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Improvement of Xylanase Activity by Error-prone PCR

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Error-Prone PCR conducted on a weakly active chimeric xylanase gene APNc produced a mutation library from which mutant forms with enhanced xylanase activity were effectively identified. From 1766 mutant clones, a clone showing strong xylanase activity was selected. DNA sequencing of the clone showed that it had one amino acid substitution: E¹²⁷ V. The E127V mutant enzyme and APNc chimeric enzyme were purified by affinity chromatography using Ni-NTA agarose. The E127V displayed thermal stability similar to APNc, up to 35 °C stable. The respective optimum pH and optimum temperature of the two enzymes were observed at pH 7.2, 45 °C for E127V, and at pH 7.2, 40 °C for APNc. The pH stability profiles showed that the two enzymes retained above 80% of their original activity between pH 3.7-8.7 for APNc, and between 5.1-10.0 for E127V. The kinetic parameters of the two enzymes were measured employing the *p*-nitrophenyl- α -D-xylobioside as the substrate. The K_m values for E127V and APNc were 0.035 mM and 0.065 mM, respectively. The K_{cat} value was increased by 10-fold in the case of the mutant E127V enzyme (0.270 s⁻¹) compared with that of the chimeric APNc enzyme (0.027 s⁻¹).