Improve the catalytic activity of GH10 xylanase at low temperatures based on structure rational design

Yan Ge, Shuai You, Wenxin Zhang, Zhiyuan Bai, Fuan Wu, Jun Wang

[1]*School* of Biotechnology, Jiangsu University of Science and Technology, Zhenjiang 212018, PR China

[2]Sericultural Research Institute, Chinese Academy of Agricultural Sciences, 212018 Zhenjiang, China

E-mail: wangjun@just.edu.cn (Prof. Dr. Jun. Wang)

Introduction

Xylanase is one of the most crucial enzymes and has been widely used in various fields. The stability and catalytic activity of a xylanase are key factors for enzyme applications. At present, scientists mainly improve the properties of xylanase through molecular modification and enzyme engineering methods. But so far, there is no general method or universal theory to improve both thermal stability and catalytic activity of the xylanase.

Methods

Based on the relationship between structure and function, in this study, the stable GH10 xylanase (GtXyn10) from Gloeophyllum trabeum via site-directed mutagenesis to improve the low-temperature catalytic performance. Through catalytic amino acid sites and substrate binding sites were predicted by hotspot wizard, two locations, N119D，N166M, were identified as the contributors to the whole enzyme molecule. Single (N119D and N166M) and double (N119D + N166M) mutants were produced and compared with the wild-type (WT).

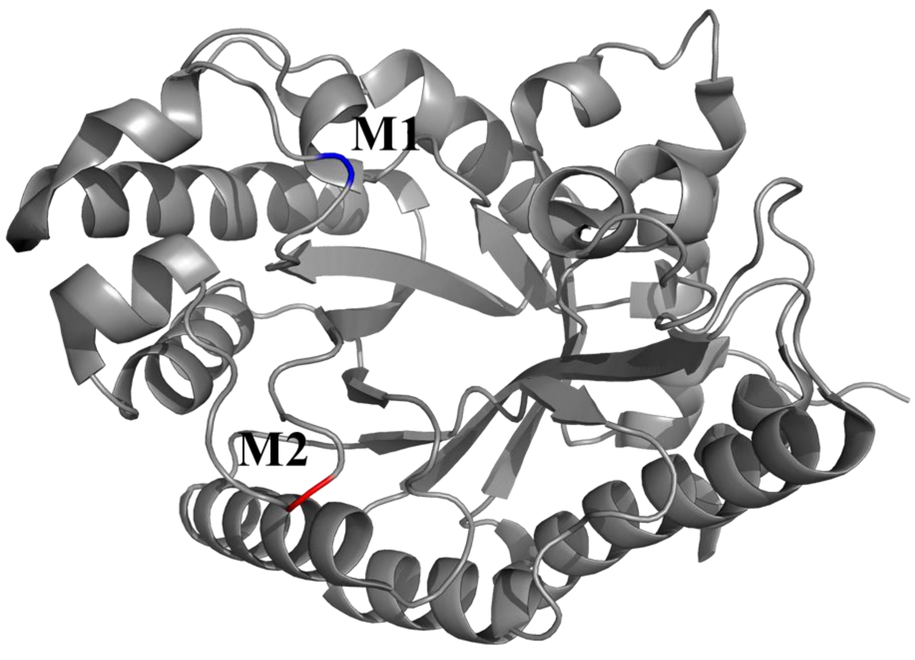

Figure 1. Schematic diagram of mutation site

Results & Discussion

Using beechwood xylan as substrate, the specific activity of recombinant high specific active xylanase mutant and wild type at 40 ℃ were 440 U/mg, 420 U/mg, 590 U/mg and 250 U/mg (Table 1). Compared with the wild type, the mutant (N119D + N166M) had the highest enzyme activity at 40 ℃ (Figure 2).

The thermal stability of all the mutants was better than that of the wild type. After 30 min treatment at 70 ℃, the remaining enzyme activities of the three mutants were 1.1, 1.5 and 1.5 times that of the wild type (Figure 2). After treatment at 80 ℃ for 20 min, the remaining enzyme activities of the three mutants were 1.2, 1.2 and 1.4 times of those of the wild type, respectively (Figure 2).The results showed that the thermal stability of all the mutants was better than that of the wild type at 70 ℃ and 80 ℃.

Table 1. Kinetic parameters and specific activity of GtXyn10 and its three mutants towards beechwood xylan .

Enzymes	K_m (mg/mL)	V_{max} (μmol/min·mg)	k_{cat}/K_m (mL/s·mg)	Specific activity (U/mg)
WT	3.2 ± 0.25	271 ± 30	52 ± 5	251 ± 11
N119D	2.6 ± 0.21	456 ± 51	106 ± 9	440 ± 27
N166M	2.4 ± 0.18	428 ± 32	260 ± 15	417 ± 23
N119D/N166M	2.0 ± 0.17	595 ± 47	361 ± 21	587 ± 76

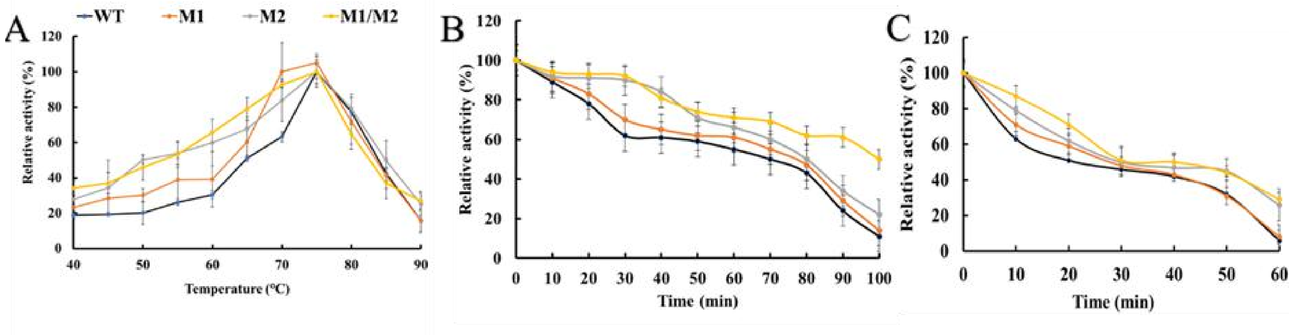

Figure 2. Activity analysis of recombinant xylanase mutants and wild type. **A** shows the optimal temperature of xylanase mutant and wild type. **B** shows thermal stability of xylanase mutant and wild type at 70℃; **C** shows thermal stability of xylanase mutants and wild type at 80 ℃.

Conclusions

Under these conditions, the specific activity of xylanase mutants at 40 ℃ was significantly higher than that of the wild type. By modifying different amino acid sites of xylanase GtXyn10, on the one hand, improves the specific activity at 40 ℃ without losing the thermal stability of the enzyme; on the other hand, such xylanase mutant with high enzyme activity, which is stable in acidic pH environment and in the range of low temperature, has a broad application prospect for lignocellulosic degradation and reducing sugar production. At the same time, this study provides significant method for the improvement of the GH10 xylanase and other glycoside hydrolases.

Acknowledgements

The authors thank the financial support from the Natural Science Foundation of Jiangsu Province (BK20190957), the National Natural Science Foundation of China (Grant Number 21978121), the National Natural Science Foundation of China (Grant Number 31472127).

References

[1] Wong KK, Tan LU, Saddler JN. Multiplicity of β-1,4-xylanases in microorganisms: functions and applications. Microbiol Rev, 1988, 52:305–317.

[2] Prade R. Xylanases: from biology to biotechnology. Biotechnol Gene Eng Rev, 1995, 13(12): 101–131.

[3] Almeida MN, Guimarães VM, Falkoski DL, et al. Direct ethanol production from glucose, xylose and sugarcane bagasse by the corn endophytic fungi Fusarium verticillioides and Acremonium zeae. Journal of Biotechnology, 2013, 168(1): 71–77.

[4] Zhang H, Li J, Wang J, et al. Determinants for the improved thermostability of a mesophilic family 11 xylanase predicted by computational methods. Biotechnology for Biofuels, 2014, 7(1): 3–13.