Effects of N-glycosylation on thermal stability and catalytic efficiency of GH10 family xylanases

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The improvement of the thermostability of the highly active xylanases is of great significance to the use of biomass materials to produce clean energy (Chen et al., 2021). In the animal feed industry, they are used as supplements to enhance digestion and conversion efficiency and eliminate anti-nutritional factors (Li et al., 2021). Therefore, the study of xylan is of great significance to the application of xylanases. It is reported that xylanases XYL10CΔN and 4XX6 have excellent catalytic efficiency. However, its thermal stability at high temperature is poor (You et al., 2021). Numerous studies had shown that N-glycosylation has a significant impact on the characteristics of the expressed recombinant enzymes, especially for thermostability and catalytic efficiency (Qin et al., 2014). N-glycosylation not only affects the thermostability of proteins but also affects their secretion. It assists in the folding and transfer of newly synthesized polypeptide chains, thereby promoting the efficient secretion of recombinant enzymes. Previous studies had shown that different N-glycosylation sites promote or inhibit the activity, thermostability, and secretion efficiency of different enzymes (Dotsenko et al., 2016). By studying its amino acid sequence, it was found that it contains five N-glycosylation sites, respectively. Therefore, N-glycosylation sites can be removed to compare changes in enzyme properties.

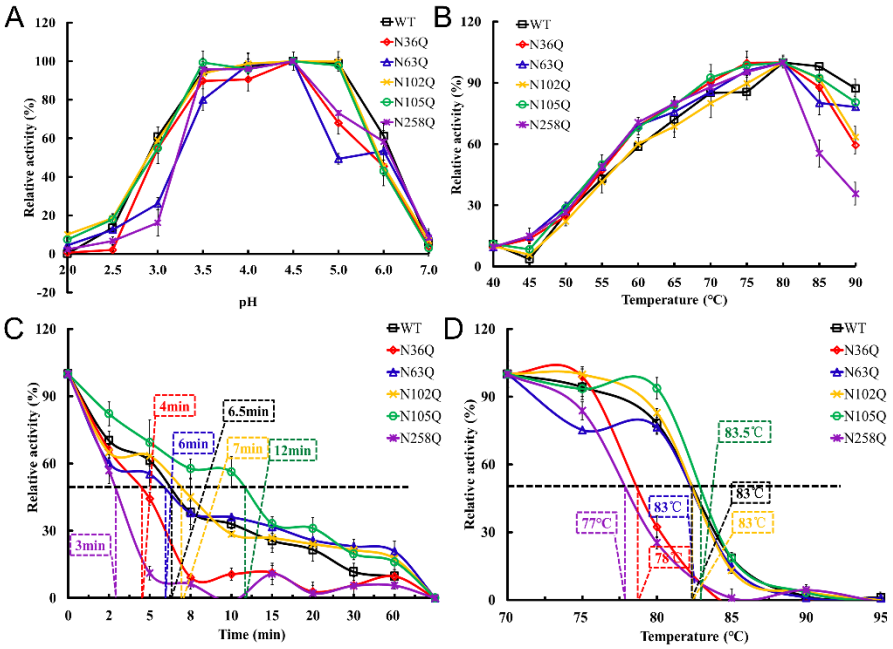

Figure 1. A: Optimum pH for XYL10CΔN and its mutants; B: Optimum temperature for XYL10CΔN and its mutants; C. $t_{1/2}$ of XYL10CΔN and mutants at 80 °C; D. T_{50} values of XYL10CΔN and mutants.

Figure 1 comprehensively compared the optimum pH, optimum temperature, $t_{1/2}$ value and T_{50} value of the XYL10CΔN and mutants, and it was found that the change trend of optimum temperature and optimum pH of wild type and mutant type is consistent. The optimum temperature and pH of all mutants were the same as those of wild type, and the relative activity of the mutant N258Q decreased rapidly at the range of 80 °C and 90 °C. When the N-glycosylation site of the enzyme was removed, the thermal stability of N105Q became stronger and the thermal stability of other mutants became weaker. T_{50} values of wild-type and mutants were also measured. Among them, the thermal stability of N36Q and N258Q mutants decreased, and the data were consistent with $t_{1/2}$.

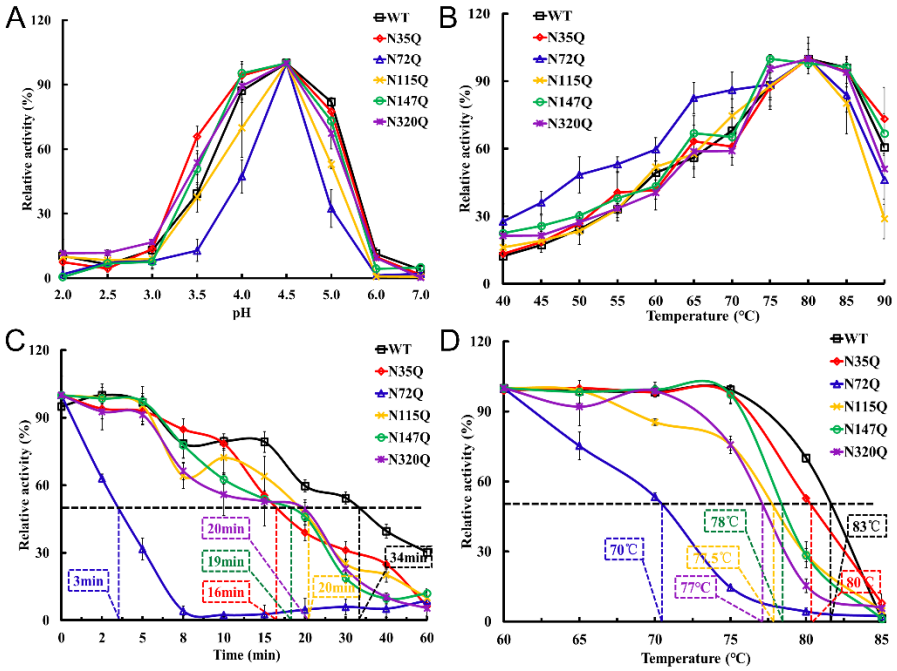

Figure 2. A: Optimum pH for 4XX6 and its mutants; B: Optimum temperature for 4XX6 and its mutants; C. $t_{1/2}$ of 4XX6 and mutants at 65 ℃; D. T_{50} values of 4XX6 and mutants.

Figure 2 shows the optimum pH, optimum temperature, $t_{1/2}$ value and T_{50} value of the 4XX6 and mutants, and it was found that the optimum temperature and pH of all mutants were the same as those of wild type, and the optimum pH range of mutant N72Q was decreased. T_{50} values showed that when the N-glycosylation sites were removed, the thermal stability of all mutants decreased. Among them, the thermal stability of mutant N72Q decreased most seriously. The experimental date of $t_{1/2}$ showed the same results.

This is the first study of the effects of N-glycosylation on thermal stability of GH10 family xylanases XYL10CΔN and 4XX6. The experimental results showed that the sugar chain at this site has an important influence on thermostability (Tian et al., 2021). This is of great help to the research on molecular modification of the GH10 family xylanases. It also has considerable potential in feed and biorefinery applications (Srivastava et al., 2015).

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