Improving the thermostability and catalytic efficiency of GH10 xylanase from *Gloeophyllum trabeum* by rational design

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Endo-β-1,4-xylanases (EC 3.2.1.8) catalyze the hydrolysis of β-1,4-glycosidic bonds in heteroxylans, which are major components of hemicellulose in the biosphere (Prade R. 1995). The enzymehas considerable potential in many applications, such as biobleaching of pulps, deinking of waste paper, improving the digestibility of animal feed, bread making, converting of plant biomass into biofuels, and producing prebiotics (Tony et al., 2010).

Xylanases are mainly distributed in glycoside hydrolase (GH) families 5, 7, 8, 10, 11, 30, and 43. Current research focuses on GH10 and GH11 family xylanase. The GH10 xylanases has better thermal stability (Beaugrand et al., 2004), but lower catalytic efficiency, which limits its industrial application. Therefore, obtaining xylanase with high thermocatalytic activity is very important for industrial production.

At present, protein engineering is widely used in the improvement of enzyme molecules, that is, by modifying or modifying genes or the protein itself to change the structure of the protein to achieve the transformation of enzyme functions. Protein engineering is mainly used for the design and modification of enzyme properties such as thermal stability, catalytic efficiency, substrate specificity and extreme environmental tolerance. The main methods involved are directed evolution, rational design and semi-rational design. Among them, rational design is a quick and effective means of transformation, and its commonly used methods mainly include module replacement and site-specific mutation. This method significantly improves the thermostability of the xylanase XynA derived from Thermoascus aurantiacus (Abou-Hachem et al., 2002). Wang Xiaoyu et al. optimized the catalytic channel of Talaromyces leycettanus xylanase TlXyn10A through rational design, which increased the specific activity by 40% and the pH stability was also significantly improved (Wang et al., 2016).

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 catalytic performance. Through catalytic amino acid sites and substrate binding sites were predicted by hotspot wizard, three locations, G29L， H32K, and Q99A were identified as the contributors to the whole enzyme molecule. The mutants were produced and compared with the wild-type (WT). Using beechwood xylan as substrate, the specific activities of mutants G29L, H32K and Q99A were 1520 U/mg, 1830 U/mg and 1720 U/mg, respectively, which were higher than that of wild-type GtXyn10 (1060U/mg) were increased by 43%, 73% and 62%, the catalytic efficiency was 300 mL/s·mg, 610 mL/s·mg and 360 mL/s·mg respectively, compared with wild-type GtXyn10 (210 mL/s·mg), increased by 43%, 1.9 times and 71% respectively. (Table 1)

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)
GtXyn10	4.2 ± 0.41	1450 ± 179	210 ± 25	1060 ± 95
G29L	4.3 ± 0.38	2080 ± 301	300 ± 41	1520 ± 132
H32K	2.4 ± 0.15	2380 ± 229	610 ± 77	1830 ± 199
Q99A	3.8 ± 0.27	2250 ± 218	360 ± 49	1720 ± 183

The optimum temperature and optimum pH have no significant changes compared to the wild type. In terms of thermal stability (Figure 1), mutants G29L, H32K and Q99A were treated at 75 °C for 30 min, and the remaining enzyme activities were 45%, 64% and 28%, respectively. Compared with wild-type enzyme GtXyn10 (62%), the thermal stability of mutants G29L and H32K did not change significantly, while the thermal stability of mutant Q99A decreased significantly.

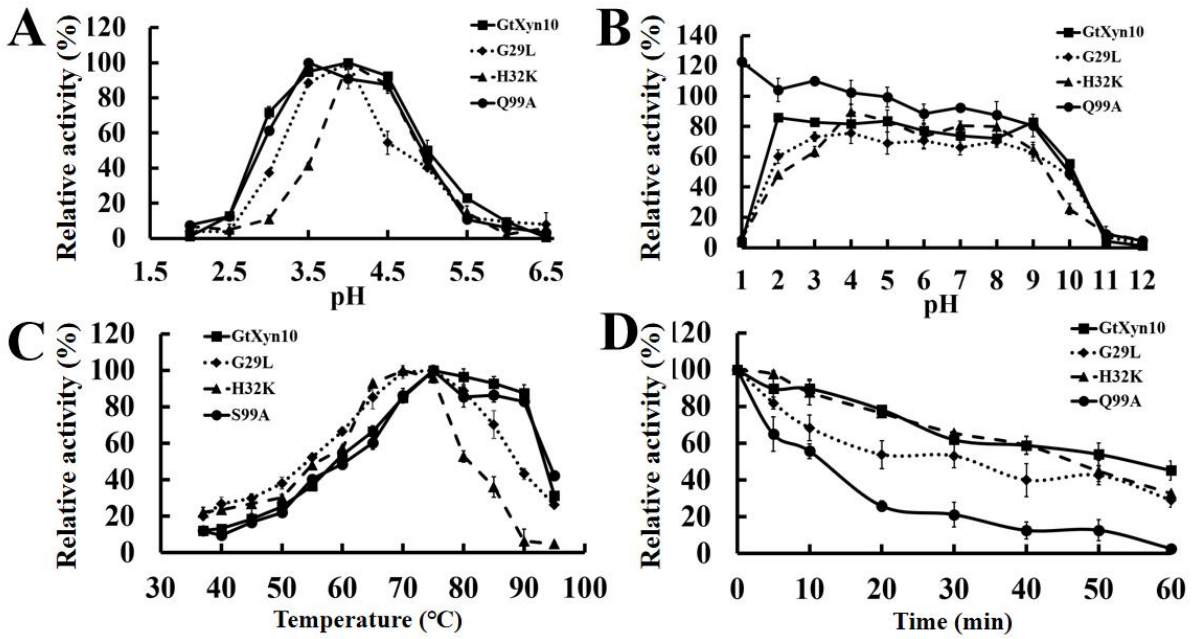

Figure 1. Relative activity analysis of recombinant xylanase mutants and wild type. **A** shows the optimal pH of xylanase mutant and wild type. **B** shows the pH stability of xylanase mutant and wild type. **C** shows the optimal temperature of xylanase mutant and wild type. **D** shows thermal stability of xylanase mutant and wild type at 75℃.

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