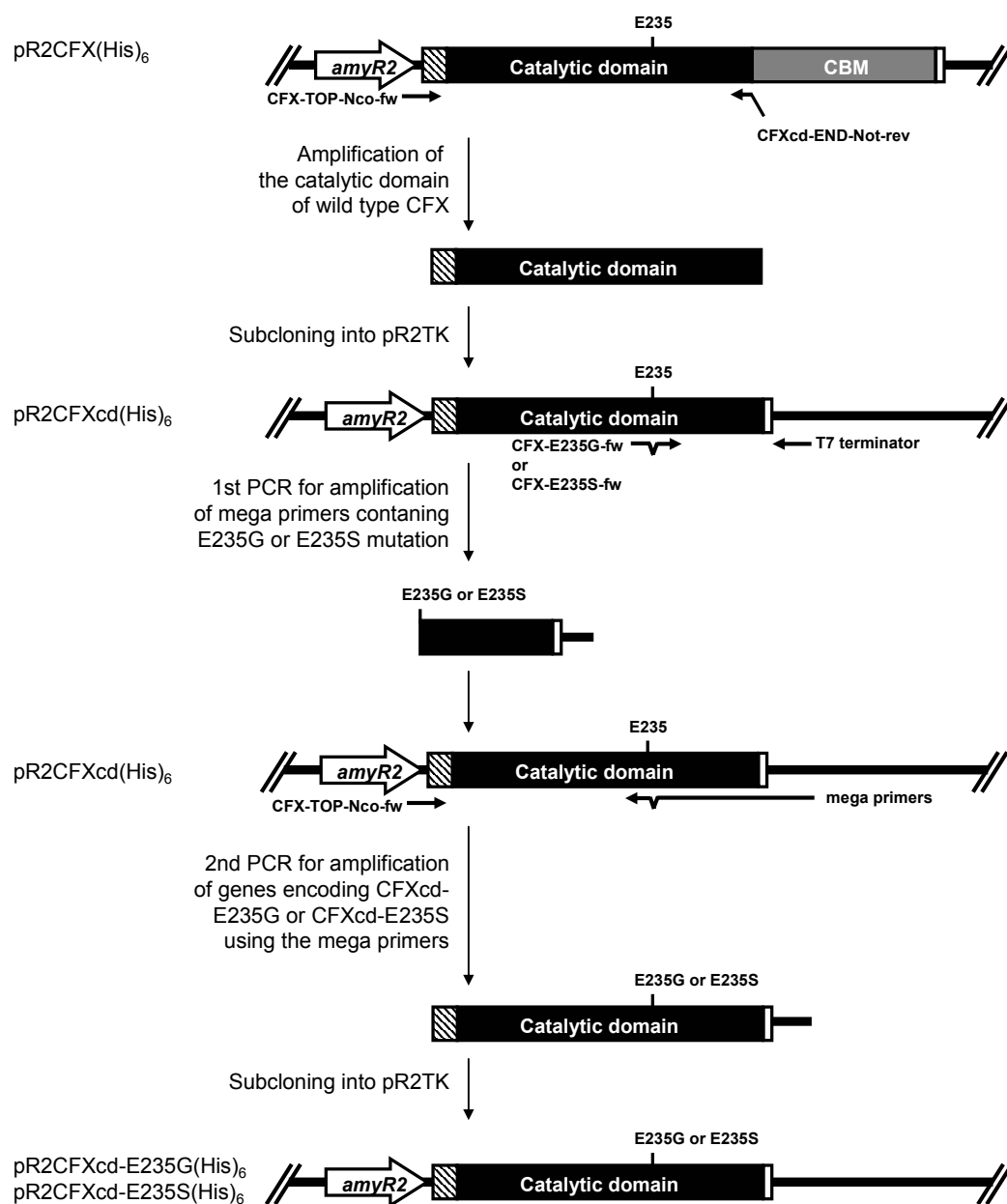


Fig. S-1 Construction strategy for pR2CFXcd(His)₆, pR2CFXcd-E235G(His)₆, and pR2CFXcd-E235S(His)₆

Selection of active glycosynthases derived from CFXcd

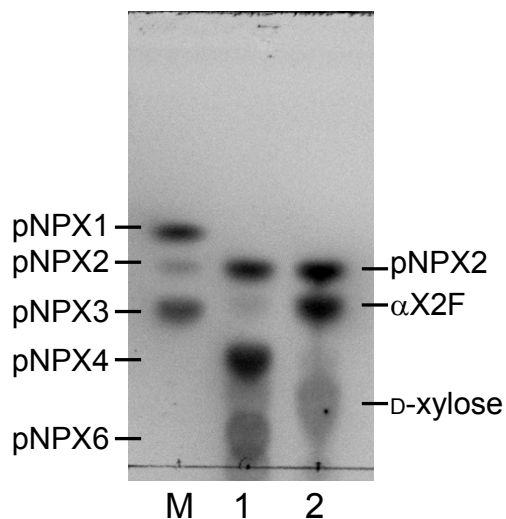


Fig. S-2 TLC analysis of the reaction mixtures using CFXcd-E235G and CFXcd-E235S mutants.

Lane M, standards for pNP xylooligosaccharides, pNPX, pNPX2, and pNPX3; Lane 1, the reaction mixture of CFXcd-E235G; Lane 2, the reaction mixture of CFXcd-E235S. α X2F and pNPX2 were used as donor and acceptor, respectively. The plate was visualized by exposure to 10 % sulfuric acid in methanol followed by charring.

Enzyme stability

In order to investigate enzyme stability, aliquots of CFXcd-E235G (in 50 mM phosphate buffer/pH 7.0) were incubated at 37 °C for 24 h and 48 h without substrates. Then, glycosynthase reactions were carried out using the preincubated enzymes. A control reaction was carried out using CFXcd-E235G which stored at 4 °C. The concentrations of substrates were 10 mM. After 12 h incubation at 30 °C, the reaction mixtures were analyzed using TLC. The amount of transfer products in the reactions using the preincubated enzymes was not so much different (Fig. S-3). This result suggests that CFXcd-E235G is stable at 37 °C for 48 h, and its activity also maintained after the reaction for the synthesis of xylooligosaccharides (30 °C, 24 h)

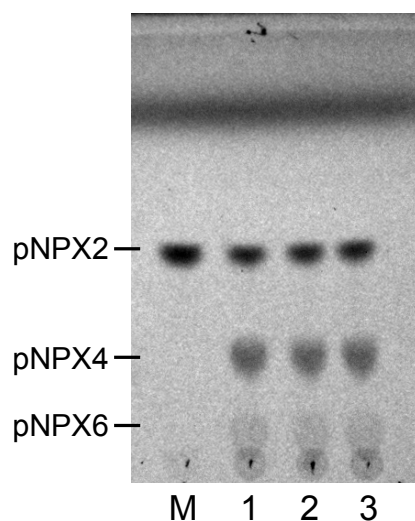


Fig. S-3 TLC analysis of the reaction mixtures using CFXcd-E235G stored at 4 °C and 37 °C . Lane 1, blank reaction without enzyme; Lane 2, the reaction mixture of CFXcd-E235G stored at 4 °C; Lane 3, the reaction mixture of CFXcd-E235G stored at 37 °C for 24 h; Lane 4, the reaction mixture of CFXcd-E235G stored at 37 °C for 48 h. α X2F and pNPX2 were used as donor and acceptor, respectively. The plate was visualized under UV light.

Time dependence during the preparative scale synthesis

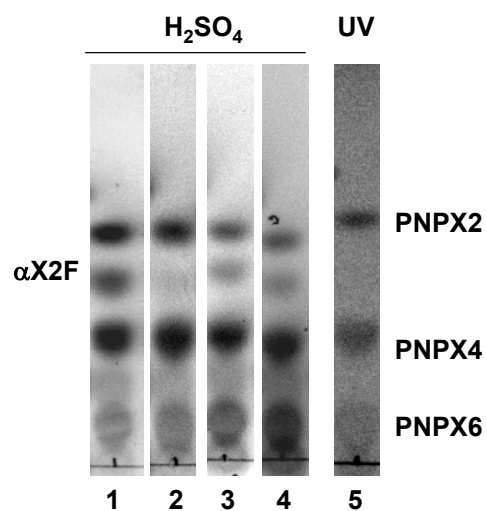


Fig. S-4 TLC of the reactions of CFXcd-E235G using $\alpha X2F$ and pNPX2 over time.

Lane 1, reaction mixture after 6 h; Lane 2, reaction mixture after 10 h; Lane 3, reaction mixture after 21 h; Lane 4, reaction mixture after 24 h; Lane 5, reaction mixture after 21 h. The plate for Lane 5 was visualized under UV light and the others by exposure to 10 % sulfuric acid in methanol followed by charring.

Mass spectrometry data for purified aryl xylooligosaccharides

4-Nitrophenyl β -D-xylopyranosyl-(1 \rightarrow 4)-bis[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranoside (PNPX4). ESI MS: Calcd for C₂₆H₃₇NO₁₉+Na⁺: 690.2. Found: 690.2

4-Nitrophenyl β -D-xylopyranosyl-(1 \rightarrow 4)-tetra[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranoside (PNPX6). ESI MS: Calcd for C₃₆H₅₃NO₂₇+Na⁺: 954.3. Found: 954.4

4-Nitrophenyl β -D-xylopyranosyl-(1 \rightarrow 4)-hexa[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranoside (PNPX8). ESI MS: Calcd for C₄₆H₆₉NO₃₅+Na⁺: 1218.5 + 23. Found: 1218.5

4-Nitrophenyl β -D-xylopyranosyl-(1 \rightarrow 4)-octa[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranoside (PNPX10). ESIMS: Calcd for C₅₆H₈₅NO₄₃+Na⁺: 1482.4. Found: 1482.8

4-Nitrophenyl β -D-xylopyranosyl-(1 \rightarrow 4)-deca[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranoside (PNPX12). ESI MS: Calcd for C₆₆H₁₀₁NO₅₁+Na⁺: 1746.5. Found: 1747.1

Benzylthio β -D-xylopyranosyl-(1 \rightarrow 4)-bis[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranoside (BTX4). ESI MS: Calcd for C₂₇H₄₀O₁₆S+Na⁺: 675.2. Found: 675.2

Benzylthio β -D-xylopyranosyl-(1 \rightarrow 4)-tetra[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranoside (BTX6). ESI MS: Calcd for C₃₇H₅₆O₂₄S+Na⁺: 939.3. Found: 939.5

Benzylthio β -D-xylopyranosyl-(1 \rightarrow 4)-hexa[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranoside (BTX8). ESI MS: Calcd for C₄₇H₇₂O₃₂S+Na⁺: 1203.4. Found: 1203.4

Benzylthio β -D-xylopyranosyl-(1 \rightarrow 4)-octa[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranoside (BTX10). ESI MS: Calcd for C₅₇H₈₈O₄₀S+Na⁺: 1467.4. Found: 1467.6

Benzylthio β -D-xylopyranosyl-(1 \rightarrow 4)-deca[β -D-xylopyranosyl-(1 \rightarrow 4)]- β -D-xylopyranoside (BTX12). ESI MS: Calcd for C₆₇H₁₀₄O₄₈S+Na⁺: 1731.5. Found: 1731.9