

Title: Crystal structure and functional characterization of a cold-active acetyl xylan esterase (*PbAcE*) from psychrophilic soil microbe *Paenibacillus* sp.

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Cold-active acetyl xylan esterases allow for reduced bioreactor heating costs in bioenergy production. Here, we isolated and characterized a cold-active acetyl xylan esterase (*PbAcE*) from the psychrophilic soil microbe *Paenibacillus* sp. R4. The enzyme hydrolyzes glucose penta-acetate and xylan acetate, reversibly producing acetyl xylan from xylan, and it shows higher activity at 4°C than at 25°C. We solved the crystal structure of *PbAcE* at 2.1-Å resolution to investigate its active site and the reason for its low-temperature activity. Structural analysis showed that *PbAcE* forms a hexamer with a central substrate binding tunnel, and the inter-subunit interactions are relatively weak compared with those of its mesophilic and thermophilic homologs. *PbAcE* also has a shorter loop and different residue composition in the $\beta 4$ - $\alpha 3$ and $\beta 5$ - $\alpha 4$ regions near the substrate binding site. Flexible subunit movements and different active site loop conformations may enable the strong low-temperature activity and broad substrate specificity of *PbAcE*. In addition, *PbAcE* was found to have strong activity against antibiotic compound substrates, such as cefotaxime and 7-amino cephalosporanic acid (7-ACA). In conclusion, the *PbAcE* structure and our biochemical results provide the first example of a cold-active acetyl xylan esterase and a starting template for structure-based protein engineering.

References

[1] Park SH, Yoo W, Lee CW, Jeong CS, Shin SC, Kim HW, et al. Crystal structure and functional characterization of a cold-active acetyl xylan esterase (*PbAcE*) from psychrophilic soil microbe *Paenibacillus* sp. *PLoS one* (2018)