Loop engineering of a thermostable GH10 xylanase to Improve low-temperature catalytic performance for better synergistic biomass-degrading abilities

Shuai You [1, 2, 3, 4], SHITTU [1], Wen-Xin Zhang [1], Zhi-Yuan Bai [1], Yi-Wen Chen [1], Jun Wang [1, 2, 3, 4, *]

[1] School of Biotechnology, Jiangsu University of Science and Technology, 212018 Zhenjiang, China;
[2] Sericultural Research Institute, Chinese Academy of Agricultural Sciences, 212018 Zhenjiang, China;
[3] Key Laboratory of Silkworm and Mulberry Genetic Improvement, Ministry of Agriculture, Sericultural Research Institute, Zhenjiang 212018, PR China;
[4] Jiangsu Key Laboratory of Sericultural Biology and Biotechnology, Zhenjiang 212018, PR China.
* Corresponding author. *E-mail*: wangjun@just.edu.cn.

Abstract: Currently, thermostable xylanases with good catalytic performance have been a research focus because of their wide application in the biorefinery and bioenergy industries. These are being achieved through protein engineering. Herein, we used directed evolution to improve thermostable xylanase catalytic performance under low temperature. A wild type XYL10C_ΔN of GH10 thermostable xylanase and its mutants (M137E, N207G and M137E/N207G) were produced in *Pichia pastoris* and biochemically characterized. All enzyme exhibited similar optimal activity at pH 4.5 and 90 ℃. Under the chosen conditions (pH 4.5, 37 ℃ and 10 min), all the mutants showed higher activities towards all the substrates used with the combined mutant (M137E/N269G) showed highest activities of 1610 U/mg, 1980 U/mg and 2130 U/mg towards beechwood xylan, sugarcane xylan and crocob xylan respectively. In comparison with wild type XYL10C_ΔN (510 U/mg against beechwood xylan, 660 U/mg against sugarcane xylan, and 770 U/mg against corncob xylan respectively). All mutants exhibited remarkedly increased in catalytic efficiency (k_{cat}/K_m) coupled with higher V_{max} and k_{cat} values compared to the wild-type enzyme XYL10C_ΔN under the same conditions. This as a result, the k_{cat}/K_m of M137E, N269G and M137E/N269G were significantly improved by approximately 2.8-fold, 2.4-fold and 4-fold respectively. Using mulberry bark and corn cob, enzymatic hydrolysis was performed for reducing sugar production. The combined mutant and cellulose showed high degree of synergy and the reducing sugar production was improved by 143% and 133% respectively. The significantly improved catalytic efficiency of mutants will pave the way for applications in different industrial areas under low temperature.

Keywords: GH10 xylanase; Directed evolution; Low-temperature catalytic performance; Thermostability; Biomass degradation