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## Loop engineering of a thermostable GH10 xylanase to improve low-temperature catalytic performance for better synergistic biomass-degrading abilities

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**Abstract:** Multi-enzyme synergistic degradation of bagasse to produce lignooligosaccharides is one of the effective ways to achieve high value utilization of sugar waste. Herein, we used directed evolution to improve xylanase catalytic performance under mesothermal condition. Three mutants (M137E, N207G and M137E/N207G) were produced in *Pichia pastoris* and biochemically characterized. Under the chosen conditions (pH 4.5 and 37 °C), all mutants towards all substrates showed highest activities of 1610, 1980, and 2130 U/mg towards beechwood, sugarcane and crocob xylan respectively, in comparison with the wild type (510, 660, and 770, respectively). The  $k_{cat}/K_m$  of three mutants were significantly improved by 2.8-fold, 2.4-fold and 4-fold respectively. Using pretreated bagasse as the substrate, the combined mutant and cellulose showed high degree of synergy and the reducing sugar production was improved by 175%. This study revealed the mechanism of xylo-oligomer blocking cellulose hydrolysis and provided a reference for high value utilization of sugar waste.

**Keywords:** GH10 xylanase; Catalytic performance; Sugar waste; Bagasse; Synergistic degradation