Catalytic performance and thermostability improvement of a GH10 xylanase by module substitution

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Xylan is the major component of hemicellulose and represents an abundant renewable biomass for biorefinery and bio-energy (Mikkonen et al, 2012). Xylanases are crucial enzymes that catalyze the hydrolysis of xylan, and have widespread applications in the animal feed, food, bio-ethanol, and paper pulp industries (Hu et al., 2013, Juturu et al, 2012). Thermostable xylanases with high catalytic efficiency are required for many industrial processes.

The common approaches are to mine new genetic resources from extreme environments, engineer the enzymes, and optimize their application (Lilyan et al., 2007, Park et al., 2014). Several xylanases from *Aspergillus fumigatus* R1 (Deshmukh et al., 2016), *Streptomyces coelicolor* A3 (Enkhbaatar et al., 2016), and *Bispora* sp. MEY-1 (Luo et al., 2009) have high catalytic activities of 18,000–38,000 U/mg. There have also been some successful cases of protein engineering. For example, Wang et al. improved the catalytic efficiency of the xylanase from *Geobacillus stearothermophilus* by 3.46-fold using saturation mutagenesis and directed evolution (Wang et al., 2013). However, there often seems to be a trade-off between enzyme activity and stability at the level of individual mutations (Seung-Goo et al., 2006). Increased enzyme activity at the cost of thermostability is not biotechnologically and practically desirable. Therefore, improving the catalytic efficiency as well as the thermostability of an enzyme is a research focus for high-temperature industrial applications (Mehta et al., 2015).

Modified hybrid enzymes may have improved catalytic efficiency and thermostability. In the present study, we employed the module substitution to improve the enzymatic properties of a GH10 xylanase, XylE, from *Penicillium canescens* (Fedorova et al., 2012). XylE shares 53% sequence identity with the hyperthermophilic XYL10C from acido-mesophilic *Bispora* sp. MEY-1 but has a much lower specific activity (18,000 vs. 50 U/mg). Based on sequence and structure alignments, we aimed to identify structural modules and exchange them between XYL10C and XylE. The hybrid enzymes were then biochemically characterized. Among 10 hybrid enzymes obtained, seven showed xylanase activity. As shown in Table 1, substitution of modules M3, M6, M9, and their combinations improved the catalytic efficiency (by 2.4 to 4.0-fold) and the specific activity (by 1.2 to 3.3-fold). As shown in figure 1, hybrids XylE-M3, XylE-M3/M6, XylE-M3/M9, and XylE-M3/M6/M9 showed enhanced thermostability (increases in the T_{50} and T_m, and extended $t_{1/2}$). Molecular dynamic simulations revealed that these hybrid enzymes form more complex hydrogen-bond networks, contributing to the reduced free energy and rigid overall structure.

Table 1. Specific activity and kinetic parameters of XylE and its hybrid enzymes assayed at 70 °C with beechwood xylan as the substrate.

Enzymes	K_m **(mg/mL)**	V_{max} **(µmol/min/mg)**	k_{cat} **(/s)**	k_{cat}/K_m **(mL/s/mg)**	**Specific activity (U/mg)**
XylE	0.75 ± 0.04	680 ± 31	430 ± 12	570 ± 18	610 ± 18
XylE-M3	0.61 ± 0.03	1390 ± 41	860 ± 16	1340 ± 22	1310 ± 45
XylE-M6	0.42 ± 0.01	1160 ± 17	740 ± 20	1760 ± 28	1150 ± 55
XylE-M9	0.46 ± 0.02	1390 ± 38	900 ± 10	1960 ± 31	1130 ± 38
XylE-M3/M6	0.62 ± 0.02	2200± 28	1410 ± 22	2280 ± 29	2010 ± 51
XylE-M3/M9	0.42 ± 0.02	880± 21	570 ± 16	1370 ± 22	730 ± 21
XylE-M6/M9	0.57 ± 0.01	1280 ± 34	820 ± 19	1440 ± 18	1270 ± 37
XylE-M3/M6/M9	0.67 ± 0.03	1760 ± 47	1170 ± 26	1750 ± 19	1500 ± 46

*: No activity dected

This study provided an effective strategy to improve enzymes' catalytic performance and identified several xylanase candidates for use in high-temperature industrial applications.

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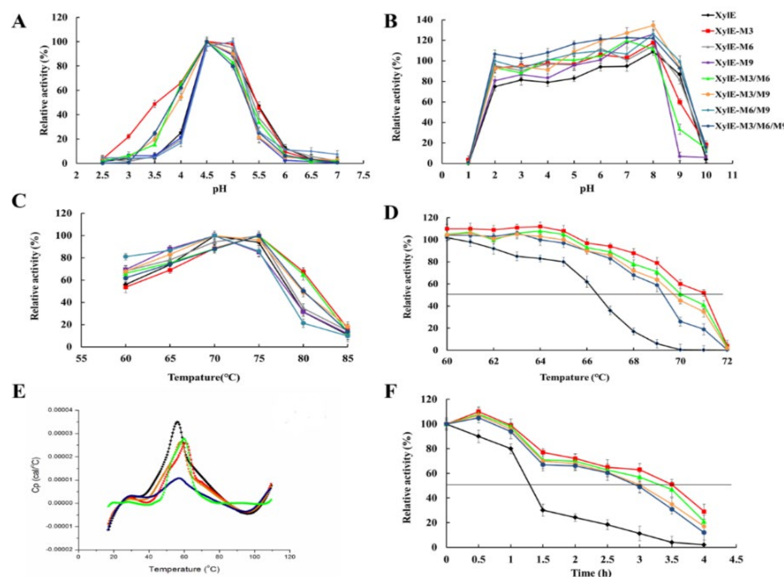

Fig. 1 Enzyme properties of the purified recombinant wild-type XylE and its hybrid mutants. A. pH-activity profiles tested at the optimal temperature of each enzyme (70 °C for XylE, XylE-M9, XylE-M3/M9, and XylE-M6/M9; and 75 °C for the others). B. pH-stability profiles. After incubation of the enzymes at 37 °C for 1 h in buffers ranging from pH 1.0 to 10.0, the residual activities were determined in 100 mM McIlvaine buffer at the optimal pH and optimal temperature of each enzyme. C. Temperature-activity profiles tested at the optimal pH of each enzyme (pH 4.5 for XylE and pH 5.0 for the others). D. Temperature-stability profiles (T_{50}). E. Thermograms determined by using differential scanning calorimetry (DSC). The calorimetric recordings for XylE and its mutants were scanned at 1°C/min in 10 mM phosphate buffered saline (PBS) (pH 6.5) at 350 μg/mL. F. Half-lives of wild-type XylE and its mutants at 65 °C.

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