Structural and Functional Studies of Carbohydrate Esterase Family 7 Enzymes

<u>Ivet Krastanova</u>^a, Alberto Cassetta^b, Doriano Lamba^b, ^aSISSA, Trieste, Italy. ^bIC-CNR, Trieste, Italy. E-mail: kivet@sissa.it

The Carbohydrate Esterase family 7 (CE-7, CAZy, April 2005) includes 26 bacterial oligometric α/β hydrolases with multifunctional deacetylase activity. Their primary role is the deacetylation of the decorated xylooligosaccharides that are transported into the bacterium cytoplasm. Therefore, the enzymes could be considered accessory ones in the plant cell wall biodegradation. Despite the crystal structure of two family members was determined, it is still unclear how the substrate reaches the catalytic site, and how the product is released from the oligomeric enzymatic assembly. To further characterize the CE-7 family, Bacillus pumilus acetyl xylan esterase (AXE) was expressed, purified and crystallized - alone and in complex with the reaction products xylose and acetate. The 3D structures were determined by X-ray analysis at 1.9Å and 2.6Å respectively, each one showing two doughnut-like hexamers with local 32 symmetry in the asymmetric unit. Snapshots of the enzymatic process were obtained. The identified xylose binding sites let us hypothesize a route connecting the active site to the exterior of the selfcompartmentalizing enzymatic assembly. The CE-7 family representative, *Thermoanaerobacterium sp.* AXE1, was also characterized, and its crystal structure determined at 1.9Å. As a result, new insights into the CE-7 family mechanism of action are suggested, and structural basis for their different sensitivity to the commonly used serine-modifying reagent, PMSF, are provided.

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