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Title: Impact of the removal of N-terminal non-structured amino acids on activity and stability of xylanases from *Opinomyces* sp. PC-2

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**Impact of the removal of N-terminal non-structured amino acids on activity and stability of xylanases from *Orpinomyces* sp. PC-2**

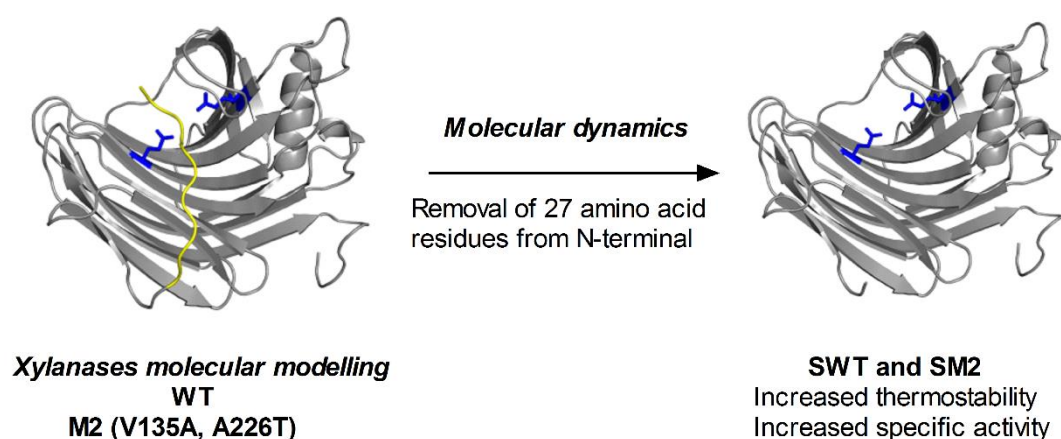
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Graphical Abstract



**Highlights**

- Molecular dynamics analyses indicated that N-terminal tail could destabilize the XynA xylanase from *Orpinomyces*.
- N-terminal tail removal positively affected the expression and activity of xylanases.
- Engineered xylanases showed high thermostability at 50 °C.

**Abstract**

Xylanases catalyze the random hydrolysis of xylan backbone from plant biomass and thus, they have application in the production of biofuels, Kraft pulps biobleaching and feed industry. Here, xylanases derived from *Orpinomyces* sp. PC-2 were engineered guided by molecular dynamics methods to obtain more thermostable enzymes. Based on these models, 27 amino acid residues from the N-terminal were predicted to reduce protein stability and the impact of this removal was validated to two enzyme constructs: small xylanase Wild-Type (SWT) obtained from Wild-Type xylanase (WT) and small xylanase Mutant (SM2) generated from M2 mutant xylanase (V135A, A226T). The tail removal promoted increase in specific activity of purified SWT and SM2, which achieved 5,801.7 and 5,106.8 U.mg<sup>-1</sup> of protein, respectively, while the WT activity was 444.1 U.mg<sup>-1</sup> of protein. WT, SWT and SM2

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