Improving the catalytic efficiency and thermostability of GH10 xylanase from *Cladophialophora carrionii* by rational design

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The xylanases with high specific activity and resistance to harsh conditions are of high practical value for biomass utilization (Abdul et al., 2018). Xylanases are hemicellulases that break down xylan into soluble pentoses. They are used in industrial applications such as paper whitening, beverage clarification and biofuel production (Olinda et al., 2022). All these biochemical properties of the xylanases offer practical potential for future applications.

Xylanases are divided into different families, two of which are the glycoside hydrolase 10 (GH10) and 11 (GH11) families. These well-characterised xylanases show different modes of action in the hydrolysis of xylans. This new type of xylanase may have potential applications in different industrial processes, as it can replace two separate enzymes and thus minimise production costs (Ehsan et al., 2021). Although GH10 xylanase has better thermal stability (Beaugrand et al., 2004), the lower catalytic efficiency limits its industrial application. Therefore, it would be important for industrial production if xylanases with high thermal catalytic activity could be obtained.

Current trends in protein engineering methods such as rational design, directed evolution, ab initio design, computational methods and including recent advances in the field over the last few years are being used to design and develop new proteins with improved properties or advanced applications (Rajeshwari et al., 2019). The main use is for the design and modification of enzymes for properties such as thermal stability, catalytic efficiency, substrate specificity and extreme environmental tolerance. Amongst these, rational design is a fast and effective means of transformation, and its common transformation methods mainly include modular substitution and site-specific mutagenesis. This method significantly improves the thermostability of the xylanase CDBFV derived from hyperthermophilic (Miao et al., 2021). Wang 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).

In this study, the catalytic performance of GH10 xylanase (Ccxyl10B) was enhanced by targeted mutagenesis to obtain a stable GH10 xylanase from *Cladophialophora carrionii* based on its structure-function relationship. The catalytic amino acid sites and substrate binding sites were predicted by hotspot wizards, and three sites, V126F, S210P, P243L and V126F/P243L/S210P were identified as contributors to the overall enzyme molecule. Mutants were generated and compared with the wild type (WT). Using beech xylan as substrate, mutants V126F, S210P, P243L and V126F/P243L/S210P had specific activities of 2359 U/mg, 1655 U/mg,1501 U/mg and 1520 U/mg, respectively, only the mutant V126F was higher than WT (1882 U/mg) were increased by 25%, the catalytic efficiency was 2672 mL/s·mg, 1905 mL/s·mg 1766 mL/s·mg and 1628 mL/s·mg respectively, compared with WT (2476 mL/s·mg), only the mutant V126F increased by 8%. (Table 1)

Table. 1 Kinetic parameters and specific activity of Ccxyl10B and its three mutants and combination 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)
Ccxyl10B	0.59±0.09	1985±112	2476±242	1882±31
V126F	0.56±0.07	2329±76	2672±223	2359±25
S210P	0.64±0.05	1661±55	1905±79	1655±43
P243L	0.72±0.06	1894±54	1766±89	1501±154
V126F/P243L/S210P	0.71±0.02	1576±23	1628±33	1520±74

The optimum temperature and optimum pH have no significant changes compared to the wild type. In terms of thermal stability (Figure 1), mutants V126F, P243L and S210P were treated at 85 °C for 30 min, and the remaining enzyme activities were 43%, 21%, 43%, and 43%, respectively. Compared with wild-type enzyme Ccxyl10B, the thermal stability of mutants V126F and V126F/P243L/S210P decreased steadily, while mutant P243L and S210P showed a more pronounced change in thermal stability.

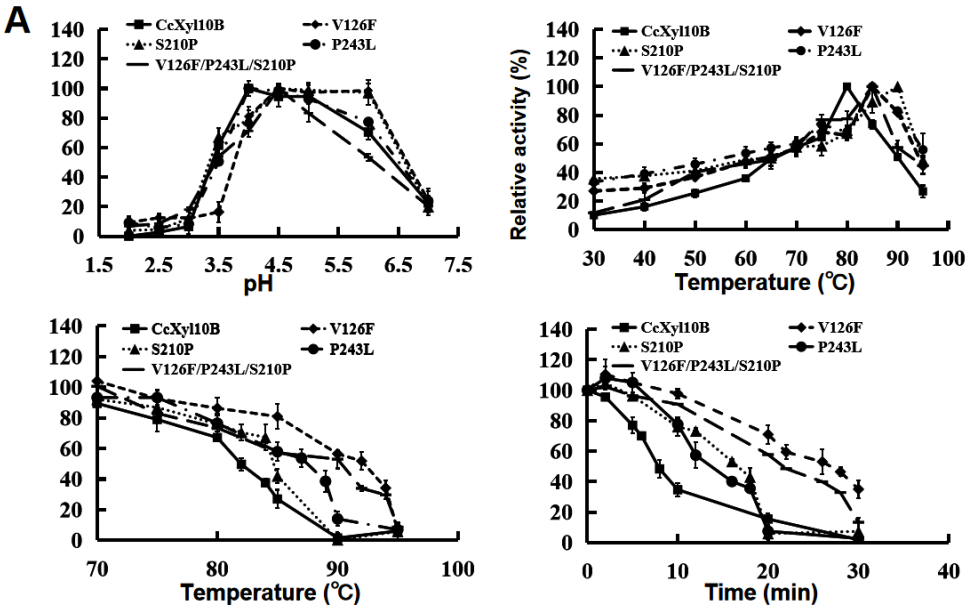

Figure. 1 Relative activity analysis of recombinant xylanase mutants and wild type. **A** shows the optimal pH of xylanase mutants and wild type. **B** shows the optimal temperature of xylanase mutants and wild type. **C** shows the thermal stability of xylanase mutants and wild type. **D** shows thermal stability of xylanase mutants and wild type at 85 ℃.

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