CHALMERS UNIVERSITY OF TECHNOLOGY

INDUSTRIAL BIOTECHNOLOGY

BIOCHEMICAL & STRUCTURAL CHARACTERIZATION OF BACTERIAL CARBOHYDRATE ESTERASE 15 (CE15) MEMBERS

Scott Mazurkewich[a], Jenny Arnling Bååth[a], Jens-Christian Navarro Poulsen[b], Rasmus Meland Knudsen[b], Lisbeth Olsson[a], Leila Lo Leggio[b] and Johan Larsbrink[a]

[a]Wallenberg Wood Science Center, Division of Industrial Biotechnology, Department of Biology and Biological Engineering, Chalmers University of Technology, SE-412 96 Gothenburg, Sweden

[b]Department of Chemistry, HC Ørsteds Institutet, Copenhagen University, Copenhagen, Denmark

BACKGROUND

Glucuronoyl esterases (GEs) are a relatively new type of enzyme which cleave an ester linkage connecting lignin to glucuronoxylan (Figure 1A). Putative GEs have been identified in many biomass degrading microbes and are now classified in the Carbohydrate Esterase 15 (CE15) family. Phylogenetic analysis of CE15 members indicates that the family has a wide degree of sequence diversity (Figure 1B). Previously, few GEs have been biochemically characterized and only three protein structures had been determined.

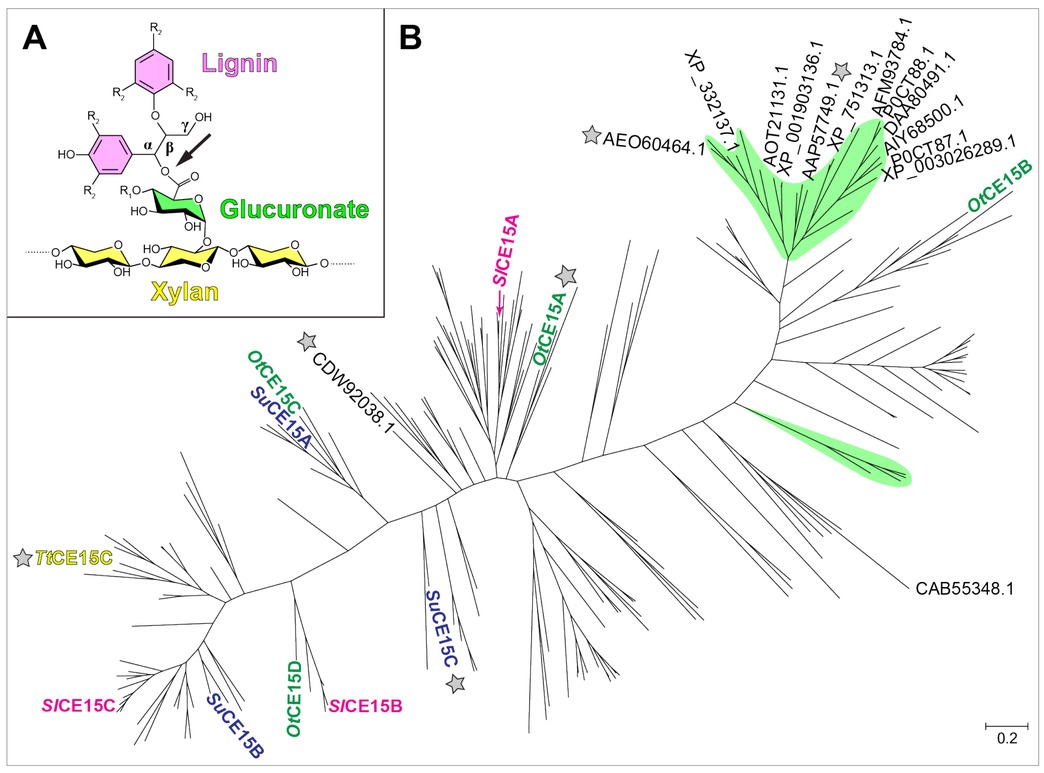

Figure 1: The glucuronoyl esterase reaction and CE15 family. *(A) General structure of LCC esters (either α- or γ- linked to glucuronic acid moieties on xylan), and site of enzymatic cleavage by glucuronoyl esterases (arrow). R1 may be either H or a methyl moiety, while R2 labels represent possible further connections to the lignin network. (B) Phylogenetic tree of all CE15 catalytic domains in CAZy. Biochemically characterized members are labelled with their respective Genbank accession numbers. Branches representing members of fungal origin are shaded in green. Stars indicate structurally determined members. Enzymes characterized in this study are labelled with their protein names, color coded in green for O. terrae, magenta for S. linguale, blue for S. usitatus, and yellow for T. turnerae.*

OBJECTIVE

Advance understanding of the CE15 family by biochemically characterizing and determining structures of bacterial CE15 proteins from across the protein family.

METHODS

Cloning, recombinant expression, and protein purification of specific targets from across the tree → **Biochemical characterization on model substrates** → **Crystallization & structure determination by X-ray diffraction**

BACTERIAL CE15 STRUCTURES

We have determined 3 bacterial CE15 protein structures (*Ot*CE15A, *Su*CE15C, and *Tt*CE15A)[1, 2]. Significantly, the bacterial CE15 members have inserted regions relative to their fungal counterparts which may modulate substrate specificity.

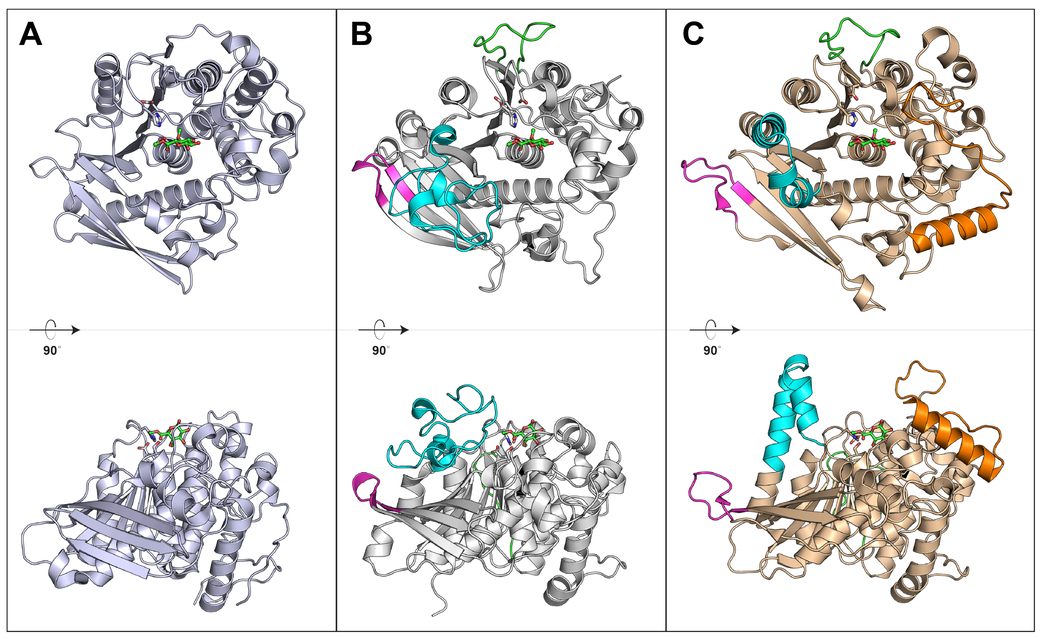

Figure 2: Comparison of protein structures across the CE15 family. *CE15 members from T. thermophila (A, StGE2, PDB: 4g4g), O. terrae (B, OtCE15A, PDB: 6gs0), and T. turnerae (C, TtCE15A, PDB: 6hsw) are shown with the methyl ester of 4-O-methyl glucuronate (green sticks) determined in the T. thermophila structure (PDB: 4g4j). The catalytic residues of each enzyme are shown in sticks. Inserted regions in the bacterial structures relative to their fungal counterparts are coloured in magenta, cyan, green, and orange.*

*Ot*CE15A COMPLEXED WITH XUX

We have been pursuing ligand complexes with our solved structures. A *Ot*CE15A structure in complex with the glucuronoxylan tetrasaccharide XUX has been determined and reveals key residues involved in xylan binding.

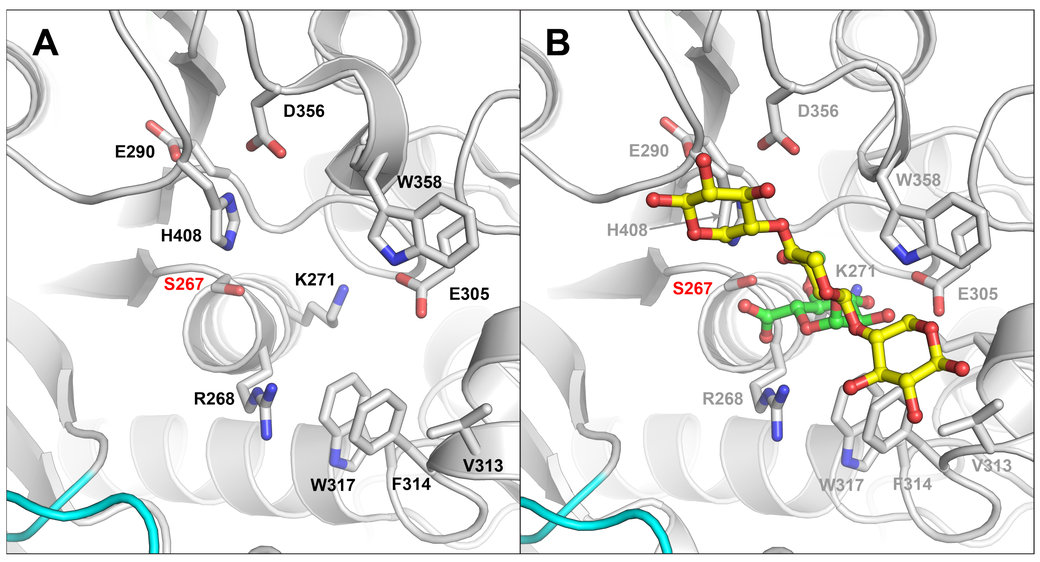

Figure 3: Active site organization of OtCE15A. *(A) The active site of the OtCE15A (PDB: 6gs0) compared with (B) enzyme in complex with the glucuronoxylan oligo XUX (unpublished structure). Key residues lining the active site pocket are shown in sticks and the catalytic serine is written in red text. The bound XUX molecule is shown in sticks and coloured as in Figure 1.*

REFERENCES

[1] Arnling Baath, J., Mazurkewich, S., *et al*. (2018). Biotechnol Biofuels **11**, 213.

[2] Arnling Baath, J., Mazurkewich, S., *et al*. (2019). JBC **294**, 6635.

Scott Mazurkewich
Post Doctoral Researcher
Department of Biology and Biological Engineering
Division of Industrial Biotechnology

CHALMERS UNIVERSITY OF TECHNOLOGY
SE-412 96 Gothenburg, Sweden
www.chalmers.se

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